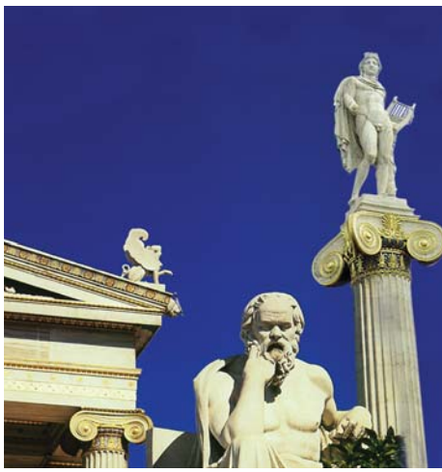
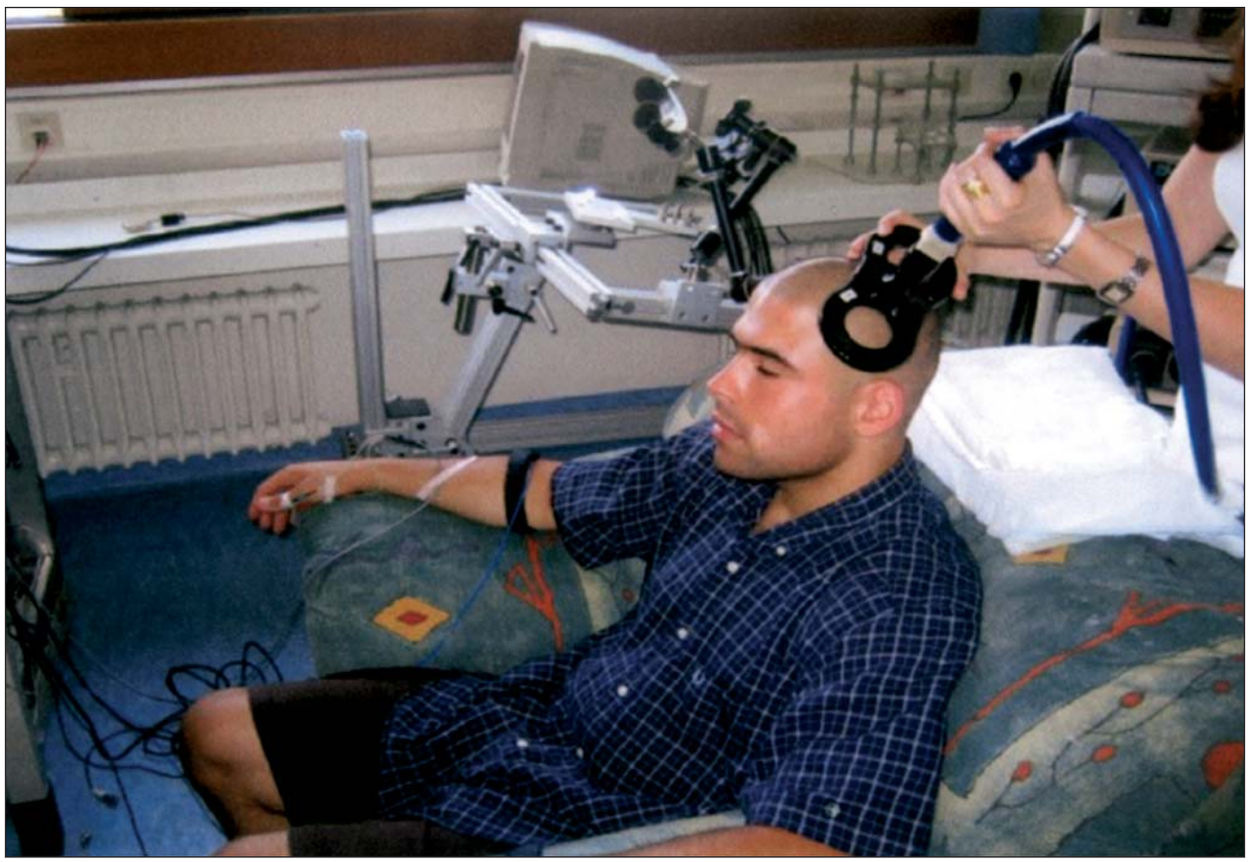


Γενετικές Έρευνες στο Πανεπιστήμιο Αθηνών

Στο εργαστήριο Εργοφυσιολογίας του Πανεπιστημίου Αθηνών συνέχισε με αδιάπτωτο ενδιαφέρον να διερευνά το θεμελιώδες πρόβλημα της κληρονομησιμότητας φυσιολογικών λειτουργιών, ιστοχημικών ιδιοτήτων, μορφολογικών γνωρισμάτων και βιολογικών ικανοτήτων. Παρατίθενται εδώ ενδεικτικά οι παρακάτω Γενετικές εργασίες οι οποίες εισάγονται με δύο Invited Editorials του περιοδικού Sports Medicine & Physical Fitness, που δείχνουν την προοπτική των σχετικών ερευνών εν όψει μάλιστα της χαρτογράφησης του ανθρωπίνου γονιδιώματος.

- EDITORIAL-1
- EDITORIAL-2
- GENES AND OLYMPIC PERFORMANCE: A CO-TWIN STUDY (2001)
- HERITABILITY IN NEUROMUSCULAR COORDINATION: IMPLICATIONS FOR MOTOR CONTROL STRATEGIES (2004)
- NATURE PREVAILS OVER NURTURE (2007)
- NATURE-NURTURE: NOT AN EITHER-OR QUESTION (2007)
- PLASTICITY OF MOTOR CORTEX IS IN PART GENETICALLY DETERMINED (2011)
- HERITABILITY OF MOTOR CONTROL AND MOTOR LEARNING (2013)
- WHY NATURE PREVAILS OVER NURTURE IN THE MAKING OF AN ELITE ATHLETE (2017).

Ιδιαίτερη μνεία αξίζει να γίνει στην καρποφόρο συνεργασία με το Πανεπιστήμιο της Ρώμης "Foro Italico", το Πανεπιστήμιο Brighton της Αγγλίας και το Πανεπιστήμιο Wursburg της Γερμανίας. Χαρακτηριστική είναι η περίπτωση που ο καθη-



γητής μνημονεύει στο σύγγραμμά του "Εργοφυσιολογία". Η Τζούλια Μισιτζή υποψήφια διδάκτορας Εργοφυσιολογίας τότε, ενδιαφέρθηκε να μελετήσει την κληρονομησιμότητα του κινητικού φλοιού του εγκεφάλου. Επειδή όμως στη χώρα μας δεν υπήρχε ο απαιτούμενος εργαστηριακός εξοπλισμός και η ανάλογη ερευνητική εμπειρία, δεν εγκατέλειψε τον υψηλό της στόχο. Εντόπισε το εξειδικευμένο εργαστήριο Λειτουργίας του Ανθρωπίνου Εγκεφάλου του Καθηγητή Joseph Classen στο Πανεπιστήμιο Wursburg της Γερμανίας, όπου όχι μετέβη και μυήθηκε στη μέθοδο διακρανικού μαγνητικού ερεθισμού (βλ. παραπάνω εικόνα), αλλά οργάνωσε και ολόκληρη επιχείρηση μεταφοράς (!) των δοκιμαζομένων διδύμων από την Ελλάδα στη Γερμανία. Σχολιάζει ο καθηγητής: *«Εκείνο που κάνει τον άνθρωπο της Επιστήμης δεν είναι η κατοχή της αλήθειας, αλλά η επίμονη αναζήτησή της».*

Η εν λόγω πρωτοποριακή εργασία της Μισιτζή δημοσιεύτηκε με ιδιαίτερο σχολιασμό στο έγκριτο περιοδικό Journal of Physiology, ενώ απέσπασε το πρώτο βραβείο σε Ευρωπαϊκό Συνέδριο Νευρολογίας.

Editorial-1
Journal of Sports Medicine & Physical Fitness 37:1, 1997

Heritability of Adaptive Variation: An Old Problem Revisited

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The study of human diversity using twins has a long history. In an explicit statement, based largely on comparisons of the two types of twins in 1876, Francis Galton[1] said that "there is no escape from the conclusion that nature prevails enormously over nurture". However, it was not until 50 years later that the twin model was first applied.[2] This model makes use of monozygotic (MZ) and dizygotic (DZ) twins. MZ twins have identical heredity and therefore any intrapair difference in a trait must be due exclusively to environmental influences, while DZ twins share on the average half of their genes like ordinary siblings and any difference observed between them can be attributed to either genes or environment of both. From comparisons of intrapair differences between MZ and DZ twins, it is possible to separate the relative contribution of genotype and environment for any attribute by deriving a coefficient of Heritability. Heritability (h^2) is defined as the proportion of phenotypic variance attributable to observed individual differences in actualized genetic potential and its proximity to unity signifies the relative share of the genotype, i.e., the closer the h^2 is to unity the stronger the assumed genetic influence.

This twin model was put to use in 1971 to determine the Heritability of adaptive variation.[3] The focus was on the genetic origin of individual differences observed in physiological responses related to O_2 transport and utilization during maximal muscular effort. The intrapair correlation for VO_2max was 0.91 in MZ vs 0.44 in DZ twins, respectively and the Heritability estimate was 93.4%. On this evidence it was concluded that the variation observed in maximal aerobic power is almost entirely due to the variety of genotypes which exist in the individuals. It should be noted that 25 pairs of preadolescent twin boys (15 MZ and 10 DZ pairs) were used purposely in the study in order to ensure that environmental influences were similar for both types of twins and, thus the fundamental assumption of environmental comparability, on which the twin method is based, was satisfied. It could be argued, however, that DZ pairs would be under more diverse environmental influences than MZ pairs, during the developmental period. Thus, a follow-up study was conducted to determine whether the small intrapair differences observed bet-

This Editorial is based on invited lecture presented at the First annual congress of the European College of Sport Science (Nice, May 27-31, 1996).

ween identical twins and the marked differences between fraternal twins persist throughout life.[4] It was reasoned that, in twins exposed to similar environments at different stages in their lives any differences between DZ as compared to MZ might be an expression of the relative strength of the genotype. On the contrary, in those exposed to contrasting environments the resulting differences could provide a measure of the responsiveness to environmental forces. Thirty-nine pairs of twins (23 MZ &16 DZ of both sexes), ranging in age from 9 to 52 years, were used as subjects in this study. The mean intrapair difference between twin pairs was significant for dizygotic twins, but not for monozygotic. These observations gave further sup-port to the hypothesis of the preponderance of the genetic effect on the phenotypic variation in maximal aerobic power and strengthened the Galtonian notion that "natural tendency inevitably asserts itself.

In a more recent twin study Fagard et al.[5] measured the peak O_2 uptake in 48 pairs of male twins (29 MZ & 19 DZ) aged 18-31 years. They reported a Heritability coefficient of 80% for peak O_2 uptake in ml $\cdot$ min^{-1} $\cdot$ kg^{-1} of mass which was reduced to 74% when adjusted for body weight, skinfold thickness and sport participation. The data were also submitted to path analysis which provides for additive genetic variance and variance attributable to nonshared and shared environmental effects. After adjustment for anthropometric characteristics and actual sport activity, the Heritability estimate of peak O_2 uptake was 66%, while 34% of the variance was attributable to non-shared environmental factors.

In sharp contrast with these results is the work of Bouchard and coworkers[6] who reported a much lower h^2 for maximal aerobic power. They measured 27 pairs of brothers, 33 pairs of DZ twins and 53 pairs of MZ twins of both sexes, aged 16 to 34 years. Heritability reached 47% for VO_2max per kg of mass, but only 17% for VO_2max per kg of fat-free mass. The intraclass correlation for MZ twins was 0.70 while for DZ twins and brothers it was 0.51 and 0.41, respectively. Considering the higher correlation found in DZ twins in comparison to the brothers the authors hypothesized that the 47% estimate was inflated by shared environmental conditions, and that the true Heritability of VO_2max per kg of mass was more likely to be about 25% of the adjusted phenotypic variation. This hypothetical estimate of genetic effect for VO_2max has been accepted by Bouchard as a true value and is reported since widely in the literature.[7]

In order to support the contention that environmental influences are stronger than genetic ones in the phenotypic variation of VO_2max, some twin studies are often cited in which the foremost assumption of equal environments is admittedly not respected. A striking example is the twin study of Howald[8] who found in his small sample no inheritance component involved in the phenotypic variation of VO_2max. However, when he excluded from the analysis of his data two pairs of MZ twins, who had been exposed to contrasting environments, the ge-

netic variance reached 68%. Hence, the results of these studies have to be viewed with caution.

A significant genetic variance has also been assessed for aerobic capacity on the basis of either the total work output during a non-stop 90 min maximal ergocycle test, or the lactacid anaerobic threshold. Using the former method of assessment Bouchard et αl.[6] found intraclass coefficients of 0.82 and 0.45 for MZ and DZ twins, respectively, and a h^2 of 72%, but could not reconcile the wide discrepancy with the VO_2max data. These findings concur with those obtained recently in our laboratory where the anaerobic threshold, defined as the running speed on the treadmill corresponding to a blood lactate concentration of 4 mmol·I, was deter-mined in MZ and DZ twins. The resemblance in the two types of twins was reflected in the intraclass coefficients which were respectively 0.83 and 0.54, as well as in the h^2 which was 80% (Table I). Taken together these studies converge on the conclusion that not only genetic influences are significant, but they are also substantial, acco-

TABLE I. Heritability estimates of various biological attibutes computed by the following formulae[2] [: Clark (σ^2_{DZ}-σ^2_{mZ}/σ^2_{DZ}). Newman (σ_{MZ}-σ_{DZ}/σ_{DZ}) and Falconer 2(σ_{MZ}-σ_{DZ}). Consistent h^2 are obtained with the former two formulae. Computations were done only if the difference between means (t′-test) and total variance (F′-test) of both types of twins was nonsignificant and the difference in genetic variance between twin types (F - test) significant[10]. (Klissouras and colleagues, unpublished observations).

Biological attribute	Hipotheses tested			Heritability estimate h^2		
	t'-test	F'-test	F-test	Clark	Newman	Falconer
Maximal anaerobic power (watt)	1.96	1.25	7.21***	0.86	0.83	0.38
Anaerobic capacity (watt in 30")	0.24	1.63	6.91***	0.86	0.76	0.31
Fatigue (%)	0.80	1.04	1.56 3.92*			
Peak blood lactate (mmol.l)	0.81	1.14	7.43***	0.74	0.71	0.99
V0 2max (l.min)	0.20	1.02	5.47***	0.87	0.87	0.59
V0 2max (ml.min.kg-)	1.21	1.10	4.07**	0.82	0.83	0.70
VO 2max (ml.min.kg LBW-)	0.69	1.17	3.82*	0.75	0.79	0.62
VE(1.min-)	0.16	1.03	10.85***	0.74	0.73	0.92
Maximal heart rate (bt.min)-	0.70	2.51	8.46***	0.91	0.77	0.62
Maximal 02pulse (ml.bt-)	0.04	1.13	4.94**	0.88	0.90	0.77
Anaerobic threshold (AT) (km.h-	1.07	1.86	3.35*	0.80	0.62	0.55
Anaerobic threshold (ml.min.kg-)	0.45	1.36		0.70	0.59	0.75
Running economy (below AT):			2.609*			
VO2 (ml.min.kg-)	1.10	1.30	3.00*	0.61	0.50	0.44
Gross V0 2(ml.kg-)	1.26	1.26		0.67	0.58	0.61
Running economy (above AT):			5.42**			
VO2 (ml.min.kg-)	1.31	1.26	4.23**	0.82	0.85	0.44
Gross V02(ml.kg-)	1.76	1.15	10.45***	0.76	0.80	0.09
Endomorphy	1.61	1.15	8.48***	0.90	0.89	1.51
Mesomorphy	0.48	1.57	15.00***	0.88	0.81	0.64
Ectomorphy	0.33	1.25		0.93	0.92	1.00

*)p<005;**) p<001;***)p<0001

unting for the most part of individual differences in maximal aerobic power and capacity.

The estimates of Heritability for the major determinants of the O_2 transport and O^2 utilization systems vary considerably at rest and during exercise. The genetic effect on heart structures and function, such as ventricular diameters, wall thickness, functional shortening, maximal heart rate and O_2 pulse ranged in various studies from nonsignificant to a h^2 level exceeding 85% of the phenotypic variance,[9-11] while the effect on cardiac output remains unknown. Regarding the O_2 utilization system, no genetic effect could be detected for the mitochondrial volume and density, while a low effect was found for maximal activity of key regulatory enzymes of glycogen breakdown and substrate oxidation.[8, 12-14]

Data available from a handful of twin studies have yielded widely divergent Heritability estimates of the phenotypic variance in histochemical, morphological and biochemical characteristics of human skeletal muscle.

These estimates range almost from zero to 100%. Komi and associates[12] took muscle biopsies from the vastus lateralis of 31 twin pairs (15 MZ & 16 DZ) of both sexes. They reported a Heritability coefficient for the proportion of type I fibers of 96% suggesting that the variation in muscle fiber distribution is almost exclusively genotype-dependent. A similar study was conducted by Bouchard and coworkers[13] using a larger sample of 35 pairs of MZ twins, 26 pairs of DZ twins and 32 pairs of brothers. The intraclass correlation for the percentage of type I fibers was about the same in MZ & DZ twins (0.55 and 0.52 respectively) and much lower in brothers (0.33). A Heritability coefficient of 6% could be computed from the data although such analysis has no meaning, since the intrapair variance between MZ & DZ twins was non-significant.[19] In spite of this finding in a very recent review of genetic determinism of fiber type proportion in human skeletal muscle, the same authors[14] suggested that from the total phenotypic variance about 15% could be explained by the error of measurement, 40% could be due to environmental factors and the remaining 45% could be attributed to genetic variance. However, this partition is purely inferential, if not speculative, and is based mostly on training studies of non-twins where the influence of the genetic factor cannot be assessed. The review of such training studies in humans and small mammals shows conflicting results.[14,15] Some demonstrated that the proportion of muscle fibers in humans is not altered in response to training and chronic electrical stimulation, while other studies observed an alteration in response to training, detraining and immobilization. Further, it seems that there is an interconversion of type IIa and IIb muscle fibers in humans in response to training as well as an interconversion of type II to type I muscle fibers in small mammals in response to increased muscular contractile activity. These results are often used eclectically to support the notion of the relative powers of either nature or nurture, genes or environment. However, genetic dependence

does not exclude environmental influences. Genetic dependence of muscle fibers does not necessarily

The h^2 has often been misinterpreted. A value of 96% found for muscle fiber distribution for example, is often interpreted to mean that 96% of an individual's type I muscle fibers is genetically determined and the remaining 4% is susceptible to environmental modification. However, this is a fallacy, since the h^2 has no etiologic role in the phenotype, nor has it sensible meaning with reference to measurement in an individual. It refers only to the population and is an estimate of the extent to which heredity affects the variation of a given attribute in a given population exposed to common environmental influences at a given time. It is also fallacious to identify the concept of Heritability with determinism. A highly heritable attribute does not mean that it is predetermined and the environment has no effect. No genes can operate in a vacuum, nor phenotypes can develop and be actualized without the action of environmental forces. Thus, when it' is stated that VO_2max is highly heritable, what is really meant is that after individuals have reached the upper limits of their VO_2max, with appropriate training, there will still be a wide interindividual variability which is genetic in origin. The levels of the absolute individual ceilings is a reflection of the actualized genetic potential of these individuals. Those with a strong genotype will fall in the extreme upper part of the normal distribution curve. Apparently training does contribute significantly to the development of VO_2max, but cannot contribute beyond a ceiling set by the genotype.[16] Superior performers in aerobic sports are endowed with a high genetic potential for VO_2max. However, this genetic potential is not a passive possibility but an active disposition realized through man's prodigious effort. The realization of the genetic potential does not occur instantly. As Bronfenbrennen and Ceci [17] eloquently put it "this dynamic potential does not spring forth full-blown like Athena out of Zeus's head from a single blow of Vulcan's hammer. The process of transforming genotypes into phenotypes is not so simple or so quick".

The twin model has often been subject to criticism due to the biases of ascertainment. There are three sources of such biases. The first refers to mis-classification of zygosity. Since the twin model is based on comparisons between the two types of twins, it is of outmost importance that twins are classified as MZ or DZ with precision. Physical similarity and characteristics are used as a first approximation of zygosity determination with an accuracy of about 90%, while genetic markers analysis increases the accuracy of diagnosis to more than 95%. A new molecular genetic method involves a probe for a tandem repeat region of DNA and is an excellent test of zygosity, since only MZ twins have exactly the same DNA "fingerprints".[18] Errors in diagnosis of zygosity will lower the h^2, because they are linely to lower the MZ correlation and increase the correlation of DZ twins. Another potential source of bias is related to the estimation of genetic variance.[19] Computa-

tions of h^2 should be carried out only if the difference in genetic variance between the twin types (F-test) is significant and the difference between means ("t"-test) and total variance ("t"-test) of both types of twins non-significant (Table I). The third bias refers to representativeness. If twins are different in means and variances from the population, results might not completely apply to the population at large. Indeed, twins are 3 to 4 weeks premature compared to singletons, 30% lighter and 17% shorter at birth, while there are differences for MZ twins in intrauterine position and blood supply to the embryo.18 However, these prenatal and early postnatal differences are not enduring and are progressively equalized under the influence of a maturational pacemaker and disappear by middle childhood.[20]

The validity of any Heritability estimate depends upon the acceptability of the underlying assumptions.[21] Four fundamental assumptions are necessarily made in the derivation of a h^2. It is assumed that a) environmental influences are comparable for both types of twins, b) no correlation exists between spouses due to assortative mating, c) genetic and environmental influences are not correlated, and d) genetic variance shows no dominance or interaction effects. Environmental comparability is tenable if special control is made for all confounding factors, such as gender, age, maturation, socioeconomic status, health condition and sport participation. This does not mean that the environmental influences are kept constant, but that they vary approximately in the same direction and to the same degree for all twins. Ideally, these environmental influences should act maximally on all twins under study, so that their genetic potential is fully actualized and a true measure of h^2 is obtained. Otherwise, any amount of unactualized potential remains unknown and the value of h^2 is limited. In this respect, twin athletes are ideal subjects for the evaluation of the relative powers of genes and environment.[22] Regarding the second assumption, if there is an assortative mating effect and it is not considered, it would underestimate the genetic influences, since such an effect will increase the resemblance between DZ twins and the families variance. However, it is doubtful whether biological criteria are used to any appreciable extent in mating; e.g., correlation coefficient between spouses is 0.30 for height and 0.18 for VO_2max.[23] The assumption that genetic and environmental influences are not correlated may be only partially true. Parents most likely give gifted children special opportunity to practice and provide them an environment conducive to the development of their propensities and dispositions, although most variation (88%) in sport activities participation is attributed to nontransmissible environmental factors with no genetic effect.[24] Finally, the additive model used to compute h^2 assumes that there is no interaction effect between genotype and environment. It is quite probable that this simple model may not be adequate to explain the observed intrapair variance of DZ twins, and that it should be modified to include an additional term (σ^2ge), signifying the mutual interaction between genotype and environment.

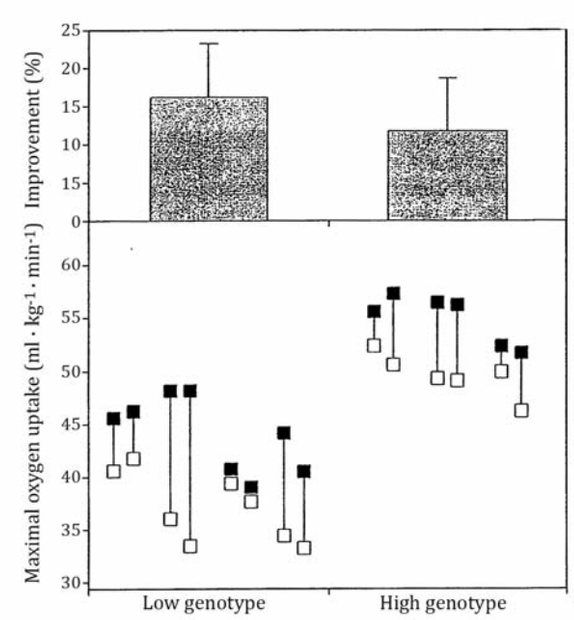

FIG. I. Training change in VO_2max expressed in O_2 ml · min^{-1} · kg^{-1} (lower panel) and in percent improvement in pairs of MZ twins with a low (<40) and a high (>50) pretraining phenotypic level in VO_2max. Open and closed squares represent values before and after training for each individual twin (Based on the original data of Prud' Homme et a/.).[26]

However, based on present evidence it is equivocal and most unlikely that a genotype-environment interaction takes place to any marked degree in adaptive variation. Analysis of variance of the results obtained for VO_2max from a co-twin study,[25] where one twin trained aerobically and his identical brother acted as a control, revealed that from the total intrapair variance in VO_2max 51% was due to genotype, 42% to training and only 7% due to the interaction between genotype and training. Using a different experimental approach Prud'Homme et al.[26] submitted both members of each pair of MZ twins to endurance training and observed a close intrapair resemblance in the magnitude of training change in VO_2max (r=0.74), concluding that the sensitivity of VO_2max to training is largely genotype-dependent. A comparable intra-class coefficient (r=0.65) was obtained from a similar co-twin study[27] but the twin resemblance in the magnitude of training change in VO_2max was not significant after 7 weeks of training and reached almost the significance level after 15 weeks.

Yet, in another co-twin training study conducted in the same laboratory, the intraclass coefficient of the twin resemblance was considerably lower (r=0.44).[28] Further, the intraclass coefficient in the Prud'Homme et al.26 study could be dramatically reduced to almost half of the reported value (0.33 from 0.74), if the two extreme cases of MZ twin pairs are not considered in the computation, on the grounds that the marked response in one case and the nonresponse in the other may be associated with the pretraining phenotypic level.[29] Further and most important, reanalysis of the data of this study by separating the twin pairs into two categories on the basis of pretraining phenotype in VO_2max shows that, the magnitude of trainingchange is almost the same for both groups of low and high genotype (Fig. 1).

Moreover, the absence of the genotype dependency of the training response has also been reported for key enzyme activity and fiber type composition of human skeletal muscle.[30] In short, scrutinization of the available evidence does not support the contention that trainability of VO_2max is genotype-dependent, nor does it warrant the inference that superior athletes are more sensitive to training.

It is clear that there is still much work to be done to broaden our understanding on the interplay of genes and environment in determining adaptive variation in man. Future research will undoubtedly face the challenge of the marvelous advancement of molecular biology and apply the new methodologies of measured-genotype approach for gene mapping and analysis of DNA sequence, for specifying the polymorphic genes accounting for the Heritability of phenotypic variance. Yet, before we go far beneath the surface of the biological adaptations and penetrate into the secret of their origin searching the underlying mechanisms which govern them, we need to resolve present uncertainties as to how much heritable and trainable these adaptations are. To this end, classic methods of quantitative genetics (twins, nuclear families, adoptees, pedigrees) used with sophisticated and rigorous experimental designs (path analysis, multivariate models) would be most valuable. Twins and particularly twin athletes, provide the basis for a general theory of the etiology of individual differences. They offer a unique and powerful method of addressing the question of genetic causation or non-genetic transmission, thus enabling us to refute or falsify, in a Popperian sense, the null-hypothesis that genetic factors do not explain the variability in biological traits and adaptation to muscular effort in man.

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Editorial-2
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Genetic Limits of Sport Performance: Quo Vadis?

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Olympic athletes are characterized by several bio-logical and behavioral abilities and traits, all integrated in a complex system. The key of understanding the limits of Olympic-level sport performance, is understanding the two major sources of individual differences in those abilities and traits; namely, genes and environment.

In this respect, a number of twin studies have been conducted over the past few years, to elucidate the genetic effect on the variation observed in several phenotypes linked to sport performance, such as functional abilities, morphological components, muscle composition, motor attributes and behavioral traits.[1] The twin model used in these studies makes use of monozygotic (MZ) and dizygotic (DZ) twins. MZ have identical heredity and any intrapair difference is due to environmental influence, while DZ share half of their genes and any intrapair difference may be attributed to both genes and environment. From comparisons of intrapair differences between MZ and DZ twins, we derive heritability estimates, which are a measure of the relative contribution of the genotype to individual differences observed. The closer the heritability estimate to unity, the stronger is the genetic influence.[2,3] Heritabilities have been reported in the range of 47% to 93% for maximal aerobic power,[2-6] 70% to 99% for maximal anaerobic power and capacity,[7-9] 66% to 97% for maximal muscular force and power,7·8.10 69% to 98% for somatic dimensions and maturation,[11-13] 66% to 87% for motor skill acquisition and neuromuscular coordination,[14,15] 40% to 71% for personality traits and cognitive abilities,[16] while for muscle fiber composition the heritability estimate approaches unity.[17]

All these twins studies converge on the conclusion that not only genetic influences are significant, but they are also substantial, accounting for individual differences in most performance phenotypes.

However, the aforesaid studies have addressed actually the etiology of individual

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differences in various phenotypes related to sport performance in the normal range of the bell curve. Data in the high end of the distribution are lacking. The studies cited have used twins who have been exposed to normal environmental influences and the heritabilities obtained denote the etiology of differences in the relevant phenotype, among individuals in the normal range; they express the genetic and environmental provenance of measured differences among individuals as they exist in a particular population. Generability of findings to top level athletes who represent the high end of the distribution is problematic.

The need to study twin athletes who have under-gone years of strenuous training and have actualized their genetic potential is thus apparent. Based on their intrapair differences we could compute "group herilabilities" of high ability in phenotypes linked to Olympic performance in various sports disciplines. Group heritability, in contrast to traditional heritability statistic, is the genetic contribution of the average difference between a selected group and the rest of the population. It could be assessed by the method of " extreme analysis", as the differential regression oi the population mean of monozygotic and dizygotic oi co-twins, on the basis of performance phenotypes, as illustrated in Figure l.[1,I8]

Olympic twin athletes constitute a powerful "experiment in nature" and can provide invaluable information on how far and hard we can push ourselves, on both an individual basis and as a species. In particulai it is envisaged to provide insights into the following fundamental questions, first raised years ago but remain both unanswered and relevant today.

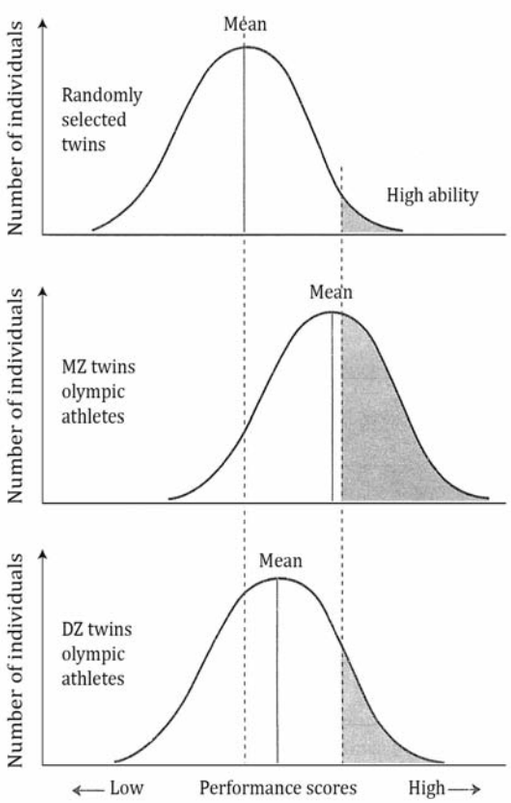

FIG 1. Performance distributions for an unselected sample of twins and Olympic athletes monozygotic (MZ) and dizygotic (DZ) co-twins. The top distribution is an idealized normal distribution for an unselected sample of twins. Individuals of high ability, are defined as those with a performance score of a predetermined standard deviation, above the sample mean of 0.0. The two distributions below are those for Olympic athletes MZ and DZ co-twins. In the event mean regresses less far towards the mean of the unselected population than does the DZ co-twin mean, it suggests heritability of high peak performance 1.18.

Genotype - training interaction

It has been postulated that in addition to superior genotypes, athletes of Olympic caliber most probably also have inherited the genotype characteristic of high response to training.[19] This contention was initially based on the wide interindividual variability observed in VO_2max of previously sedentary humans exposed to endurance training and subsequently supported by some limited evidence obtained by co-twin studies and a familial aggregation response.[20]

TABLE I. Analysis of variance in VO_{2max}ml · min^{-1} · kg^{-1}. Estimates of variance, in actual figures, were computed in the following way (n=number of twin pairs): Heredity=(mean sq. heredity - mean sq. interaction)/2; Training=(mean sq. training - mean sq. interae-tion)/n. Eight male pairs of MZ twins aged 10-16 years participated in the 1976 study where one twin in each pair trained for 10 weeks; while nine male pairs of MX twins aged 11-14 years participated in the 2003 study where one twin in each pair trained for 24 weeks.

Sources of variations	Weber, Kartodihardjo and Klissouras, 1976		Danis, Kyriazis and Klissouras, 1976	
	Mean squares	Variance in % of total variance	Mean squares	Variance in % of total variance
Training	221.72	42	278.48	37
Heredity	69.04	51	43.55	46
Interaction	4.39	7	16.60	17

Findings from a handful of other studies have cast serious doubt on the proposition that there is a genotype-environment interaction in VO_2max and other phenotypes related to sport performance. Using a different experimental approach Klissouras and associates were unable to find that trainability of VO_2max is genotype-dependent.[21,22] Split-twin experiments, in which one twin trains and his identical partner acts as a control, make it possible to separate the observed intra-pair variance into its three components: that due to heredity, that due to training and that due to the interaction between heredity and training. Treatment of the results by analysis of variance revealed that the interaction between genotype and training contributed only 7% of the total variance in one study and 17% in the other (Table I).

It seems that there is also a nil or minor genotype-training interaction in muscle strength and muscle hypertrophy after resistance training,[23] as well as in key enzyme activity and fiber type composition of human muscle after intermittent training.[9]

Given these results, it remains uncertain whether the heterogeneity in trainability of some basic performance phenotypes is genotype-dependent and forces us to rethink a long held belief. In addition, and equally important, findings from these studies can hardly be applied to athletes. The reason is that previously sedentary humans were used and hence the focus had been centered on the etiology of individual differences in the normal range of the distribution curve.

Genotypes set a limit to phenotypes

The use of cross-sectional and longitudinal studies in disentangling this hypothesis, has the obvious limitation that the genetic factor is operant to an unknown degree in different individuals. Using monozygotic twins as subjects, however, obviates this problem since each subject is accompanied by a genotypically identical control. It is reasoned, that if athletic training, confined to one twin and extended over a period of years, fails to raise his functional capacity from a low to a superior level, then its upper limit might be assumed to be set by his genotype.

In an early co-twin study the trained twin was unable to surpass an average level of VO_2max, despite hard and prolonged training. The reason for this seemed

to hinge on his low pretraining VO_2max, as judged from that of his identical counterpart.[24] This observation suggested at the time that vigorous athletic training cannot contribute to functional adaptability beyond a limit set by the genotype. However, this conclusion based on limited data from only a pair of twins with apparently low genetic potential can hardly be generalized and applied to superior performance.

Beyond the ken of physiology

The difference between an Olympic winner and a non winner may not lie entirely in their physiological functions, histochemical quantities and morphological dimensions. It may be as Bannister phrased it, that: "psychological and other factors beyond the ken of physiology set the razor's edge of defeat or victory and determine how closely the athlete approaches the absolute limits of performance".[25]

A co-twin study of Olympic twin athletes in 20-km walking race is revealing.[26] The two twins are genetically identical, have been exposed for some 20 years to the same training and the same coach (actually, their own brother), and have both reached top-level athletic performance. Yet, one has been three times an Olympic winner while the other won a World Championship only when his co-twin was not participating.

When one looks at various biological variables during maximal effort one finds them to be practically overlapping in the two twins. A substantial difference, however, is found in their personality profile, with special respect to the experience and expression of anger.[26]

The Olympic winner had an exaggerated response to frustration and showed excessive sensitivity to criticism and negative evaluations, as well as excessive control over his emotions and behavior, while his anger was never openly expressed. The emotional reactions of his brother were, however, at the opposite extreme: he was not frustrated, was insensitive to criticism and only moderately able to control his anger through the cognitive elaboration of his frustrations. It seems likely that this major and basically only difference between the twins may be responsible for their difference in performance, and one could reason that the unexpressed anger in the champion may have enhanced his competitive drive and his autonomic function. It could also be inferred that such a drive may explain his better tolerance to acidosis during heavy exercise.

At any rate, this fairly unique example of performance difference in otherwise identical twins shows that along with genetic predisposition and appropriate training, a major role in top level performance is presumably played by personality traits.

Gene hunting

Group heritabilities estimated for Olympic twin athletes, as illustrated in Figure

1, could also pave the way for identification of human gene polymorphisms associated with high ability phenotypes. For multi-factorial phenotypes, such as VO_2max, the goal is not to find the single major gene but the polygenes that contribute to their variance. Several genes have been identified as putative factors.[27,28] However, given that there are 32 000 human genes, the task of identifying multiple polymorphisms that contribute to the variation observed in Olympic-level athletic performance is daunting. Indeed, there is a long way to go before we begin to understand which genes and pathways are contributing to human variation in sport performance.

What next?

It is clear that there is a paucity of data regarding the genetic limits of sport performance, and that the results derived from the very few twin studies conducted up to the present, almost exclusively in children and sedentary individuals, are variable, providing inconclusive evidence. This call for the need to rely on data stemming from elite twin athletes, whose organism has been challenged maximally with chronic overloads and their individual phenotypes could be influenced by factors affecting gene expression.

Clearly, all functional capacities and physiological processes in man, as in all other species, must have a genetically determined ceiling. Additionally, we find ceilings characteristics of individual genotypes at different levels, and the question arises as to what extent physical training can raise an individual's capacity above a certain level, towards the species maximum value: a value characterizing Olympic performance. A definitive and clear answer to this fundamental question could come from the comparison of intrapair differences in identical and nonidentical twin elite athletes, who have undergone years of heavy physical training.

This is the focus of an on-going international project that has been undertaken with a European Olympic Committee (EOC) Medical and Scientific Commission grant and with a large collaborative effort, involving research groups in different countries. The initial stage of the project aims at ascertainment, i.e. identification and direct contacts of top-level twin athletes in the various sports, attribution of their status and recruitment to the study. The second stage involves planning and direct testing of the twins, including zygosity determination by DNA analysis, assessment of environ-mental influences, training, competition and performance profile, as well as measurements on a wide spectrum of behavioral and biological polygenic factors, such as: neuromuscular function, histochemical properties and metabolic activity of muscle, cardiorespiratory responses to exercise, echocardiography, psychological characteristics and cortical function using non-invasive electrophysiological and imaging techniques, which have made investigations of the intact human brain possible.[29] A case report offers an example of the relevance and extent

of the procedures involved, while giving an idea of the far reaching implications of the systematic and comprehensive evaluation of Olympic twin athletes.[26]

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Genes & Olympic Performance: A Co-Twin Study

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An Olympic gold medalist in a 20 km competitive walking race and his identical twin brother, also an Olympic athlete in the same event but with inferior performance, were tested in order to obtain some further insight into the relative importance of genetic factors in modulating athletic excellence. Both twins had undergone the same strenuous, long-term training for 19 years since the age of 15 under the guidance of the same coach. An assessment of their bio-behavioural profiles at 40 years of age, i.e. 7 years after they ceased training, revealed that intrapair differences were negligible in physiological attributes but divergent in personality traits measured. Respective values for the Olympic winner and his identical counterpart were as follows: Body mass index 23.2 and 22.7, cardiac mass index 85.4 and 84.4 $g \cdot m^2$, squat jumping 25.3 and 27.3 cm, VO_2 at running speed 9 $km \cdot h^{-1}$ 33.1 and 33.6 $ml \cdot kg^{-1} \cdot min^{-1}$, VO_2 max 57.1 and 58.6 $ml \cdot kg^{-1} \cdot min^{-1}$ (72.5 $ml \cdot kg^{-1} \cdot min^{-1}$ for the Olympic winner at age 22yrs), reaction to anger 97 and 9 and anger expression 2 and 76 in percentile of the State-Trait Anger Expression Inventory. Findings suggest that although genetic constitution and years of physical training are prerequisites for making an Olympic athlete, success may be largely influenced by personality traits.

Key words: Genes, Olympic performance, physical training, personality traits, twins.

Introduction

Many attempts have been made to shed light upon the relative powers of genes and environment in making an Olympic champion, and various approaches have been used: cross-sectional or longitudinal studies, and twin studies.

Cross-sectional studies have shown that peak human performance represents several independent biological and behavioural traits integrated in a complex system; we know from such studies that the physiologic limit to human performance is linked to a multiplicity of bodily functions, processes and adaptations, such as

biophysical disposition of energy output, function of the neuromuscular apparatus, somatic traits and morphological characteristics [5-9,16,18,21,31,38-40]. Moreover, longitudinal studies reveal that appropriate training elicits chronic adaptation to functional, morphological and metabolic processes, and other determinants of superior sport performance [11,15,19,20,35].

These have the obvious limitation that the genetic factor is operant to an unknown degree in different individuals; and hence twin studies can be of help [14,32]. In the classical twin design, the heritability coefficient, expressing the amount of phenotypic variance due to genetic differences, is derived by comparing the similarity of monozygotic (MZ) to that of dizygotic (DZ) twin partners (any difference between MZ twins being attributed to environmental influence, with DZ twins serving as controls). A very effective experimental approach is the so-called co-twin control study, in which the actual adaptability or trainability is tested by exposing identical twin partners to different environmental influences.

Using the classical twin method, most investigators [13, 22, 24,25,26,27, 29,30,42], but not all [3,4], found high heritability estimates for several biological attributes associated with peak sport performance, such as VO_2 max maximal anaerobic power, muscle fiber distribution, maximal contractile force, speed of muscle contraction, metabolic capacity, and somatic structure. However, these findings do not necessarily mean that top performance is genetically determined. First, the twins used in these studies were generally in the normal range of the spectrum and their data cannot be construed to apply to individuals at the upper end of the distribution. These twins were usually not athletes, and the data reported are far from reflecting individual ceilings and actualized genetic potential. Moreover, a high heritability does not imply that the relative attribute is unaltered, fixed and predetermined and that environment has no effect, since genes do not operate in a vacuum, nor can phenotypes be actualized without the action of environmental forces. That the actualization of a phenotype does not occur instantly, but may rather take several years, is a well established fact [12,43].

In this context, we present a case report, resulting from a larger epidemiological study [33], that, because of its uniqueness and experimental nature, may prove particularly enlightening. Our study refers to a pair of Olympic twin athletes in 20 km competitive walking race, who, although genetically identical and exposed to the same environmental influences and the same training with the same coach, were markedly different in performance.

Methods

Subjects

Subjects were two 40-year-old identical twin brothers, who underwent strenuous

athletic training for 19 years to become outstanding competitors in 20 km walking race and one of them Olympic medal winner in three successive Olympiads. Their zygosity was established through direct observation of relevant anthropological markers as well as by DNA fingerprinting. As is known, this method consists of the comparison of a number of DNA regions (markers) known to be highly variable in the general population, and of assessing the probability that these would be identical in the two subjects if they were unrelated. Whereas 5 to 10 such markers are usually enough for identification purposes, as many as 21 were analyzed in our twins. The molecular analysis shows the twins to be identical for each of these 21 DNA markers, the biological compatibility between the two being assessed at 99.9998%. The twins can therefore be considered to be MZ, the probability of dizyg-osity being lower than $2 \cdot 10^{-6}$.

Training, competition and performance profile

Both twins had highly trained during adolescence (from age 15 to 18) for 10 km competitive walking, and thereafter (19 to 33) for 20 km, under the coaching of their older brother with an identical training programme. Table 1 shows the training, competition and performance profile of the twins from the age of 19 to 33. More generally, living style and related variables were very similar in the two twins, who have been living together from the time of their birth.

At the age of 33 years, 7 years before the present measurements were carried out, both twins ceased training and entered business leading a sedentary life. They only participated in moderate-intensity physical activities as assessed by Standard Activity Questionnaire [37]. During their sport career they walked yearly an average of 5,125 km for 243 days. Their mode of training consisted of endurance (59% of the time), specific work (15%), strength (9%), and technique (17%). They competed an average of 14 times per year and had re-

TABLE 1. The amount of training in kilometers and days per year, the number of competitions per year, and the best performance time each year in 20 km competitive walking race for A (the Olympic winner) and his identical twin brother (B) during their sport career in the adult age (19 to 33 years)

Training per year			Competitions per year	Performance time in 20 km	
Year	Km	days	No	A	B
1976	4028	267	13	1.29.17	1.32.47
1977	3910	222	9	1.25.32	1.30.40
1978	4265	245	14	1.24.57	1.27.50
1979	4870	236	1	1.22.59	1.25.28
1980	4795	223	2	1.21.47 [1]	1.25.48 [s]
1981	4550	230	1	1.22.26	1.24.22
1982	4624	225	7	1.22.06	1.29.44
1983	5025	240	21	1.20.09	1.24.26 [6]
1984	5930	264	1	1.20.09 [2]	1.23.54
1985	5750	259	4	1.21.43	1.23.43
1986	6410	260	1	1.21.17	1.25.04
1987	6310	255	3	1.20.45 [3]	1.24.17
1988	5395	250	1	1.20.14 [4]	1.25.07
1989	5060	230	5	1.22.01	1.30.22
1990	6235	245	1	1.23.05	1.28.23

[1]Cold medal in Moscow Olympic Games
[2]Silver medal in tos Angeles Olympic Games,
[3]Gold medal in World Championship,
[4]Silver medal in Seoul Olympic Games,
[5]1th place in Moscow Olympic Games,
[6]Cold medal in World Championship

markable sport achievements. One of them (A) was an Olympic medal winner at three successive Olympiads (gold medalist in 1980, silver medalist in 1984 and 1988) as well as world champion in 1987 while the other (B) finished at the 11th place in the 1980 Olympic Games and came first in the World Championship of 1983, which was when, twin A did not participate, thus possibly giving his brother a chance to win.

Measurements

As described below, both twins were measured alternatively during the same session at the age of 40 years. However, the discussion will also consider some earlier measurements made with the same methodological approach when the Olympic winner was 22, at a time of his peak sport performance in the pre-Olympic period.

Anthropometry

Assessment was made at the nearest 0.1 kg for body weight, 5 mm for height and 0.2 mm for skinfold thickness taken at two sites (biceps and triceps) using the Harpenden constant pressure caliper (10g/mm2) for the estimation of body fat and applying the Lohman formula [28]. Conventional spirometry was used for lung volume measurements.

Echocardiography

Two-dimensional echocardiography and Doppler echocardiography were performed using a commercially available Hewlett-Packard instrument (Sony 1000) with a 3.5 MHz transducer. The extent and distribution of left ventricular (LV) wall thickening was assessed primarily in the parasternal long-axis and short-axis views. Measurements of the maximal wall thickness of LV was obtained from M-mode echocardiogram derived under direct LV two-dimensional anatomical visuali-zation; measurements of LV wall thickness were also verified on the two-dimensional images in order to increase their accuracy [34]. Other cardiac dimensions were assessed from the M-mode echocardiograms, according to the recommendations of the American Society of Echocardiography [36]. Left ventricular mass (LVM) was calculated using the formula proposed by Devereux and Reichnek [10] while cardiac mass index was derived by dividing LVM by body surface area. Parameters of left ventricular filling were obtained with Doppler echocardiography. The electrocardiogram was recorded in lying position with 12-standard leads.

Dynamometry

The maximal isometric force, was assessed by means of an isometric dynamometer equipped with electronic sensors which register the signal relative to the force production of the leg extensor muscles of the dominant limb. Moreover, the power out-

put of the leg extensor muscles was evaluated during single and consecutive vertical jumps for a 15 s period [2].

Ergospirometry

After a familiarization period with the experimental procedure, subjects first walked horizontally on a motor-driven treadmill at three submaximal speeds (7, 9, and 11 km·h^{-1}) in order to evaluate their metabolic efficiency during walking. The walk at each speed lasted 5 min and was followed by rest pauses of 3, 5, and 6 min, respectively, during which time ar-terialized blood samples were taken from the ear lobe for the assessment of the anaerobic lactate threshold at 4 mM·l^{-1}. Subjects then ran at a starting speed of 9 km·h^{-1} increasing progressively 1 km·h^{-1} each min up to exhaustion for the determination of VO_2 max while blood samples were taken as above during recovery for the assessment of maximal lactate concentration. Oxygen uptake and heart rate were continuously monitored during submaximal and maximal exercise testing. Oxygen uptake was measured breath by breath using a respiratory mass spectrometer (QP 9000, Clinical and Scientific Equipment Ltd, England), heart rate was monitored by a Sport Tester (Polar Electro Finland), and blood lactate concentration was measured with the aid of the enzyme electrode in the EB10 plus according to the enzymatic amperometric principle of measurement (Eppendorf - Netheler - Hinz GmbH, Germany).

Personality traits

The State-Trait Anger Expression Inventory (STAXI) was administered to the twins, under the same conditions and on the same day, to provide systematic measurements representative of anger experience and expression and more ge-

TABLE 2. Physiological data and % intrapair differences obtained from the twins; the Olympic winner (Twin A) and his identical counterpart (Twin B)

Variable	Twin A	Twin B	Difference (%)
Anthropometry			
Body weight, kg	76.8	72.6	5.5
Height, cm	182.0	182.0	0.0
Body mass index	23.2	22.7	2.1
Body surface area, m^2	1.98	1.99	2.0
Body fat, %	11.9	13.4	-11.2
Vital Capacity, l	5.64	5.44	3.5
FEV/VC x 100 (%)	75.5	75.9	-0.5
Echocardiography			
Maximal wall thickness, mm	9.0	9.0	0.0
Left ventricular diameter (diastole), mm	52.0	52.0	0.0
Left ventricular diameter (systole), mm	36.0	30.0	16.7
Left ventricular mass, g	169.0	168.0	0.6
Left ventricular mass index, g x m^2	85.4	84.4	1.2
Left atrium, mm	36.0	35.0	2.7
Aortic root, mm	32.0	32.0	0.0
Ejection fraction, %	53.0	68.0	-283
Stroke volume, ml	98.4	118.9	-20.8
Atrial peak flow velocity, cm x sec-1	50.0	47.0	6.0
Dynamometry			
Maximal isometric force, kg	160.9	170.4	-5.6
Squat jumping, cm	23.4	25.9	-9.6
15 s jumping, w x kg-1	19.9	21.7	-8.3
Submaximal exercise			
V02 ml x kg-1 at 9 km x h-1	33.1	33.6	-1.5
Heart rate, beats x min-at 9 km x h-1	130.0	128.0	1.5
V02 ml x kg-1 at 11 km x h-1	40.93	42.60	-4.1
Heart rate, beats x min-at 11 km x h-1	154	159	-3.2
Walking velocity (km x h-1) at 4mm x I-1	11.1	11.2	-0.9
%V02 max at 4mM x I-1	78.1	76.0	2.6
Maximal exercise			
V02ml x kg-1 x min-1	57.1	58.6	-2.6
Heart rate, beats x min-1	182.0	182.0	0.0
02 pulse, ml x beat	24.2	23.5	2.9
Pulmonary ventilation, l x m-1	156.7	154.8	1.2
Respiratory exchange ratio	1.12	1.11	0.9
Blood lactate, mM x I-1	10.3	8.4	18.4

nerally to assess individual personality [41]. In answering the 44 items of the inventory, the twins classified their own anger feelings -experienced, expressed, hidden, and controlled - on a scale ranging from 1 to 4, and intensity and frequency were evaluated. In the inventory, anger is understood as an emotional reaction to conditions evoked at diverse levels and is based on two theoretical structures, anger experience and anger expression. Anger experience has two main components: state anger and trait anger. State anger is characterized by subjective feelings of different intensity and is usually accompanied by muscular tension and the activation of the autonomic nervous system.

Trait anger is the disposition to perceive a wide variety of situations as being irritating or frustrating and is accompanied by the tendency to respond to similar situations with a more frequent increase of anger state. Anger expression includes three principal aspects: 1) anger expression towards others or surrounding objects, 2) containing or suppressing anger, and 3) control of anger expression.

Results

Physiological data obtained from the Olympic winner and his genetically identical counterpart are given in absolute values and intrapair differences in Table 2. lntrapair differences were small for most anthropometric data, with the Olympic winner being slightly heavier and his brother slightly fatter, albeit body fat was in both twins at the minimal level observed in elite endurance athletes [40].

The twins had normal echocardiographic parameters for highly trained athletes even though colour-Doppler echocardiography showed in both a mild mitral regurgitation with normal leaflets (Fig. 1). Their intrapair differences were limited to negligible values for maximal wall thickness, left ventricular and diastolic diameter, left ventricular mass index, left atrium and atrial peak mitral flow velocity, while the Olympic winner had a strikingly lower stroke volume and ejection fraction than his ' brother.

In all dynamometric measurements - namely the maximal isometric force of the leg extensor muscles, the squat, as well as the 15 sec jumping - the Olympic winner did not perform as well as his brother.

The intrapair difference in submaximal physiological response, as assessed by oxygen uptake and heart rate during walking at a speed of 9 km χ h-1, was insignificant. Minor was also the intrapair difference for the lactate anaerobic threshold expressed as % VO_2 max at 4 $mM \cdot 1^{-1}$. The metabolic cardiorespiratory response to maximal effort was about the same in both twins, with the exception of blood lactate concentration which was appreciably higher in the Olympic winner.

The psychological profile of the twins as assessed by the STAXI, is shown in Fig. 2. Both twins had average scores for state-anger and anger expression towards others or surrounding objects, very high scores for anger suppression, and very

low scores for temperament provoked by anger. However, there were marked intrapair differences in the remaining psychological attributes. The Olympic winner scored extremely high (almost 100% in the percentile scale) for reaction to anger and anger control, while his brother scored extremely low (almost zero) for anger reaction and average for anger control. Finally, the Olympic winner showed a tendency not to express his feelings of anger (almost zero in the percentile scale) while his brother scored appreciably above average (76%) in this attribute.

Discussion

Cross-sectional and longitudinal studies of elite athletes have indicated that peak human performance results from several biological, behavioural and other traits integrated in a complex system, but the relative roles of genetic and environmental forces remain unclear. These were addressed in a number of twin studies that, taken together, point to the existence of significant genetic influences in most of the phenotypes related to human performance [23].

However, the twins in these studies fell generally in the middle of the normal distribution curve being usually not exposed to athletic training, so that the heritability estimates in these studies do not provide a true measure of genetic variance in high human performance. In this regard, only twin athletes who have undergone years of heavy physical training and have reached the upper limit of their potential constitute ideal subjects. Quite obviously, such subjects are very rare.

Therefore, the pair of Olympic twin athletes we tested provides a unique opportunity, particularly in view of the fact that, although genetically identical and identically trained for years, their achievement was distinctly different, one being three times an Olympic winner while the other was about 4.4% slower and only managed to win when his co-twin was not competing.

Possible determinants of aerobic performance in elite athletes, like competitive racewalking, are VO_2 max, fractional utilization of VO_2 max at the threshold for lactate release, and walking economy [7,16,21 J. It appears that differences between the high- and the low-performing twin in all these factors were within the experimental error (about 5%) and are not expected to reflect performance time difference (about 4.4%), which corresponds close to 1 km distance in a 20 km racewalking. It should be noted that measurements were made at the age of 40 (7 years after the twins had ended their competitive career) and an expected age-related decline in VO_2 max was observed [17,35]. At the time of his peak performance, at age 22, the high performing twin had a VO_2 max of 72.4 ml χ kg-1·min^{-1} (unpublished data from the same laboratory) and considering the concordance observed for VO_2 max in MZ twins exposed to a comparable environment, we can reasonably assume that his brother must have had at that time a very similar value. A marked difference of 18.4% was noted in the ability of the Olympic twin to produce a higher

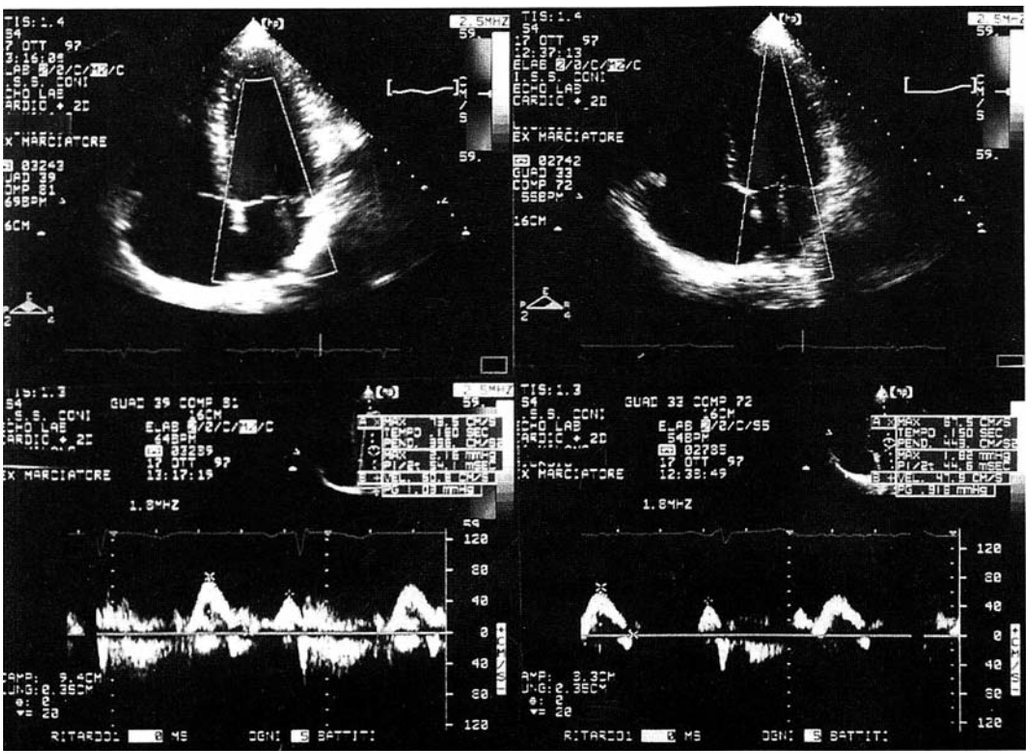

FIG. 1. M-mode echocardiogram of the Olympic winner (left) and his identical co-twin brother. Both showed a mild mitral regurgitation with normal leaflets

lactate concentration in the blood during maximal effort. However, this anaerobic component was not reflected in the percentage of VO_2 max at the threshold for lactate release as mentioned earlier, and hence it is highly unlikely that the anaerobic processes could have contributed to the enhancement of the endurance performance of the Olympic winner [1].

Several echocardiographic studies have described maximal left ventricular hypertrophy and morphologic alterations in trained athletes. Long-term systematic and intense training increases the diastolic dimension of the left ventricular cavity, the thickness of the left ventricular wall and left ventricular mass, characteristic features of the 'athlete's heart'. The degree of adaptation in left ventricular remodelling seems to be proportional to the type and intensity of training and depends on the sporting discipline [20, 34]. Racewalking has a moderate or only marginal effect on left ventricular morphology; no significant sustained hemodynamic overload occurs in the race-walkers and only mild changes in cardiac output. Both twins had normal cardiac dimension, but the Olympic winner had surprisingly a lower stroke volume and injection fraction than his brother during the resting state. It cannot be inferred from the data obtained if such a difference persists during maximal effort. In addition, we realize the limitation of calculating stroke volume and ejection from echocardiograph.

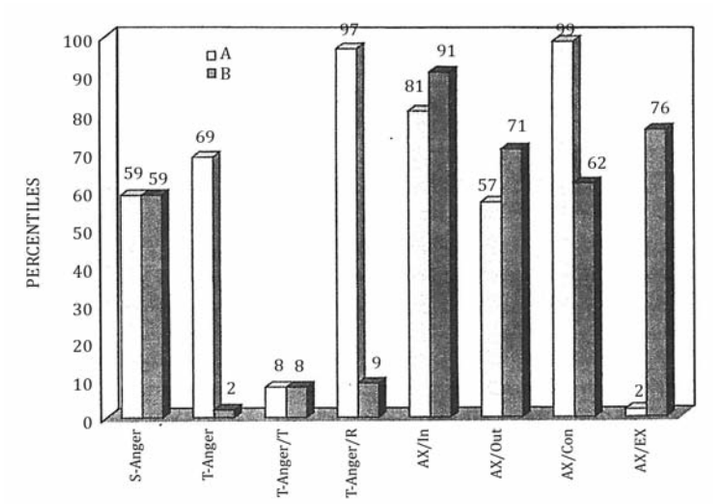

FIG. 2. The psychological profile of the Olympic winner (A) and his co-twin brother (B) as measured by the State-Trait Anger Expression Inventory. S-Anger stands for state-Anger, T-Anger for trait-Anger including T-Anger/T (temperament provoked by anger, and T-Anger/R (reaction to anger), AX/In for anger suppression, AX/Out for anger expression towards others or surrounding objects, AX/Con for anger control and AX/EX provides a general index of anger expression frequency without taking into account whether it is an expression of . AX/In or AX/Out.

Finally, personality measures were also taken in the two twins. In general, the heritability of personality variables hardly exceeds 0.5, so that some amount of variation is to be expected in identical twins as well. The focus of the analysis was on anger-related variables, considered to be possibly associated to sport performance. The psychological profile of the twins with respect to anger expression, which reflects the emotional reaction to conditions evoked at diverse levels and is considered an important personality trait, was similar for some components but completely different for others. The twins were comparable in anger expression towards others and surrounding objects, anger suppression and temperament evoked by anger. However, the Olympic winner had an exaggerated response to frustration and showed excessive sensitivity to criticism and negative evaluations, as well as excessive control over his emotions and behaviour, while his anger was never openly expressed. The emotional reactions of his brother were however, at the opposite extreme: he was not frustrated, insensitive to criticism and only moderately able to control his anger through the cognitive elaboration of his frustrations. It seems likely that this major and basically only difference between the twins may be responsible for their difference in performance, and one could reason that the unexpressed anger in the champion may have enhanced his competitive drive and his autonomic function. It could also be inferred that such a drive may explain his better tolerance to acidosis during heavy exercise. At any rate, this fairly unique example of performance difference in otherwise identical twins shows that genetic factors as well as training alone are not sufficient to make a champion, and that the personality profile plays relevant a role in athletic performance.

Acknowledgements

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Heritability in Neuromuscular Coordination: Implications for Motor Control Strategies

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The aim of this study was to assess the relative power of genetic and environmental contribution to the variation observed in neuromuscular coordination. ***Methods:*** Using the twin model and comparing intrapair differences between monozygotic (MZ) and dizygotic (DZ) twins, we derived heritability estimates (h^2). Forty healthy male twins, 10 MZ and 10 DZ pairs, aged 21.5 ± 2.4 and 21 ±2.1 yr, respectively, performed a series of elbow flexions in one degree of freedom with different velocities attempting to accurately reach a target. Neuromuscular coordination was evaluated for both accuracy and economy of movement and assessed by kinematics and EMG activity. ***Results:*** The heritability in movement accuracy as assessed by the displacement from the target at 70% maximal velocity was 0.87. The accuracy at 30% and 50% of maximal velocity showed that the intrapair variation of MZ twins did not differ significantly from that of DZ twins. High heritability indexes of 0.85 and 0.73 were found for neuromuscular coordination as expressed by movement economy, assessed by the relative EMG activity of biceps long head at 70% and 50% of maximal velocity; no genetic dependence was found for low velocities. ***Conclusions:*** In this study, heredity accounted for most of the existing differences in neuromuscular coordination in fast movements. This implies that movement strategies, which are organized in the CNS and control fast movements, are also strongly genetically dependent.

Key Words: GENETIC VARIATION, TWINS, ACCURACY, ECONOMY, ELECTROMYOGRAPHY

Neuromuscular coordination can be defined as a necessary tool for the successful completion of motor tasks characterized by accuracy and economy. Comprehensive development of coordination is essential for other biological attributes such as speed, jumping ability, and agility and may enhance learning of movement technique, which is a prerequisite in all sports disciplines.

Although physiological parameters of neuromuscular coordination have been

well reported (2,11,17,18,25), studies related to the genetic and environmental determinants of this trait are limited (16,23,29). The few studies related to the heritability of neuromuscular coordination have used a variety of tasks as evaluating methods, creating difficulties in the comparability of the results. Further, tasks used in these studies involve the interaction of several other biological and behavioral factors, which are inseparably integrated with neuromuscular coordination, making its isolation difficult. The results of these studies confirm the allegation that such tasks do not measure exactly the same qualities.

The investigation of the problem of genetic and environmental conditioning of neuromuscular coordination is very important, not only from a theoretical but also from a practical point of view, because this trait is a basic characteristic of athletic performance. The heritability of most other attributes in neuromuscular performance has been extensively studied and found to be moderate to high (8,22,23,26,28). The determination of the heritability of neuromuscular coordination would assist in the selection of outstanding athletes as well as those who require a high phenotype in motor skills, such as musicians, dancers, surgeons, and pilots.

Obviously, training and experience does contribute significantly to the development of neuromuscular coordination. Phenotypes cannot be developed and actualized without environmental forces. Even a highly heritable attribute is not predetermined and can be affected by the environment (19). Therefore, the aim of this study was to assess the relative power of genetic and environmental contribution to the variation observed in neuromuscular coordination by selecting a sufficiently homogeneous sample of monozygotic (MZ) and dizygotic (DZ) twins, and comparing the intrapair differences between the two types of twins. Neuromuscular coordination in this study was examined by kinematics and electromyographic activity during a single joint movement, in one degree of freedom. This method of assessing neuromuscular coordination has become standard in neurophysiologic studies because displacement from a target, velocity, and EMG records reflect the intricate temporal patterning of the forces in space and time, and hence the details of the control processes (2,11,21).

METHODS

Subjects. Forty healthy male twins, 10 MZ and 10 DZ pairs (age 21.5 ± 2.4 and 21 ± 2.1 yr, weight 81.8 ± 22.3 and 76.7 ± 15.2 kg, height 178.4 ± 7.6 and 180.2 ± 7.0 cm) were fully informed about the study and the measurement protocol before giving their written consent. To rule out differences in morphology, particular attention was paid to forearm-hand length, which was similar for both types of twins (46.5 ± 1.9 and 47.1 ± 2.0 cm for MZ and DZ, respectively, with negligible intrapair

differences). A questionnaire was administered to ensure that all twin pairs had similar physical activity profiles, training history, and occupational physical loading of the upper extremity. Only subjects without severe diseases or current medication affecting neuromuscular coordination, reports of tiredness, acute infections, and sensory or musculoskeletal complaints were included in the study. The participation of the subjects and the research project was approved by the Institutional Review Board.

Determination of zygosity was based on morphological characteristics, testimony from the obstetrical clinics and serological examination of genetic markers. Discordance for a single antiserum was regarded as sufficient evidence of dizygosity (19).

Experimental procedures. Measurements were performed in a quiet room at 22-23°C to avoid changes in EMG intensity. Both siblings were tested within 2 h to avoid possible effects of diurnal variation in neuromuscular coordination. None of the twins performed any vigorous activity or consumed alcohol or caffeine during the 24 h before the tests. All were informed of the importance of having the same adequate sleep during the night preceding the tests, and all were familiarized with the procedures.

Twins were seated, facing a target, with the arm steadily supported in an interface in such a way that the shoulder was stabilized. This diminished co-contraction and limited performance of the desired movement to one degree of freedom. The interface was adjustable to account for segmental differences of the upper limb and to ensure the same starting position between twins (Fig. 1). The trunk and arm formed an angle of 90°, and the forearm was positioned so that the movement of the dominant hand was performed in a sagittal plane. A wrist cuff was attached proximal to the styloid process to prevent undesirable movements of the wrist. The starting position of the elbow was at supination and 40° of flexion (with the fully extended position taken as zero). The index finger was extended and used as a pointer, and the other fingers and the thumb were flexed. Tasks were executed with an inertial load of 2.2% of body weight, corresponding to the forearm and hand weight, attached to the wrist with Velcro straps (30).

Subjects were asked to flex the elbow at four different speeds, as accurately as possible, from the initial position to the target (a 2-cm diameter ball, fixed on a free-standing stand). They were instructed to relax at the beginning and move first at slow speed, then at their preferred speed, then at high speed, and finally to move as fast as possible. On a verbal "get ready" signal, subjects positioned their arm at the starting position, and on the signal "go" they tried to reach the target with their index finger. Each subject performed five trials at 10-s intervals for each experimental speed, with 30-s rest between speeds. This number of trials was required because of the variability observed even in the best-trained subjects (21).

Finally, all subjects were instructed to avoid oscillation at the end of the movement.

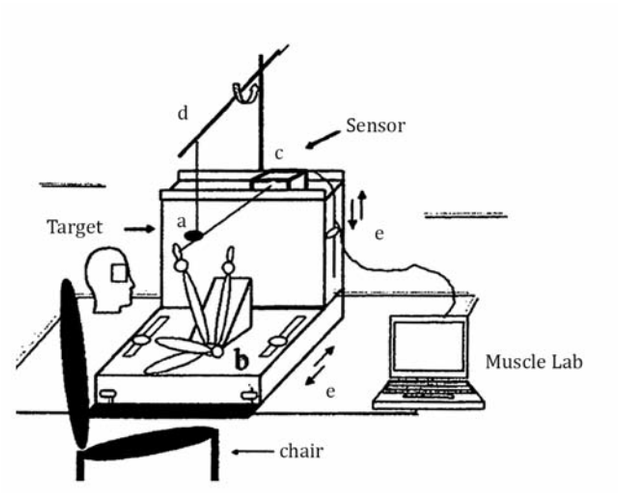

FIGURE 1. Test apparatus and subject's position during the experiment: a, predetermined target; b, solid triangle base for forearm support; c, sensor (linear encoder); d, rotated girder beam; e, graded gliding mechanism to accommodate segmental differences.

Kinematic and physiological variables studied. Movement time, movement amplitude, displacement from the target, average and peak velocity, and average and peak EMG root mean square (rms) activity of the biceps long head was recorded in each trial. The displacement of the load during the elbow flexion was monitored with simple mechanics and sensor arrangement (MuscleLab-Bosco System) attached to the index finger pulp and connected to a personal computer (Fig. 1). The load was mechanically linked to a sensor that glided on a track bar and was connected to an electronic device. When the load was moved, a signal was transmitted by a sensor at every 3 mm of displacement, allowing calculation of the corresponding velocity (4). The reliability of the velocity during dynamic elbow flexion, assessed in a pilot study of 12 subjects, was high (correlation coefficient 0.96), and the coefficient of variation ranged from 3.7 to 19%, comparable to previous studies (4,5).

The signals from the biceps long head were recorded during each trial with bipolar surface electrodes (AE-131, Circular sEMG Neurodyne Medical Co., interelectrode distance 1.2 cm) prepared with adhesive tape and electrolyte gel, and fixed longitudinally over the muscle belly. Before electrode placement, the skin surface was prepared to reduce the skin-electrode interface impedance. An amplifier (Bioship Grenoble, gain 600, input impedance 2 GΩ, CMMR 100 db, band-pass filter 6-1500 Hz) was used. The Muscle-Lab converted the amplified raw EMG signal to an average rms signal via its built-in hardware circuit network (frequency response 450 kHz, averaging constant 100 ms, total error ± 0.5%). EMGrms was expressed as function of time, and because the EMGrms (mV) signals were recorded in association with the biomechanical parameters, they were simultaneously sampled at 100 Hz (Fig. 2). The reliability of measurements using Muscle-Lab exceeds 0.90, whereas the coefficient of variation ranges from 12.3 to 15% (5,6). In three subjects, EMG record distortion resulted from the swinging of the cables, and in one pair from excessive fat. Hence, 16 pairs of twins (eight MZ and eight DZ) were used for the heritability estimate of movement economy.

Measurement of maximal velocity and EMG activity during maximal isometric contraction. Before the main task, all twins performed a dynamic elbow flexion, with their dominant hand at maximal velocity without a target. Three trials

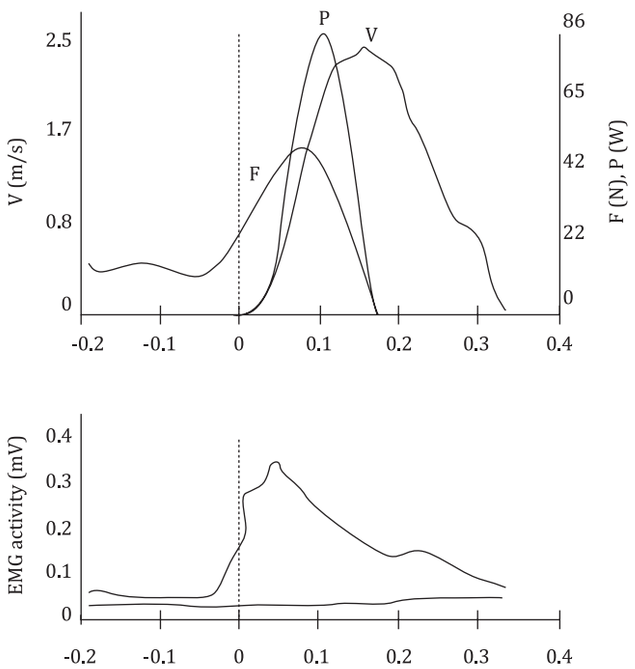

FIGURE 2. A typical kinematic and muscle activity profile of one subject, showing velocity, force, power, and EMG activity from the biceps long head. The recovery EMG signal was slightly above the prebaseline at the end of the aiming movement, signifying that when at target position biceps are not completely relaxed.

were made with 30-s intervals, and the highest peak value was used for statistical analysis. EMG measurements were made of the biceps long head during maximal isometric contraction while the elbow was at 90° flexion, positioned with a standard goniometer. Twins performed three trials at 1-min intervals, and the statistical analysis was based on the highest peak value. These maximal values for both velocity and EMG activity were used for the calculation of the peak relative values at different submaximal velocities, which made possible the normalization and thus the comparability of the results (12).

Data processing. Neuromuscular coordination expressed as movement accuracy was assessed from the relationship between movement speed and displacement from the target, whereas the relationship between movement speed and EMG activity was used to assess neuromuscular coordination as a function of movement economy. As a first approximation, the R2 was used to determine the degree of correlation and prediction for these relationships. Subsequently, examination of three prediction models confirmed that the semilog (log y = a + bx) model had the best fit for both relationships in almost all cases. Based on the exponential equation y = aebx, where y = accuracy or economy, a = intercept a, e = constant, b = coefficient b, and χ = velocity, movement accuracy and movement economy were determined at 30%, 50%, and 70% of the maximal velocity without a target. These three velocities were selected so that it would be possible to evaluate the intrapair differences for all twins at the same relative velocity. The lower velocity, 30% of maximal, was selected because it is considered to be representative of everyday voluntary movements, and the upper velocity, 70% of maximal, because it corresponds to the maximal velocity performed with the target; 50% was used as an intermediate velocity.

Statistical analysis. Heritability was assessed by the twin model, which makes use of MZ and DZ twins. MZ twins are genetically identical, whereas DZ twins share only 50% of their genes. On the basis of the intrapair difference between MZ and DZ twins, heritability (h^2), which denotes the degree to which individual differences in a given variable are attributed to genetic differences, was estimated. The single-

factor ANOVA for each variable was used to determine the significance of the differences between the mean monozygotic and dizygotic intrapair variance, taking into consideration genetic type and pair factor (8). The variance ratio (F) derived from the single-factor ANOVA determined whether further analysis was necessary. The following Clark equation (10,19), based on intrapair variance was used to estimate heritability: h^2 = (s2 DZ - s2 MZ/ s2 DZ) X 100. The computation of h^2 was carried out, provided that the difference in genetic variance between the twin types (F-test) was significant and the difference between means (t'-test) and total variance of both types of twins (F'-test), which shows the homogeneity of the sample, was nonsignificant (9). Intraclass correlations between MZ and DZ twins were also computed.

RESULTS

For the purpose of this study, neuromuscular coordination was expressed as the accuracy and economy of movement performance. Movement accuracy was defined as displacement from the target during elbow flexion in three different velocities (30%, 50%, and 70% Vmax). Movement economy was shown by the recordings of EMG activity of biceps long head at the same velocities. A clearer picture of neuromuscular coordination could have been given if more than one elbow flexor and/or an agonist-antagonist pair about the elbow joint had been examined. The focus of this study, however, was to determine heritability, which is based on intrapair differences. For this reason, the comparison of the normalized peak EMGrms activity of biceps for a given movement speed could be used as an indication of movement economy.

Heritability in neuromuscular coordination expressed as movement accuracy. Displacement data obtained from the target deviation were averaged across five trials for each velocity for all MZ and DZ twin pairs. Figure

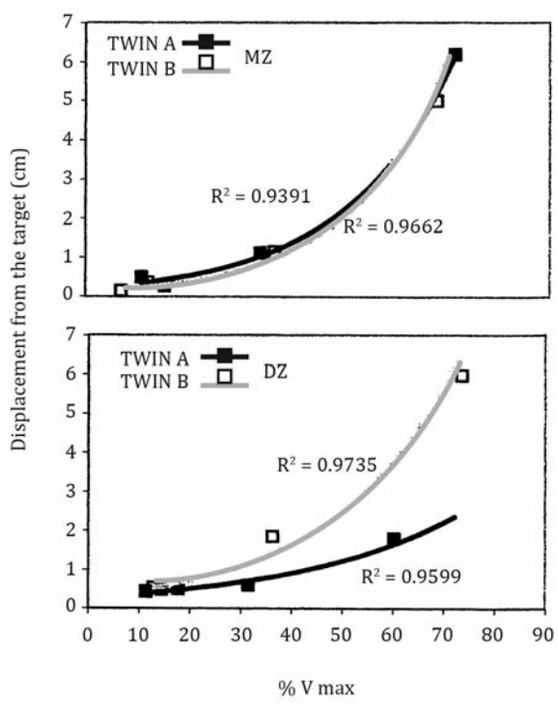

FIGURE 3. Relationship between performing velocity expressed as percent of its maximal value and the corresponding displacement from the target. Values are means of five trials at each velocity from a typical MZ and DZ twin pair.

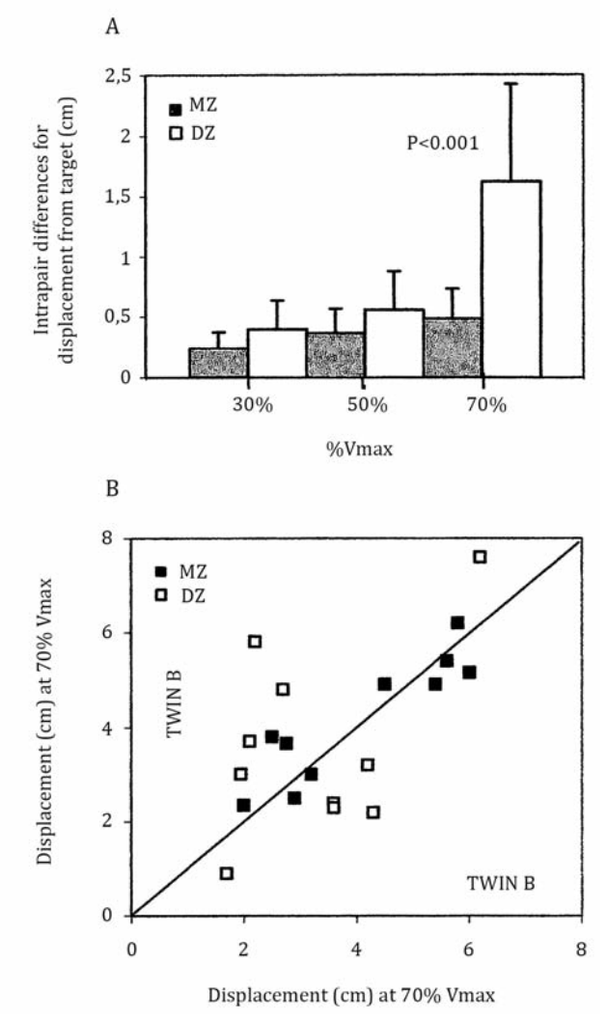

FIGURE 4. Mean and standard deviation of intrapair differences for displacement from target at 30%, 50%, and 70% of maximal velocity (A). Intrapair values for displacement from target at 70% of maximal velocity for MZ and DZ twins (B).

3 shows representative findings of movement accuracy observed from a typical MZ and DZ twin pair. Most of the resulting velocities ranged from 10% to 70% Vmax and only in few cases did not reach the upper value of 70%. Remarkably, most MZ twin correlation lines were almost identical, whereas the lines of DZ twins had marked differences that increased with movement velocity. Figure 4 presents means and standard deviations of intrapair differences in displacement from the target for 30%, 50%, and 70% Vmax for both MZ and DZ twins. The differences in DZ twins become more apparent in Figure 5, where values for monozygous twins are almost identical, whereas those for dizygous twins are widely scattered.

The respective intrapair correlations for MZ and DZ twins was 0.54 and 0.46 for 30% Vmax, 0.64 and 0.55 for 50% Vmax, and 0.91 and 0.43 for 70% Vmax. The differences between means and total variance of both types of twins and the difference in genetic variance of the displacement from the target with velocity corresponding to 30% and 50% Vmax were not statistically significant. For 70% Vmax, differences between means and total variance of both types of twins were not significant, whereas the genetic variance between the twin types was significant ($F = 8$, $P < 0.001$) (Table 1). Therefore, computation of h^2 was carried out only in this latter case, in which genetic factors explained 87% of the total variance. Similar heritability estimates were obtained with equations proposed by Newman et al. (24) and Falconer and Mackey (13): 0.84 and 0.96, respectively.

Heritability in neuromuscular coordination expressed as movement economy. Regarding the qualitative observations in EMG records, the onset of biceps long head was visually determined for each trial. The values of the noise amplitude calculated at two standard deviations from the normalized baseline mean value and done via the built-in hardware circuit network were used as an onset criterion. Low velocity movements related with low peak EMG bursts, whereas for most of the cases, the activation onset and the peak of the EMG burst was not as clear as in

faster movements. On the contrary, EMG records from faster movements had larger amplitudes lasting approximately 150 ms at the onset of the movement, whereas peak EMGrms recorded close to 100 ms (Fig. 2). Further, peak EMGrms coincided as a rule with peak force, whereas the rate of force rise was associated with a parallel change in the rise of motoneuron activity, as revealed by the initial slope of the agonist EMG burst.

Data for movement economy were treated in the same manner as for movement accuracy. Correlation lines of the relationship between movement speed and EMG activity showed an intrapair similarity for MZ twins and a divergence for DZ twins, which became wider with increasing movement velocity. Intrapair difference for EMG activity between MZ and DZ twins was not significant for 30% Vmax but was significant for 50% ($P < 0.05$) and for 70% ($P < 0.001$) of maximal velocity. More specifically, average intrapair difference between DZ was 0.054 ± 0.03 and between MZ 0.026 ± 0.01 for 50% of Vmax, and for 70% Vmax0.127 ± 0.06 and 0.038 ± 0.01 for DZ and MZ, respectively.

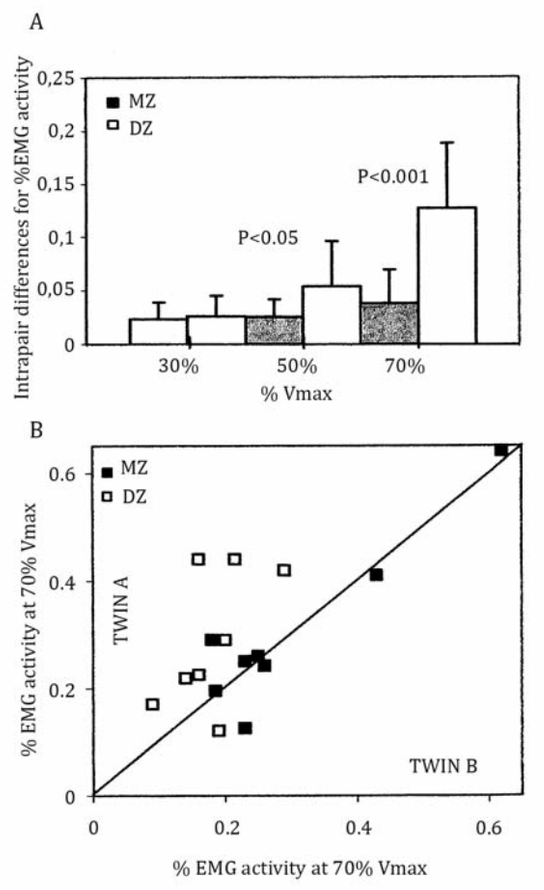

FIGURE 5. Mean and standard deviation of intrapair differences for % EMG activity of biceps long head at 30%, 50%, and 70% of maximal velocity (A). Intrapair values of EMG activity at 70% of the maximal velocity for MZ and DZ twins (B).

The intrapair correlation for MZ twins was 0.93 for 70% Vmax and 0.90 for 50% Vmax, whereas for DZ twins the corresponding values were 0.64 and 0.56. In the slow relative velocity of 30% Vmax, the intrapair correlation was similar for both types of twins.

After testing the statistical hypotheses of genetic variance (Table 1), we computed heritability estimates using the Clark equation for both 50% and 70% Vmax, which were 0.73 and 0.85, respectively. Similar values were found by the Newman equation (0.79,0.84) and the Falconer equation (0.78, 0.74) for 50% and 70%Vmax.

DISCUSSION

The findings of this study demonstrated that heredity accounts for the major part of existing differences in neuromuscular coordination of fast movements expressed either as movement accuracy or movement economy. These findings will be discussed in light of evidence obtained from relevant twin studies and with a focus on implications for motor control strategies in movements performed at different velocities under the same accuracy requirements. The rigorousness of the twin me-

thod, which was used for the assessment of phenotypic variation in neuromuscular coordination, has been discussed recently elsewhere, where it was shown to be an acceptable and valuable tool in elucidating genetic causation (19).

In our study, the heritability estimate in movement accuracy with a high velocity corresponding to 70% of maximal value was 0.87 (assessed by displacement from a target). On the contrary, the accuracy in slow velocities equivalent to 30% and 50% of maximal in MZ twins was not significantly different from that of DZ twins.

A significant genetic effect has been reported previously in a variety of tasks, such as pursuit rotor tracking, tapping speed, and stabilimetry with h^2 varying according to the task and ranging from 0.56 to 0.86 (16,23,29). It is obvious that each of these tasks requires the involvement of other bio-logical and behavioral factors, like balance, power, proprioception, rhythm, motor learning, and perception. These factors are inseparably integrated with neuromuscular coordination and pose difficulties in its study. Moreover, the tasks used in these limited previous studies are based on less constrained movements and hence would require more com-plex and sophisticated measurement apparatuses that would simultaneously measure movements with many degrees of freedom (15,18).

In the present study, neuromuscular coordination was examined by kinematic and electromyographic records during a simple, single joint movement in one degree of free-dom. This method has become standard in neurophysiologic studies, because displacement from a target, performance velocity, and EMG activity reflect the intricate temporal patterning of the forces in space and time, and hence provide details of the control processes (2,7,11,18,21,25). Such details cannot be fully described by movement time or performance error scores, as was done in the aforementioned studies. Further, an attempt was made to isolate neuromuscular coordination using a motor task that involved a movement of a single joint, in a specially designed manipulandum, which on the one hand facilitates the measurement and therefore improves the quality of the data obtained, and on the other minimizes the involvement of factors that may contaminate the results (2,11).

Furthermore, the use of a simple but perfectly representative aiming movement limited the differences that would probably arise from the influences of motor learning, a factor that by itself constitutes an object of study in intrapair differences. Therefore, the genetic influence in neuromuscular coordination should be more easily detectable when interfering factors inherent in the tasks are eliminated. This assertion has been supported by several twin studies that examined other abilities such as balance, speed, pace, kinematic structure of running, speed, and rhythm (26,29).

For two reasons, probably the most appropriate task used so far to test neuromuscular coordination is tapping speed: first, this activity is based on the fundamental speed-accuracy relationship, and second, confounding factors could be con-

TABLE 1. Testing statistical hypothesis for the derivation of h^2 regarding movement accuracy (dd) and movement economy (EMG) at 30%, 50% and 70% of maximal velocity.

	30%Vmax		50% Vmax		70% Vmax	
Hypotheses	dd	EMG	dd	EMG	dd	EMG
t'-test	0.64 (t < tc)	1.35 (t<tc)	0.56 (t< tc)	1.26 (t<tc)	0.98 (t < to)	1.04 (t< tc)
P-test	2.39(F'<Fc)	4.09 (F' < Fc)	1.17 (F'<Fc)	2.83 (F'<Fc)	1.40(F'<Fc)	1.91 (F' < Fc)
F-test	2.8	1.34	2.3	3.8*	8**	7**

*P< 0.05; **/P< 0.001.
t'-test signifies the difference between means of the twin pairs, P-test the difference of total variance of both types of twins, and Rest the difference in genetic variance between the twin types.

trolled to a greater extent compared with other tasks (14,23). Studies that used this task to assess intrapair differences, however, have not taken into account the interaction between accuracy and speed, whereas in our study movement accuracy was derived from the same relative maximum velocity of each twin and thus the intrapair comparisons of this variable should be more reliable.

A fundamental principle of movement behavior was verified in this study (14). When the movement was performed at slow velocity, the accuracy was good, its variance was small, and a linear relationship was observed as a function of velocity. On the contrary, when high velocity was required to reach the target, accuracy decreased exponentially with a concomitant increase in its variability at the endpoints. In this latter case, twins seemed to actualize their potential ability approaching their peak performance. Therefore, a hypothesis relative to the system can be suggested, considering that approximately at 50% to 55% of the maximal velocity, an accelerated increase in the displacement from the target occurred (Fig. 3). As the movement time decreases, forces exerted against the bones of the forearm increase because of the increased activity both in agonists and antagonists as well as the recruitment of Type II motor units. Thus, the inaccuracy of a movement increases as movement time decreases, primarily because of the increased "noisy" processes in the CNS that are generated in producing stronger muscle contractions. This, in conjunction with the present study's homogenous trained sample, could support that the relative velocity of 50-55% of maximal is the threshold of movement accuracy and that beyond this point errors increase exponentially because of motor unit recruitment and intrinsic forces on the arm.

From the previous discussion, it may be seen that every-day activities are performed with accuracy, economy, and minimal differences between individuals. A question arises at this point: why is the variability genetically dependent in fast movements and not in slow movements? It is reasonable to postulate that a mechanism in neuromuscular coordination allows most people in everyday life to perform skilled movements accurately at slow speeds, whereas only few people can

perform skilled movements at high speeds with accuracy. In this latter case, the neuromuscular system probably imposes a functional mechanism leading to physiological limits and hence to full expression of an individual's genetic potential. This observation is important from not only a theoretical but also a practical point of view. The execution of everyday conscious movements, which are mainly of slow to moderate velocity, can be performed economically and accurately by almost everyone, and hence it is not necessary to assess individual capabilities in the workplace, which demands movements of slow to moderate velocities. On the contrary, movements of very high velocity are completed before performers can utilize the feedback produced by the action to alter its course (27). In our study, movement time ranged from 0.65 to 0.50 s for medium velocity and 0.31 to 0.20 s for fast-velocity movements. These latter movements allowed only reflexes to be activated and contribute in part to the action, whereas information from the conscious loop is incapable of influencing the correction of the movement. Because the duration of many movements in sports activities is less than 0.10 s, the role of motor programs in the CNS becomes very important in neuromuscular coordination. Moreover, that heredity accounts for the major part of existing differences in neuromuscular coordination in fast movements implies that movement strategies organized in the CNS to control fast movements, as well as reflexes, are strongly genetically dependent. This contention is in harmony with previous findings (20).

Finally, these results could have implications for the possible selection of outstanding performers in sports that require a high neuromuscular-coordination phenotype, as well as in those whose profession requires fast movements performed with great accuracy and economy, such as dancers, musicians, pilots, and surgeons. We should not ignore the fact, however, that even a highly heritable attribute does not mean that it is predetermined and that the environment has no effect.

Regarding the heritability of neuromuscular coordination expressed as movement economy and assessed by the relative EMG activity of the biceps long head in selected velocities, high heritability indexes of 0.85 and 0.73 were found for velocities of 70% and 50% of maximal value, whereas no genetic dependence was found for low velocities. No study so far has reported heritability in neuromuscular coordination using the EMG activity, which in fact is prerequisite for such an assessment.

For the single joint movement of the elbow used in this study, the only torques acting on the forearm are those produced by the elbow flexor and extensor muscles and gravity. These movements were initiated with a burst of EMG activity in the agonist flexor muscle (long head of biceps) and sometimes concluded by a second agonist EMG burst, which "placed" the limb in position. Further, the increase in movement velocity over a constant amplitude against a constant load resulted in an increase in the rate of EMG rise, peak value, and area of the first agonist burst.

This suggested that, depending on the change in velocity, motoneuron excitation pulse patterns can be generated by specifying their height, width, and relative timing. These observations are in concordance with previous studies investigating movement coordination (3).

It could be argued that neuromuscular coordination could be better assessed if more than one elbow flexor and/or agonist-antagonist pair about the elbow joint were examined. For the purpose of the present study, however, which compares intrapair differences, the normalized peak EMGrms activity of biceps for a given movement speed should be sufficient to indicate movement economy. In this context, a lower EMG magnitude for a given velocity will imply either a lower recruitment of the muscle fibers or a greater distribution of activity among the different elbow flexors. This depends upon the individual's skill in performing the task, because a more precise and accurate control of the increase in force is obtained when the CNS selects a slower recruitment of motor units in the agonist muscle. Moreover, for a given velocity, a lower EMG magnitude of biceps could also imply lower amplitude for the antagonists in order to provide a braking force to stop movement upon reaching the target (11).

The results also showed that low velocity movements correlated with low peak EMG bursts. On the contrary, in fast to very fast movements, the first EMG burst of the biceps was observed at the onset of the movement, lasted approximately 150 ms, and their intensity was augmented while the movement velocity increased, a finding that has been shown in previous studies (2,11,17,18,25). Further, our data demonstrated a high correlation between peak movement velocity and EMG activity of biceps. This correlation was expected because the forces exerted on the bones by the tendons and hence the developed torques becomes greater as velocity increases.

Our findings may be compatible with more than one theoretical framework. If the CNS prescribes certain patterns of muscle activation to match required patterns of muscle torque, the dual-strategy hypothesis can be used. This implies that the movements are controlled with rectangular pulses of excitation sent to motoneuronal pools, such that the width and amplitude of each pulse and the delay between the pulses to the agonist and antagonist pools are modulated according to the task parameters (2,11). Alternatively, if EMG are considered consequences of both central commands and reflex effects from receptors sensitive to movement kinematics, the equilibrium-point hypothesis, which assumes that the CNS manipulates equilibrium states of the system effector-load, can be used (1). Because in this study we did not examine delayed events, like the antagonist burst, but examined only the first EMG burst of biceps, which lasted about 100-150 ms at the onset of the movement, we can support that the hypothesis of the dual-strategy explains better the regulations of this first burst, especially in very fast movements. This possible

explanation, in conjunction with the finding that heredity accounts for the major part of existing differences in neuromuscular coordination of fast movements, fits the hypothesis that variation in movement strategies which are organized in the CNS and control fast movements are highly genetically dependent.

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Nature prevails over Nurture

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Quantitative genetics using the twin model offers a unique and powerful method of disentangling the relative power of Nature and Nurture, genes and environment, in the variation observed in phenotypes related to sport performance. The model makes use of monozygotic (MZ) twins who have identical heredity and dizygotic (DZ) twins who share half of their genes. From comparisons of intrapair differences between MZ & DZ twins we derive heritability (If2) estimates, which signify the extent to which heredity affects the variation of a given phenotype. There is accumulating evidence to show that individual differences in most functional abilities, morphological characteristics, motor attributes, personality and cognitive traits linked to superior sport performance are substantially influenced by genetic factors. H^2 reported by twin studies suggest that genetic influence is so ubiquitous and persuasive that ive ask not what is heritable but ivbat is not heritable. However, a high b2 of a given phenotype does not exclude environmental influence. Nature and Nurture are indeed inseparable and phenotypes reflect the effects of genes as well as those of epigenetic influences, the most potent of which is training. Training can produce results within the variability allowed by the genotype, but cannot erase individual differences which are due to innate ability. Deliberate effortful practice is a prerequisite for the actualization of an athlete's genetic potential. If the environmental forces were optimized, the only decisive factor to peak sport performance would be the gentoype. Yet, though genes and training may set the biophysical limits to human performance, there is evidence that it is behavioral features which determine the ultimate frontiers of sport performance. The postulate that in addition to superior genotypes athletes of olympic caliber have also inherited genes which mediate a high response to training, is not tenable. To unravel the complex etiology of individual differences in sport performance we need to continue using techniques from quantitative genetics for the selection of candidate genes and tools from molecular genetics, now available, for identification of genes of performance phenotypes. Although there is a long way to go before we have a clear picture of the human gene map for sport performance traits, a number of laboratories and scientists concerned by the role of genes and DNA sequence variation in sport performance is rising.

KEY WORDS: Environment, Genes, Nature, Nurture, Nature-Nurture Interplay, Sport Performance, Training, Twins.

If a man does not keep pace with his companions,
perhaps it is because he hears a different drummer.
Let him step to the music which he hears,
however measured or far away.

-Thoreau

The term Nature-Nurture was coined by Francis Galton to refer to the two major sources of individual differences, heredity and environment. He introduced the term in his landmark paper "The history of twins as a criterion of the relative powers of nature and nurture", where he reported a study of life histories of two groups of twins (Galton, 1876): "It is, that their history affords means of distinguishing between the effects of tendencies received at birth, and of those that were imposed by the circumstances of their after lives; in other words, between the effects of nature and nurture". These comparisons led Galton to argue for the inheritance of the traits that lead to human eminence. He used the following eloquent parable to illustrate this notion:

> *"Many a person has amused himself by throwing bits of stick into a tiny brook and watching their progress; how they are arrested, first by one chance obstacle, then by another and again, how their onward course is facilitated by a combination of circumstances. He might ascribe much importance to each of these events, and think, how largely the destiny of the stick had been governed by a series of trifling accidents. Nevertheless all the sticks succeed in passing down the current, and in the long run, they travel at nearly the same rate. The one element that varies in different individuals, but is constant in each of them, is the natural tendency; it corresponds to the current in the stream, and inevitably asserts itself... There is no escape from the conclusion that Nature prevails enormously over Nurture".*

There is mounting scientific evidence to show that Galton's argument off the preponderance of Nature on phenotypic variation applies equally well to biological and behavioral abilities and traits associated with superior sport performance. This review will describe some of the work to date that has been done to elucidate the relative powers of Nature and Nurture as pertaining to sport performance, by focusing in the following five areas:

- *The twin model.* This most informative research design has been used for disentangling influences associated with Nature and Nurture.
- *The relative power of Nature.* Data from twin studies are critically analyzed and

the concept of heritability is clarified in order to elucidate the importance of genetic influence on phenotypes linked to superior sport performance.

- *The relative power of Nurture.* Findings from co-twin studies are examined to reveal the potency of environmental forces in the actualization of genetic potential.
- *Nature-Nurture interplay.* Evidence from co-twin and family studies is scrutinized to disclose to what extent training responses are genotype-dependent.
- *Beyond heritability: Good gene hunting.* Advances in identifying genes that contribute to the variance of sport performance phenotypes and potential abuses will be briefly reviewed.

The Twin Model

The twin model was first applied in a systematic way to the study of human diversity in the early 1920s (Merriman, 1924). The model makes use of monozygotic (MZ) and dizygotic (DZ) twins. Monozygotic twins have identical heredity and therefore any intrapair difference in a measurable attribute must be due exclusively to environmental influences. Dizygotic twins, on the other hand, share half of their genes, like ordinary siblings and any difference observed between them in a trait must be attributed to both genes and environment. From comparisons of intrapair differences between identical and fraternal twins, it is possible to separate the relative contribution of genotype and environment for any polygenic attribute.

In DZ twins the variance of the differences in an attribute between partners is partly dependent on genetic variability (σ^2g), partly due to environmental effects (σ^2e) and partly affected by the error of measurement (σ^2m):

$$\sigma^2_{DZ} = \sigma^2_{DZg} + \sigma^2_{DZe} + \sigma^2_{DZm} \quad (1)$$

For MZ twins there is no genetic variability and the intrapair difference is attributed solely to nongenetic influences, namely environment and error of measurement:

$$\sigma^2_{MZ} = \sigma^2_{MZe} + \sigma^2_{MZm} \quad (2)$$

Equations 1 and 2 can be combined and by eliminating the environmental effect (σ^2e), which is assumed to be equal for MZ and DZ twins, we derive the following equation, which denotes the variance in dizygous twins due to genetic difference:

$$\sigma^2_{DZg} = (\sigma^2_{DZ} - \sigma^2_{DZm}) - (\sigma^2_{MZ} - \sigma^2_{MZm}) \quad (3)$$

Further, if we arrange the above equation in a ratio form, and refer to the term (σ^2DZg) as heritability (h^2) we have (Holzinger, 1929, Klissouras, 1971):

$$h^2 = \frac{(\sigma^2_{DZ} - \sigma^2_{DZm}) - (\sigma^2_{MZ} - \sigma^2_{MZm})}{(\sigma^2_{DZ} - \sigma^2_{DZm})} \times 100 \quad (4)$$

Heritability (h^2) is defined as the proportion of phenotypic variance attributable to observed individual differences in actualized genetic potential and its proximity to unity signifies the relative share of the genotype, i.e., the closer the h^2 is to unity the stronger the assumed genetic influence. It must be emphasized that *heritability has no etiologic role in the pheno-type, nor has it sensible meaningful reference to the ability of an individual. It is only an estimate of the extent to which heredity affects the variation of a given trait.*

The validity of any heritability estimate depends upon the biases of ascertainment and the acceptability of underlying assumptions on which the twin model is based. Both of these are of paramount importance and should be carefully considered in each twin study.

BIASES OF ASCERTAINMENT

The twin model has often been subject to criticism due to the biases of ascertainment. There are three sources of such biases:

- Misclassification of zygosity
- Representativeness, and
- Estimation of genetic variance

Misclassification of zygosity

Since the twin model is based on comparisons between the two types of twins, it is of outmost importance that twins are classified as MZ or DZ with precision. Direct observation of anthropological markers is used as a first approximation of zygosity determination with an accuracy of about 90%. Blood and serum examination renders greater precision. Discordance for a single antisera is regarded as sufficient evidence of zygosity, while in concordant sets the median probability of monozygosity is more than 95%. A new molecular genetic method involves the comparison of a number of DNA regions (markers) known to be highly variable in the general population, and assessing the probability that these would be identical in two individuals if they were unrelated. This is an excellent test of zygosity, since only MZ twins have exactly the same DNA "fingerprints". Errors in diagnosis of zygosity will underestimate the real value of h^2, because they are likely to lower the MZ correlation (resemblance) and increase the correlation of DZ twins.

Representativeness

If twins are different in means and variances from the population, results might not completely apply to the population at large. Indeed, twins are 3 to 4 weeks premature compared to singletons, 30% lighter and 17% shorter at birth, while there are differences for MZ twins in intrauterine position and blood supply to the embryo (Plomin, De Fries, McClearn, & McGuffin, 2001). However, these prenatal and

early postnatal differences are not enduring, progressively get equalized under the influcence of a maturational pacemaker and disappear by middle childhood (Wilson, 1979).

Estimation of genetic variance

Another potential source of bias is related to the estimation of genetic variance (Christian, 1979). Computations of h^2 should be carried out only if the difference in genetic variance between the twin types (F-test) is significant and the difference between means (t-test) and total variance (F-test) of both types of twins non-significant (Table I).

FUNDAMENTAL ASSUMPTIONS

The validity of any h^2 depends upon the acceptability of the underlying assumptions. Four fundamental assumptions are necessarily made in the derivation of a h^2. It is assumed that:

- Environmental influences are comparable for both types of twins
- No correlation exists between spouses due to assortative mating

TABLE I. Heritability estimates (b2) of various biological attributes related to human performance, computed using the following formula: Clarke [σ^2DZ - σ^2DZ /σ^2DZ]; Newman [σDZ - σMZ/I - σ^2DZ]; and Falconer [2(rMZ - rDZ)]. Computations were done after testing the hypotheses of genetic variance, and only if the difference between the twing types (F-test) was significant and the difference between (test) and total variance (F-test) of both types of twin non-significant (Klissouras, 1997).

Biological attribute	Hypotheses tested			Heritability estimate (h^2)		
	t-test	F-test	F-test	Clark	Newman	Falconet
Maximal aerobic power (watt)	1.96	1.25	7.21***	0.86	0.83	0.38
Anaerobic capacity (watt in 30")	0.24	1.63	6.91***	0.86	0.76	0.31
Fatigue (%)	0.80	1.04	1.56			
Peak blood lactate (mmol.l^{1})	0.81	1.14	3.92*	0.74	0.71	0.99
VO2max (1.min^{-1})	0.20	1.02	7.43***	0.87	0.87	0.59
VO2max (ml.min^{-1}.kg^{-1})	1.21	1.10	5.47***	0.82	0.83	0.70
VO2max (ml.min.kg LBW-^{1})	0.69	1.17	4.07**	0.75	0.79	0.62
VE (l.min^{-1})	0.16	1.03	3.82*	0.74	0.73	0.92
Maximal heart rate (bt-min^{-1})	0.70	2.51	10.85***	0.91	0.77	0.62
Maximal O_2 pulse (ml.br-^{1}))	0.04	1.13	8.46**	0.88	0.90	0.77
Anaerobic threshold (AT) (km.h-^{1})	1.07	1.86	4.94**	0.80	0.62	0.55
Anaerobic threshold (ml.min^{-1}.kg- ^{1})	0.45	1.36	3.35*	0.70	0.59	0.75
Running economy (below AT):						
$V0_2$ (ml.min^{-1}.kg^{-1})	1.10	1.30	2.609*	0.61	0.50	0.44
Gross VO_2 (ml.kg^{-1})	1.26	1.26	3.00*	0.67	0.58	0.61
Running economy (above AT):						
VO_2 (ml.min^{-1}.kg^{-1})	1.31	1.26	5.42**	0.82	0.85	0.44
Gross VO_2 (ml.kg^{-1})	1.76	1.15	4.23**	0.76	0.80	0.09
Endomorphy	1.61	1.15	10.45***	0.90	0.89	1.51
Mesomorphy	0.48	1.57	8.48***	0.88	0.81	0.64
Ectomorphy	0.33	1.25	15.00***	0.93	0.92	1.00

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

- Genetic and environmental influences are not correlated, and
- Genetic variance shows no dominance or interaction effects.

Environmental comparability

Environmental comparability is tenable if special control is made for all confounding factors, such as gender, age, maturation, socioeconomic status, health condition and sport participation. This does not mean that the environmental influences are kept constant, but that they vary approximately in the same direction and to the same degree for all twins. Ideally, these environmental influences should act maximally on all twins under study, so that their genetic potential is fully actualized and a true measure of h^2 is obtained. Otherwise, any amount of unactualized potential remains unknown and the value of h^2 is limited. In this respect, twin athletes are ideal subjects for the evaluation of the relative powers of genes and environment (Parisi, Casini, Di Salvo, Pigozzi, Pittaluga, Prinzi, & Klissouras, 2001).

Assortative mating

Regarding the second assumption, the possible existence of an assortative mating effect which was ignored would underestimate the genetic influences, since such an effect increases the resemblance between DZ twins and the families variance. However, it is doubtful whether biological criteria are used to any appreciable extent in mating; for example, correlation coefficient between spouses is 0.30 for height and between 0.14 and 0.22 for VO_2max (Monotoye, & Gayle, 1978; Lesage, Simoneau, Jobin, Leblanc, & Bouchard, 1985; Bouchard, et al., 1998). Spouses correlate about 0.10 for personality traits and about 0.45 or general cognitive ability (Jensen 1978).

Disposition to sports participation

The assumption that genetic and environmental influences are not correlated may be only partially true. Parents most likely give gifted children special opportunity to practice and provide them an environment conducive to the development of their propensities and dispositions. Twin studies have shown that the heritability for sport participation ranges from 49-83% (Beunen, & Thomis, 1999; Maia, Thomis, & Beuven, 2002; Frederiksen, & Christensen, 2003) and for participation in intense leisure time activity from 50-62% (Kaprio, Koskenvuo, & Sarna, 1981; Lauderdale Fabsitz, Meyer, 1997; Sholinsky, Ramakrishna, & Goldberg, 1997), suggesting that a portion of the phenotypic variability seen in the population with respect to sport and activity participation may be genetically mediated.

Genotype-environment interaction

Finally, the additive model used to compute h^2 assumes that there is no interaction

effect between genotype and environment. It is quite probable that this simple model may not be adequate to explain the observed intrapair variance of DZ twins, and that it should be modified to include an additional term (σ^2ge), signifying the mutual interaction between genotype and environment. However, based on existing evidence it is equivocal and most unlikely that a genotype-environment interaction contributes to any marked degree to adaptive variation (see later).

The Relative Power of Nature

EARLY TWIN STUDIES ON VO_2MAX

The twin model was put in use in the early 70s to determine the heri-tability of adaptive variation (Klissouras, 1971). The focus was on the genetic origin of individual differences observed in maximal aerobic power or VO_2max which represents the upper limit of adaptational response of the organism to physical exertion. VO_2max measured in ml-min-1 is a good indicator of the capacity of the cardiorespiratory system to transport oxygen and of the muscle system to utilize it; when expressed in $ml \cdot kg^{-1} \cdot min^{-1}$, it is a criterion of aerobic fitness. There was ample evidence at the time to suggest that VO_2max is affected on the one hand by extrinsic factors such as training, altitude and prolonged periods of complete inactivity, and on the other by intrinsic factors, such as sex and age. However, the wide interindividual variability of VO_2max in a homogenous population remained a puzzle, and we wondered to what extent genetic differences may account for existing individual differences.

Twenty-five pairs of male twins (15 MZ and 10 DZ) raised in the ecological setting of the same metropolis participated in that study. Their zygosity was determined on the basis of morphological traits and a serological examination. They ranged in age from 7 to 13 years. The lower age limit was set because younger children were unable to exert themselves maximally and satisfy the criteria set for attainment of VO_2max. The upper limit was set for, as children grow older, the assumption we have made of a shared environment becomes less certain.

Twins performed a series of runs of progressively increasing intensity on a motordriven treadmill. Measurements were made in physiological responses related to oxygen transport and utilization systems. It was found that the difference of the intrapair variance between MZ and DZ twins was significant beyond the 1 % probability level for maximal aerobic power and maximal heart rate, and beyond the 5% level of confidence for maximal blood lactate concentration, a rough index of the subjects to maintain exercise at high intensity. Thus, we proceeded with the computation of the more elaborate heritability estimates and found that the variability of these parameters is genetically determined by 93.4%, 81.4% and 85.9% respectively. Figure 1 from this study shows that the scores of the MZ twins tends

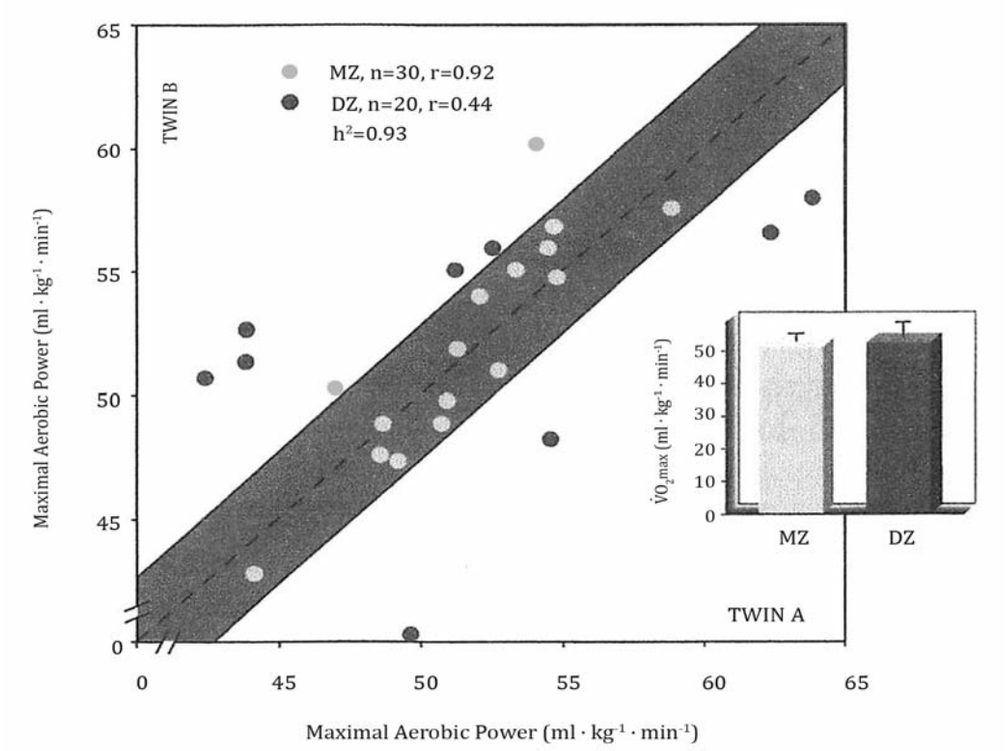

FIG. 1. Intraclass correlation in pairs of monozygotic (MZ) and dizygotic (DZ) twins for maximal aerobic power. Shaded area represents the magnitude of error of measurement. The insert shows the insignificant difference between means of both types of twins (Klissouras 1971).

to cluster around the line of identity and fall within the shaded area which represents the magnitude of the error of measurement, whereas the scores of the DZ twins are widely scattered. On the grounds of the evidence obtained it was concluded that heredity alone accounts almost entirely for existing differences in functional adaptability, as assessed by maximal aerobic power, in a fairly homogenous group of individuals.

It should be noted that young twins were used purposely as subjects in that early study in order to ensure that environmental influences were similar for both types of twins and, thus, the fundamental assumption of environmental comparability, on which the twin method is based, was satisfied. It could be argued, however, that dizygotic pairs would be under more diverse environmental influence than monozygotic pairs during the developmental period. For this reason, we conducted a follow-up study to determine whether the small intrapair differences observed in VO_2max between identical twins and the marked differences between fraternal twins persist throughout life (Klissouras, Pirnay, & Petit, 1973).

It was reasoned that, in twins exposed to similar environments at dif-ferent stages in their lives, any differences between DZ pairs as compared with MZ pairs must be an expression of the relative strength of the genotype. On the contrary, in those twins exposed to contrasting environments the resulting differences may

provide a measure of the responsiveness to environmental forces. Thirty-nine pairs of twins (23 MZ and 16 DZ of both sexes), ranging in age from 9 to 52 years of age were used as subjects in this study (Klissouras, Pirnay, & Petit, 1973). The mean intrapair difference in maximal aerobic power between twin pairs was 10 ml · kg^{-1} · min^{-1} for DZ twins and 2.5 ml · kg^{-1} · min^{-1} for MZ. There was about 16 times more intrapair variance in DZ than in MZ (F ratio=16.45, $p<0.001$). These observations provided further support to the hypothesis of the preponderance of the genetic effect on the phenotypic variation in maximal aerobic power and strengthened the Galtonian notion that *natural tendency inevitably asserts itself.*

In sharp contrast with these results is the work of Bouchard and col-leagues (Bouchard, Lesage, Lortie, Simoneau, Hamel, Boulay, Perusse, Theriault, & Leblanc, 1986a) who reported a much lower h^2 for maximal aerobic power. They measured 27 pairs of brothers, 33 pairs of DZ twins and 53 pairs of MZ twins of both sexes, aged 16 to 34 years. Heritability reached 47% for VO_2max per kg of mass. The intraclass correlation for MZ twins was 0.70 while for DZ twins and brothers it was 0.51 and 0.41, respectively. Considering the higher correlation found in DZ in comparison to the brothers, the authors hypothesized that the 47% estimate was inflated by shared environmental conditions, and that the true h^2 of VO_2max per kg of mass was more likely to be about 25% of the adjusted phenotypic variation. This hypothetical estimate of genetic effect for VO_2max has been accepted erroneously as a true value and has been reported since widely in the literature.

In order to support the contention that environmental influences are stronger than genetic ones in the phenotypic variation of VO_2max, some twin studies were cited where the foremost assumption of equal environments was admitedly not respected and the genetic variance, as a bias of ascertainment, was not considered, A striking example is the twin study of Howald (1976) who found no inheritance component involved in the phenotypic variation of VO_2max. However, when he did not consider in the analysis of his data two pairs of MZ twins, who had been exposed to contrasting environments, the genetic variance reached 68%. Hence, the results of such studies where methodological considerations are ignored or violated must be viewed with caution. For the application of the twin model it is imperative, as explained earlier, to control for potential biases of ascertainment and test all the underlying fundamental assumptions on which the twin model is based.

PATH MODEL ANALYSIS ON VO_2max

One of the criticisms of the classic twin study method is that it fails to separate the variance attributable to non-shared and shared environmental effects. For this reason more recent studies have applied to twin and nuclear family data, the path genetic analysis, where a phenotype of the twin brothers is modeled as being determined by additive genetic effects, common environmental effects and specific

environmental effects. Phenotypic interaction effects can be also addressed (Neale & Gardon, 1992).

Fagard, Bielen and Amery (1991) measured the peak O_2 uptake in 48 pairs of male twins (29 MZ and 19 DZ) aged 18-31 years. They all performed a graded uninterrupted exercise test on the bicycle ergometer to exhaustion. By use of path model analysis, the genetic variance of measured peak O_2 uptake was estimated at 77% after adjustment for weight and skinfold thickness, and at 66% after additional adjustment for weekly hours of sport participation. The remaining variance was attributable to non-shared environmental factors. The estimate of genetic effect of peak O_2 uptake in $ml \cdot kg^{-1} \cdot min^{-1}$ calculated with the heritability coefficient (h^2) was 80% and reduced to 74% when adjusted for body weight, skinfold thickness and sport pariticipation.

Maes, Beunen, Vlietinck, Neall, Tomis, Eynole, Lyssens, Simons, Derom, and Derom (1996) measured maximal oxygen uptake during a maximal exercise test on a treadmill for 105 10-yr-old twin pairs and their parents. Genetic models were fitted to data to quantify the contribution of genetic and environmental factors of the variation observed in VO_2max. The genetic component for VO_2max variation was 87% for females and 69% for males.

Thus, the elaborate path model of analysis demonstrated, as earlier twin studies, that there is a high heritability for VO_2max. Moreover, it was shown that genetic influence was mainly additive and environmental influence was nonshared, without evidence for major impact of genetic dominance of shared environment.

FAMILIAL AGGREGATION OF VO_2MAX

The genetic influence on VO_2max has been investigated using also families where between family are compared with within family variances. In the HERITAGE Family Study, VO_2max was measured in 429 sedentary individuals (170 parents and 257 of their offsprings) aged between 16 and 65 years and it was found that there was about 2.6 to 2.9 times more variance between families than within families in VO_2max. About 40% of the variance in VO_2max was accounted for by family lines, which means that VO_2max aggregates in families (Bouchard et al., 1999). In this same study applying to the data the familial correlation model it was found that heritabilities ranged from 51 to 59% depending on the type of adjustement performed (age, sex and weight). However, it should be pointed out that twin studies are indispensable and needed to determine the relative importance of genetic and environmental influences underlying familial resemblance.

GENETIC VARIATION IN PHENOTYPES LINKED TO SPORT PERFORMANCE

A number of twin studies have been conducted to elucidate the genetic effect on the variation observed in several phenotypes linked to sport performance, such as

functional abilities, morphological components, muscle composition, motor attributes and behavioural traits.

Functional abilities

In addition to maximal aerobic power, a significant genetic variance has also been assessed for aerobic endurance on the basis of either the total work output during a non-stop 90 min ergocycle test, or the lactacid anaerobic threshold. Using the former method of assessment Bouchard and co-workers found intraclass coefficients of 0.82 and 0.45 for MZ and DZ twins, respectively, and a h^2 of 72% (Bouchard, Lesage, Lortie, Simoneau, Hamel, Boulay, Perusse, Theriault, & Leblanc, 1986). These findings concur with those obtained in our laboratory where the anaerobic threshold, defined as the running speed on the treadmill corresponding to a blood lactate concentration of 4 $mmol \cdot 1^{-1}$, was determined in MZ, and DZ twins. The resemblance in the two types of twins was reflected in the intraclass coefficients which were respectively 0.83 and 0.54, as well as in the h^2 which was 80% (see Table I).

The heritability of maximal anaerobic power, as assessed either by lactate production or by mechanical power output, was very high and varied between 70% and almost unity (Klissouras, 1971; Komi, Klissouras, & Karvinen, 1973; Jones, & Klissouras, 1986; Simoneau, Lortie, Boulay, Mar-cotte, Thibauld, & Bouchard, 1986; Calvo, Vallejo, Estruch, Arcas, Joviette, Viscor, & Ventura, 2002).

A strong genetic effect has also been reported for individual differences in neuromuscular performance. Thomis, Van Lemputte, Maes, Blimkie, Claessens, Marchal, Williams, Vlietinck, and Beunen (1997) used genetic model analysis to quantify genetic and environmental contributions to individual differences observed in maximal isometric strength of elbow flexors and its key determinants. They reported heritability estimates of 66% to 78% for maximal isometric torque, 84% to 86% for limb-segment length, and 92% for muscle cross-sectional area. They concluded that the observed variaton in isometric strength, body dimensions and muscle area is highly genetically determined. These observations support earlier twin findings reported by Komi, Klissouras, and Karvi-nen (1973) and Jones and Klissouras (1986). These latter authors studied maximal isometric force and maximal muscular power for the arm flexors in nine MZ and eight DZ male twin pairs (11-17 years). They found that genetic variation accounts for 97 % and 83 % for maximal force and maximal power, respectively.

Morphological components

The variation observed in most morphological characteristics related to sport performance seems also to be strongly affected by the genotype. Kovar (1977) studied in twins the variation observed in somatotype and found the highest h^2 for the ectomorphic (87%) component which is related to weight-height ratio, and the lowest for endomorphy (69%), which is an indicator of fat amount, whereas h^2 in

mesomorphy, which indicates the degree of muscularity, reached a value equal 75%. We made similar observations in our laboratory (see Table I).

The work of Orvanova (1984) has demonstrated that, stature, body and segmental length yield generally higher h^2 than measures of skeletal breadth, and body height higher h^2 than body segments taken separately. Lykken, McGue, Tellegan, & Bouchard (1992) found that MZ twins correlated by 0.94 in height, and DZ twins by 0.50, yielding a h^2 of 88%.

To assess the relative importance of genetic and environmental effect of body mass index (weight in kilograms divided by the square of the height in meters), Stunkard, Harris, Petersen, and McClearn (1990) studied samples of identical and fraternal twins, reared apart or reared together. The samples consisted of 93 pairs of identical twins reared apart, 154 pairs of identical twins reared together, 218 pairs of fraternal twins reared apart, and 208 pairs of fraternal twins reared together. The intrapair correlation coefficiency of the values for body mass index of identical male twins reared apart was 0.70 and for those reared together 0.74, while for fraternal the respective coefficients were 0.15 and 0.33. Similar estimates were derived for women. They concluded that genetic factors appear to be major determinants of the body mass index and they may account for as much as 70% of the variance, whereas the childhood environment has little or no influence. Similar conclusions were reached by other investigators (Bodurtha, Mosteller, Hewitt, Nance, Eaves, Miskowitz, Katz, & Schieken 1990; Tambs, Mourn, Eaves, Nell, Midthjell, Lund-Larsen, Naess, & Halmen 1991; Herskind, McGue, Sorensen, & Harvald, 1996).

Moreover, Sklad (1977) and Maridaki and Klissouras (1998) showed that the variation in biological maturation, which is related to sport performance as assessed by the skeletal age, is also under strong genetic influence; the h^2 was in the range of 0.80 to 0.98.

Data available from a handful of twin studies have yielded widely divergent heritability estimates for the phenotypic variance in morphological characteristics of human skeletal muscle. These estimates range almost from zero to 100%. Komi, Viitasalo, and Havu (1977) took muscle biopsies from the vastus lateralis of 31 twin pairs (15 MZ and 16 DZ) of both sexes. They reported a heritability coefficient for the proportion of type I fibers of 96% suggesting that the variation in muscle fiber distribution is almost exclusively genotype-dependent. A similar study was conducted by Bouchard, Simoneau, Lortie, Boulay, Marcotte, Thibauld, and Bouchard (1986), using a larger sample of 35 pairs of MZ twins, 26 pairs of DZ twins and 32 pairs of brothers. The intraclass correlation for the percentage of type I fibers was about the same in MZ & DZ twins (0.55 and 0.52 respectively) and much lower in brothers (0.33). A heritability coefficient of 6% could be computed from the data although such analysis has no meaning, since the intrapair variance between MZ and DZ twins was non-significant (see above biases of ascertainment).

In spite of their findings in a review of genetic determinism of fiber type pro-

portion in human skeletal muscle, Simoneau and Bouchard (1995) suggested that from the total phenotypic variance about 15% could be explained by the error of measurement, 40% could be due to environmental factors and the remaining 45% could be attributed to genetic variance. However, this partition is purely inferential, if not speculative, and is based mostly on training studies of non-twins where the influence of the genetic factor cannot be assessed.

Motor attributes

Studies related to genetic variation of motor development, motor performance and motor learning date back to the early 1930s (McNemar, 1933; Newman, Freedman, & Holzinger, 1937). It seems that simple phylogenic motor activities, such as walking and running are more conditioned by heredity than complicated and ontogenic activities such as throwing and balancing.

Although there is some scatter of the heritability and the intraclass coefficients reported by different investigators, there is general agreement that identical twins are significantly more similar than fraternal twins in motor learning and motor skill (Sklad, 1975; Kovar, 1981; Bouchard, Malina, & Perusse, 1997).

Williams, and Gross (1980) observed that heritability was low in the early days of practice in a stabilometer balance task, but increased and stabilized at about 65% towards the end of a 6-day practice. Similar observations were made by Fox, Hershberger, and Bouchard (1996) who tested MZ and DZ twins during three successive sessions, of roughly 30 min each, on the pursuit rotor task. They noted a trend towards increased heritability from 0.66 to 0.74 and concluded that *"the main findings of our study unequivocally show the important contribution of genotypic factors underlying individual differences in skill acquisition".*

Missitzi, Geladas, and Klissouras (2004) studied the heritability of neuromuscular coordination. Neuromuscular coordination was examined by kinematic and electromyographic records in MZ and DZ twins during a simple, single joint movement in one degree of freedom. This method was used because displacement from a target, performance velocity, and EMG activity reflect the intricate temporal patterning of the forces in space and time, and hence provide details of the control processes. They found that at high velocities the heritability for movement accuracy was 0.87 and for movement economy 0.85. They suggested that the variation in movement strategies which are organized in the CNS and control fast movements are highly genetically dependent.

Personality and cognitive abilities

Personality traits if expressed at high level may be facilitators of or detractors from superior proficiency in sport performance, while cognitive abilities are of paramount importance in information processing speed (Singer, & Janelle, 1999).

TABLE II. Example of Trait Adjectives Defining the Five-Factor Model (John & Srivastana, 1999).	
Factor	**Example of factor definers**
Extraversion vs introversion	Active, Assertive, Enthusiastic, outgoing
Neuroticism vs emotional stability	Anxious, Selfpitying, Tense, Worrying
Agreebleness vs antagonism	Generous, Kind, Sympathetic, Trusting
Conscientiousness vs lack of direction	Organized, Planful, Reliable, Responsible
Openess vs closeness to experience	Artistic, Curious, Imaginative, Wide interests

Personality traits are enduring individual differences in behavior that are stable across time and across situations (Pervin, & John, 1999). Since the dawn of psychology experts have disagreed about the structure of personality, but during the past two decades consensus has been growing towards a Five-Factor Model defined with the trait adjectives shown in Table II.

In a meta-analysis of twin and adoption studies, Loehlin (1992) summarized the behavioral genetics research on personality traits organized according to the Five-Factor Model. Figure 2 shows that genetic influence is substantial for all personality traits and to the extent that non-additive genetic effects are important, heritability is in the 40-50% range. For each personality trait, a little over 40% of the variation is due to additive genetic factors, about 25% due to non-shared environmental influences, less than 10% due to shared environment influences and the remaining 25 % is simply error of measurement (Bouchard, 1999).

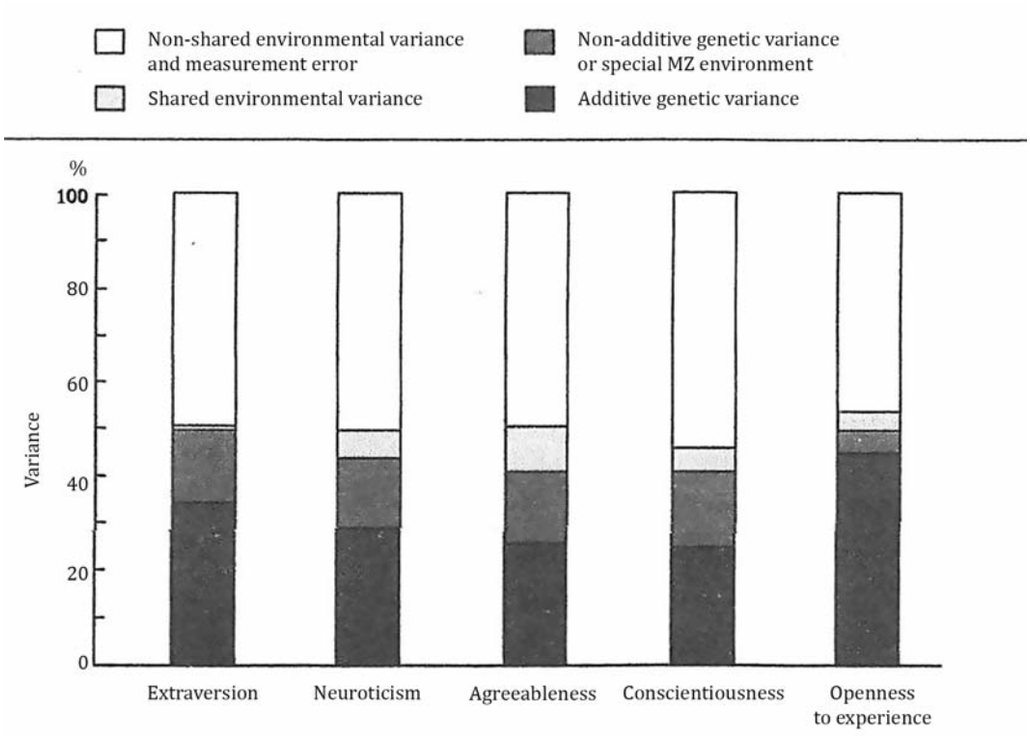

FIG. 2. Summary of twin and adoption research studies in personality traits organized according to the Five-Factor Model (Loehlin 1992).

Research suggests that as much as half of the variation in general cognitive ability or "g" among individuals may be attributed to genetic factors, In studies involving more than 10.000 pairs of twins, the average "g" correlations are 0.85 for identical twins and 0.60 for same-sex fraternal twins, and the heritability is 50% (Plomin, De Fries & McClearn, 1997).

Twin studies of identical and fraternal twins reared apart provide additional support for genetic influence on specific cognitive abilities. Heritability estimate of perceptual speed is in the range of 53 to 58 per cent and of spatial visualization of 46 to 71 per cent (Plomin, Happe, & Caspi 2004).

EPITOME

As indicated in the preceeding section genetic studies converge on the conclusion that individual differences in most phenotypes linked to superior sport performance are substantially influenced by genetic factors. Figure 3 summarizes heritability estimates reported by twin studies referred to in earlier paragraphs and they suggest that genetic influence is so ubiquitious and persuasive in most determinants of sport performance that we ask not what is heritable, but what is not heritable.

However, the concept of heritability is often misunderstood. A heritability estimate of 93 per cent found in our early study, for maximal aerobic power, as an

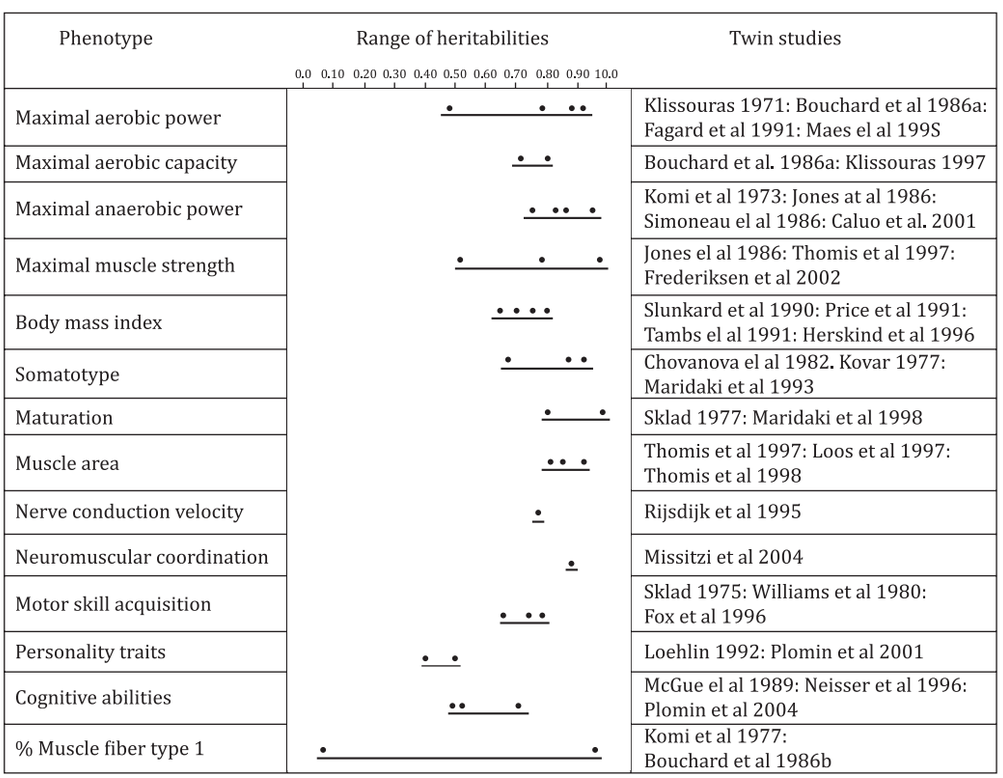

FIG. 3. Summary of twin studies on the heritability of phenotypes related to superior sport performance. Each dot represents one study.

example, is often misinterpreted to mean that 93 per cent of an individual's VO_2max is genetically determined and the remaining 7 per cent is susceptible to environmental modification. This is a fallacy. Heritability has no etiologic role in the phenotype, nor has it sensible meaning with reference to measurement in an individual. It is a statistical measure, expressed as a percentage, and refers only to the population. It describes the extent to which heredity affects the variation of a given attribute in a given population exposed to common environmental influcences at a given time.

High heritabilities obtained for some determinants of sport performance have been overinterpreted to mean that peak performance is genetically determined. A high heritable attribute does not mean that it is predeter-mined and the environment has no effect. It only indicates that observed individual differences in the given attribute are due to genetic differences and are highly predictable. Thus, when it is stated that VO_2max is highly heritable, what is really meant is that after individuals have reached the upper limits of their VO_2max, with appropriate training, there will still be a wide interindividual variability which is genetic in origin.

Let us now turn to the potency of environmental forces, in the actualization of the genetic potential, for disentangling further the influences associated with Nature and Nurture.

Relative Power of Nurture

A high heritability of a given phenotype does not exclude environmental influence. The suggestion, for example, that the variation in muscle fiber distribution is genotype-dependent does not necessarily mean that the proportion of muscle fibers is unaltered, fixed and predetermined. In fact, it has been demonstrated that muscle fibers will change their phenotypic properties in response to sustained changes in functional demands. Adaptive responses encompass changes in gene expression, ultimately resulting in reversible fiber type transitions (Pette, 2005). The proportion of muscle fibers in humans is altered in response to training, detraining, immobilization and microgravity (Saltin, & Gollnick, 1983; Mikesky, Giddings, Mathews, & Gonyea, 1991; Antonio, & Gonyea, 1993; Hawke, & Garry, 2001). Further, it has been shown that there is an interconversion of type IIa and IIb muscle fibers in humans in response to training as well as an interconversion of type II to type I muscle fibers in small mammals in response to increased muscular contractile activity (Pette, & Staron, 1990; Booth, & Thomson, 1991; Staron, & Johnson, 1993; Kraemer, Fleck, & Evans, 1996; Kadi, & Thornell, 1999).

The development of a phenotype reflects the effects of genes as well as those of epigenetic influences. Apparently, no genes can operate in a vacuum nor can phenotypes develop without the action of environmental forces.

Apparently genes are active during life; they switch each other on and off; they

respond to the environment. As Ridley (2004)put it; they are both case and consequence of our actions. Somehow the adherents of the "nurture" side of the argument have scared themseves at the power and inevitability of genes, and missed the greatest lesson of all: the genes are on their side.

Is a phenotypes ceiling set by the genotype or by training? The use of cross-sectional and longitudinal studies in disentangling this hypothesis has the obvious limitation that the genetic factor is operant to an unknown degree in different individuals. Using monozygotic twins as subjects, however, obviates this problem since each subject is accompanied by a genotypically identical control. We tested a pair of monozygotic twins aged 21 years, an athlete and his identical sedentery brother, over a 17-month period. The athlete, being a member of the varsity football and hockey on ice teams, underwent strenuous athletic training, year-round, designed to develop both maximal aerobic and anaerobic powers. It was reasoned in selecting the co-twin analysis, that if athletic training, confined to one twin and extended over a period of years, failed to raise his functional adaptability from a low to a superior level, then its upper limit might be assumed to be set by the genotype (Klissouras, 1972).

In this co-twin study the trained brother had marked adaptations in metabolic, cardiac and muscular functions. For example, he had almost 40% higher VO_2max and 70% higher blood lactate concentration after maximal exercise, than his untrained brother. The most striking observation however, was not the percent change but the absolute values. The untrained twin had a VO_2max of 35 ml·kg^{-1}·min^{-1}, whereas the trained twin had a peak value of only 49 ml·kg^{-1}·min^{-1}. This latter value is comparable to an average maximum value of about 50 ml·kg^{-1}·min^{-1} for untrained college men of the same age, well below values reported for top athletes. So despite hard and prolonged training, the trained twin was unable to surpass an average level of VO_2max. The reason for this seems to hinge on his low pretraining as judged from that of his identical counterpart. This observation strongly suggests that rigorous athletic training cannot contribute to functional development beyond a limit set by the genotype.

Training can produce results only within the variability allowed by the genotype. The standard deviation of maximum oxygen uptake in a large homogeneous population with a similar degree of training is about 13%. Suppose then that the mean value of maximum oxygen uptake in a given population is 40 ml-·kg^{-1}·min^{-1}; individual values for 95% of the population (2 SD) will range anywhere between 29.6 and 50.4 ml·kg^{-1}·min^{-1}. With rigorous training the individual with a maximal value of 29.6 will be able to reach a level of about 40 ml·kg^{-1}·min^{-1}. Such an individual can never expect to achieve any distinction in sport performance which is dependent upon the maximal aerobic power. Training will never erase individual differences which are due to innate ability.

ACTUALIZATION OF GENETIC POTENTIAL

Superior performers are endowed with high genetic potential for their specific sport. Genetic potential is not a passive possibility, but an active disposition, actualized through hard, prolonged and prodigious effort. *Genes apparently are not like switches which can he turned on to have effects.* The actual realization of the genetic potential does not occur instantly, but may rather take several years. As Bronfenbrenner, and Ceci (1993) eloquently put it *"... this dynamic potential does not spring forth full-blown like Athena out of Zeus's head from a single blow of Vulcan's hammer. The process of transforming genotypes into phenotypes is not so simple or so quick".*

It is true that a talented athlete, endowed with superior natural ability, may manifest high performance with a minimal amount of practice or without environmental support but eminent achievement cannot be accomplished without a high level of appropriate deliberate practice (Ericsson & Charness, 1994). Deliberate effortful practice is a prerequisite for the actualization of the athlete's genetic potential. If the environmental forces were optimized, the only decisive factor to peak sport performance would be the genotype.

Yet, though genes and training may set the biophysical limits to human performance, and the prerequisites to enter, metaphorically speaking, the gate of the olympic stadium, it is behavioral features, which determine the ultimate frontiers of sport performance.

In this context, we reported a case study resulting from a larger epidemiological study that, because of its uniqueness and experimental nature, may prove particularly enlightening. Our study refers to a pair of Olympic twin athletes in 20 km competitive walking race, who, although genetically identical and exposed to the same environmental influences and the same training with the same coach, were markedly different in performance (Klis-souras, Casini, Di Salvo, Faina, Marini, Pigozzi, Pittaluga, Spataro, Taddei, &Parisi, 2001).

Both twins had highly trained during adolescence (from age 15 to 18) for 10 km competitive walking, and thereafter (19 to 33) for 20 km, under the coaching of their older brother with an identical training programme. More generally, living style and related variables were very similar in the two twins, who had been living together from the time of their birth. During their sport career they walked yearly an average of 5.125 km for 243 days. Their mode of training consisted of endurance (59% of the time), specific work (15%), strength (9%), and technique (17%). They competed an average of 14 times per year and had remarkable sport achievements. One of them was an Olympic medal winner at three successive Olympiads (gold medalist in 1980, silver medalist in 1984 and 1988) as well as world champion in 1987, while the other finished at the 11th place in the 1980 Olympic Games and came first in the World Championship of 1983, which was when his brother did not participate.

Therefore, the pair of Olympic twin athletes we tested provides a unique case, particularly in view of the fact that, although genetically identical and identically trained for years, their achievement was distinctly different, as one three times an Olympic winner while the other was about 4.4% slower and only managed to win when his co-twin was not competing.

Possible determinants of aerobic performance in competitive racewalking are VO_2max, fractional utilization of VO_2max at the threshold for lactate release, and walking economy (Hagberg & Coyle, 1983; Joyner, 1991; Coyle, 1995). Differences between the high- and the low-performing twin in all these factors were within the experimental error (about 5%) and were not expected to reflect performance time difference (about 4.4%), which corresponds close to 1 km distance in a 20 km race walking. A marked difference of 18.4% was noted in the ability of the Olympic twin to produce a higher lactate concentration in the blood during maximal effort. However, this anaerobic component was not reflected in the percentage of VO_2max at the threshold for lactate release as mentioned earlier, and hence it is highly unlikely that the anaerobic processes could have contributed to the enhancement of the endurance performance of the Olympic winner (Billat, Pinoteau, Petit, Renoux, & Koralsztein, 1994).

Finally, personality measures were also taken in the two twins. In general, the heritability of personality variables, as was pointed out earlier, hardly exceeds 0.5, so that some amount of variation is expected in identical twins as well. The focus of the analysis was on anger-related variables (Spielberger, Jacobs, Russel, & Grane, 1983), considered to be possibly associated to sport performance. The psychological profile of the twins with respect to anger expression, which reflects the emotional reaction to conditions evoked at diverse levels and is considered an important personality trait, was similar for some components but completely different for others (Figure 4).

The twins were comparable in anger expression towards others and surrounding objects, anger suppression and temperament evoked by anger. However, the Olympic winner had an exaggerated response to frustration and showed excessive sensitivity to criticism and negative evaluations, as well as excessive control over his emotions and behaviour, while his anger was never openly expressed. The emotional reactions of his brother were, however, at the opposite extreme: he was not frustrated, was insensitive to criticism and only moderately able to control his anger through the cognitive elaboration of his frustrations.

It seems likely that this major and basically only difference between the twins may be responsible for their difference in performance, and one could reason that the unexpressed anger in the champion may have enhanced his competitive drive and his autonomic function. It could also be inferred that such a drive may explain his better tolerance to acidosis during heavy exercise. At any rate, this fairly unique

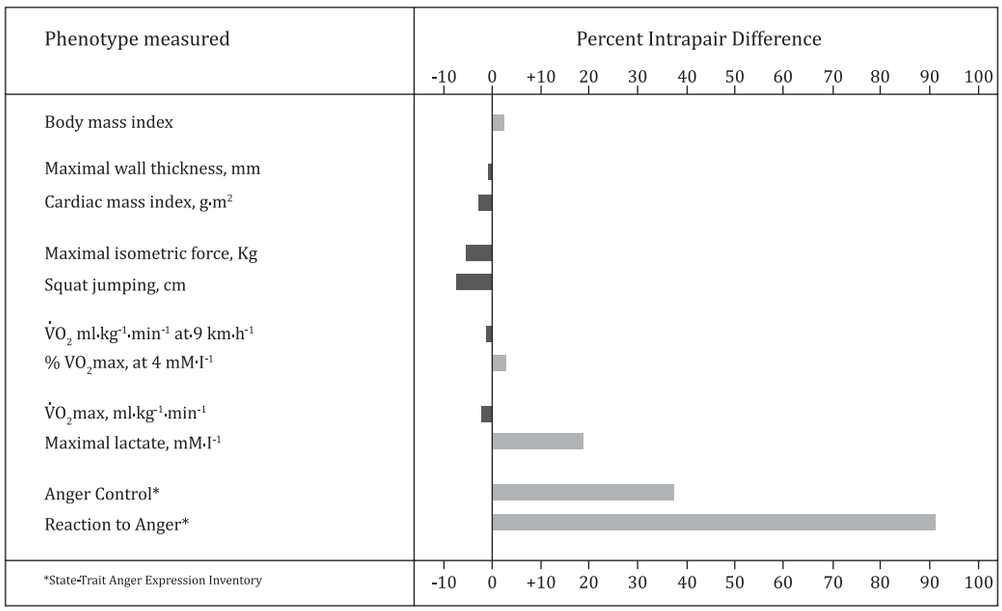

FIG. 4. Intrapair differences in physiological attributes and personality traits of an olympic gold medallist in a 20 km competitive walking race and his identical twin brother, also an Olympic athlete in the same event but with inferior performance (Klissouras, Casini, Di Salvo, Faina, Marini, Pigozzi, Pittaluga, Spataro, Taddei, & Parisi,2001).

example of performance difference in otherwise identical twins shows that genetic factors as well as training alone are not sufficient to make a champion, and that the personality profile plays relevant a role in superior sport performance.

Nature-Nurture Interplay

From the preceeding discussion it appears that elite athletes are endowed with superior genetic potential, which, when actualized with appropriate training, is expressed with sport-specific phenotypes, determinants of superior performance. A question of considerable theoretical and practical importance is whether different genotypes respond to a given training stimulus with a change of different magnitude. In other words, one wonders whether there is an interaction between Nature and Nurture. It has been postulated that in addition to superior genotypes, athletes of Olympic caliber most probably also have inherited the genotype characteristic of high response to training (Bouchard & Malina, 1984). This postulate was initially based on the wide inter-individual variability observed in VO_2max increase of previously sedentary humans exposed to endurance training and subsequently supported by some evidence obtained by co-twin studies.

Two relevant studies were conducted in Bouchard's laboratory at Laval University. In the first study 10 MZ twin pairs (6 female and 4 male) aged 20+2.9 years were submitted to a 20-week endurance training. VO_2max improved by 16%, with considerable interindividual differences in training gains as illustrated by a range of 0 to 41%. Intraclass correlations computed with the amount of training gain in VO_2max ml $\cdot$ kg^{-1} $\cdot$ min^{-1} was 0.74, indicating that members of the same twin pair

yielded a fairly similar response to training, i.e. 74% of the variance in the training response seemed to be genotype-dependent (Prud' Homme, Bouchard, Leblanc, Landry, & Fontaine, 1984). In the second study 6 pairs of MZ twins (3 males and 3 females) 21 ±4 years of age were submitted to a 15 week endurance training. Changes in VO_2max were 4.6 times more similar within twin pairs than between pairs, as revealed by the F-ratios of the interaction effect. The intraclass correlation of the twin resemblance in the response to training reached 0.65 for VO_2max ml $\cdot$ kg^{-1} $\cdot$ min^{-1} (Hamel, Simoneau, Lortie, Boulay, & Bouchard, 1986).

The contention of the inheritance of VO_2max trainability is strengthened with data obtained in the HERITAGE Family Study (Bouchard, An, Rice, Skinner, Wilmore, Gagnon, Pérusse, Leon, & Rao, 1999). It has been demonstrated that there is a familial aggregation of ΔVO_2max response to exercise training. There were families with a predominantly low-response phenotype and others with large concentrations of high responders. There was 2.5 times more variance between families than within families and this was attributed to genetic influence. A heritability estimate of 47% was reported in that study.

Moreover, it was revealed that the VO_2max response to training ('VO_2max) expressed as ml $\cdot$ kg^{-1} $\cdot$ min^{-1} was not related to the initial level of VO_2max expressed also in ml$\cdot$kg$^{-1}\cdot$min^{-1}. There were nonsignificant correlation coefficients ranging from 0.03 to 0.16 computed separately for fathers, mothers, sons and daughters. The authors, in their discussion, stated that "The familial factors underlying VO_2max in sedentary families are quantitatively similar to those underling its response to exercise training. However, even though they are qunatitatively about the same, the familial and genetic factors underlying the two phenotypes appear to be different, as indicated by the lack of a relationship between baseline VO_2max and VO_2max response."

In a subsequent analysis of the data from the same laboratory it was found that when the relative (%) changes in VO_2max were correlated with initial levels, the relationship was significant (r=-0.38) (Skinner, Jaskolski, Jaskolska, Krasnoff, Gagnon, Leon, Rao, Wilmore, & Bouchard, 2001). Thus, it may be important how the increase is expressed when we search for the cause of heterogeneity in response to training.

Findings from a handful of other studies have cast serious doubt on the proposition that there is a genotype-environment interaction in VO_2max and other phenotypes related to sport performance. Using a different experimental approach Klissouras and associates were unable to find that train-ability of VO_2max is genotype-dependent. Split-twin experiments, in which one twin trains and his identical partner acts as a control, make it possible to separate the observed intra-pair variance into its three components: that due to heredity, that due to training and that due to the interaction between heredity and training.

In an initial study eight twin boys underwent a 10-week training program of the same amount and intensity, while their identical brothers restricted their activities to normal daily routines (Weber, Kartodihardjo, & Klissouras, 1976). VO_2max of all twins was measured before and at the end of the 10-week period. The mean VO_2max for all experimental and control twins was 51.9 $ml \cdot kg^{-1} \cdot min^{-1}$, with non-significant intra-pair differences. The inter-pair variability ranged from 41.1 to 58.6 $ml \cdot kg^{-1} \cdot min^{-1}$, so that the interaction hypothesis could be tested. The mean VO_2max after training was 59.4 $ml \cdot kg^{-1} \cdot min^{-1}$, with adjustments for changes observed in the non-trained twins, and the range was 45.2 to 69.3 $ml \cdot kg^{-1} \cdot min^{-1}$. Treatment of the results by analyis of variance revealed that the interaction between genotype and training contributes only 7% to the total variance (Table III). The same Table shows also the findings from a more recent split-twin study with nine male pairs of MZ, where one twin from each pair undertook training for 24 weeks (Danis, Kyriazis, & Klissouras, 2003). The contribution of the genotype-training interaction explained still a relatively small (17%) part of the total variance.

It seems that there is also a nil or minor genotype-training interaction in muscle strength and muscle hypertrophy after high resistance training. Thomis, Beunen, Maes, Blimkie, Van Leemputte, Claessens, Marchal, Willems, and Vlietinck (1998) submitted 25 male monozygotic twin pairs, aged 22.4 ±3.7 years, to a 10-wk resistance training program for the elbow flexors.

The MZ intra-pair resemblance in training responses showed a moderate correlation in one repetition maximum (0.46) and isometric strength (0.30) increases in MZ twins, while nonsignificant low (0.07 to 0.30) and negative correlations (-0.04 to -0.40) were found for dynamic strength and muscle cross-sectional area. Further, no evidence of genotype-training interaction was found for key enzyme activity and fiber type composition of human skeletal muscle (Simoneau, Lortie, Boulay, Marcotte, Thibault, & Bouchard, 1986).

On the basis of the aforementioned studies it appears that the hypothesis that the heterogeneity in trainability is inherited is not tenable. In addition, findings

TABLE III. Analysis of variance in VO_2max, ($ml \cdot min^{-1} \cdot kg^{-1}$). Estimates of variance, in actual figures, were computed in the following way (n=numbers of twin pairs): Heredity = (mean sq. heredity - mean sq. interaction)/2; Training = (mean sq. training - mean sq. interaction)/n. Eight male pairs of MZ: twins aged 10-16 years participated in the 1976 study where one twin in each pair trained for 10 weeks, while nine male pairs of MZ twins aged 11-14 years participated in the 2003 study where one twin in each pair trained for 24 weeks.

	Weber, Kartodihardjo, & Klissouras, 1976		**Danis, Kyriazis, & Klissouras, 2003**	
Sources of variation	**Mean squares**	**Variances in per cent of total variance**	**Mean squares**	**Variances in per cent of total variance**
Training	221.72	42	278.48	37
Heredity	69.04	51	43.55	46
Interaction	4.39	7	16.60	17

from these studies can hardly be applied to athletes. The reason is that previously sedentary humans were used and hence the focus had been centered on the etiology of individual differences in the normal range of the distribution curve.

Beyond Heritability: Good Gene Hunting

Quantitative genetics using experiments of Nature such as twinning have made it possible to disentangle the effects of Nature and Nurture. Today we know the heritability of many phenotypes related to superior sport performance. One of the most formidable challenges for scientists is to identify human gene polymorphism responsible for the substantial heritability of their phenotypes. For multifactorial phenotypes, such as VO_2max, the goal is not to find the single major gene but the polygenes that contribute to their variance.

Two approaches have been used to detect specific genes related to sport performance and phenotype; the family-based linkage analysis and the candidate gene association approach (Brutsaert & Parra, 2006). The candidate gene studies seek to associate measured genotypes with phenotypes.

One of the first genes identified as a putative factor of VO_2max and sport peformance was the agiotensin-converting enzyme (ACE) gene. This enzyme plays a key role in generating angiotensin, which is a powerful vasconstricting hormone that acts at various sites in the cardiovascular system. There is conflicting evidence as to the role that ACE Insertion (I)/Deletion (D) polymorphism plays in sport performance. There is an I form and D form of the ACE gene in each individual. Individuals with the II genotype have lower ACE activity and with DD higher, while individuals with ID have intermediate levels of activity. Some studies show an association between the ACE I/D genotype and performance phenotypes (Gayagay, Yu, Hambly, Boston, Hahn, Celermajer, & Trent, 1998; Myerson, Hemingway, Budget, Martin, Humphries, & Montgomery, 1999; Alvarez, Terrados, Ortolano, Iglesias-Cubero, Reguero Batalla, Cortina, Fernandez-Garcia, Rodriguez, Braga, Alvarez, & Coto, 2000; Williams, Rayson, Jubb, World, Woods, Hayward, Martin, Humphries, & Montgomery, 2000), while others failed to find such association (Taylor, Mamotte, Fallonet, Van Bockxmeer, 1999; Rankinen, Wolfarth, Simoneau, Majer-Lenz, Rauramaa, Rivera, Boulay, Chagnon, Perusse, Keul, & Bouchard, 2000). Hence, the significance of the ACE I/D polymorphism in explaining variation in sport performance remains controversial. In this regard Brutsaert and Parra (2006) pointed out that "this may prove to be paradigmatic for the candidate gene approach in general", and cited Cambell and Rudan (2002) who argue that "measured gene studies are observational not experimental, and so there is the problem of false association due to chance, bias or confounding".

Many other genes have been identified as putative factors. However, given that there are 32,000 human genes, the task of identifying mulitple polymorphisms that contribute to the variation observed in superior sport performance is daunting.

An annual report of the Human Gene Map for Performance produced by a team of scientists led by Claude Bouchard, provides periodically a compendium of all genes and markers associated with performance. The 2005 map includes 140 automosal genes and quantitative trait loci, five X chromosome assignments and 17 mitochondrial DNA markers (Rankinen, Bray, Hagberg, Perusse, Roth, Wolfarth, & Bouchard, 2006). Indeed, there is a long way to go before we begin to understand which genes and pathways are contributing to human variation in sport performance.

GENETICALLY MODIFIED ATHLETES: TO WHAT END?

Technology now available to study how genetic characteristics shape sport performance may some day revolutionize almost every facet of sport Advances in this area may provide further insights into the molecular and genotype mechanisms governing the limits of sport performance, and may hold great promise in improving athletic training.

However, such advances may also have profound implications on the practice of sport. The next technological advance will be implants and transplants. It will be possible to insert, modify and activate specific genes. The technology is already available to insert a gene that will overproduce insulinlike growth factor in skeletal muscle. Using this gene therapy technique on mice resulted in a 15-30% increase in muscle size (Goldspink, 2004). Inserting genes, it is also possible to shut down myostatin, the protein that acts as a break and puts an upper limit on muslce growth and strength. Muscle mass could increase as much as 40% by blocking myostatin (Whittemore, Song, Li, Agha-janian, Davies, & Girgenrath, 2003).

There is a fear that athletes may use, misuse and abuse genetic technology to gain a competitive edge. The frightening possibility exists, that limits of sport performance may be determined less by an athlete's innate endowment and commitmet to training, and more by genetic interventions engineered for faster-acting, more powerful muscles, greater oxygen transport, and more rapid circulation.

Genetic doping threatens the spirit, the very essence of sport. It could eliminate what sport is all about. In the words of Wadder (2001): *"We stand at the brink of an uncertain future. The unpredictability and the velocity of change are not an excuse for reserving judgement about some profound distinctions, that should fundamentally govern our perspective on the role of sport in our society...". The distinction between "the triumph of character and the triumph of chemistry".*

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Nature-Nurture: Not an either-or question

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"On the path to excellence the immortal gods set sweat;
it is long, steep and rough at first. But when one reaches the top,
then it is easy, for all the difficulty.
Hesiod

The athletes who take part in the Olympic Games are in peak physical condition. To attain this, they undergo years of physical training. And some people think that, if they had had the same advantages of youth and training, they too could become Olympic athletes. Could they? Is training really the big variable or is a superior athlete born, not made?

Ericsson's basic premise, in his target article of this issue, is that exceptional performers are *made not born.* We have argued in our target article that the question can't be answered with a clear-cut yes or no, but systematic twin studies performed in a number of laboratories over the years indicate the tremendous importance of being born with the right genes.

We may accept Ericsson's argument that "in well-established domains of exercise even the most 'talented' cannot reach an international level in less than around a decade of experience and intense preparation". Further, we have no reason to reject Ericsson's concept of *deliberate practice* and there is indeed evidence to support his suggestion that "expert performers keep pushing themselves during training to go beyond their current adaptations to reach new and more far-reaching changes"; by applying, we may add, the well established physiological principles of progressive overload and specificity. Furthermore, there is growing evidence to suggest that a central governor may regulate sport performance (Noakes 2000), and as Bannister (1956) has written *"Though physiology may indicate respiratory and cardiovascular limits to muscular effort, psychological and other factors beyond the ken of physiology set the razor's edge of defeat or victory and determine how closely the athlete approaches the absolute limits of performance".*

Thus, the question "Is an athlete born or made?" should be rephrased to read "Does everybody have the genetic material which with an appropriate training can tune to produce a superior athlete?" And the answer is "No". This is not to say that training has no purpose but rather that, even with training, each of us has a ceiling

of performance dictated by our genes. It seems that training will never erase individual differences which are due to innate ability. Training can exert its (indeed) profound effect only within the fixed limits of heredity. If environmental factors are optimized, the only decisive factor to peak human performance would be the genotype.

Methodological Criticism

The Twin Model: A Powerful Tool

We have reached this general conclusion mainly by using the classical twin study. The most common methodological criticism of this approach concerns the equal environments assumption, namely that both monozygotic and dizygotic twins had been exposed to similar environmental circumstances. It is axiomatic that twin studies in which the foremost assumption of equal environments has been ignored or violated, must be viewed with caution. However, well conducted twin studies, in which the assumptions of the twin model (see page 39 in our target article) have been respected, clearly show that Nature has a major impact on phenotypes related to sport performance. It seems that Nature leads and Nurture follows.

The twin model is not only of "historical value", as commented by Malina. Two novel approaches have been used to detect genes related to phenotypes and sport performance; the family based linkage analysis and the candidate gene association approach. They are two relatively new promising approaches with their own pros and cons. However, they are both highly reductive, complementary to and not substitutes for the twin model. Whatever is old is not historic and obsolete; linkage analysis is not new and it has long lasting history which has been recently revived (Brutsaert & Parra, 2006). As a matter of fact, a heritability estimate related to sport performance may be far removed from single genes but not from gene combinations, since performance is polygenic in nature.

Quantitative genetics, using the twin model, offer a unique and powerful method of disentangling the relative power of genes and environment and their interaction, according to specific experimental protocols, in the variation observed in the phenotypes linked to superior performance. This model reveals little about the causal spectrum and genetic architecture of twins and its empirical application entails difficulties related to the subjects' shortage and the underlying, many times untested assumptions which accompany it.

Paradigms proposed in this issue by: a) Ericsson, b) Araujo and c) Aber-nethy and Cote deal with the effect of different aspects of the environment on formulating high performance and are mutually exclusive. On the contrary the theoretical frame around the twin model is the only tri-dimensional, and comprehensive one. It can be used to acquire empirical data from an ecological, psychological, physiological

perspective and any of their combination. As Hawke points out in his commentary the gene-environment interplay has been left largely unaddressed and this inevitably focuses our interest on Nature and Nurture. It is ironic, however, that our stance, grounded on abundant evidence, is characterized by some commentators as reductive or dualistic while at the same time they propose paradigms embodied with high degree of *monadism* and methodological solitude.

The estimation of heritability is not a division of Nature and Nurture. It is only a statistical approach of the relative influence in the phenotypic variation at the population level. Such an analysis may serve as an early step in carrying out research for identification of genes involved in physiological traits and polymorphisms that have been associated with athletic performance. It may also serve as a precursor in elucidating mechanisms underlying individual differences in the development of relavent phenotypes. Moreover, such identification of genetic variants which influence athletic performance may be added to the existing battery of physiological, biochemical and psychological tests that form the current basis for selecting talented young athletes for further training. However, as MacArthur and North (2005) have noted *"there is still no evidence that any of these variants have any substantial predictive value for prospectively identifying potential elite athletes. The detailed analyses of physiological parameters currently used actually represent integrated measurements of the effects of multiple genes and environmental influences on the phe-notype, whereas genetic tests examine only single isolated determinants."*

Holistic Approach

Factors involved in sport performance are extremely complex and some commentators argue that we need a holistic, an interactionist approach to study this phenomenon. We recognize the inseparability of Nature and Nurture, since neither genes can operate in a vacuum, nor phenotypes can develop and be actualized without the action of environmental forces. Hence, there is a need to explore the hyphen in the phrase Nature-Nurture. As Dobzhansky (1964) has stated: *"The Nature-Nurture problem is far from meaningless. Asking right questions is, in science, often a large step toward obtaining right answers. The question about the roles of the genotype and the environment in human development must be posed thus: To what extent are the differences observed among people conditioned by the differences of their genotypes and by the differences betiveen the environments in which people were born, grew and brought up?"*

Moreover, a holistic approach implies to link, to unite psychology and biology. Such an interdisciplinary integration would allow for cross-fertilization, the fruitful application of methods and data from one domain to the other. We recognize that this is a formidable task that requires the command of both different disciplines, but can be accomplished with collaboration of scientists from both fields. Moreover, it is imperative to overcome dualism, the last bastion of the mind-body dichotomy.

Nature and Nurture cannot willingly be integrated. *"No theory ever agrees with facts in its domain, yet it is not always the theory that is to blame. Theories become clear only after incoherent parts of them have been used for a long time" (Feyerabend, 1986).*

TALENT: KNOWNS AND UNKNOWNS

Although our lead article had not explicitly addressed the issue of talent, it certainly has some implications for it. Most commentators and particularly Button and Abbott have chosen to focus on talent identification and development, and in so doing, they have extended and enriched the discussion on Nature-Nurture and Sport Performance.

In an excellent review of relevant research, Howe Davidson and Sloboda (1998) argued that talent has several properties. First, they suggested that talent originates in genetically transmitted structures and hence is partly innate. Talent may not be evident at an early stage, but there will be some advance indicators that enable trained people to identify its presence, before exceptional levels of nature performance have been demonstrated. These early indicators of talent provide a basis for predicting those individuals who are likely to excel at some later stage. Only a minority is talented in any single domain, for if all children were talented, there would be no way to predict or explain differential success. Finally, talent is domain-specific.

These properties highlight the complex and multidimensional nature of talent. It appears that there are physical, physiological, psychological and sociological predictors of talent (Williams & Riley, 2000), while for individuals to reach their full potential, they must possess and exhibit the motivation and learning strategies to interact effectively with the developmental opportunities offered by the environment (Abbott & Collins, 2004).

We argue that genes are ability multipliers and precursors of high achievement. A prodigy, a highly-gifted, an exceptionally able child is the precursor of adult excellence. Top performance is an epiphenomenon of talent. A talented athlete is endowed with superior natural ability, that is a biophysical disposition combined with eagerness and power of hard work. A talented individual may manifest high performance with a minimal amount of practice or without environment support, but exceptional performance cannot be achieved without appropriate training. In this respect we concur with Simonton (1999) who stated: *"Just because a trait claims a genetic foundation does not automatically mean that the trait appears all at once. On the contrary, many characteristics, even if under demonstrably genetic control, take many years, even decades to emerge".*

OLYMPIC TWIN ATHLETES: THE NATURE-NURTURE EXPERIMENT

Twin studies reported in our target article have addressed the aetiology of individual differences in various phenotypes related to sport performance in the normal

range of the bell curve. Twin athletes who represent the high end of the distribution rarely have been used.

Using ordinary twins who have been exposed to normal, but similar, environmental influences we have derived heritabilities for most phenotypes linked to high performance. These heritabilities denote the aetiology of differences in these phenotypes between individuals in the normal range; they express the genetic and environmental provenance of measured differences among individuals as they exist in a particular population. The implications of heritability data are commonly misunderstood. As Plomin and Thompson (1993), and Plomin and DeFries (1998) point out, the degree of heritability for a given trait is not set in stone. The relative influence of genes and environment can change. Heritability describes "what is" in a population, it does not predict "what could be", nor does it prescribe "what should be". Heritability denotes probabilistic genetic influence for a population, not predeterminism or immunability for an individual.

The same authors raise the issue whether genetic factors affect high ability and how the magnitude of this genetic influence compares with the magnitude of genetic factors that contribute to individual differences in the normal range. They suggest that this question can be addressed using a new approach that generates an estimate of what is called *group heritability,* in contrast to the traditional heritability statistic, which is referred to as *individual heritability.* Group heritability is

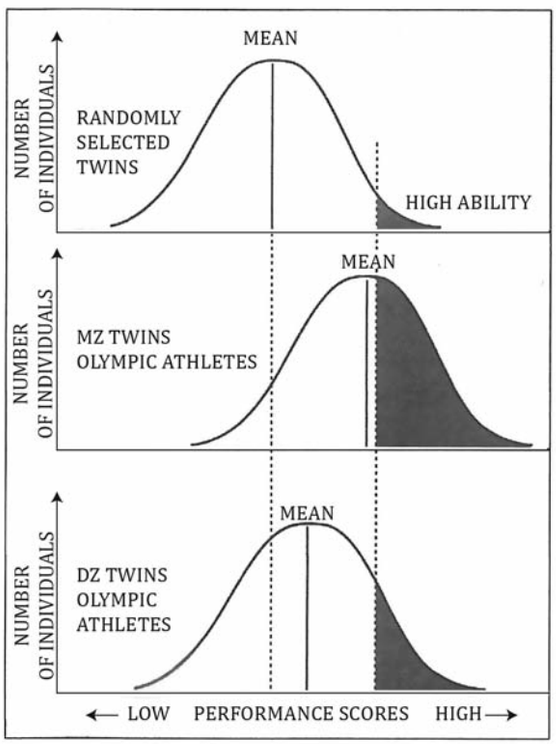

FIG. 1. Performance distributions for an unselected sample of twins and Olympic athletes monozygotic (MZ) and dizygotic (DZ) co-twins. The top distribution is an idealized normal distribution for an unselected sample of twins. Individuals of high ability, are defined as those with a performance score of a predetermined standard deviation, above the sample mean of 0.0. The two distributions below are those for Olympic athletes MZ and DZ co-twins. In the event that the MZ co-twin mean regresses less far towards the mean of the unselected population than does the DZ co-twin mean, it suggests heritability of high peak performance (based on Plomin & Thompson, 1993).

the genetic contribution to the average difference between a selected group and the rest of the population. It is assessed by the method called DF extremes analysis, as the differential regression of the population mean of the co-twins of monozygotic and dizygotic twins selected on the basis of a quantitative measure of high ability (DeFries & Fulker 1985, 1988; Plomin, DeFries, McClean & McGuffin, 2001).

We are currently applying this new genetic technique, which is illustrated in Figure 1, using Olympic twin athletes who have undergone years of strenuous athletic training and have actualized their genetic potential. We expect that such an analysis will yield estimates of group heritability of high ability in phenotypes linked to Olympic performance in various sport disciplines, with far-reaching implications to the age old Nature-Nurture problem.

ANIMAL STUDIES: IT'S ALL IN THE GENES

Given the complexity of performance traits which are determined by the interplay of "Nature" (genetic endowment for high activity) and "Nurture" (environmental factors such as lifestyle and training), animal models with minimal genetic as well as environmental variation have been used for determining the genes that underlie the individual variations in exercise capacity. Artificially divergent selection of rats was aimed at creating low-capacity (LCR) and high-capacity runners (HCR) as an experimental tool for separating genetic and environmental influences on particular phenotypic determinants, such as aerobic capacity. The animals remain sedentary throughout their lifetimes and, therefore, differences in exercise performance between LCR and HCR rats reflect their genetically determined intrinsic exercise capacities (Hawley & Spargo, 2006).

Six generations of selection produced LCR and HCR that differed in aerobic endurance capacity (evaluated as distance run to exhaustion), as shown in Figure 2, by 171% (LCR: 310+8 m; HCR: 839+21 m); while after 11 generations of selective breeding intrisic endurance capacity had diverged by a massive 347% (Wisloff, Najjar, Ellingsen, Haram, Swoap, Al-Share, Fernstrom, Rezaei, Lee, Koch & Britton, 2005).

Endurance capacity is considered to be more dependent on the oxidative capacity of the skeletal muscle compared to VO_2max (Bassett & Howley, 2000). Interestingly, in female rats artificially selected for endurance running, the 12% higher VO_2max in HCR compared to LCR at generation 7 (Henderson, Wagner, Favret, Britton, Koch, Wagner, & Gonzalez, 2000) was solely due to enhanced muscle oxygen extraction and utilization.

However, as VO_2max differences between LCR and HCR continued to increase with successive generations (50% higher VO_2max in HCR) the HCR rats of the 15th generation showed significantly higher values of maximal cardiac output (by 42%) and oxygen delivery (by 41%) than LCR, suggesting that, progressively, the incre-

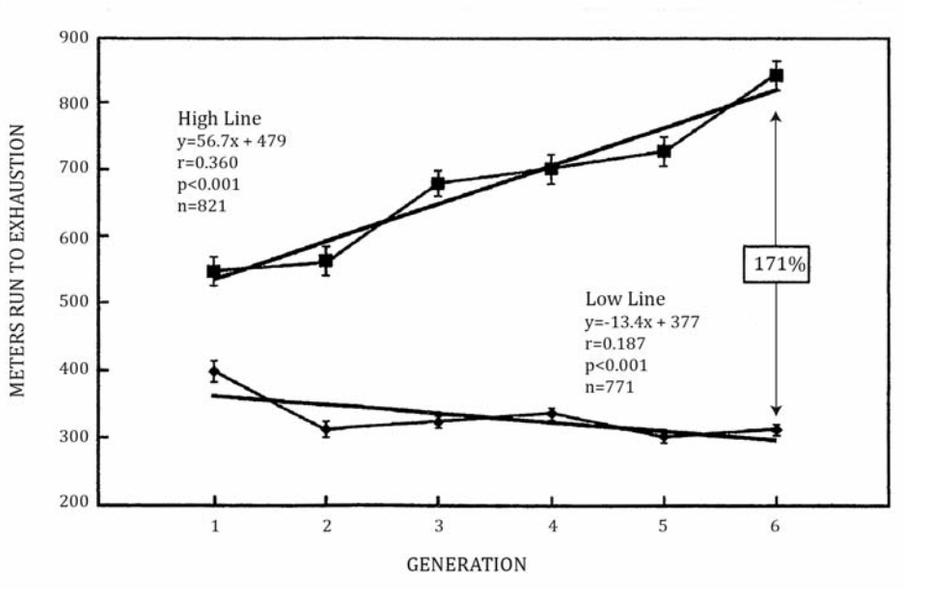

FIG. 2. Divergent response to selection across six generations for the low and high lines. Each point represents the average distance run (±1 SE) to exhaustion for females and males combined at each generation. The regressions were derived from data on all rats (low line n=771; high line n=821). On average the high line increased 56.7 m/gen-eration, and the low line decreased 13.4 m/generation (Koch & Britton, 2001).

ased capacity for oxygen transfer at the tissue level was accompanied by an increased rate of convective oxygen delivery to the tissues (Gonzalez, Kirkton, Howlett, Britton, Koch, Wagner, & Wagner, 2006).

Apparently the inherent maximal aerobic capacity depends on the stage of "evolution" that the organism is in. Nevertheless, such results are expected to speed the progress towards understanding the genetic factors underlying exercise endurance in animal models, which in the long term will be translated to the human model (Lightfoot, 2006).

SPORT FOR ALL: NURTURE, NOT NATURE

We have argued in our target article that genetic influence in superior sport performance is so ubiquitous and persuasive that we ask not what is heritable but what is not heritable. The question arises whether the preponderance of Nature on phenotypic variation applies equally well to factors associated with daily physical activity and by implication to sport for all, which requires physical activity of moderate intensity. A number of genetic epidemiological studies using large samples of twins and structural equation modeling for data analysis, have investigated the hereditary effects on physical activity. In a just published survey Carlsson, Andersson, Lichtenstein, Michaelsson and Ahlbom (2006) found that variation in physical activity due to heritage, was 57% in males and 50% in females. In the study of De Geus, Boomsma and Snieder (2003) heritability of physical activity reached 79% while in the study of Stubbe, Boomsma and De Geus reached 85 %.

Few studies have investigated the genetic effects on different levels of physical activity. In an early most quoted twin study from the Finish Twin Registry, compri-

sed of a very large number of twins and data analyzed using up-to-date analytical strategies, Kaprio, Koskenvuo and Sarna (1981) reported high heritability estimates across the lifespan for physical activity and suggested that genes may be more important for high physical activity.

Similarly, data from the Vietnam Era Twin study (Lauderdale, Fabsitz, Meyer, Sholinsky, Ramakrishnan, & Goldberg, 1997) indicated that genetic effects are more important for participation in vigorous compared with moderate activity. The twin odds ratio was more pronounced for high than for low physical activity. On the contrary, data from the Swedish Twin Registry (Carlsson, Andersson, Lichtenstein, Michaelsson, & Ahlbom, 2006) and from a Portoguese Twin Population (Maia, Thomis, & Beunen, 2002) indicate that the twin odds ratio was almost equally pronounced for both high and low physical activity.

These discrepancies may be due to different definitions and methods of ascertainment of physical activity participation, but make it unclear whether the genetic effect is less important for low physical activity.

We sought to ascertain the heritability of fast and slow movements, through which the level of physical activity is mediated, and hence shed some light on the genetic and environmental influence on sport for all participation. Using the twin model and comparing intrapair differences between monozygotic (MZ) and dizygotic (DZ) twins, we derived heritability estimates (h^2), which signify the relative strength of the genotype in phenotypic variation. Forty male twins in their early twenties (10MZ and 10DZ pairs) performed a series of elbow flexions at different speeds, as accurately as possible, from the initial position to a target. When the load was moved, a signal was transmitted by a sensor at every 3 mm of displacement, allowing calculation of corresponding velocity. On the basis of evidence obtained we concluded that variability in fast movements is genetically dependent, but not in slow movements; and considering available literature on twin and family studies it was postulated that most people in everyday life can perform skilled movements accurately at slow movements, whereas only few people can peform skilled movements at high speed with accuracy (Missitzi, Geladas & Klissouras, 2004).

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Plasticity in human motor cortex is in part genetically determined

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Non-technical summary

Neuronal plasticity refers to the ability of the brain to change in response to different experiences. Plasticity varies between people, but it is not known how much of this variability is due to differences in their genes. In humans, plasticity can be probed by a protocol termed paired associative stimulation and the changes in the motor system that are brought about by such stimulation are thought to be due to strengthening synapses which connect different neurons. We examined pairs of sisters which were either genetically identical (monozygotic) or different (dizygotic). We found that the variability within the monozygotic sister pairs was less than the variability within the dizygotic sister pairs. That plasticity in human motor cortex is in a substantial part genetically determined may be relevant for motor learning and neurorehabilitation, such as after stroke.

Abstract

Brain plasticity refers to changes in the organization of the brain as a result of different environmental stimuli. The aim of this study was to assess the genetic variation of brain plasticity, by comparing intrapair differences between monozygotic (MZ) and dizygotic (DZ) twins. Plasticity was examined by a paired associative stimulation (PAS) in 32 healthy female twins (9 MZ and 7 DZ pairs, aged 22.6 ±2.7 and 23.8 ±3.6 years, respectively). Stimulation consisted of low frequency repetitive application of single afferent electric stimuli, delivered to the right median nerve, paired with a single pulse transcranial magnetic stimulation (TMS) for activation of the abductor pollicis brevis muscle (APB). Corticospinal excitability was monitored for 30 min following the intervention. PAS induced an increase in the amplitudes of the motor evoked potentials (MEP) in the resting APB, compared to baseline. Intrapair differences, after baseline normalization, in the MEP amplitudes measured at 25-30 min post-intervention, were almost double for DZ (1.25) in com-

parison to MZ (0.64) twins (P = 0.036). The heritability estimate for brain plasticity was found to be 0.68. This finding implicates that genetic factors may contribute significantly to interindividual variability in plasticity paradigms. Genetic factors may be important in adaptive brain reorganization involved in motor learning and rehabilitation from brain injury.

Introduction

The adult brain maintains the ability to modify its organization through physiological mechanisms, such as synaptic plasticity, in response to various injuries (Donoghue et al. 1990; Sanes et al. 1992; Brazil-Neto et al. 1993), environmental changes (Pascual-Leone et al. 1995; Pearce et al. 2000; Stefan et al. 2000; Latash et al. 2003; Perez et al. 2004) and even repetitions of simple movements (Classen et al. 1998). In a changing environment, brain plasticity enables the nervous system to ensure that proper activation of muscles may be acquired and maintained to serve the behavioural goal. Major advances have been made within the past 20 years in understanding the mechanisms involved in brain plasticity (Sanes & Donoghue, 2000; Nudo, 2006). In motor plasticity paradigms, several behavioural factors (e.g. initial level of proficiency, rate of improvement and final level of attainment) have been identified as influencing the variability of individual response to the plasticity inducing protocol (Wassermann 2002; Muller-Dahlhaus et al. 2008; Sale et al 2008), the different functional outcomes after neurological injury (Noyes et al. 1983), and the effectiveness of rehabilitation or training (Fox et al. 1996). However, little is known about the magnitude of genetic determinants of the variability observed in these complex phenotypes.

Recently, a genetic component has been observed for brain plasticity, as individuals with the val66met polymorphism in the brain derived neurotrophic factor (BDNF) gene show less increase in the motor evoked potentials (MEPs) after motor training (Kleim et al. 2006). Cheeran and colleagues (2008) extended this observation, by using paired associative stimulation (PAS), a protocol intended to model synaptic plasticity in humans (Muller-Dahlhaus et al 2010). These authors found that the susceptibility to TMS probes was significantly influenced by the BDNF polymorphism in the normal population, suggesting that BDNF signalling is a major factor influencing synaptic plasticity (Cheeran etal. 2008). Although these studies have provided proof-of-principle evidence that synaptic plasticity may be genetically influenced, independent and complementary information could be gained from twin studies. Without previous assumptions of the genes involved, a twin study design allows the discrimination between environmental and genetic effects of any genotype.

Therefore, the aim of this study was to assess the relative power of genetic and environmental contribution to the variation observed in brain plasticity by selec-

ting a sufficiently homogeneous sample of monozygotic (MZ) and dizygotic (DZ) twins and comparing the intrapair differences between the two types of twins. Plasticity in this study was examined by paired associative stimulation, which has been shown to alter excitability, in the human motor cortex, by mechanisms related to synaptic long term potentiation (LTP).

Methods

Subjects

Thirty-two healthy female twins (9 MZ and 7 DZ pairs, aged 22.6 ±2.7 and 23.8 ±3.6 years, respectively) from a university student population were invited to participate in this study. Twins were fully informed about the protocol before giving their written consent. Since environmental comparability is a fundamental assumption made in the twin model, special attention was given to a large variety of potential confounding factors. For this purpose a questionnaire was administered regarding physical activity profiles, sport participation, socioeconomic status, occupational physical loading of the upper extremity, and health condition, to ensure that environmental influences were comparable in both types of twins. Since all twins were women, the questionnaire also included information about the age of menarche and about menstrual cycle (duration, timing and flow). All volunteers were right handed, except one twin pair, who were left handed according to the Oldfield handedness inventory (Oldfield, 1971). Only healthy subjects were allowed to participate in the study. The protocol was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics committees of the Universities of Wurzburg and Athens.

Zygosity was assessed at first approximation through direct observation of relevant morphological characteristics, physical similarities and the testimony of the obstetrical archives (Chen et al. 1999; Kasriel 8c Eaves, 1976), and subsequently confirmed by serological examination of genetic markers in all twins. Discordance for a single antiserum was regarded as sufficient evidence of dizygosity (Sutton et al. 1962).

BDNF genotyping technique

Genotyping was carried out twice in 14 subjects. Genomic DNA was extracted from leukocytes by standard DNA extraction procedure. A 113 bp segment was amplified by polymerase chain reaction (PCR), using the following primers: 5'-GAGGCTTGACATCATTGGCT-3' and 5'-CGTGTACAAGTCTGCGTCCT-3'. Target sequences were amplified in a 50 μλ reaction solution containing 100 ng genomic DNA; 1 U Taq polymerase (Bioron, Ludwigshafen, Germany); 20 mM Tris-HCl (pH 8.4); 50 mM KC1; 1.5 mM MgCl2; 200 mM each of dATP, dCTP, dGTP and dTTP; and 10 pmol of each primer.

After an initial denaturation of the DNA templates for 5 min at 95°C, 3 0 cycles were performed, each consisting of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. After the last cycle, samples were incubated at 72°C for 5 min. Samples were then digested overnight with 3 U of Nlalll (MBI Fermentas, Burlington, Ontario, Canada). The fragments were separated on a 3% agarose gel at 100 V, and fragments were visualized with ethidium bromide (Neves-Pereira et al. 2002).

Stimulation

Focal transcranial magnetic stimulation (TMS) was performed using a flat figure of eight shaped magnetic coil (outer diameter of each wing: 70 mm) connected with a Magstim 200 monophasic magnetic stimulator (Magstim, Whitland, Dyfed, UK). The coil was held tangentially to the . skull with the handle pointing backward and laterally at a 45 deg angle to the sagittal plane. Electrical mixed nerve stimulation was performed with an electric stimulator (model D7AH, Digitimer, Welwyn Garden City, UK) using a standard stimulation block (cathode proximal) at a stimulation width of 200 μs.

Recording

Electromyographic activity was recorded from the abductor pollicis brevis (APB) muscle using Ag-AgCl surface electrodes (Fischer Medizintechnik, Nurnberg, Germany), with the active electrode mounted on the muscle belly and the inactive electrode placed over the base of the metacarpophalangeal joint of the thumb. Raw signals were amplified using a model 1902 amplifier (Cambridge Electronic Design, Cambridge, UK) and bandpass filtered between 1 Hz and 2 kHz. EMG signals were digitized at 5 kHz by an A/D converter (model 1401 plus, Cambridge Electronic Design) and stored in a laboratory computer for display and later analysis.

Experimental procedures

Measurements were made in each pair with a difference of no more than two hours and between 10.00 h and 16.00 h, to minimize possible circadian influences, in a quiet room at 21-22oC. None of the twins performed any vigorous activity or consumed alcohol and caffeine during the 24 h prior to the tests and all were informed of the importance of having adequate sleep, during the night preceding the tests.

Subjects were seated comfortably in an armchair. At first the optimal site of the magnetic coil for eliciting motor evoked potentials (MEPs) in the resting APB was assessed over the motor cortex at a moderately suprathreshold stimulation intensity (usually 50% of the maximal stimulator output) and marked directly on the scalp with a soft tip pen. At the optimal site (hot spot), the resting motor threshold (RMT) was determined as the minimum stimulation intensity needed to produce a response of at least 50 μV in the relaxed APB in at least 5 out of 10 consecutive

trials of the maximal stimulator output (Rossini et al. 1994). Thereafter, the stimulus sufficient to evoke a peak amplitude of 1 mV of the motor evoked potentials in the relaxed APB was determined (SI1mV)· SI1mV was 1.3 ±0.1 times the resting motor threshold. Taking all experiments into consideration, SIlmV was 53 ± 9% of the maximal stimulator output. This procedure took ~15 min to complete.

For intervention, a paired associative stimulation (PAS) protocol, the principles of which were described previously (Stefan et al. 2000; Wolters et al. 2003; Classen et al. 2004), was employed. This consisted of low frequency (0.1 Hz), repetitive application of single afferent electrical stimuli delivered to the median nerve at the level of the wrist at 300% of the perceptual threshold, paired with single pulse transcranial magnetic stimulation (TMS) at ~1.2-1.3 times RMT delivered to the hot spot at a fixed interstimulus interval (ISI) of 25 ms. An ISI of 25 ms was used because this interval has been shown in previous experiments to be effective in inducing cortical plasticity in a high percentage of subjects (Stefan et al. 2000).

PAS-induced changes of corticospinal excitability were fully expressed in some studies (Stefan et al. 2000) while in others (Morgante et al. 2006; Weise et al. 2006), with subtly different protocols, maximal increase was noted only after a delay of some 20 min. To account for this effect and to ensure the ability to test for intrapair differences at the full expression of PAS-induced plasticity, corticospinal excitability was monitored for 30 min following the intervention. One hundred and eighty pairs were delivered at 0.1 Hz over 30 min. For amplitudes of MEPs of the resting muscle, 60 trials were collected before and 180 after intervention, using a stimulus intensity of SI1mV and a stimulation rate of 0.1 Hz. Identical stimulus intensities were used before and after intervention (Fig. 1). Throughout the experiment, complete muscle relaxation was continuously monitored by visual and auditory feedback.

The reliability of the measurements on MEPs was assessed in 17 female subjects (5 DZ, 2 MZ pairs and 1 DZ triplet) in a pilot study on two separate days with a week time interval, and was measured using an intraclass correlation analysis of variance (ANOVA) design. Intraclass reliability for the whole sample was found to be 0.75 (P — 0.01), which is in agreement with previous studies (Kamen, 2004). No differences in correlation coefficient were found between MZ and DZ twins (0.73 and 0.77, respectively).

Data analysis

MEPs were measured peak to peak in each individual trial. Changes in the average of MEP amplitudes between each epoch were compared by analysis of variance (ANOVA). A generalized linear model with Bonferroni correction was used to account for the number of multiple comparisons being performed simultaneously. In the current study design, P values would be required to be smaller than 0.0083 to declare significance. To assess the genetic variation on plasticity in human motor

cortex, for each subject the average of the MEP amplitudes before the intervention were subtracted from the average of the MEP amplitudes, on the grounds that we did not observe any relationship between the initial pre-interventional values and the change (post-pre interventional values).

Heritability estimates

Heritability (h^2), which denotes the degree to which individual differences in a given variable are attributed to genetic differences, was estimated on the basis of the intrapair difference between MZ and DZ twins. MZ twins are genetically identical, whereas DZ twins, like ordinary siblings, share only 50% of their segregating genes. In this way it is possible to separate the relative contribution of genotype and environment for the observed differences in plasticity of human motor cortex. A single-factor analysis of variance (ANOVA) was done to determine the significance of the differences between the mean monozygotic and dizygotic intrapair variance, taking into consideration genetic type and pair factor. The variance ratio (F) derived from the single-factor ANOVA determined whether further analysis was necessary. The following Clark equation based on intrapair variance was used to estimate heritability: 100, where is the variance of intrapair differences in DZ twins and is the variance of intrapair differences in MZ twins (Khssouras et al. 2007). The computation of h^2 was carried out, provided that the difference in genetic variance (within groups mean square) between the twin types (F test) was significant and the difference between means (t' test) and total variance (within plus between groups mean square) of both types of twins (F test), which shows the homogeneity of the sample, was non-significant (Christian, 1979). In this way it was assured that plasticity is independent of the type of twin. Given our total sample size of η = 32 it

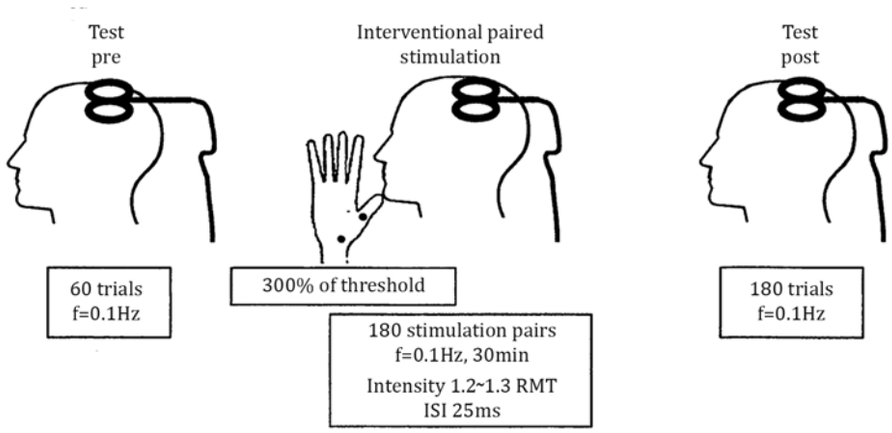

FIGURE 1. Experimental design. Test amplitudes were elicited by single-pulse TMS before and after the intervention. During interventional stimulation, 180 pairs of stimuli consisting of electrical stimuli delivered to the median nerve followed by TMS over the optimal site for activating the APB muscle were applied using a constant interstimulus interval and an interpair interval of 0.1 Hz

appears that with type I error probability at 0.05, and the smallest expected difference between MZ and DZ set at h^2 — 0.50 a power level of at least 95% was secured in this analysis (Dixon & Massey, 1985).

Results

Characteristics of the subjects

Only modest non-significant differences in age, weight, height and physical activity profiles were seen between MZ and DZ twins (22.6 2.7 and 23.8 3.6 years, 55.8: 6.5 and 60.5 11.3 kg, and 164.9 4.6 and 167.5 6.2 cm for MZ and DZ, respectively). Physical activity was also similar within pairs, as well as between zygosity groups. Intra-pair differences were present in menstrual cycle (duration, timing and flow), but were similar in MZ and DZ pairs (data not shown).

Taking all experiments into consideration, resting motor threshold was 41.2 6.7% (mean ± s.D.), stimulus intensity was 53.1 9% of the maximal stimulator output, perceptual threshold of electrical stimuli was 2.6 0.5 mA and intensity of the electrical stimulation was 7.8 1.6 mA. For all these parameters as well as for attention during the experiments, no statistically significant differences were present between zygosity groups (Table 1).

Effect of paired associative stimulation (PAS)

Following PAS, the amplitudes of MEP responses recorded from APB muscle increased. The increase amounted, from a mean of 0.99 ± 0.39 mV to 1.21 ± 0.57 mV or on average, of 22% (P — 0.04) 5 min after the intervention, and to 1.42 ± 0.75 mV or of 43% (P = 0.0002) when the measurement was taken 25-30 min after the intervention, consistent with previous observations using a similar protocol (Weise et al. 2006). The percentage increase varied between subjects and ranged from +9 to +210% of the baseline value. In about two-thirds of all experimental sessions the increase was at least 30%.

The build-up of the change in the resting amplitudes was examined by delivering probing TMS pulses for a period of 30 min following the intervention. Resting amplitudes following intervention were binned in epochs of duration 5 min. Including the pre-interventional epoch consisting of 60 consecutive trials, this resulted in seven epochs (one before and six after intervention). A repeated measures ANOVA was performed on the binned data and revealed a significant effect for epoch (0-6) (F=5.9, P= 0.001). Pre-planned contrasts

TABLE 1. Characteristics of stimulation in monozygotic and dizygotic twins

	MZ (18)	DZ (14)
Resting motor threshold (%)	40.1 ± 7.4	42.3 ± 7.8
Stimulus intensity (%)	53.6 ±10.0	52.5 ± 8.7
Peripheral threshold (mA)	2.5 ± 0.5	2.7± 0.6
Electrical stimulation intensity (mA)	7.7 ±1.7	7.9 ±1.4
Attention (number of errors)	2.6±0.5	2.8 ±0.6

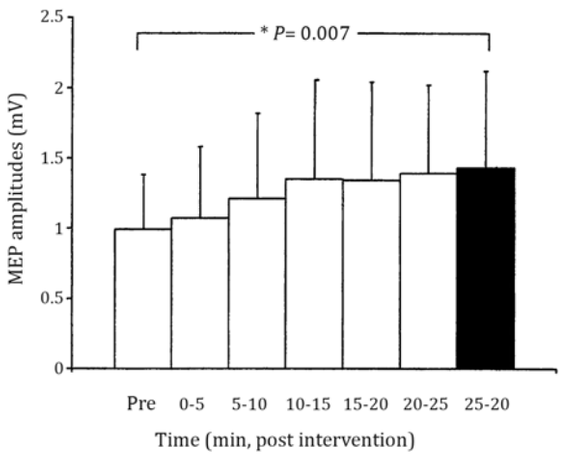

FIGURE 2. Motor evoked potential amplitudes. Average data (means and s.d.) from the resting amplitude increase, in the APB, for all subjects. Significant difference after Bonferroni correction was found only when the pre-interventional was tested against the last post-interventional epoch (paired f test; with the P value estimated at 0.007).

were computed using Student's t test with the Bonferroni correction to account for the number of multiple comparisons being performed simultaneously. Compared with the pre-interventional measurement, the mean MEP amplitudes at the first five post-interventional epochs were higher, but the results after Bonferroni correction did not reach statistical significance. In contrast, significant differences were identified between pre-interventional and the last (25-30 min) post-interventional epoch (P = 0.007; Fig. 2).

Genetic variation

For the derivation of heritability index, analysis of variance was made in the last post-interventional epoch which remained significant after the Bonferroni correction.

The comparison within any pair showed a greater similarity of the individual profile in plasticity in MZ as compared with DZ pairs. MEP amplitudes were averaged for pre- and post-interventional values for all MZ and DZ twins. Intrapair differences between MZ and DZ twins calculated either from values obtained at 25-30 min after intervention, or on values obtained at 25-30 min after sub-

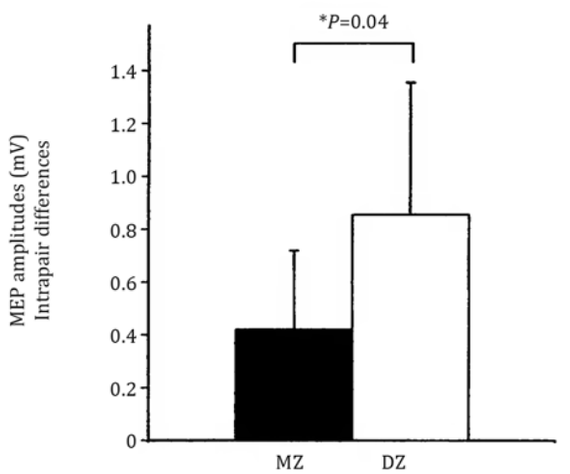

FIGURE 3. Intrapair differences (mean and s.d.) for motor evoked potential amplitudes in MZ and DZ twins, 25-30 min post-intervention after subtraction of pre-interventional values. Asterisk indicates significant difference (paired f test; "P = 0.05).

traction of pre-intervention values, were almost double for DZ twins in comparison to MZ (0.64 for MZ and 1.25 for DZ, P= 0.036, and 0.42 for MZ and 0.85 for DZ, respectively, P = 0.04). Figure 3 displays intrapair differences in MEP size for MZ and DZ twins, 25-30 min post-intervention after subtraction of pre-intervention values. Differences in DZ twins become more apparent in Fig. 4, where values for monozygous twins are closer to the line of identity, while those for DZ twins are widely scattered for both 25-30 min post-intervention, and 25-30 min post-intervention after subtraction of pre-intervention values. The lower variability in MZ twins can be seen over the whole time course as smaller standard deviations (Fig. 5).

Previous studies have found the allelic state of the BDNF gene to influence the outcome of PAS (Cheeran et al. 2008). By definition, MZ twins share the polymorphism of BDNF while this is not necessarily true for DZ twins. Hence it is possible mat the closer intrapair differences found for MZ twins may be in part due to the same allelic state of BDNF, while the wider intrapair differences for DZ twins may have been due to a different allelic state of BDNF. To address this possibility, the allelic state of 14 twins (4 MZ and 3 DZ pairs) from our total sample was examined using the method applied by Neves-Pereira et al. (2002). Of these twins, 10 were Val/Val carriers (3 DZ and 2 MZ pairs), 2 Val/Met (1 MZ pair) and 2 Met/Met (1 MZ pair). In subjects who carried met in one or more alleles (n = 4),

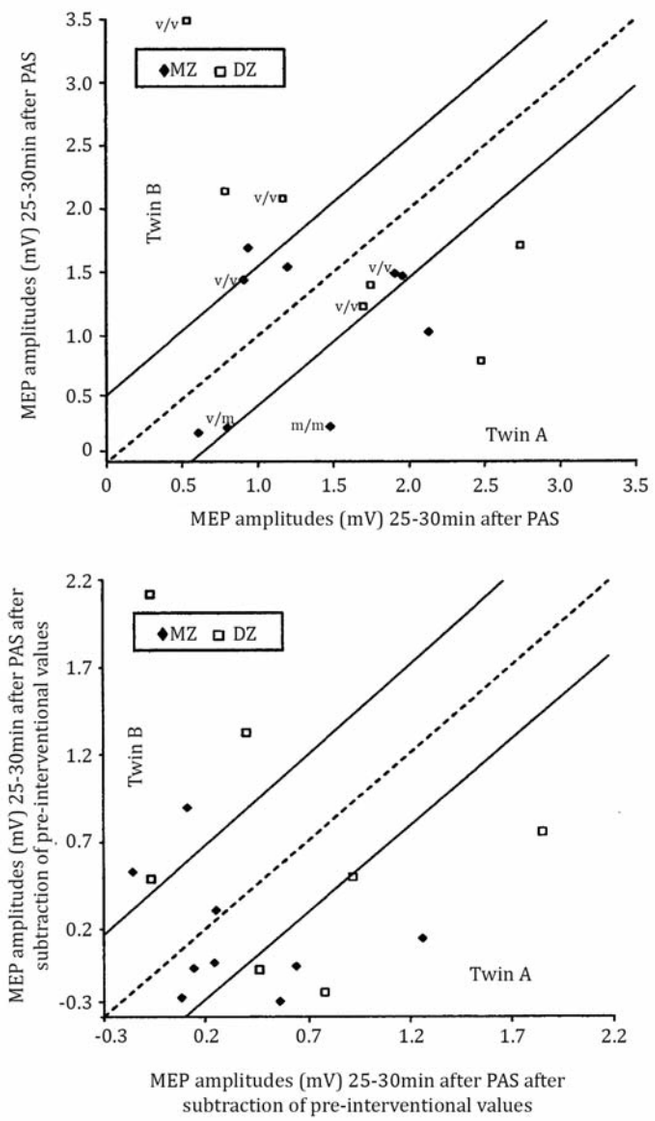

FIGURE 4. Individual values of motor evoked potential amplitudes in MZ and DZ twin pairs, 25-30 min post-intervention (upper graph) and after subtraction of pre-interventional values (lower graph). BDNF allelic state is also indicated for some twin pairs, of whom 3 DZ and 2 MZ pairs are Val/Val carriers (v/v), 1 MZ pair Val/Met (v/m) and 1 MZ pair Met/Met (m/m).

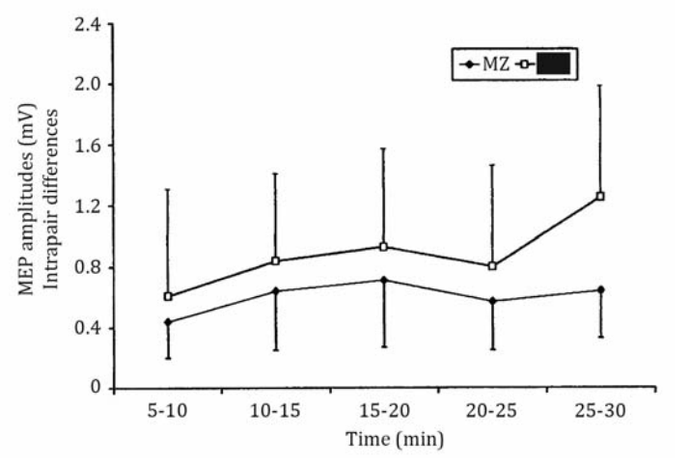

FIGURE 5. Intrapair differences (mean and s.d.) for motor evoked potential amplitudes over the whole time course for DZ and MZ twins

PAS led to virtually no enhancement of excitability from a mean of 0.67 ± 0.41, to 0.68 ± 0.52 or on average a 1% increase. In contrast, excitability was enhanced in those having Val/Val alleles («=10) from a mean of 1.05 ± 0.31 mV to 1.72 ± 0.82 mV, an increase of 67% (P = 0.007). Sisters of the same pair had always the same allelic state in all pairs with known BDNF allelic state, even in heterozygous twins (Fig. 4).

For the derivation of heritability index in the plasticity of human motor cortex, ANOVA was employed using the difference of the absolute values between post- and pre-intervention values in order to compare the absolute change of cortical excitability and to determine the significance of the differences between the mean monozygotic and dizygotic intrapair variance, taking into consideration genetic type and pair factor. While the differences between means and total variance of both types of twins were not statistically significant, the genetic variance between the twin types was significant (F — 3.32, P = 0.05). Therefore computation of h^2 was carried out in which genetic factors explained 68% of the total variance (Table 2).

Discussion

To our knowledge, this study is the first to use a twin study design to investigate the extent to which individual variation in cortical plasticity is influenced by genetic and environmental factors. The comparison between MZ and DZ twins in plasticity of human motor cortex, which was defined as the change in corticospinal excitability after PAS intervention, demonstrated that externally induced plasticity is in a substantial part (68%) genetically dependent.

A number of other factors that contribute to the observed variation in the plasticity of motor cortex, such as the subject's age (Miiller-Dahlhaus et al. 2008), the time of the day (Sale et al. 2008) and the menstrual

TABLE 2. Testing statistical hypotheses for the derivation of h^2 in plasticity of motor cortex

Hypotheses	Plasticity of brains' motor cortex
t′ test	0.83 (0.1 t′ <t_c)
F′ test	3.1 (12.3 and 16.1 F′ < Fc
F test	3.32*, *P <0.05
Heritability (h^2)	0.68

t' test signifies the difference between the means of the twin pairs, F' test the difference of total variance of both types of twins, and F test the difference in genetic variance between the twin types, tc, the subscript denotes the critical value, fc, the subscript denotes the critical value.

cycle (Inghilleri et al. 2004) were excluded as underlying the observed differences between monozygotic and heterozygotic twins. Female twins were not matched for phase of menstrual cycle, but since monozygotic twins do not seem to have the same menstrual cycle (duration, timing and flow), intra-pair differences in monozygotic twins were similar to those in heterozygotic twins. Thus, an influence of the menstrual cycle on PAS variability is highly unlikely, and in any case, if this factor had influenced our results, the heritability estimate would be underestimated, because it will lower the MZ resemblance and increase it in DZ twins.

Recent studies in human brain have shown that a single nucleotide polymorphism, BDNF val66met, may be associated with reduced hippocampus volume and episodic memory (Egan et al. 2003; Pezawas et al. 2004), modulation of training-dependent increases in the amplitude of motor-evoked potentials and motor map reorganization (Kleim et al. 2006) and influencing synaptic long-term potentiation and motor learning (Fritsch et al. 2010). Training-dependent increases of excitability (Kleim et al. 2006) or motor performance increments (Fritsch et al. 2010) were reduced in healthy subjects with a val66met polymorphism in the BDNF gene, as compared to subjects without the polymorphism. Extending these studies, Cheeran and colleagues (2008) investigated whether the susceptibility to TMS-induced plasticity is significantly influenced by the BDNF polymorphism. The response of Met allele carriers differed significandy in all protocols compared with the response of Val/Val individuals, suggesting that this was due to the effect of BNDF on the susceptibility of synapses to undergo LTP/LTD. In our subgroup of 14 subjects in whom we were able to ascertain the BDNF gene polymorphisms, we identified four individuals who carried at least one Met allele. PAS-induced response in these four subjects was virtually absent, whereas the remaining subjects carrying the Val/Val allelic state responded with a significant increase. Our observations confirm those of Cheeran and co-workers (2008) who found a significant increase of the MEP amplitudes in APB after PAS in Val/Val, but no increase in non-Val/Val, individuals exposed to plasticity inducing brain stimulation protocols. Based on the assumption that Met alleles would occur at the same frequency as in the cohort of 14 subjects, two DZ pairs could be heterozygous for BDNF gene alleles. Given the large difference in responsiveness toward the PAS protocol in non-Met and Met carriers, it is possible that the BDNF polymorphism may have substantially contributed to the wider intrapair difference for DZ twins. It should be noted, however, that BDNF gene polymorphism is only one example of genetic susceptibility. Polymorphisms of other genes whose product is involved in synaptic plasticity, such as the 'kidney and brain protein' KIBRA (Papassotiropoulos et al. 2006) or catechol-O-methyltransferase (COMT) (Jacobsen et al. 2010), could be of similar or even greater relevance. Hence, more studies are needed to determine which gene polymorphisms may underline the difference between MZ and DZ twins.

A recent twin study has demonstrated a major influence of genes on cortical excitability in humans, with heritability estimates of 0.80 for intracortical inhibition and 0.92 for facilitation (Pellicciari et al. 2009). Importandy the same study did not demonstrate a main genetic influence on variation of the size of MEPs evoked by single-pulse TMS, in agreement with our findings in pilot experiments demonstrating similar intra-pair variation of baseline excitability in monozygotic twins. Animal studies show that GABAergic intracortical inhibition powerfully modulates synaptic efficacy (Hess et al. 1996). Furthermore, intracortical disinhibition is known to be involved in PAS-induced plasticity (Stefan et al. 2002). Therefore, greater intra-pair similarity of intracortical excitability in monozygotic twins may have contributed to enhanced intra-pair similarity of PAS-induced plasticity. This mechanism would indicate a less direct influence of genes involved in regulating synaptic efficacy.

The heritability estimate of corticomotor plasticity found in the present study (0.68) was lower than the heritability estimates of Pellicciari and co-workers (2009) relating to intracortical excitability measures. The lower degree of heritability estimates of plasticity may suggests that some additional, non-genetic factors contribute to plasticity variation in genetically identical humans. Such variation could be attributable to environmental and epigenetic (Fraga et al. 2005; Wang et al. 2005) influences. Thus external and internal factors may also affect to some extent the plasticity of motor cortex by altering the pattern of epigenetic modifications, thereby modulating individually genetic information.

PAS-induced facilitation was maximal at the time interval of 25-30 min where it reached 43%, in agreement with previous observations (Weise et al. 2006; Morgante et al. 2006). This pattern of progressive increase in MEP size possibly suggests that there is a latent interval until the optimal strengthening of the synaptic efficacy is consolidated and becomes apparent. Although there was an increment in MEP size comparative to baseline in all post-intervention epochs, after Bonferroni correction only the increment in the last measurement (25-30 min) reached statistical significance with the intra-pair differences between MZ being significant less than DZ twins. It could be postulated that both the difference in the degree of MEP amplitude, as well as in the amount of the intrapair differences, during this testing period may be due to a different rate of the increase in excitability. If this were the case, probably a functional mechanism would lead to physiological limits for the particular environmental influence and hence to full expression of an individual's genetic potential.

Our findings, along with those demonstrating a major influence of genes on cortical excitability in humans (Pellicciari et al. 2009), underline the importance of genetic contributions to physiological measures. Therefore, it could be of relevance to include genetic variation as a potential covariate in the analysis of experimental data. As noted above, plasticity induced by paired associative stimulation may pro-

be long-term potentiation of excitatory synapses in motor cortex (Muller-Dahlhaus et al. 2010), a mechanism strongly implicated in motor learning (Rioult-Pedotti et al. 2000). Therefore, it appears tempting to speculate that the same genetic variation that modulates the PAS response could influence motor learning. In agreement with this hypothesis recent evidence obtained in both humans and animals indicates that LTP formation and motor learning are both affected by the BDNF val66met polymorphism (Fritsch et al. 2010). Moreover, it may be that athletes of Olympic calibre in addition to their superior genotypes may also have inherited to some degree the cortical ability to better respond to motor training.

Finally, our findings may also be relevant to understanding why people express different adaptive central nervous system response patterns to various injuries. Functional deficiencies and recovery outcomes differ widely between patients with identical peripheral injuries (Kapreli et al. 2007) possibly as a result of different expressions of central motor plasticity. The fact that heredity accounts for a substantial part of the existing differences in plasticity of human motor cortex, in conjuction with the implication that movement strategies, which are organized in the CNS, are strongly genetically dependent (Missitzi et al. 2004), may also suggest that this influence is a factor in the development of or compensation of certain neurological injuries.

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Author contributions

J.M.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, revising it critically for important intellectual content. R.G.: acquisition of data, analysis and interpretation of data, drafting the article. N.G.: analysis and interpretation of data, drafting the article, revising it critically for important intellectual content. P.P.: analysis and interpretation of data, drafting the article. N.K.: analysis and interpretation of data, revising the manuscript critically for important intellectual content. J.C.: conception and design, analysis and interpretation of data, drafting the article, revising it critically for important intellectual content. V.K.: conception and design, analysis and interpretation of data, drafting the article, revising it critically for important intellectual content. All authors approved the final version to be published. The experiments were done in The Human Cortical Physiology and Motor Control Laboratory, Department of Neurology, University of Wurzburg, Wiirzburg, Germany; Ergophysiology Research Laboratory, Department of Sport Medicine and Biology of Physical Activity, University of Athens, Athens, Greece; Section of Histology, Center of Basic Research, Biomedical Research Foundation, Academy of Athens, Athens, Greece.

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Heritability of Motor Control and Motor Learning

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ABSTRACT

The aim of this study was to elucidate the relative contribution of genes and environment on individual differences in motor control and acquisition of a force control task, in view of recent association studies showing that several candidate polymorphisms may have an effect on them. Forty-four healthy female twins performed brisk isometric abductions with their right thumb. Force was recorded by a transducer and fed back to the subject on a computer screen. The task was to place the tracing of the peak force in a force window defined between 30% and 40% of the subject's maximum force, as determined beforehand. The initial level of proficiency was defined as the number of attempts reaching the force window criterion within the first 100 trials. The difference between the number of successful trials within the last and the first 100 trials was taken as a measure of motor learning. For motor control, defined by the initial level of proficiency, the intrapair differences in monozygotic (MZ) and dizygotic (DZ) twins were 6.8 ± 7.8 and 13.8 ± 8.4, and the intrapair correlations 0.77 and 0.39, respectively. Heritability was estimated at 0.68. Likewise for motor learning intrapair differences in the increment of the number of successful trials in MZ and DZ twins were 5.4 ± 5.2 and 12.8 ± 7, and the intrapair correlations 0.58 and 0.19. Heritability reached 0.70. The present findings suggest that heredity accounts for a major part of existing differences in motor control and motor learning, but uncertainty remains which gene polymorphisms may be responsible.

INTRODUCTION

Practice with feedback is a fundamental variable that influences motor skills. However, although everyone can improve with practice, some improve more than others. Moreover, people without previous experience perform certain activities better than others who have been practicing for years. Even in groups showing similar attainment, retrospective studies show individual differences in accumulated prac-

tise (Starkes et al. 1996). These differences in skill might arise from among other factors, different degrees of proximity of initial performance to the target performance, different conformity to optimal training, or gene-mediated differences in responses to training (Yarrow et al. 2009).

Until recently, the question of the relative importance of genetic and environmental influences on motor control and motor learning was open, as previous studies have been confounded by a number of other biological and behavioral factors (Sklad 1972; Williams and Gross 1980; Fox et al. 1996). However, recently behavioral evidence was found that the brain derived neurotrophic factor (BDNF) val66-met polymorphism may be a key factor influencing practice-induced plasticity and motor learning (Kleim et al. 2006; Cirillo et al. 2012) suggesting a major genetic influence. Evidence obtained in both humans and animals confirmed this behavioral finding and has additionally supported the hypothesis that the same polymorphism also modulates the formation of long-term potentiation (LTP), a major candidate mechanism of motor learning (Fritsch et al. 2010). Using a paired associative stimulation protocol (PAS) (Stefan et al. 2000), which likely probes LTP of excitatory synapses in motor cortex (Miiller-Dahlhaus et al. 2010), we found that variability in PAS-induced plasticity was smaller between monozygotic (MZ) as compared to dizygotic (DZ) twins (Missitzi et al. 2011). We also showed that the susceptibility to PAS-induced plasticity was significantly influenced by the BDNF val66met polymorphism (Missitzi et al. 2011) in agreement with previous work (Cheeran et al. 2008). Based on this previous work, we hypothesized that the same genetic variation that influences PAS-induced plasticity could influence motor learning.

We undertook to assess the relative power of genetic and environmental contribution to the variation observed in motor control and learning using the classical twin method based on a comparison of MZ and DZ twins. Motor control was examined by the initial level of proficiency and motor learning by the improvement between the initial level of proficiency and the final level of attainment after dynamic training with feedback in force control.

METHODS

Subjects

Forty-four healthy female twins, (13 MZ and 9 DZ pairs, aged 24.6 ± 2.9 and 23.5 ± 3.2 years, respectively) were selected from a university student population to voluntarily participate in this study. With the addition of six more pairs, the population of subjects was identical to that studied in Missitzi et al. (2011). Special control was made for all confounding factors, as environmental comparability is a fundamental assumption made in the twin model. To ensure that environmental influen-

TABLE 1. Subjects demotraphics scores.		
	MZ	**DZ**
Age (years)	22.9 ± 2.8	23.7 ± 3.7
Weight (kg)	56.3± 6.5	59.2 ± 10.3
Height (cm)	165.5 ±4.8	167.1 ± 6.1
Instrument playing (h/week)	1.0 ± 0.5	1.5 ± 0.2
Keyboard writing (h/week)	3.9 ± 1.0	4.1 ± 2.3
Oldfield handedness score	0.9 ± 0.0	0.8 ± 0.0
Physical activity score	8.5 ± 2.1	8.3 ± 2.9
All p>0.05		

ces are comparable in both types of twins a questionnaire was administered regarding physical activity profiles, sport participation, occupational physical loading of the upper extremity, such as playing a musical instrument or using the computer (Baecke et al. 1982), socioeconomic status, and health condition (Table 1). None of the subjects had a history of serious medical, neurological or psychiatric illness, or used illegal, neuroactive recreational drugs as probed by a standardized questionnaire. All volunteers were right handed, except one twin pair, which was left handed according to the Oldfield handedness inventory (Oldfield 1971). The study was approved by University Institutional ethics committee and written informed consent was obtained from all participants. Zygosity was assessed through direct observation of relevant morphological characteristics, physical similarities, as well as the testimony of the obstetrical archives (Kasriel and Eaves 1976; Chen et al. 1999) and confirmed by serological examination of genetic markers in all twins. Discordance for a single antiserum was regarded as sufficient evidence of dizygosity (Sutton et al. 1962). BDNF genotyping was carried out twice in 14 subjects (four DZ and three MZ pairs) with a method described previously (Missitzi et al. 2011).

Experimental procedures

Subjects complied with pretest instructions that restricted alcohol and caffeine consumption during the 24 h prior to the tests and all were informed of the importance of having adequate sleep, during the night preceding the tests. To minimize possible circadian influences, experiments were started no more than 2 h apart in each pair, between 10:00 am and 4:00 pm.

Experiments were performed in a quiet room with an ambient temperature of 21-22°C. Subjects were asked to perform brisk isometric abductions with their right thumb. Force was recorded by a force transducer (Grass CP122A, Grass Instruments CO, West Warwick, RI) and the force signal was fed back to the subject on a computer screen. Prior to the main task, the subject's maximum force was established, and a target force window was defined as a range between 30% and 40%, of the individual maximum force, displayed as two horizontal lines on the computer screen. Because in each experiment the display was scaled to the subject's individual maximum force, the target window had the same geometrical size for all subjects (Fig. 1).

Assessment of motor control

Motor control was defined as the initial level of proficiency. Each subject performed

two blocks consisting of 50 isometric thumb abductions each, separated by 30 sec, at a frequency of 0.5 Hz. The total number of successful attempts achieved in the two blocks was used to assess motor control.

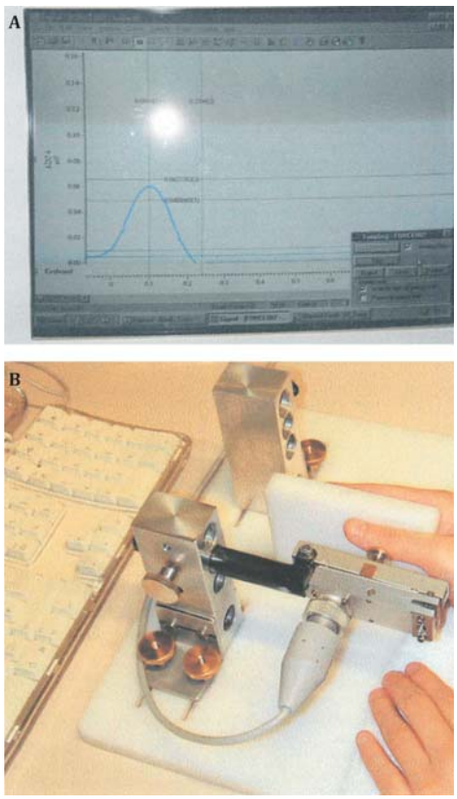

FIGURE 1. Test apparatus and the recording from one succesive hit displayed between the two horizontal lines on the computer screen.

Assessment of motor learning

Motor learning was defined by the difference between the initial and last level of proficiency and was assessed by the difference between the total number of successful hits achieved in the last two training blocks, and the total number of successful hits achieved in the first two training blocks. Each subject had to perform a total of 500 metronome-paced (0.5 Hz) isometric thumb abductions, exactly at the target location on the screen, in a series of10 training blocks that were separated by 60 sec and consisted of 50 abductions each.

Heritability estimates

Heritability (h^2) which is defined as the proportion of phenotypic variance attributable to observed individual differences in actualized genetic potential was estimated on the basis of the intrapair difference between MZ and DZ twins. MZ twins have identical heredity, whereas DZ twins, like ordinary siblings, share half of their segregating genes. In this way, it is possible to separate the relative contribution of genotype and environment for the observed differences in motor control and learning (Klissouras et al. 2007). Data obtained were analyzed using the single-factor analysis of variance (ANOVA) for each variable, to determine the significance of the differences, between the mean MZ and DZ intrapair variance, taking into consideration genetic type and pair factor. The variance ratio (F) derived from the single-factor ANOVA determined whether further analysis was necessary. The following Clark equation based on intrapair variance was used to estimate heritability: h^2 = (s2 DZ - s2 MZ/s2 DZ) χ 100, where s2 DZ is the variance of intrapair differences in DZ twins and s2 MZ is the variance of intrapair differences in MZ twins. The computation of h^2 was carried out, provided that the difference in genetic variance (within groups mean square) between the twin types (F-test) was significant and the difference between means (f-test) and total variance (within plus between groups mean square) of both types of twins (f-test), which shows the homogeneity of the sample, was nonsignificant (Christian 1979). It is assured, therefore, that parameters assessed are independent from the twin type.

Statistical analyses were performed using SPSS (12.0 for Windows, SPSS Inc., Chicago, IL) and statistical functions built in Excel 2002 (Microsoft Corporation,

Redmond, WA). Since our total sample size was η = 44 it follows that with type I error probability at 0.05, and the smallest expected difference between MZ and DZ set at $h^2 = 0.50$, a power level of at least at 95% is secured in this analysis (Dixon and Massiu 1985).

RESULTS

Characteristics of the subjects

MZ and DZ twins did not differ in any of the demographic variables (Table 1) Furthermore, physical activity as assessed with a physical activity score (Baecke et al. 1982) was also similar within pairs, as well as between zygosity groups (Table 1).

Taking all experiments into consideration, initial performance assessed by the two first series was 60.8 ± 11.2. During training the force trajectories gradually became smoother and the number of hits into the force target zone increased. The outcome of training assessed by the two last series was 73.8 ± 10 (23%, P < 0.001, Fig. 2). The number of hits increased similarly in groups MZ (from 60.4 ± 9.4 to 72.2 ± 9.4, P < 0.001, paired two-tailed ί-test) and DZ (61.8 ± 12.6 to 76.8 ± 10.6, P < 0.001).

Heritability of motor control

Motor control was defined by the initial level of proficiency. The number of successful attempts of the first two blocks of exercise ranged from 45 to 80 hits into target windows for MZ and 43-75 for DZ twins. For most MZ twins' performance were almost identical, whereas for DZ twins there were marked differences. Figure 3 presents means and standard deviations of intrapair differences in motor control as defined by the initial level of proficiency. Average intrapair differences between DZ twins were 13.8 ± 8.4 and between MZ 6.8 ± 7.8. The difference becomes more apparent in Figure 4, where the intrapair values for MZ are closer, and for DZ twins are more scattered. The respective intrapair correlation for MZ and DZ twins was 0.77 and 0.39. Statisti-

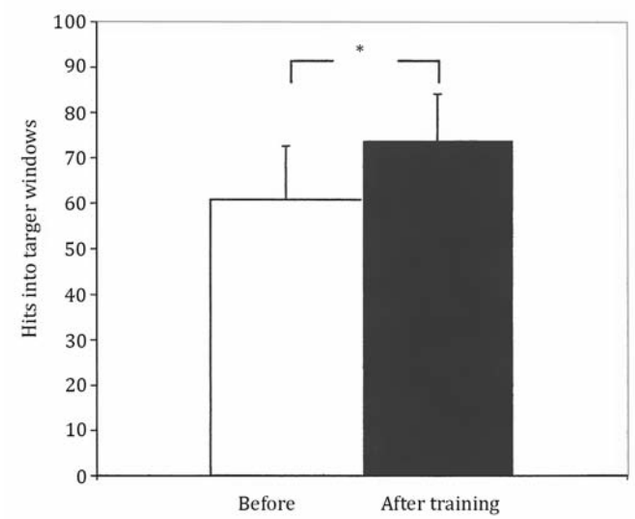

FIGURE 2. Mean and standard deviation in hits into target windows before (100 trials in two blocks) and after training (100 trials in two blocks). Asterisk indicates significant difference (paired t test; *P = 0.05).

cal analysis of the data revealed that the differences between means and total variance of both types of twins were not significant, whereas the genetic variance between the twin types was significant $P < 0.05$. Therefore, computation of h^2 was carried out and revealed that genetic factors explained 68% of the total variance.

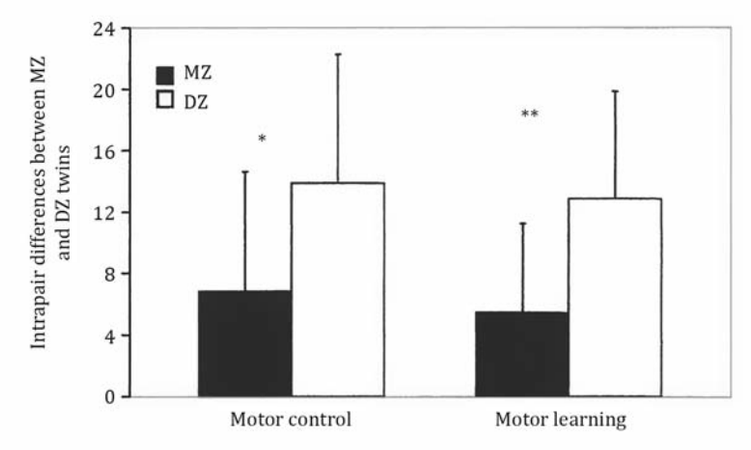

FIGURE 3. Mean and standard deviation of intrapair differences between MZ and DZ twins in motor control and motor learning. Asterisks indicate significant differences (paired f test; *P = 0.05 and *P= 0.01, respectively).

Heritability of motor learning

Motor learning was defined by the difference between the initial and last level of proficiency. Data obtained from the difference between the total number of successful hits achieved in the last two training blocks, and the total number of successful hits achieved in the first two training blocks were averaged for all MZ and DZ twin pairs. The results ranged from increments of 2-33 hits into target windows for MZ and 2-40 for DZ twins. A correlation was found (0.54) between the increment of force control and the baseline motor capacity. The correlation was similar in MZ as in DZ twins. Therefore, dependence on the initial level of proficiency is unlikely to explain the effect of zygosity.

In a subgroup of the present cohort, PAS-induced plasticity was assessed (reported in Missitzi et al. [2011]). Using Pearson's correlation coefficient we examined a potential relationship between the increment of force control and the baseline normalized magnitude of corticospinal excitability following PAS and we found a small but significant correlation ($r = 0,21$, $t = 0.73 > tc = 0,66$ $P < 0.01$).

Motor learning showed a greater intrapair similarity for MZ twins than for DZ twins. Intrapair differences between the two types of twins were more than double in DZ twins (12.8 ± 7) compared to MZ twins (5.4 ± 5.2; $P < 0.01$, Fig. 3). The difference becomes more apparent in Figure 4, where it can be seen that values of MZ twins are closer to the line of identity than values of DZ twins. The respective intrapair correlation for MZ and DZ twins was 0.58 and 0.19.

Statistical analysis of the data revealed that the differences between means and total variance of both types of twins were not significant, whereas the genetic variance between the twin types was significant beyond the 0.01 level of confidence. Therefore, computation of h^2 was carried out and it was found that genetic factors explained 70% of the total variance.

Genotyping results

Recent study has found that LTP formation and motor learning are both affected

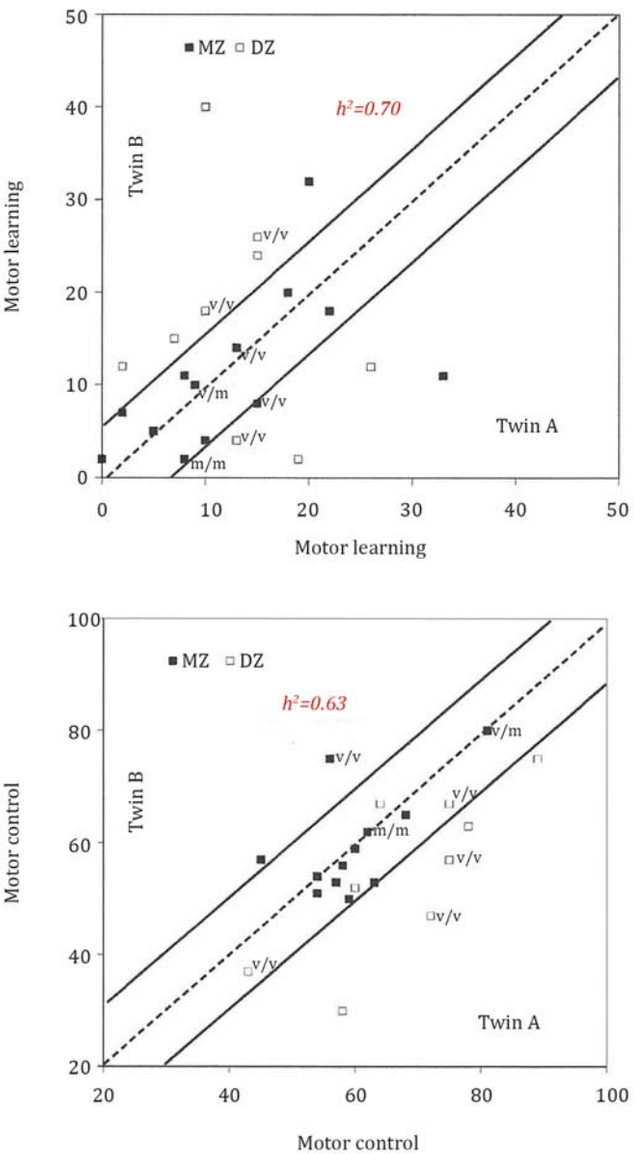

FIGURE 4. Individual values of motor learning and motor control in MZ and DZ twin pairs. BDNF allelic state is also indicated for some twin pairs, of whom three DZ and two MZ pairs are Val/Val carriers (v/v), one MZ pair Val/Met (v/m) and one MZ pair Met/Met (m/m).

by the BDNF val66met polymorphism (Fritsch et al. 2010). By definition, MZ twins share the polymorphism of BDNF while this is not necessarily the case for DZ twins. Hence, it is possible that the closer intrapair differences found for MZ twins may be in a part due to the same BDNF haplotype, whereas the wider intrapair differences in DZ twins may have been due to a different BDNF haplotype. We were able to ascertain the BDNF haplotype in 14 twin pairs. As reported elsewhere (Missitzi et al. 2011), using the method applied by Neves-Pereira et al. (2002), it was found that from this group of 14 twins, 10 were Val/Val carriers (3 DZ and 2 MZ pairs), 2 Val/Met (1 MZ pair) and 2 Met/Met (1 MZ pair). Sisters of the same pair had always the same haplotype in all pairs with known BDNF allelic state, even in heterozygous twins Regarding force control, subjects who carried Met in one or more alleles (N = 4) reached 71.2 ± 10.6 hits into target windows and did not show any significant difference from those having Val/Val who had outcomes of 63.4 ± 13.4 hits into target. With respect to learning, subjects who carried Met in one or more alleles (N = 4) improved by 9% (7 ± 3.4 hits; $P < 0.01$) after training with feedback. In contrast, those having Val/Val alleles (N = 10) improved on average by 20% (12.2 ± 6.6 hits, $P < 0.001$ Fig. 4).

DISCUSSION

The current study demonstrated that existing interindi-vidual differences on both

force control and motor learning are under genetic influence, with heritability being 0.68 and 0.70, respectively.

Motor control

Previous studies have reported a significant genetic effect for motor control, using a variety of tasks, such as pursuit rotor tracking, tapping speed, and stabilometry with heritability ranging from 0.56 to 0.86, depending on the task (Williams and Gross 1980; Fox et al. 1996; Maes et al. 1996). Nevertheless, by performing these tasks, the involvement of other biological and behavioral factors, like balance, power, proprioception, rhythm, perception, and motor learning, which may influence motor control, is inevitable, making its isolation difficult. An attempt was made, however, to examine neuromuscular coordination by kinematic and electromyographic recordings during a simple, single joint movement in one degree of freedom. A comparison of intrapair differences between MZ and DZ twins in neuromuscular coordination of fast movements, expressed either as movement accuracy or movement economy, demonstrated that heredity accounts for the major part (87%) of existing differences (Missitzi et al. 2004). The heritability estimate of motor control found in this study (0.68) was lower than that found previously relating to neuromuscular coordination measures, an observation that may be explained by the fact that different tasks challenge multiple sensory and motor capacities differently and are each subject to different her-itability (J. Missitzi, A. Misitzi, N. Geladas, J. Classen, and V. Klissouras, unpubl. data).

Motor control was examined in the current study by performing brisk isometric abductions with the thumb, a movement of a single joint, in one degree of freedom in a simple protocol, used previously (Stefan et al. 2006). Simplicity of the motor task enhances the chances for thorough control of experimental factors and may thus minimize the influence of confounding factors (Corcos et al. 1989; Almeida et al. 1995). Despite the apparent simplicity of the motor task, it challenges the orchestration of multiple sources of sensory information, exteroceptive (vision) as well as proprioceptive, namely, the tendon organs' sensitivity, the discharge of the major part of all muscle spindle afferents and the excitement of cutaneous receptors, through ensemble coding mechanisms, but without knowing the exact contribution of each (Edin and Valbo 1990; Jones and Piateski 2006) within the motor output system. Recendy, it has been shown that isometric muscle contractions can produce a perception of joint displacement in the same direction as the joint would move if unrestrained (Walsh et al. 2009). In addition, hemiparetic participants seem to rely primarily on sense of effort rather than proprioceptive feedback for gauging lower limb force production for both isometric and isotonic contractions (Simon et al. 2009). Therefore, in this study along with afferent information, centrally generated motor command signals, which have been found to have a genetic basis (Missitzi

et al. 2004), is likely to play a major role. Hence, any part of the system concerned with the generation of the force production task, collection or processing of afferent information or the generation of efferent signals may be genetically influenced.

It has been shown that BDNF val66met polymorphism is associated with reduced hippocampus volume and function, episodic and working memory, less gray matter volume throughout the prefrontal cortex (Egan et al. 2003; Hariri et al. 2003; Pezawas et al. 2004; Dempster et al. 2005; Tan et al. 2005; Ho et al. 2006). As the task used in this study probed motor, attentional, memory, and visuospatial systems, one might hypothesize BDNF polymorphism to play a role in the intrapair differences in motor control found between MZ and DZ twins. However, the mean number of successful attempts achieved from subjects who carried Met in one or more alleles (N — 4) did not show any significant difference from those having Val/Val (N = 10). Therefore, the wider intrapair differences in DZ twins in the motor control task employed here are unlikely to be due to a different haplotype of BDNF polymorphism. Alternatively, the BDNF polymorphism may play a role of motor skill acquisition in the short term (see below), but less so in the long term. On this view carriers of the BDNF gene Met allele would be at a disadvantage in the rapidity of motor skill acquisition, but able to compensate for this disadvantage, perhaps by other genes or by an advantageous effect of the Met allele in later stages of motor skill encoding (McHug-hen et al. 2011), such as in consolidation. However, it should be noted that on one hand the sample for detecting genotyping was small and further investigation is needed to exclude this possibility and on the other hand polymorphisms of other genes whose product is involved in motor control such as DRD2/ANKK1 Ankyrin repeat and kinase domain 1 (Munafo et al. 2005) or GCH1 GTP cyclohydrolase 1 (Tegeder et al. 2006), or GLRA1 Glycine receptor 1 (Elmslie et al. 1996) or other (Mishra et al. 2007) could be more relevant.

Hence, as motor control presents a high heritability, efforts to find a causative gene are worthwhile to continue and determine which gene polymorphisms or a combination thereof may underline the difference between MZ and DZ twins.

Motor learning

A significant genetic variance component (0.70) was also found for motor learning. Previous studies reported a similar heritability index for the initial level of motor learning which increased further with practice (Williams and Gross 1980; Fox et al. 1996). In this study, both groups of twins started with a similar level of performance without significant differences and both improved significantly over the 10 sessions of motor practice. However, as motor practice continued although everyone improved some improved more than others. It is of importance to note that the results from the first two series, which were determined as the initial performance as well as from the improvement after the practice differ a lot between the parti-

cipants and that some twins presented particularly good performance from the start which was maintained and increased over the course of the training. In our subgroup of 14 subjects in whom we were able to ascertain the BDNF gene polymorphisms, we found as above-mentioned four individuals who carried at least one Met allele. Training led to a significantly smaller increase in motor learning in these four subjects; on the contrary, the remaining subjects carrying the Val/Val allelic state responded with an almost double increment. In accordance with this, the results of a recent study showed that the BDNF val66met polymorphism impairs motor skill acquisition in humans and mice (Fritsch et al. 2010). Furthermore, in a recent functional magnetic resonance imaging (MRI) study, McHug-hen and associates (McHughen et al. 2010) examined a single-nucleotide polymorphism of the human BDNF gene in relation to brain motor system function, short-term plasticity, and short motor learning and found that Val/Met polymorphism subjects of BDNF genotype showed poorer short-term learning and retention on motor behavior tests relative to Val/Val subjects. Furthermore, previous study gives an indirect support of these results, as a significant increase in the motor evoked potentials amplitudes in abductor pollicis brevis after PAS was found in Val/Val, but no increase in non-Val/Val individuals exposed to plasticity inducing brain stimulation protocols; which is supposed to be under the same neural substrate as motor learning (Cheeran et al. 2008). The twin cohort of this study on motor control and motor learning includes a cohort of twins in whom the genetic influence on externally induced plasticity was studied. In the previous study, it was demonstrated that the change in corticospinal excitability after an intervention with PAS was genetically dependent in a substantial part 68% (Miss-itzi et al. 2011), almost the same as in the results found in the current study for motor learning (70%).

As these studies examine the heritability of early motor learning and heritability of human brain short-term plasticity; two parameters which are thought to be supported by the same mechanisms (Asanuma and Pavlides 1997; Rioult-Pedotti et al. 2000) specifically in early phases of human motor learning (Rosenkranz et al. 2007), through unmasking of preexisting mtracortical connections and increasing the efficacy of existing synaptic connections by LTP-like plasticity, we could speculate that the same genotype influences their genetic variation to a similar degree. This would be entirely consistent with the fact that both studies activated the same muscle and was conducted to almost the same sample of twins. In the previous study, PAS intervention activated both intracortical pathways that were also active with the voluntary activity in the current study, together with the median nerve of the abductor pollicis brevis. This supports theoretical models that have been proposed as the basis of motor learning, as well as that the same mechanisms support plasticity of motor cortex and motor learning.

Our conclusion that plasticity of motor cortex and motor learning are associated

through shared genes is in line with a similar genetic influence on intelligence and change in cortical thickness (Brans et al. 2010) and with the dependence of learning and memory formation on the plasticity of neural circuits (Escobar et al. 2008).

In this survey, the sisters of the same pair of our subgroup who have been genotyped had always the same allelic state in all pairs, even in heterozygous twins (Fig. 4). Therefore, it is difficult to draw conclusions about the influence of the BDNF polymorphisms on the difference between MZ and DZ twins. However, based on the assumption that Met alleles would occur at the same frequency as in the cohort of 14 subjects, two DZ pairs could be heterozygous for BDNF gene alleles. Given the large difference in responsiveness toward the training protocol in non-Met and Met carriers, it is conceivable that the BDNF polymorphism may have substantially contributed to the wider intrapair difference for DZ twins.

In addition, by comparing the present results related to the motor skill acquisition, with previous ones on externally induced LTP-like plasticity (Missitzi et al. 2011) we found a correlation between motor learning and the PAS-induced plasticity results. This finding appears to provide evidence that the two measures are related, although the weakness of the correlation between them suggests a rather indirect relationship or the presence of significant other factors modulating the relationship between them. Individuals carrying at least one Met allele of the BDNF polymorphism exhibited both reduced ability for motor learning (this study) and brain plasticity (Missitzi et al. 2011). These observations, along with recent evidence that BDNF val66met polymorphism may be a major factor influencing practise-induced brain plasticity, motor learning as well as modulating the formation of LTP (Kleim et al. 2006; Fritsch et al. 2010; Cirillo et al. 2012) strengthen and enhance the possibility that BDNF gene polymorphism may influence both motor learning and neuronal plasticity and be partly responsible for the differences observed between the two types of twins. It has to be noted, however, that BDNF gene polymorphism is only one example of genetic susceptibility and that some controversy exists about the role of the BDNF polymorphism for motor learning (Li Voti et al. 2011; Freundlieb et al. 2012) while there is evidence for an interaction of the genes encoding BDNF and Catechol-O-methyltrans-ferase (COMT) with respect to human cortical plasticity, and that genotype-related differences in neurophysiology, translate into behavioral differences (Witte et al. 2012). Hence, more studies are needed to determine which gene polymorphisms and under which circumstances may underline the difference between MZ and DZ twins.

Recent evidence shows that after brief periods of movement training there are not only changes in motor function but also persistent changes to the way we perceive the position of our limbs (Feldman 2009; Ostry et al. 2010). Moreover, force field learning might in principle lead subjects to modify their estimates of limb po-

sition and to interpret somatosensory feedback during subsequent perceptual testing (Kording and Wolpert 2004). Therefore, the smallness of the correlation found between motor learning and PAS-induced plasticity may indicate isolated motor adaptation in brain plasticity, as PAS does not require active involvement of the participant in the context of movement production, which is required for the sensory shift. Heritability indexes, however, for brain plasticity and motor learning were almost the same, regardless of the somatosensory system participation in motor learning, perhaps because individual variation in proprioception seems to be influenced also by genetic factors (J. Missitzi, A. Misitzi, N. Geladas, J. Classen, and V. Klissouras, unpubl. data).

Although recent studies have provided evidence that synaptic plasticity (Cheeran et al. 2008) as well as motor learning (McHughen et al, 2010) are genetically influenced, independent and complementary information could be gained from twin studies, especially in these multifactorial characteristics which are unlikely to be influenced by a single gene. Our findings elucidate the genetic effect on individual differences in force control and motor learning and support studies that integrate genomics with developmental biology, for understanding the molecular and genetic mechanisms that govern the limits of athletic performance. Adaptive changes are essential for the consolidation of a memory of performance and therefore for the lasting ability of performing highly skilled movements, like those required for Olympic performance (Nielsen and Cohen 2008) As the same external intervention does not induce the same adjustments and it does not lead to the same activation levels, either for learning a motor skill or master a task to perfection, it may be that athletes of Olympic caliber in addition to their superior genotypes may also have inherited to some degree the cortical ability to better respond to motor training. These results not only could provide an insight for performance variation in sports which require high phenotype in motor skills, and learning but also in professions which require movements executed with precision and economy, such as pilots, dancers, musicians, and surgeons.

The present findings may be relevant to understand why people express different adaptive central nervous system response patterns to various injuries. Functional deficiencies and recovery outcomes differ widely between patients with identical peripheral injuries (Kapreli et al. 2007) possibly as a result of different expressions of motor learning. The fact that heredity accounts for a substantial part of the existing differences in human motor learning capacity may also imply that this influence is a factor in the development of or compensation of certain neurological injuries. On the basis of the aforementioned studies and based on our findings, we consider it likely that the differences in rehabilitation after an injury as well as in any type of motor skill acquisition in sports, or in professional, artistic, and recreational activities may be in part genetically influenced.

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Conflict of Interest

None declared.

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Why Nature prevails over Nurture in the making of the elite athlete

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ABSTRACT

While the influence of nature (genes) and nurture (environment) on elite sporting performance remains difficult to precisely determine, the dismissal of either as a contributing factor to performance is unwarranted. It is accepted that a complex interaction of a combination of innumerable factors may mold a talented athlete into a champion. The prevailing view today is that understanding elite human performance will require the deciphering of two major sources of individual differences, genes and the environment. It is widely accepted that superior performers are endowed with a high genetic potential actualized through hard and prodigious effort. Heritability studies using the twin model have provided the basis to disentangle genetic and environmental factors that contribute to complex human traits and have paved the way to the detection of specific genes for elite sport performance. Yet, the heritability for most phenotypes essential to elite human performance is above 50% but, below 100%, meaning that the environment is also important. Furthermore, individual differences can potentially also be explained not only by the impact of DNA sequence variation on biology and behaviour, but also by the effects of epigenetic changes which affect phenotype by modifying gene expression. Despite this complexity, the overwhelming and accumulating evidence, amounted through experimental research spanning almost two centuries, tips the balance in favour of nature in the "nature" and "nurture" debate. In other words, truly elite-level athletes are built - but only from those born with innate ability.

Keywords: nature, nurture, genes, twin studies, heritability, trainability, sport performance

BACKGROUND

The making of champions and achieving elite performance in sport has long been the subject of intense debate - from both a theoretical and a practical perspective. The "nature" versus "nurture" debate dates as far back as the 5th century BC, with one of the first known accounts for the relative nature versus nurture contribution to health and "regimen" having been presented by Hippocrates (460-370 BC), universally honored as the father of medicine, In his Book 1 *Περί Διαίτης (Dietetics)* he noted:

> *"Eating alone will not keep a man well; he must also take exercise. For food and exercise, while possessing opposite properties, yet contribute mutually to maintain health. For it is the nature of exercise to use up material, while of food and drink to restore them. And it is necessary, as it appears, to determine exactly the powers of various exercises, both natural exercises and artificial, and which of them contribute to the development of muscle and which to wear and tear ; and not only this, but also to proportion exercise to the quantity of food, to the predisposition of the person, to his age, to the season of the year, to the changes of the winds, to the geographical place in which the person resides, and to the climatological conditions of the specific year".*

Hippocrates made reference not only to nurture (to include foods and exercise, in addition to other significant environmental conditions) as a requisite for *positive health,* but also to an individual's "genetic predisposition" - in other words, heritability.

Centuries later, Galton (who conceived standard deviation as the measure to quantify normal variation) seemed to be the first academic to give an opinion as to which is more important, with the Galtonian model advocating a hereditary ceiling to physical and mental capacities [1] and objecting "pretensions of natural equality". This implied that performance is limited by heritable characteristics, which are insurmountable by any amount of practice and training. In his landmark paper "The history of twins as a criterion of the relative powers of nature and nurture" Francis Galton used the following eloquent parable to illustrate the notion of the preponderance of nature on phenotypic variation [2]:

> *"Many a person has amused himself by throwing bits of stick into a tiny brook and watching their progress; how they are arrested, first by one chance obstacle, then by another and again, how their onward course is facilitated by a combination of circumstances. He might ascribe much importance to each of these events, and think how largely the destiny of the stick had been governed by a series of trifling accidents. Nevertheless, all the sticks succeed in passing down*

the current, and in the long run, they travel at nearly the same rate. The one element that varies in different individuals, but is constant in each of them, is the natural tendency; it corresponds to the current in the stream, and inevitably asserts itself... There is no escape from the conclusion that Nature prevails enormously over Nurture".

If one accepts that differences between elite and less accomplished performers reflect inherent abilities (so-called "talent"), then it is reasonable to assume that any improvement in performance beyond a fixed maximal level is unmodifiable by extrinsic environmental factors. Empirical evidence has repeatedly refuted this assumption across a wide and diverse range of attributes, including physical performance and motor skills. In particular, improved performance in sport - evidenced by faster times on the track and greater distances in the field events - has been clearly demonstrated, given that sporting performance is measured and recorded under strict standardised conditions at national and world level. However, these improvements in sporting performance over the years can adequately be explained by increases in the duration and intensity of training, new training methods, and even changes to equipment and rules. A recent example of the contribution of extrinsic factors other than nature to performance improvement is the SUB2 marathon project (www.sub2hrs.com); the first dedicated international research initiative made up of specialist multidisciplinary scientists from academia, elite athletes and strategic industry partners with the aim to promote high performance marathon running without doping. While there are no guarantees the SUB2 marathon project will succeed in delivering a 1:59:59 marathon within 5 years, the SUB2 team boast a 100% marathon success record in 2016 and the second fastest marathon in history at the 2016 Berlin Marathon with a time of 2:03:03.

The theoretical framework for *"deliberate practice",* on the otherhand, presents this idea as the means to expert performance and limits the role of innate/inherited characteristics on optimal performance. Ericsson et al [3] argued that commitment to deliberate practice and effort, over a specific period of time, is the distinguishing factor between the qualitative differences that exist between expert and normal performance. Elite performance, they claim, is the "product of a decade or more of maximal efforts to improve performance in a domain through an optimal distribution of deliberate practice", thus rejecting the Galtonian model of innate ability in the making of champions. Domain-specific talent - especially when identified at a young age - is perceived as supporting and motivating early practice and attainment of high levels of deliberate practice, eventually resulting in elite performance. This is in stark contrast to the notion that talent in itself reflects inherent exceptional abilities. Ericsson went on to further develop his model, proposing a specific volume of 10,000 hours of training to be accumulated over a period of approxima-

tely 10 years, as necessary for achieving expert levels [4]. Despite the widespread appeal and popularity of Ericsson's idea as reflected in the emergence of a string of popular books such as Outliers [5], The genius in all of us [6], Bounce [7], the evidence to support a "genetic talent myth" is lacking. The most compelling opposition to Ericsson's idea is the finding that performance is poorly related to deliberate practice time. For example, only 28% of the variance in performance in the sport of darts could be explained by accumulated training [8]. The theory that performance is constrained by accumulated hours of deliberate practice is also further weakened by studies showing that elite athletes rarely complete the necessary 10,000 hours of training before reaching world-class level [9, 10]. The lack of measures of variance (standard deviation or ranges) presented by Ericsson et al [3] also significantly weakens their argument for an association between training and performance, as applicable to every individual. A number of studies examining the relationship between training and performance especially of skill-based activities, revealed significant individual variation as reflected by a large standard deviation and coefficient of variation [11,12]. For example, Gobet and Campilelli [11] investigated markers of talent, environment, and critical period for the acquisition of expert performance in Argentinian chess players (N = 104), ranging from weak amateurs to grandmasters. Their findings reaffirmed the importance of practice for the attainment of high levels of performance but also revealed large variability. Notably, some players needed 8 times as much practice to reach master level than others. The authors concluded that practice was necessary, but not a sufficient condition for the acquisition of expertise and that some additional factors seemed to differentiate chessplayers and non-chessplayers. It is our contention that these additional factors/characteristics, once identified, will emerge to be substantially heritable in nature.

The large variability in all essential attributes and/or responses is precisely what would be predicted and underpins the present day concept of precision/personalised medicine, where major international consortia are attempting to correlate genomic and other high-throughput "omics" data in order to identify individual differences in the response to treatment of major medical conditions such as cancer, and type 2 diabetes. The development of biomarkers for personalised oncology is a striking example of how this large inter-individual variability in response can potentially be harnessed to improve efficacy of treatment. In the last decade there have been significant advances in the development of biomarkers for novel drug targets and new treatment strategies for patients with advanced-stage cancers are moving away from the traditional treatment strategy to one with a biomarker driven treatment algorithm based on the molecular profile of the tumor. As such, predictive biomarkers are increasingly being used to match targeted therapies with patients, and prevent toxicity of standard therapies [13].

THE EVIDENCE FROM GENOMICS, GENETICS AND EXERCISE BIOLOGY

Heritability studies on physical performance and functional adaptability provided strong evidence of a significant genetic component to various parameters that ultimately determine elite performance. Over the past two decades there has been a clear shift in terms of how sports and exercise genetics research has been conducted and the ever-increasing focus on determining specific genes linked to performance. Early family studies/twin models (see Table 1) have provided the basis for disentangling the genetic and environmental factors that contribute to complex human traits, and subsequent genetic association studies in unrelated individuals have further paved the way to detect specific genes for elite sport performance. The pioneering studies on twins [14] revealed that as much as 93% of variability in maximal aerobic capacity (VO_2max) is genetically determined in 25 pairs of monozygotic (n=15) and dizygotic (n=10) twins. The model developed by Klissouras assumed comparable environmental influences between the two sets of twins and the absence of gene-environment interactions. Klissouras also found that VO_2max and maximal heart rate are heavily dependent on genes, which accounted for 81% and 86% of the variation of traits, respectively. Subsequent studies applying path analysis of twin and nuclear family data also reported a high genetic component for VO_2max, namely 77% [15], 69% to 87% [16], and 71% [17], while a more recent meta-analysis of eight twin studies generated a weighted VO_2max heritability estimate of 72% [18]. Notable exceptions are the lower heritability estimates of 40% and 51%, for VO_2max reported by Bouchard and colleagues [19, 20]. Heritability estimates have also been reported for other phenotypic traits linked to sporting performance, such as 99% for maximal anaerobic power [21], 66-92% for muscle cross-sectional area and body dimensions [22, 23], 93-100% for muscle fiber distribution [24], 85% and 73% for neuromuscular coordination at 70% and 50% of maximal velocity, respectively [25], 68% for motor control and motor learning [26], 68% for motor cortex plasticity [27], 80% for intracortical inhibition and 92% for intracortical facilitation [28], 40-50% for personality traits, and 38-71% for specific cognitive abilities [29]. The twin study approach has also demonstrated that the acquisition of motor skills is significantly heritable [26, 30]. For example, Fox and colleagues studied learning in a sample of monozygotic and dizygotic twins who had been reared apart [30]. Specifically, these authors found that heritability of performance at a rotary pursuit task, in which subjects learned to track a rotating target with a stylus, was high even at baseline (66%) and increased with practice (74%), and concluded that the effect of practice is to decrease the effect of environmental variation (previous learning) and increase the relative strength of genetic influences on motor performance. Taken together, virtually all individual differences in functional capacities, morphological dimensions, motor attributes, personality traits and cognitive abilities are moderately to substantially heritable. This

is in line with the most comprehensive meta-analysis of virtually all twin studies published in the past 50 years, on a wide range of traits and reporting on more than 14 million twin pairs across 39 countries, that provide compelling evidence that all human traits are heritable [31]. Estimates of heritability clustered strongly within functional domains, with the largest heritability estimates for traits classified under the ophthalmological domain (h^2=0.712, s.e.m.=0.041), followed by the ear, nose and throat (h^2=0.637, s.e.m.= 0.064), dermatological (h^2=0.604, s.e.m.=0.043) and skeletal (h^2=0.591, s.e.m.=0.018) domains. Substantially lower heritability estimates were reported for traits in the environment, reproduction and social values domains (i.e., 0.290-0.313).

Table 1, includes some of the most important milestones in genomics, genetics, and exercise biology.

As the twin studies/heritability estimate approach has received scathing criticism [32, 33, 34], it is helpful to fully explain the concept of heritability, which is often misunderstood. For example, a heritability estimate of 93% for a given trait such as VO_2max is often misinterpreted to mean that 93% of this phenotype is genetically determined and the remaining 7% is susceptible to environmental modification. Heritability has no etiologic role in a phenotype, nor is it meaningful in terms of measurement in an individual. It is a statistical measure, expressed as a proportion, and refers only to the population under study. More specifically, it describes the extent to which heredity affects the variation of a given attribute in a given population exposed to common environmental influences at a given time. A high heritable attribute does not mean that a phenotype is predetermined and the environment has no effect. It only indicates that the observed individual differences in the given attribute are due to genetic differences and are highly predictable [35]. In addition, a frequently overlooked limitation of the early twin studies is that nearly all heritability estimates have been derived using twins exposed to normal environmental influences and represent the normal range of the bell curve and not elite-level athletic twins. The necessity for a nature-nurture investigation using Olympic twin athletes who have actualised their genetic potential with strenuous athletic training and represent the high end of the distribution is clearly required, in that it may provide new insight and

TABLE 1. Some key milestones in genomics, genetics, and exercise biology*

1971	Vassilis Klissouras / Twin Studies of $\dot{V}O_2$max [14]
1984	Claude Bouchard / Twin Studies of trainability of $\dot{V}O_2$max [39]
1999	Claude Bouchard / Heritage Family Study [40]
2000	Hugh Montgomery/ Candidate Gene Approach -ACE [45]
2001	The Human Genome Project (HGP) - Initial sequencing and analysis of the human genome (http://web.ornl.gov/sci/techresources/Human_Genome/index.shtml)
2003	The ENCODE Project - large public research consortium aimed at identifying all functional elements in the human genome sequence (www.encodeproject.org)
2004	Kathy North/ ACTN3 Speed Gene [46]
2007	Yannis Pitsiladis/Genetics of East African Runners [41]
2008	The 1000 Genomes Project - the largest public catalogue containing human variation and genotype data (www.internationalgenome.org)
2016	GAMES / The first GWAS of athletic performance [48]
2016	The Athlome Project - call for international collaborated efforts in genetic discovery for elite human performance, muscle injury prevention and adaptive training [49]

*See Bouchard and Malina, 2014 [61] for a detailed account of the history of genomics, genetics, and exercise biology

may have far-reaching implications to the nature and nurture debate [36]. Further limitations of research on twins are also addressed by Ericsson [4], who advocates *"twin studies of the acquisition of elite performance are unlikely ever to resolve the issue of heritability of elite performance".* The frequency of twins (even a single member of a twin pair) who attain an elite level of performance in various areas of expertise is much lower than would be expected by chance. The under-representation of twins among eminent individuals may be a consequence of how twins are reared and that deliberate practice by one or both twins may not be encouraged by their parents and/or the other twin during childhood and adolescence, due to its solitary non-social nature. Despite these valid criticisms, twin studies have been an integral part of science for nearly a century and have enhanced significantly our understanding of the extent to which certain traits are inherited.

Despite the mainly indirect evidence favouring a more prominent role of nature over nurture, deliberate practice and environmental factors are undoubtedly both critical to sporting excellence, but they do not in themselves produce elite athletes. Wang et al [37] defined world-class performance as "a polygenic, multifactorial trait, determined by the interaction of genes and the environment". The value of training is by no means refused but rather it is proposed that training is defined as the realisation of one's genetic potential [38]. One of the authors (JB), who spent two decades training and reached world-class level in the 400 metres track event in athletics, strongly advocates the case for inherent talent as a prerequisite for elite performance. Having trained with a large group of athletes, only few went on to reach world- and Olympic-level. When all other extrinsic factors (the nurturers) are consistent - the time spent training, the type of training, the facilities, the training environment - what will ultimately distinguish elite performers is their genetic make-up.

The concept of individual differences in the response to exercise training or trainabiliry was also defined empirically more than three decades ago in a series of experimental studies with pairs of monozygotic twins and evidence reported in support of a strong genotype dependency of the ability to respond to regular exercise [39] (Table 1). In the HERITAGE Family Study that ensued [40], it was observed that the heritability of the VO_2max response following 20 weeks of standardised exercise training reached 47% after adjustment for age, sex, baseline VO_2max and baseline body mass and composition. Notably, there was 2.5 times more variance in individual differences in training response between families than within families. Neither candidate gene studies nor genome-wide explorations have, to date, yielded any validated gene targets and variants as originally anticipated.

Despite some early progress, the question remains as to which genetic variants are those that irrefutably define elite athletic performance and trainability, as numerous attempts to discover candidate genes have largely proved inconclusive, even when genetic superiority was widely assumed as in the African runners ph-

enomenon [41, 42] or conversely the lack of African-American swimmers excelling on the world stage [43]. It is not surprising, given the complex associations between genotypes and phenotypes and the intricacies of the human genome. As of 2008, over 200 genes were associated with human physical performance, with more reported since [44]. Among the genes reported, the angiotensin-1-converting enzyme insertion/deletion (ACE I/D) and the α-actinin-3 (ACTN3) R577X polymorphisms have been the most extensively studied (see Table 1) and, in general, consistently associated with elite endurance and sprint performance [45, 46]. In a recent review [37], we argue that the limited progress achieved today in the field of sport and exercise genomics is due to limitations in the number of genetic variants studied in small and often heterogeneous cohorts, resulting in "spurious and conflicting results". There is an evident need for larger collaborative efforts involving clearly defined phenotypes, control of sources of variability, and rigorous replications in order to produce any meaningful results, which has led to the formation of the "Athlome Project Consortium" (www.athlomeconsortium.org). This international collaborative initiative brings together a large databank, expertise and state-of-the-art "omics" technologies from around the world, aiming to understand genetic variation underlying athletic performance, adaptation to exercise training, and injury predisposition. The review by Wang et al [37] presents the current cohorts and projects involved in the Athlome Consortium and highlight the need for a paradigm shift of the status quo to the era of sport and exercise genomics. In particular, an unbiased exploration of the human genome is needed utilising the full power of genomics, epigenomics, and transcriptonomics, in combination with large-scale, replicable study designs [47]. Notable highlights in this regard from the Athlome Consortium is the first published GWAS of athletic performance [48] and the declaration of the sequencing of 1000 of the world's greatest athletes in the 1,000 Athlomes project [49]. Specifically, GWASs were undertaken on 2 cohorts of elite endurance athletes (GENATHLETE and Japanese endurance runners) and their respective controls, from which a panel of 45 candidate single nucleotide polymorphisms (SNPs) was identified, and tested for replication in 7 additional cohorts of endurance athletes and controls from Australia, Ethiopia, Japan, Kenya, Poland, Russia, and Spain. This first of its kind study of elite athletes, was based on a total of 1,520 endurance athletes (835 of them had competed in World Championships or Olympic Games) and 2,760 controls. This initial GWAS attempt failed to identify a panel of genomic variants common to these elite endurance athlete groups due, to the study being underpowered to identify alleles with small effect sizes, and/or due to the use of an earlier generation gene microarray with only 195,000 gene markers (Illumina® CardioMetabochip, Illumina USA), as opposed to some 40 million common polymorphic sites possible, let alone the absence of other genomic features only accessible with full genome sequencing. The 1,000 Athlomes Project

is therefore timely, as it aims to sequence 1,000 genomes of sprinters and distance runners of the highest level from West and East African descent (i.e. world record holders, Olympians and World Champions). It is envisaged that the large amount of genotype data to be generated from the 1,000 Athlomes Project will serve as a reference panel for future performance studies and guide other extreme phenotype studies in biomedical science.

Undoubtedly, and as previously outlined, science has evolved significantly throughout the last two centuries, with the early 19th century Galtonian model leading to research on heritability of athletic performance via family/twin studies which, in turn, gave rise to studies on gene identification through hypothesis-driven and hypothesis-generating genetic association studies in unrelated individuals. Following the ENCODE (Encyclopedia of DNA Elements) Project (www.encodeproject.org) and the 1000 Genomes Project (www.internationalgenome.org) (Table 1), over 88 million genetic variants have been characterised and over 99% of SNPs reported with a frequency of >1% for a variety of ancestries [50]. As such, the now widely accepted view of human genomics is far more realistic, complex and exciting with extremely large but finite numbers of variants with almost infinite possible permutations.

A more complete understanding of the interplay between the molecular basis of elite human performance and the environment will require deciphering the epigentic response to environmental stimuli; the changes in gene function that cannot be explained by alterations in the DNA sequence. Several animal and human studies have already provided novel insights into how internal and external environmental factors can influence physiologic processes by regulating gene activity and expression. For example, genome-wide epigenetic changes can be induced by acute and chronic exercise in skeletal muscle [51], adipose tissue [52] and the brain [53, 54]. For example chromatin modifications seem to be involved in triggering the gene expression responses required for physiological and functional adjustments in neurons mediating cognitive processing of stressful events [55]. Epigenetics may therefore be an attractive hypothesis to explain the paradoxical findings obtained in twin investigations where identical twins differ for some traits not because of their genes or because they are exposed to different environments [56, 57]. Epigenetic differences in genetically identical humans have been demonstrated repeatedly [58], and epigenetic markers appear to be at the interface between environmental stimuli and long-lasting molecular, cellular and behavioral phenotypes [59]. Our knowledge of sport and exercise epigenetics remains limited, and complex mechanisms that modulate gene expression are largely unknown [60]. A great challenge for sport and exercise genomics for the future is to dissect the role of epigenomic alterations in facilitating physiological, metabolic, cognitive, emotional and behavioural changes that empower Olympic athletes to push performance beyond perceived limits.

CONCLUSIONS

While the influence of nature (genes) and nurture (environment) on elite sporting performance remains difficult to precisely determine, the dismissal of either as a contributing factor to performance is unjustified. It is accepted that a complex interaction of a combination of innumerable factors may mold a talented athlete into a champion. In their most basic form, these factors amount to genetics, training and preparation. The contribution of each is absolutely necessary in the making of a world-class athlete. The essential role of practice and training is widely and indisputably recognised. Individual variation, however, in terms of starting performance levels, subsequent response to training, and final performance levels attained with the same amount of training, clearly illustrate the prominent role of genetic factors and their interaction with training and the environment. The overwhelming and accumulating evidence, amounted through empirical and experimental research spanning over almost two centuries, tips the balance in favour of nature in the "nature" and "nurture" debate. In other words, truly elite-level athletes are built - but only from those born with innate ability. This conclusion is in line with the prophetic text by Galton, some 150 years ago who wrote "there is nothing in what I am about say that shall underrate the sterling value of nurture, including all kinds of sanitary improvements; may, I wish to claim them as powerful auxiliaries to my cause; nevertheless, I look upon race as far more important than nurture." [62]

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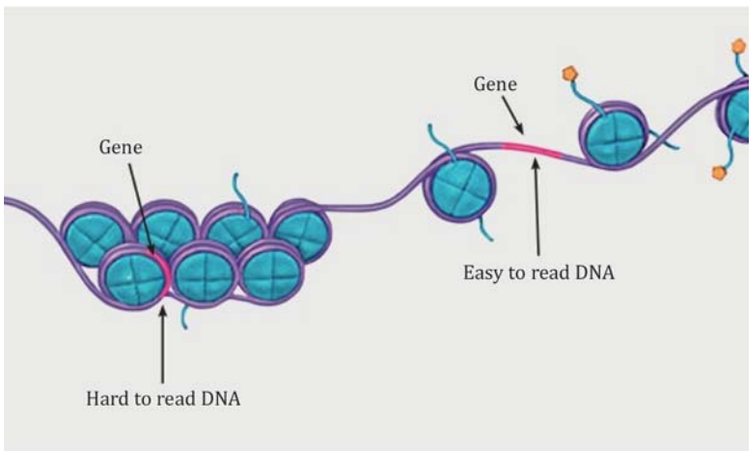
Στροφή σε Επιγενετικές Έρευνες

Ερωτήθηκε ο καθηγητής Κλεισούρας, αν έχουν καταλήξει οι γενετικές έρευνες χρησιμοποιώντας το μοντέλο των διδύμων σε κάποια συμπεράσματα. Και ακόμα του ζητήθηκε να σχολιάσει την προοπτική των ερευνών αυτών εν όψει μάλιστα των ραγδαίων εξελίξεων στη γονιδιωματική. Η απάντησή του:

«Το μοντέλο των διδύμων υπήρξε γονιμοποιός πολλών ερευνών, που όλες συγκλίνουν στο συμπέρασμα ότι οι προσδιοριστικοί φαινότυποι της αθλητικής απόδοσης βρίσκονται υπό ισχυρό γενετικό έλεγχο. Ακόμα, το μοντέλο αυτό υπήρξε πρόδρομος αναζήτησης και ταυτοποίησης γονιδίων που σχετίζονται μ' εκείνους τους φαινοτύπους που ορίζουν τις Ολυμπιακές επιδόσεις, παρόλο που δεν έχει ακόμα εδραιωθεί οριστική και αναμφισβήτητη σύνδεση γονότυπου-φαινοτύπου.



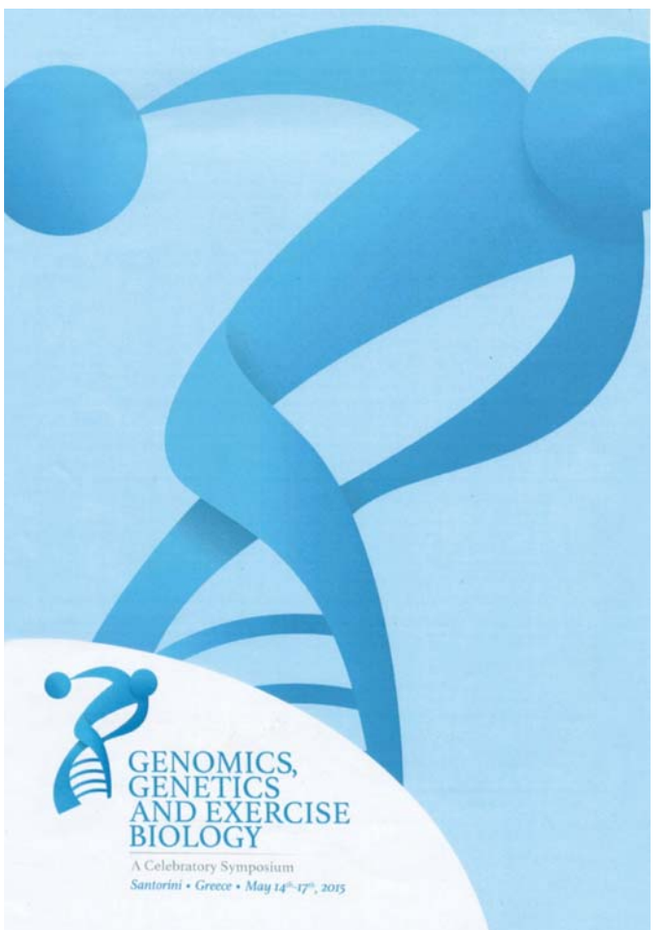
Όσον αφορά την προοπτική των ερευνών, με πρωτοβουλία του Καθηγητή Γιάννη Πιτσιλαδή ο οποίος πρωταγωνιστεί στο πεδίο αυτό, συνδιοργανώσαμε πρόσφατα στην Σαντορίνη ένα Συμπόσιο όπου εκλήθησαν οι επιφανέστεροι ερευνητές στη ***Γενετική, Γονιδιωματική & Βιολογία της Άσκησης*** *(βλ. αφίσα παρακάτω) και εξετάσαμε ενδελεχώς την παρούσα κατάσταση και την κατεύθυνση που πρέπει να πάρει η έρευνα στο μέλλον. Έτσι, αποφασίσαμε τη δημιουργία ενός κονσόρτσιουμ ερευνητικής συνεργασίας που έχει ήδη δραστηριοποιηθεί και παράγει έργο όπως φαίνεται από τις παρακάτω δημοσιεύσεις που παρατίθενται αυτούσιες στη συνέχεια».*

- **DIRECT-TO-CONSUMER GENETIC TESTING FOR PREDICTING SPORTS PERFORMANCE AND TALENT IDENTIFICATION: CONSENSUS STATEMENT**
  Πρόκειται για ομόφωνη διακήρυξη του κονσόρτιουμ που αφορά τον γενετικό έλεγχο διάγνωσης αθλητικών ταλέντων.

- **FUTURE OF GENOMIC RESEARCH IN ATHLETIC PERFORMANCE AND ADAPTATION TO TRAINING**
  Αφορά το μέλλον γονιδιωματικής έρευνας στον Αθλητισμό.

- **ATHLOME PROJECT CONSORTIUM: A CONCERTED EFFORT TO DISCOVER GENOMIC AND OTHER "OMICS" MARKERS OF ATHLETIC PERFORMANCE**
  Αναφέρεται στη σύσταση, σύνθεση, στόχους, τομείς δραστηριοποίησης και στην προοπτική του κονσόρτιουμ.

- **EPIGENETICS: A PATH TO ELITE ATHLETIC PERFORMANCE**
  Αναφέρεται στην Επιγενετική που είναι το τωρινό πεδίο ερευνητικής δράσης του τιμώμενου καθηγητή και δείχνει την πορεία της επιστημονικής του σκέψης και διαδρομής. Την ενασχόλησή του με την Γενετική βάση της αθλητικής απόδοσης στη **χαραυγή** της σταδιοδρομίας του και με την Επιγενετική στο **λυκόφως** της



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Direct-to-Consumer Genetic Testing for Predicting Sports Performance and Talent Identification: Consensus Statement

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ABSTRACT

The general consensus among sport and exercise genetics researchers is that genetic tests have no role to play in talent identification or the individualised prescription of training to maximise performance. Despite the lack of evidence, recent years have witnessed the rise of an emerging market of direct-to-consumer marketing (DTC) tests that claim to be able to identify children's athletic talents. Targeted consumers include mainly coaches and parents. There is concern among the scientific community that the current level of knowledge is being misrepresented for commercial purposes. There remains a lack of universally accepted guidelines and legislation for DTC testing in relation to all forms of genetic testing and not just for talent identification. There is concern over the lack of clarity of information over which specific genes or variants are being tested and the almost universal lack of appropriate genetic counselling for the interpretation of the genetic data to consumers. Furthermore independent studies have identified issues relating to quality control by DTC laboratories with different results being reported from samples from the same individual. Consequently, in the current state of knowledge, no child or young athlete should be exposed to DTC genetic testing to define or alter training or for talent identification aimed at selecting gifted children or adolescents. Large scale collaborative projects, may help to develop a stronger scientific foundation on these issues in the future.

INTRODUCTION-DIRECT-TO- CONSUMER MARKETING

The general consensus among sport and exercise genetics researchers in the genetic tests, based on current knowledge, have no role to play in talent identification or the individualized prescription of training to maximize performance. Despite the lack of evidence, recent years have witnessed the rise of an emerging market of direct-to-consumer marketing (DTC) tests that claim to be able to identify children's athletic talents. Targeted consumers include mainly coaches and parents. Early talent identification is seen as a starting point to success and on the basis of the results of the genetic tests parents and coaches are led to believe that they can acquire knowledge to plan and invest in a child's future. It is vitally important that sport and exercise medicine practitioners are fully aware of the state of the evidence in relation to genetic testing and the limitations of current knowledge. This article reviews the issues around the currently available evidence behind the genetic testing, comments on the ethical considerations and makes recommendations about such tests.

STATEMENT ON BACKGROUND TO THE CONSENSUS PROCESS

A group of world experts in the field of genomics, exercise, sport performance, disease, injury and antidoping gathered with the International Federation of Sports Medicine (FIMS) Scientific Commission for a symposium to discuss the current state of knowledge and to share ideas. One key concern was the misuse of research evidence and the misinformation about genetic testing, particularly when marketed directly to the public, coaches or parents. This is known as DTC testing for the purpose of talent identification and to assess potential for future sports performance. There have been a variety of documents that have addressed issues for DTC Genetic Testing in relation to screening for disease, or to identifying genetic carriers, including those from the European Workshop on Genetic Testing Offer in Europe, the Human Genetics Commission (UK), American College of Medicine Genetics among others. [1-3] However, these documents relate mainly to testing of disease states or heritability of conditions and no organization has specifically addressed the issue in regard to the world of sport for talent identification.

The sports medicine community has a duty of care to protect the health and well-being of athletes based on the current scientific knowledge.

The consensus statement was developed across four areas:

1. Genetics-expert opinion of the scientific evidence in the field of genomics, exercise, sport performance from the participants of the Genomics, Genetics and Exercise Biology Symposium.
2. Sports medicine-consideration of the impact of DTC testing for young athletes and the need for education for sport and exercise medicine participation by the FIMS Scientific Commission.

3. Ethical and Legal=independent international expert review of the document
4. An internet review of DTC tests commercially available—In June 2015, internet searches were conducted from within the UK to identify commercially-available sport and exercise-related genetic tests for humans, a follow-up to a similar analysis conducted in June 2013.[4] As in previous reports, four English language internet search terms GENETIC, TEST, EXERCISE and SPORT were used in a simple search in two popular internet search engines (Google and Bing), as a potential consumer might do. In addition, other commercially available sport and exercise-related genetic tests, of which the authors were already aware, were included in the results. The websites of the commercial operations identified were explored manually and, if available, details about the numbers and identities of genetic variants being tested were identified. The recorded number of variants tested, and the names of the genes corresponding to the variants tested, required some subjective interpretation for their relevance to sport and exercise where this was not clear on the websites. For example, genetic tests marketed in relation to body composition phenotypes, but not clearly marketed as having a direct interaction with exercise, were not included. In addition, in some instances gene names but not specific variant details were identified, so some assumptions have been made regarding the precise variants being tested in those cases. This statement does not relate to genetic testing for disease or specifically for cardiovascular conditions predisposing to sudden death related to exercise or sports performance.

SANTORINI 2015 CONSENSUS QUESTIONS

What are the issues around DTC genetic testing?

The science of genomics has advanced over the past decade at a rate unimagined by the medical scientific community. Not only is genetic testing becoming more commonplace in the clinical setting, but it has also reached the general public. Testing has also become much cheaper. From the $2.7billion it cost to sequence the first whole human genome, it now costs less than $1000 and continues to fall.[5] For analysis of specific variants this is even less, which is why companies can offer genetic testing to the public on a commercial basis. However, while the price of sequencing or genotyping has dramatically dropped, the interpretation of what the results mean is still at an early stage.[6-10] Any genetic test should be evaluated against four main criteria: analytic validity, clinical validity, clinical utility and the associated ethical, legal and social implications.[11]

The pace of advance in sequencing and genotyping technology has far exceeded the pace of change in related regulation. Testing is poorly regulated with no worldwide agreement as illustrated by the following examples. Legislation currently va-

ries from country to country in Europe. While France, Germany, Portugal and Switzerland have specific legislation that defines that genetic tests can only be carried out by a medical doctor, there is currently no regulation in the UK. [12] A new draft European Union law is still under negotiation between member states. It would require companies to provide scientific evidence for claims, and restrict or ban sales of genetic tests directly to consumers.1 The In Vitro Diagnostics (IVD) Regulation passed first reading in the European Parliament in 2014 and is currently under negotiation at the Council, representing member states.[13] This new law would require companies to provide evidence of the clinical validity of their genetic tests and would require medical supervision of testing. Australia has recently amended the Therapeutic Goods Act (July 2014) to regulate the supply and advertising of DTC genetic testing. This testing is prohibited in Australia, except where specifically approved by the Therapeutic Goods Administration (TGA), which includes proof that it is being performed in an accredited laboratory with sufficient clinical validity and utility. Companies can take tests to market without any independent analysis to verify their claims. In the USA, The Food and Drug Administration (FDA) has the authority to regulate genetic tests, but has only regulated the relatively small number of genetic tests sold to laboratories as kits. Although the FDA plans to expand its regulation to all genetic tests, this has not yet occurred.[14] A report by USA Government Accountability Office (GAO) to the US Senate highlights the problem: "A genetic test is considered by the FDA to be a medical device only if it is manufactured as a freestanding 'kit' and sold to a laboratory. Presently, though, most genetic tests are not sold as kits but are manufactured in-house by clinical laboratories. In these cases, the laboratory itself decides whether a test has sufficient 'clinical validity' (ie, is sufficiently effective at measuring what it purports to measure). Although all clinical laboratories must be approved under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and meet general standards applicable to all laboratories, there is no genetic testing specialty under CLIA." The absence of monitored quality control at the laboratory is also an issue. In the GAO report, samples of DNA from the same people were sent under different names and to different laboratories yet different genetic variants were reported for the same individual.[15]

Of concern also to exercise and sport geneticists is that there are DTC health-related tests aimed at giving nutritional and lifestyle information based on a limited genetic analysis, sometimes called 'nutrigenetic' tests. In this case, the individual is often encouraged to purchase multivitamin and mineral products. The GAO report concluded that the "results encourage the purchase of supplements that are overpriced, make unproven medical claims, and may even be harmful".[15]

What DTC tests are currently available?

Thirty-nine companies were identified as providing DTC genetic tests that were

marketed in relation to sport or exercise performance or injury. For 21 of the 39 companies (54%), it was not possible to identify the specific DNA sequence variants tested. For the 18 companies that did present information about their genetic tests on their websites, the most commonly-tested variant was the ACTN3 R577X polymorphism that was tested by 16 of those 18 companies (89%). The second most commonly-tested variant was the ACE I/D polymorphism that was tested by 11 of those 18 companies (61%). The median number of variants tested by the 18 companies was 6, ranging from 1 to 27.

Who are they aimed at, who can request them and what do they claim to show?

DTC tests are aimed at individuals, coaches, parents, athletes and sports teams but indeed anyone who is prepared to pay for the test, and willing to send a saliva sample or buccal smear, can request a test. Since the sample collection process is simple it can be completed at home by any individual and mailed to a laboratory anywhere in the world. The claims of DTC websites in relation to sport performance and talent identification are numerous and concerning as they are largely without scientific foundation. Samples of these claims are shown in the box 1 below.

Since the last comparable survey of DTC[4] the number of companies providing DTC genetic tests appears to have almost doubled from 22 identified in 2013 to 39 identified in 2015. Only 14 of the original 22 companies identified appear to still operate commercially, meaning that eight have apparently ceased to operate while 25 new companies have emerged during the past 2 years. It was observed that some of the companies listed in box 1 appear to either be linked to each other in some way (perhaps rebranded for different markets or countries/cultures), or linked to local 'clinics' (not included in box 1) via which the genetic tests are marketed. Several of the companies use their clients' genetic test results as opportunities to offer other aspects of their commercial activities for which additional fees are charged, such as training advice and especially nutritional supplements. However, the evidence to support linking specific training advice

BOX 1. Examples of claims from direct-to-consumer marketing websites

- Discover how your genes contribute to your athletic traits;
- Personalise your training based on your sports genetics results;
- Take advantage of your inherent strengths and overcome your limitations;
- Gives parents and coaches early information on their child's genetic predisposition for success in team or individual speed/power or endurance sports;
- Genetic predisposition determination can be valuable in outlining training and conditioning programmes necessary for athletic and sport development;
- Test results may be used later in development with other athletic performance;
- We use your DNA results to help you lose fat, get lean, build muscle, get fitter;
- Genetic test of athletic abilities describes:
  - better or equal disposition to engage in either endurance sports or power sports;
  - the score of genetic predisposition to engage in either endurance or power sports on 8-point scale;
  - the regulation of blood supply, work capacity and metabolic processes in your muscles;
  - the type of muscle fibres—fast-twitch or slow-twitch;
  - the availability of energy in cells;
  - the availability of constant energy supply in your muscles during exercise;
  - the presence and extent of protection of your skeletal muscles against fatigue.

and nutritional supplements based on genetic data is extremely weak. Of the companies we identified, 54% of the companies offering DTC genetic tests related to exercise and sport do not publicly state which genetic variants they rely on. While commercial pressures undoubtedly exist, it is impossible for anyone—consumer, academic scholar or others —to scrutinise the service provided by the companies if the detail is not presented to the public. Quite literally millions of genetic tests could theoretically be conducted, so the choice of which variants are tested—and how the results are interpreted— is absolutely fundamental to the usefulness of the test. The reasons for such apparent secrecy are presumably commercial sensitivity in part, although it is tempting to conclude that failing to publicise the tests conducted is a tacit admission that the scientific evidence supporting the genetic variants chosen is weak.16,[17]

The UK Human Genetics Commission, which was disbanded in 2012, developed guidelines in relation to marketing of DTC genetic tests. These suggest that the test provider should comply with any legislation or voluntary codes for advertising of medical tests and that they should also comply with more general guidance (including legal guidance) covering consumer advertising.[2] At a minimum, advertising should:

- Accurately describe both the characteristics and the limitations of the tests offered;
- Not overstate the utility of a genetic test;
- Make sure that any claim made about the clinical validity of a test is supported by relevant evidence published in peer reviewed
- Recognise that the test provider should be aware of the risk of bias when quoting evidence and ensure that evidence is presented.

Furthermore they suggest that the evidence of the association between a genetic marker and a trait should be validated at genome-wide significance level ($p<5{\cdot}10^{-8}$) in more than one large case-control study and in a cohort of the ethnic/geographic background relevant to the client. This is particularly relevant to talent identification or performance testing where the studies to date are limited in ethnicity and geographic background. In 2008, the Federal Trade Commission (FTC) in the USA issued warnings to consumers that "no standards govern the reliability or quality of at-home genetic tests. The FDA and Centers for Disease Control and Prevention recommend that genetic tests be done in a specialised laboratory and that a doctor or counsellor with specialised training interpret the results." Perhaps it is unsurprising then that the GAO report in 2010 to the US Senate is titled: 'Direct-To-Consumer Genetic Tests—Misleading Test Results Are Further Complicated by Deceptive Marketing and Other Questionable Practices'.[15]

What are the ethical and legal issues around consent and data protection for companies providing this testing?

There is a consensus in the medical scientific community that genetic tests should be carried out only after the person concerned has given free and informed consent. This can only be provided when a consumer/patient has received sufficient relevant information about the genetic test in such a manner that they are able to understand the risks, benefits, limitations and implications of the genetic test, whose consequences may be indirect and long term. Thus, for example, test data may also have implications in the future for purchase or provision of health or life insurance.

In the UK, the Human Genetics Commission produced guidelines around DTC Genetic Testing services but these had no statutory authority.[2] It includes clear guidance on consent and includes the following: "Separate, specific, informed consent should be requested by the test provider if the test provider wishes to perform further tests that are not covered by the original consent or if biological samples are to be stored by the test provider after the consumer has been provided with the genetic test results. Likewise, separate informed consent should be requested by the test provider before biological samples are used for any secondary purposes, for example, research, or before any third party is permitted access to biological samples."

In relation to children it offers the following guidance "Genetic tests in respect of children when, according to applicable law, that child does not have capacity to consent should normally be deferred until the attainment of such capacity, unless other factors indicate that testing during childhood is clinically indicated. If postponement would be detrimental to the child's health, or the management of the child's health may be altered significantly depending on the test result, then testing should be organised by a health professional who has responsibility for ensuring that any medical intervention or screening indicated will be arranged and proper arrangements made for any subsequent care." These principles of the Human Genetics Commission are applicable to 'lifestyle/behavioural' traits such as performance capacities if they are deemed by to be 'high impact', which is open to debate. For example, if the tests are performed to determine selection and future sporting careers then this may be deemed to have a 'high impact' on the individual—depending on parental or guardian use of the data—but this requires further clarification in the light of specific cases. The American Society for Human Genetics has recently published a position statement that recommends that DTC testing "be discouraged in children until such a time when companies that provide DTC GT can assure quality, accuracy and validity of their testing and assure that there is adequate pretesting and post-testing counselling".[18]

Genetic information is potentially sensitive and as such requires the highest level of security and confidentiality. It is imperative that any personal data and ge-

netic information that are linked to an individual should be subject to privacy protection and security, and cannot be shared without the explicit consent of the individual, in accordance with current professional guidance and applicable laws on data protection and confidentiality. It is also important to consider what should occur if a DTC provider should cease trading or be taken over by a third party.

What are the ethical issues of genetics-based talent identification programmes?

Genetic information by its very nature means that it is familial. It reveals facts about persons beyond those who have consented to tests, whose results may have direct health implications for other family members. Furthermore the risks of genetic testing for talent identification may not be immediately obvious because the risks may be psychological, social and financial. The psychosocial consequences might include impaired self-esteem, social stigma and, in terms of sport selection, may include employment limitation. The testing may also impact on personal relationships within families or have a life-altering impact on the behaviour of the individual taking the test.

Consumers of the test (coaches, parents, etc) may secure services that they falsely believe will steer children as to which sports most effectively can be pursued according to their genetically derived data. Such predictions are associated with ethical problems that vary according to the individual tested. These range from the narrowing of athletic participation opportunities, a heightening of the dangers of early specialisation, and a failure to engage with what could be activities that provide a lifetime of satisfaction (in the absence of athletic success). These might be thought of as infringements of children's rights to an open future,[19-21] that parents have a duty to protect. Finally, the use of DTC Genetic Testing is irresponsible when it is provided without genetic counselling. Notably, the UK Human Genetics Commission and the European Society of Human Genetics recommend that genetic tests be provided with appropriate genetic counselling so that test data can be interpreted in the light of the particular individual, their circumstances and the relative predictive power of the test outcomes.

What is the current scientific evidence for genetic testing for talent identification for sport?

The genetic variants tested most frequently by the companies providing DTC genetic tests related to sport and exercise in 2015 were those in the ACTN3 and ACE genes, which presumably reflects the fact that more research has been conducted on those polymorphisms than any others in the context of sport and exercise. Although the true role of the ACTN3 R577X and ACE I/D variants in skeletal muscle metabolism and strength traits remains controversial,[22] in meta-analyses the ACE

II genotype was associated with physical performance (OR=1.23; 95% CI 1.05 to 1.45), especially endurance performance (OR 1.35; 95% CI 1.17 to 1.55), while ACTN3 RR genotype was associated with speed and power performance (OR=1.21; 95% CI 1.03 to 1.42).[23] ORs of approximately 1.5 are very small, however and virtually meaningless for talent identification in isolation. For example, while an OR of 1.2 for ACTN3 RR genotype might imply a 20% greater likelihood of being an elite sprinter than other genotypes, in the UK's ~65 million population there are an estimated 20 million people of RR genotype— but only a tiny fraction of those people are elite athletes. Indeed, the degree of interindividual variability in sprinting performance that can be explained by ACTN3 genotype, for example, which has been estimated to reach -2-3%,[24,25] while based on the broader scientific literature is probably less than 1%. Hence, while there is a little replicated scientific evidence regarding these ACTN3 and ACE polymorphisms on a commercial basis, and one can understand individuals interested in exercise and sport wishing to learn about their own genetic composition within these two well-studied genes, the consensus is that the predictive value of such tests in the context of training responses or talent identification in sport is virtually zero.[26]

There is limited information that can be gleaned from discrete, single marker genetic tests at common polymorphisms. It is totally unwarranted for companies to sell DTC Genetic Testing based on a single variant as there is absolutely no evidence to claim they provide information on which personal exercise training or sport decisions can reasonably be made. Most of the companies identified as offering defined DTC genetic tests assess a panel of multiple genetic variants (median 6 variants, range 1-27). However, when considering genetic variants beyond those that are reasonably well-studied, the level of scientific evidence to support the choice of any particular polymorphism is extremely weak or non-existent.[26-28] While commercial pressures undoubtedly exist, it would be more responsible to wait for better and stronger scientific evidence before offering genetic tests commercially. Moreover, counselling that puts the genetic information—including the limitations of its usefulness—into proper context is absolutely necessary.

What are the recommendations that can be made from a scientific perspective on the role of DTC in talent identification?

Based on the published scientific evidence, the information provided by DTC is virtually meaningless for prediction and/or optimisation of sport performance. There is currently no evidence that existing genetic tests provide information that is useful regarding either predisposition for a particular sport, prediction of the training response likely to occur to a particular training programme, or predisposition to exercise-related injury.[29] It is unknown at this time whether genetic testing, even when knowledge and test validity improves dramatically, will provide information

that is not captured within other, traditional non-genetic tests of physiological, anthropometric, medical and performance characteristics that are already used routinely in sport and exercise science and medicine. The key issue is that the question can only be resolved by a comprehensive and highly focused research programme.

THE CONSENSUS SUMMARY

The science around genetic testing is an emerging field. With regard to predicting future sporting performance, the scientific foundation is extremely limited and largely non-existent. There is concern among the scientific community that the current level of knowledge is being misrepresented implicitly for commercial purposes. There remains a lack of universally accepted guidelines and legislation for DTC testing in relation to all forms of genetic testing and not just for talent identification. The exercise science and sports medicine community has a duty of care to provide the most up-to-date advice on issues relating to health and well-being of athletes. This also relates to advising sports teams, athletes, parents and children about the absence of scientific evidence and current limitations of genetic testing in predicting future sport performance. There is concern over the lack of clarity of information over which specific genes or variants are being tested and the almost universal lack of appropriate genetic counselling for the interpretation of the genetic data to consumers. Furthermore independent studies have identified issues relating to quality control by DTC laboratories with different results being reported from samples from the same individual. DTC companies must also better address issues around consent, privacy and ownership of data if a company should cease trading or be taken over by a third party.

While further evidence will undoubtedly emerge around the genetics of sport performance in the future, the data are currently very limited. The ACTN3 genotype is the most commonly tested by DTC companies. However, even for this genotype, its contribution to the degree of inter-individual variability in sprinting performance is trivial. Consequently, in the current state of knowledge, no child or young athlete should be exposed to DTC genetic testing to define or alter training or for talent identification aimed at selecting gifted children or adolescents. Large scale collaborative projects, such as the Athlome Project, may help to develop a stronger scientific foundation on these issues in the future but, currently, there is no place for DTC testing for predicting sports performance and talent identification.

An abbreviated consensus statement outlining the key issues and recommendations are available in online supplementary appendix A.

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The Future of Genomic Research in Athletic Performance and Adaptation to Training

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ABSTRACT

Despite numerous attempts to discover genetic variants associated with elite athletic performance, an individual's trainability and injury predisposition, there has been limited progress to date. Past reliance on candidate gene studies focusing predominantly on genotyping a limited number of ge netic variants in small, often heterogeneous cohorts has not generated results of practical signifi cance. Hypothesis-free genome-wide approaches will in the future provide more comprehensive coverage and in-depth understanding of the biology underlying sports-related traits and related genetic mechanisms. Large, collaborative projects with sound experimental designs (e.g. clearly de fined phenotypes, considerations and controls for sources of variability, and necessary replications) are required to produce meaningful results, especially when a hypothesis-free approach is used. It remains to be determined whether the novel approaches under current implementation will result in findings with real practical significance. This study will briefly summarize current and future direc tions in exercise genetics and genomics.

World-class sporting performance is a polygenic, multifactorial trait, determined by the interaction of genes and the environment. This study will provide a brief summary of the current status in exercise genetics reflecting the fact that very little of practical significance is known and will focus on future directions in exercise genetics and genomics.

Overview of the Current Status

There have been numerous efforts over the past few decades to discover performance-related genes [1-12]. Commonly used gene discovery methods in genetic research have included family-based studies (e.g. twin studies and linkage analyses) and association studies in unrelated population samples (i.e. candidate gene and hypothesis-free approaches). There have been numerous attempts to quantify the heritable component of athletic performance from twins and family aggregation studies. For example, the heritability of athletic status, trainability, and exercise behaviour is estimated to be 66, 47, and 62%, respectively [13-15]. Linkage analyses have revealed a number of chromosomal regions that contain predisposing genes associated with performance-related phenotypes such as maximal oxygen uptake and the response to training [16-19], maximal power output [18,19], cardiac output [20], physical activity levels [13,21,22], and muscle strength-related traits [23, 24]. Early family studies showed that genetic factors contribute to overall performance and performance-related traits. On the other hand, genetic association studies that are conducted in unrelated case-control samples and directly compare the allele frequencies of marker(s) in the candidate regions or spanning throughout the whole genome have a greater power in identifying variants of modest effect and of lower frequency. As of 2008, over 200 genes were reported to be significantly associated with human physical performance [6], and there have been many more associations reported since. To date, most of the genetic findings in athletic performance have been generated using the candidate gene association approach (in small, often heterogeneous cohorts), and the majority of associations have been inconclusive due primarily to (i) the variants genotyped are not causal and provide incomplete linkage with other functional variants, (ii) studies are underpowered, (iii) population stratification, and (iv) phenotypic and locus heterogeneity. False-positive discoveries can also occur in studies examining multiple genes or splitting the cohorts into subgroups for separate analysis [25] without controlling for multiple testing. Among all the genes reported, the angiotensin-1-converting enzyme insertion/deletion (ACE I/D) polymorphism and the a-actinin-3 (ACTN3) R577X polymorphism have been the most extensively studied and, in general, have consistently been associated with elite endurance (ACE I allele) and sprint performance (ACTN3 R allele). The gene discovery methods, their drawbacks and advantages, and the outcome from using these approaches related to sports genetics are presented elsewhere in more detail [26-28].

Despite the inconsistent findings reported in the exercise genetics literature, this genetic knowledge has already been used by companies to produce sports-related testing kits that are offered to coaches, parents, and athletes. The first testing kit available on the market dated back to 2004 [29], not long after Yang et al. [30] had reported the potential influence of ACTN3 on sports performance. A recent re-

view examined the issues surrounding genetic testing for athletic ability, searched and summarized the number of commercially available testing kits [31]. The consensus view in the scientific community is that current genetic findings have no predictability in personalized training and talent identification; therefore, current genetic testing using this information is misleading and must not be used for the purposes indicated. The search conducted as part of that review showed that, among 39 identified companies providing genetic tests, 21 did not reveal the specific genetic variants under testing [31]. The remaining 18 companies presented the variant details [31]. The number of variants tested by the 18 remaining companies ranged from 1 to 27, with the top 2 commonly tested variants being ACE I/D (11 of 18) and ACTN3 R577X (16 of 18) polymorphisms [31]. Genetic testing is further reviewed in the paper by Rahim et al. of this book.

Athletic performance is a complex trait that requires different genetically driven components of human biological systems to coordinate effectively with the environment so as to excel. It is undoubtedly the case that multiple genes are associated with the performance trait and/or its components, though current progress in understanding the genetic architecture underlying these traits is very limited. Exercise genomic science requires an urgent paradigm shift [32] involving a collection of different approaches to discover the genetic basis of human performance, talent selection, train-ability, and injury prevention. Ethical issues around genetic testing are paramount particularly in young children and need to be considered carefully at all times [29,31].

The Paradigm Shift to the Genomics Era

Previous candidate gene studies have focused on a limited number of variants in small and often heterogeneous cohorts resulting inevitably in spurious and conflicting results. It is necessary that exercise genomics are not restricted to the current and common practice of focusing on candidate genes, typically defined by the author preferences or from biases in the published literature, and the reliance on small, statistically underpowered, observational studies [32]. It is vital that exercise genomic science needs to shift to an unbiased exploration of the genome using all the power of genomics, epigenomics, and transcriptomics in combination with large observational (preferably prospective) and experimental study designs with the emphasis firmly on replication [32]. Therefore, large, collaborative efforts are necessary for meaningful progress to be made in the area of exercise genomics.

A group of main investigators in sports and exercise science gathered together in a symposium held in Santorini, on May 14-17, 2015, reviewed the main findings in exercise genetics and genomics, and explored and discussed future trends and possibilities. At the end of the symposium, the participants agreed to launch a large collaborative initiative (named 'the Athlome Project Consortium', www.athlome-

con-sortium.org) to share and bring together resources for future investigations. This unique initiative is envisaged to help bring about a real paradigm shift to large collaborative projects using all available state-of-the-art advances (i.e. 'omics' technologies) as recently described [33]. Briefly, the main goals of the Athlome Project are:

1. To establish an ethically sound international research consortium (Athlome Project Consortium) and biobank resource systematically across individual centres;
2. To discover genetic variants associated with exercise performance, adaptive response to exercise training, and skeletal-muscle injuries using the genome-wide association study (GWAS) approach, targeted sequencing, or whole-genome sequencing, where possible;
3. To validate and replicate the genetic markers from the discovery phase across sex and ethnicity;
4. To conduct functional investigations following replicated findings (e.g. study the replicated single-nucleotide polymorphisms, SNPs, and their linkage disequilibrium regions, in vitro expression studies and knockouts of nearby genes) to better understand the associated biology.

Understanding genetic variation underlying athletic performance, adaptation to exercise training (in both human and animal models), and exercise-related musculoskeletal injuries is the primary focus of the Athlome Project Consortium. In addition, other 'omics' approaches, such as epigenomics, transcriptomics, proteomics and metabolomics, will need to be adopted to help dissect the molecular mechanisms of elite athletic performance, response to training, and injury predisposition. Amongst the numerous challenges will be the application of effective statistical analyses to minimize confounding effects due to artefacts on the exceedingly large data sets originazing from different research centres, populations, and analytical platforms.

Current investigators and research centres participating in the Athlome Project Consortium have been described elsewhere [33] and are illustrated below.

Eastern Europe Population Studies (The Russian and Belarusian Cohorts, GELAK, GELAV, and GUAP)

The Russian and Belarusian cohorts, the Genetics and Epigenetics of Lithuanian Athletes from Kaunas (GELAK) and Vilnius (GELAV), and the Genome of Ukrainian Athletes Project (GUAP) have consolidated to identify genetic and epigenetic variations associated with high-level sports performance. The cohort comprises East Europeans (from Belarus, Lithuania, Russia, and Ukraine; in total $\eta = 8,228$ athletes and $\eta = 4,121$ controls). The athletes are grouped into international (including participants in Olympics and World Championships), national, regional, or local/non-

competitive categories. These include biathletes, distance runners, cyclists, triathletes, kayakers, rowers, canoers, modern pentathletes, orienteers, skiers, speed skaters, short-trackers, walkers, weightlifters, bodybuilders, powerlifters, strongmen, sprint runners (<400 m), sprint swimmers (50-100 m), decathletes, heptathletes, combat athletes, field athletes, bobsleigh athletes, rhythmic and artistic gymnasts, figure skaters, fencers and team ball sport players. A portion of the participants have been evaluated with a variety of quantitative performance- and health-related assessments, including strength/power-related measurements, agility/speed-related measurements, balance, flexibility and coordination measurements, endurance-related measurement, skeletal muscle biopsy, and health-related measurements.

The principal investigators are: Ildus I. Ahmetov (Volga Region State Academy of Physical Culture, Sport and Tourism, Russia), Svitlana B. Drozdovska (National University of Physical Education of Ukraine, Ukraine), Colin N. Moran (University of Stirling, UK), Valentina Ginevicienė (Vilnius University, Lithuania), Andrei A. Gilep (Institute of Bioorganic Chemistry NASB, Belarus).

ELITE Study

The Exercise at the Limit - Inherited Traits of Endurance (ELITE) consortium is a global initiative with the main objective to map the role that genetics plays in athletic ability versus environmental factors, such as training. Study participant (n >500) selection is based on a physiological variable relevant for both health and sport performance, i.e. maximum oxygen uptake (VO_2max). The main inclusion criterion is VO_2max >75 ml/kg/min for men and >63 ml/kg/min for women, respectively. The consortium is continuously expanding and is recruiting athletes from all over the globe (with the main focus on Caucasians, North East Africans, East Asians and South Americans) who are successful in endurance sports (running, cycling, cross-country skiing, triathlon, and rowing). Analyses currently include enhanced whole-exome sequencing and GWAS (1.7 million SNPs). The combination of analytical methods will enable findings and differentiation between common variants with small effects and novel rare variants with larger effects. The aim is also to investigate gender and ethnic differences.

The principal investigators are: Euan A. Ashley, C. Mikael Mattsson, Matthew Wheeler, Daryl Waggott (Stanford University, USA).

Elite East African Athlete Cohort

The consortium also aims to study the East African running success by analysing data from previously recruited subjects: (i) 76 endurance runners (64 men) and 38 sprint and power event athletes (18 men) from the Ethiopian national athletics teams, 315 controls from the general Ethiopian population (281 men), 93 controls

from the Arsi region of Ethiopia (80 men), and (ii) 291 elite Kenyan endurance athletes (232 men) and 85 control participants (40 men). Seventy (59 men) Kenyan athletes had competed internationally and achieved outstanding success.

The principal investigators are: Yannis Pitsiladis (University of Brighton, UK), Robert Scott (University of Cambridge, UK).

GAMES Study

An international consortium (GAMES) was established to compare allele frequencies between elite endurance athletes and ethnicity-matched controls. GWASs were undertaken on 2 cohorts of elite endurance athletes (GENATHLETE and Japanese endurance runners) and their respective controls, from which a panel of 45 candidate SNPs was identified. These markers were tested for replication in 7 additional cohorts of endurance athletes and controls from Australia, Ethiopia, Japan, Kenya, Poland, Russia, and Spain. The study is based on a total of 1,520 endurance athletes (835 of them had competed in World Championships or Olympic Games) and 2,760 controls. The principal investigators are: Claude Bouchard, Tuomo Rankinen (Pennington Biomedical Research Centre, Louisiana State University, USA), Noriyuki Fuku (Jun-tendo University, Japan), Yannis Pitsiladis (University of Brighton, UK), Bernd Wolfarth (Humboldt University, Germany), Alejandro Lucia (Universidad Europea de Madrid, Spain).

GENATHLETE Study

The study was launched in 1993 with the aim of identifying DNA variants that are present at different frequencies between elite endurance athletes and sedentary controls. Male endurance athletes and controls were recruited from Canada, Finland, Germany, and the USA. The cohort assembled to date includes 315 elite endurance athletes and 320 matched controls. Selection criteria for the all-male endurance athlete sample include that they had to be athletes of national or international caliber with a VO_2max of at least 75 ml/kg/min. The mean value for the 315 athletes is currently 79 ml/kg/min while the mean for the 320 control subjects reached 40 ml/kg/min. Multiple candidate genes have been studied using the resources of GENATHLETE. A genome-wide screen for common variants has been performed on GENATHLETE (see GAMES cohort above), and further studies are focusing on nuclear and mitochondrial DNA sequencing.

The principal investigators are: Claude Bouchard, Tuomo Rankinen (Pennington Biomedical Research Centre, Louisiana State University, USA), Bernd Wolfarth (Department of Sports Medicine, Charité Medical School, Germany), Louis Pérusse (Laval University, Canada), Rainer Rauramaa (University of Eastern Finland, Finland).

GENESIS Study

The Genetics of Elite Status in Sport (GENESIS) consortium aims to identify mole-

cular genetic characteristics associated with successful sports performance. The cohort (current η > 1,200) is mainly composed of UK athletes. Sports include marathon running and other track-and-field athletics, cycling and team sports (e.g. soccer). The Rugby Gene Study is a major subcomponent of GENESIS and focuses on rugby (both union and league codes). Objectives of GENESIS are: (i) to increase the current cohort size substantially; (ii) to apply hypothesis-free approaches to identify molecular genomic markers; (iii) to expand GENESIS from genomics to other 'omics', and (iv) to combine the 'omics' data with athlete health and performance data to maximize the practical impact of GENESIS. The principal investigators are: Alun G. Williams, Stephen H. Day, Georgina K. Steb-bings (Manchester Metropolitan University, UK), Robert M. Erskine (Liverpool John Moores University, UK), Hugh E. Montgomery (University College London, UK).

Gene SMART Study

The Gene SMART (Skeletal Muscle Adaptive Response to Training) study aims to identify the gene variants that predict the skeletal muscle response to both a single bout and 4 weeks of high-intensity interval training in 3 different training centres. While the lead training and testing centre is located in Victoria University, Melbourne, 2 other centres have been launched at Bond University, Gold Coast, Australia, and the University of Sao Paulo, Brazil. The cohort is comprised of moderately trained, healthy male participants (aged 20-45 years, body mass index <30). Participants are undergoing similar exercise testing and exercise training in 3 different laboratories. Dietary habits are assessed by questionnaire and nutritionist consultation. Activity history is assessed by questionnaire, and the current activity level is assessed by activity monitoring. A number of muscle and blood analyses are to be performed, including genotyp-ing, mitochondrial respiration, transcriptomics, proteomics, and enzyme activity before, during and after training, where appropriate. Currently ~40 participants have finished the study, and the aim is to train a total of 250 participants. The Gene SMART study also includes baseline and posttraining testing and sampling for all participants. The principal investigators are: David Bishop, Nir Eynon (Victoria University, Australia).

GOINg Study

The recently established Genomics of Injuries (GOINg) consortium aims to identify DNA variants that modify the risk of anterior cruciate ligament injuries. It is the only consortium within the Athlome Project to specifically investigate exercise-associated musculoskeletal injuries. The plan is to screen current known loci for anterior cruciate ligament injury susceptibility in larger data sets in an attempt to determine whether they remain as susceptibility loci across all populations using the hypothesis-driven candidate gene case-control study design. Care will be taken to use the same criteria to accurately phenotype, with respect to ancestry, sporting

and occupational details, injury profile and mechanism(s) of injury, other injury history and family history, as well as other appropriate medical history and medication use. The actual functional significance of the identified variants will also be investigated. This initial phase will be followed by sequencing, and the research objectives will be eventually expanded to include other 'omics'. Thus far, an anterior cruciate ligament rupture consortium has collected DNA samples and clinical as well as physical and occupational activity information from subjects from South Africa, Poland, Australia, Russia and Italy.

The principal investigators are: Malcolm Collins, Alison September, Michael Post-humus (University of Cape Town, South Africa), Nir Eynon (Victoria University, Australia), Pawel Cieszczyk (University of Szczecin, Poland).

J-HAP Study

The Japanese Human Athlome Project (J-HAP) focuses on the study of genes associated with physical performance and its related phenotypes (e.g. muscle mass, muscle fibre type, VO_2max). The cohort is comprised of Japanese athletes (currendy >2,400, mainly international and national levels) and healthy Japanese controls (currently > 1,000). These athletes are mainly track-and-field athletes and swimmers competing in endurance- and sprint/power-oriented events. Multiple 'omics' approaches will be used to determine genes in talent identification in the Japanese population. Among the collected Japanese athlete and control samples, approximately 200 muscle biopsies were obtained from both athletes and controls in order to investigate genetic variants associated with muscle fibre type distribution.

The primary investigators are: Noriyuki Fuku (Juntendo University, Japan), Naoki Kikuchi (Nippon Sport Science University, Japan), Eri Miyamoto-Mikami (National Institute of Fitness and Sports in Kanoya, Japan).

NTR Study

The Netherlands Twin Register (NTR) is a population-based cohort recruiting both newborn and adult multiples and their family members with continuous longitudinal data collection. In the past 25+ years, around 40% of all twins and multiples in the Netherlands have taken part in the NTR research projects. Family members and spouses of twins also took part, leading to a total of over 185,000 participants across multiple research projects. The longitudinal information that has been collected extends from genotype to biomarkers, gene expression to rich behavioural information including biennial reports on (competitive) sports participation and performance level and on injuries related to sports. In its sports research track, the NTR aims to understand the interplay between genetic and environmental factors shaping individual differences in sports participation and performance. In the NTR, participants are recruited as newborns and followed into young adulthood; 520 have played competitively at a regional and 189 at a national level. Main sports that

Dutch adolescents/ young adults engage in are swimming, tennis, bicycling, soccer, and field hockey. The longitudinal data collection of the NTR is ongoing and securely funded for the next 5 years.

The principal investigators are: Eco de Geus, Meike Bartels (VU University and VU Medical Centre, the Netherlands).

POWERGENE Study

The POWERGENE consortium aims to characterize the elite sprint/power athlete genotype. The internationally competitive (Olympic/World Championship qualifiers) sprint/power athletes are from: Australia, Belgium, Greece, Italy, Jamaica, Japan, Lithuania, Poland, Spain, the USA, Brazil, and Russia. They will be compared with subelite athletes (national qualifiers), endurance athletes, team athletes and controls. The current cohort consists of female (n = 264) and male (n = 481) specialist power athletes across 3 major ethnicities (i.e. European, West African and East Asian ancestries). Sprint/power athletes include those individuals competing in track (<800 m) and field (jump, throw) events, cycling (track), swimming (<200 m), gymnastics (artistic), weightlifting, judo, speed skating and power lifting. Endurance athletes (n = 586) include track and road running specialists (>800 m), rowers, cyclists, swimmers (>200 m), triathletes, and ironmen. Team sports (n = 862) include football (soccer), cricket, hockey, volleyball, and basketball.

The principal investigators are: Yannis Pitsiladis (University of Brighton, UK), Kathryn North (Murdoch Children's Research Institute, Australia), Nir Eynon (Victoria University, Australia).

Super-Athletes: Genes and Sweat

The study aims to (i) identify genetic variants associated with elite athletic performance, (ii) study potential ethnic differences, and (iii) study the functional significance of the identified variants. A GWAS will be carried out in 3,000 consenting elite athletes, tested negative for doping substances at the Anti-Doping Laboratories, Federazione Medico Sportiva Italiana (FMSI) and Anti-Doping Lab Qatar (ADLQ), using Illumina genotyping technologies. Examining genotype frequency distribution of elite athletes from European countries (where most of the FMSI samples will be obtained) against those from South Asian and African countries (where most of the ADLQ samples are expected to be obtained) would help to identify potential ethnic differences in the genetic predisposition to athletic performance. Subsequently, the urine metabolome in a subset of these athletes (1,000 subjects) will be assessed and related to the athlete's sporting discipline.

The principal investigators are: Mohamed El-Rayess, Costas Georgakopoulos, Mohammed Alsayrafi (ADLQ, Qatar), Francesco Botre (FMSI, Italy), Karsten Suhre (Weill Cornell Medical College in Qatar, Qatar), Mike Hubank (University College London, UK).

Epigenetics of Elite Athletic Performance Study

It is clear from animal and human studies that epigenetic marks play a role in the modulation of gene expression in relevant tissues. There are also indications that epigenetic marks can be altered by acute and chronic exercise in skeletal muscle and adipose tissue where they have been studied. Thus, individual differences in any exercise-related traits can potentially be explained by not only the impact of DNA sequence variation on biology and behaviour, but also by the effects of epigenomic signalling on gene expression. We are formulating the hypothesis that elite athletic performance is influenced by epigenomic alterations, facilitating morphological, physiological, metabolic, cognitive, emotional and behavioural changes that empower the athlete to push performance beyond existing boundaries. We envisage testing this hypothesis by recruiting twin athletes competing at the Olympic or World Championship levels.

The principal investigators are: Vassilis Klissouras (University of Athens, Greece), Yannis Pitsiladis (University of Brighton, UK).

Rat Models of Exercise and Health

The purpose of the low-capacity rat/high-capacity rat model is to serve as a resource for the in-depth study of rat models to resolve the extremes of exercise and health. By connecting clinical observation with a theoretical base, the working hypothesis *is that variation in capacity for energy transfer is the central mechanistic determinant between disease and health (energy transfer hypothesis).* As an unbiased test of this hypothesis, this study showed that two-way artificial selective breeding of rats for low and high intrinsic endurance exercise capacity also produced rats that differed for numerous disease risks, including the metabolic syndrome, premature ageing, fatty liver disease, obesity, and Alzheimer's disease. Exercise capacity is a result of intrinsic capacity plus adaptation to all aspects of physical activity. To capture this biology, rats for low and high response to 8 weeks of treadmill running exercise were selectively bred. Thus, the study has models that represent the 4 'corners' of exercise capacity. These contrasting animal model systems may prove to be translationally superior relative to more widely used simplistic models for understanding disease conditions. The rat models may be thoroughly explored to discover causal mechanisms and develop effective therapeutics. These rats are being studied at over 50 institutions in 11 countries.

The principal investigators are: Steven Britton, Lauren Koch (University of Michigan, USA).

1,000 Athlomes Project

The 1,000 Athlomes Project aims to sequence 1,000 genomes of sprinters and distance runners of West and East African descent. Phase 1 of the project has already

commenced and involves the sequencing of 12 sprinters and 12 distance runners of the highest level (i.e. world record holders, Olympians and World Champions). Phase 2 (2016-2018) will involve increasing the sample size for sequencing to 100 genomes. The pool of the runners to be sequenced will be expanded to 1,000 by 2020 (phase 3). An important aim of this sequencing project is to document the genotype distribution of elite East and West African athletes. The large amount of genotype data to be generated from the 1,000 Athlome Project will serve (1) as a reference panel for future performance studies and (2) to guide other extreme phenotype studies in medical science.

The principal investigators are: Masashi Tanaka (Tokyo Metropolitan Institute of Gerontology, Japan), Yannis Pitsiladis (University of Brighton, UK).

Conclusion

The Athlome Project Consortium is a unique and highly ambitious attempt to oversee an essential paradigm shift in the area of exercise genomics. By presenting here the main study cohorts and projects that are currently included in the Athlome Consortium, it is our intention to show a global view of the main studies and initiatives that will be performed in the foreseeable future in the field of sports genomics and that are likely to provide exciting new findings of real practical significance. It is timely, therefore, that joint efforts are being made by the main investigators in sports and exercise genetics to uncover genetic variations underlying human physical performance. Although extraordinary challenges remain in the coming years, this unique collaborative initiative has the greatest chance to succeed where individual efforts have failed, to increase our understanding of the biology of exercise performance and related performance traits.

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Athlome Project Consortium: a concerted effort to discover genomic and other "omic"markers of athletic performance

Yannis Pitsiladis, Masashi Tanaka, Nir Eynon, Claude Bouchard, Kathryn North, Alun Williams, Malcolm Collins, Colin Moran, Steven Britton, Noriyuki Fuku, Euan Ashley, Vassilis Klissouras, Alejandro Lucia, Ildus Ahmetov, Eco de Geus, Mohammed Alsayrafi, and Athlome Project Consortium

University of Brighon, UK, Tokyo Metropolitan Institute of Gerontology, Japan, Victoria University, Australia, Pennington Biomedical Research Center, USA, University of Melbourne, Australia, Manchester Metropolitan University, UK, University of Cape Town, South Africa, University of Stirling, UK, University of Michigan Medical School, USA, Juntendo University, Japan, Stanford University, USA University of Athens, Greece, Universidad Europea de Madrid, Spain, Volga Region State Academy of Physical Culture, Russia, VU University, Netherlands, Anti-Doping Lab, Qatar

Despite numerous attempts to discover genetic variants associated with elite athletic performance, injury predisposition, and elite/world-class athletic status, there has been limited progress to date. Past reliance on candidate gene studies predominantly focusing on genotyping a limited number of single nucleotide polymorphisms or the insertion/deletion variants in small, often heterogeneous cohorts (i.e., made up of athletes of quite different sport specialties) have not generated the kind of results that could offer solid opportunities to bridge the gap between basic research in exercise sciences and deliverables in biomedicine. A retrospective view of genetic association studies with complex disease traits indicates that transition to hypothesis-free genome-wide approaches will be more fruitful. In studies of complex disease, it is well recognized that the magnitude of genetic association is often smaller than initially anticipated, and, as such, large sample sizes are required to identify the gene effects robustly. A symposium was held in Athens and on the Greek island of Santorini from 14-17 May 2015 to review the main findings in exercise genetics and genomics and to explore promising trends and possibilities. The symposium also offered a forum for the development of a position stand (the Santorini Declaration). Among the participants, many were involved in ongoing collaborative studies (e.g., ELITE, GAMES, Gene SMART, GENESIS, and POWERGENE). A consensus emerged among participants that it would be advantageous to bring together all current studies and those recently launched into one new large collaborative initiative, which was subsequently named the Athlome Project Consortium.

genetics; performance; sports genomics

AT THE OUTSET, the Athlome Project aims to collectively study the genotype and phenotype data currently available on elite athletes, in adaptation to exercise training (in both human and animal models) and on exercise-related musculoskeletal injuries from individual studies and from consortia worldwide. To achieve this, several steps are set out:

1) To establish an ethically sound international research consortium (Athlome Project Consortium) and biobank resource systematically across individual centers;
2) To discover genetic variants associated with exercise performance, adaptive response to exercise-training, and skeletal-muscle injuries using the genome-wide association study (GWAS) approach, targeted sequencing or whole genome sequencing, where possible;
3) To validate and replicate the genetic markers from the discovery phase across sex and ethnicity; and
4) To conduct functional investigations following replicated findings [e.g., study the replicated single nucleotide polymorphisms (SNPs) and their linkage disequilibrium regions, in vitro expression studies and knockouts of nearby genes] to better understand the associated biology.

During the development of the initial phase of the Athlome Project, in determining the genetic variations related to elite athletic performance and injury the Athlome Project Consortium. The participating cohorts and the focus of each are depicted in Fig. 1.

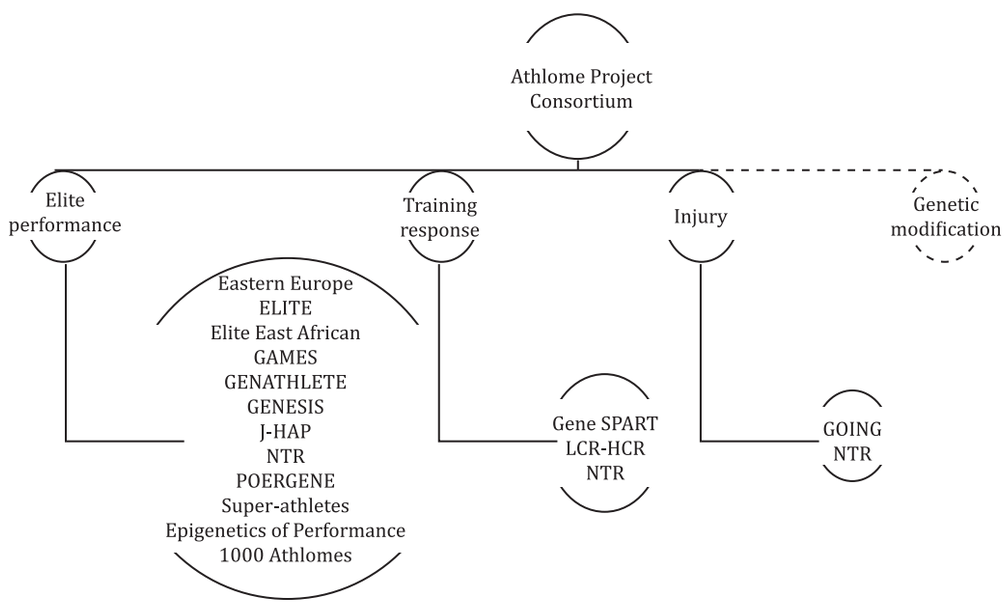

FIG. 1. The Athlome Project Consortium. Genomic, epigenomic, transcriptomic, proteomic, and metabolomic studies are being conducted by the participating centers to address questions in the 3 main research areas: elite performance, training response, and injury. Future investigations planned include studies on genetic modification

Eastern Europe Population Studies (The Russian and Belarusian Cohorts, GELAK, GELAV, and GUAP)

The Russian and Belarusian cohorts, the Genetics and Epigenetics of Lithuanian Athletes from Kaunas (GELAK) and Vilnius (GELAV), and the Genome of Ukrainian Athletes Project (GUAP) have consolidated to identify genetic and epigenetic variations associated with high-level sports performance. The cohort comprises East Europeans (from Belarus, Lithuania, Russia, and Ukraine; in total η = 8,228 athletes and η = 4,121 controls). The athletes are grouped into international (including participants in Olympics and world championships), national, regional, or local/non-competitive categories. These include biathletes, distance runners, cyclists, triathletes, kayakers, rowers, canoers, modern pentathletes, orienteers, skiers, speed skaters, short-trackers, walkers, weightlifters, bodybuilders, power-lifters, strongmen, sprint runners (< 400 m), sprint swimmers (50-100 m), decathletes, heptathletes, combat athletes, field athletes, bobsleigh athletes, rhythmic and artistic gymnasts, figure skaters, fencers, and team ball-sport players. A portion of the participants have been evaluated with a variety of quantitative performance- and health-related assessments, including strength/power-related measurements, agility/speed-related measurements, balance, flexibility and coordination measurements, endurance-related measurement, skeletal muscle biopsy, and health-related measurements.

Principal Investigators: Ildus I. Ahmetov [Volga Region State Academy of Physical Culture, Sport and Tourism, Russia (RUS)], Svitlana B. Drozdovska [National University of Physical Education of Ukraine, Ukraine (UKR)], Colin N. Moran [University of Stirling, United Kingdom (UK)], Valentina Ginevicienė [Vilnius University, Lithuania (LTU)], Andrei A. Gilep [Institute of Bioorganic Chemistry NASB, Belaurs (BLR)].

ELITE http://elite.stanford.edu

The Exercise at the Limit - Inherited Traits of Endurance (ELITE) consortium is a global initiative with the main objective to map the role that genetics plays in athletic ability vs environmental factors, such as training. Study participant (n > 600) selection is based on a physiological variable relevant for both health and sport performance, i.e., maximum oxygen uptake (VO_2max). The main inclusion criterion is VO_2max >75 $ml \cdot kg^{-1} \cdot min^{-1}$ for men and > 63 $ml \cdot kg^{-1} \cdot min^{-1}$ for women, respectively. The consortium is continuously expanding and is recruiting athletes from all over the globe (with main focus on Caucasians, North East Africans, East Asians, and South Americans) who are successful in endurance sports (running, cycling, cross-country skiing, triathlon, and rowing). Analyses currently include enhanced whole exome sequencing and GWAS (1.7 million SNPs). The combination of analytic methods will enable findings and differentiation between common variants with small

effects and novel rare variants with larger effects. The aim is also to investigate sex and ethnic differences.

Principal Investigators: Euan A. Ashley, C. Mikael Mattsson, Matthew Wheeler, Daryl Waggott (Stanford University, USA).

Elite East African Athlete Cohort

The consortium also aims to study the East African running success by analyzing data from previously recruited subjects: 7) 76 endurance runners (64 men) and 38 sprint and power event athletes (18 men) from the Ethiopian national athletics teams, 315 controls from the general Ethiopian population (281 men), 93 controls from the Arsi region of Ethiopia (80 men) and 2) 291 elite Kenyan endurance athletes (232 men) and 85 control participants (40 men). Seventy (59 men) Kenyan athletes had competed internationally and achieved outstanding success.

Principal Investigators: Yannis Pitsiladis (University of Brighton, UK), Robert Scott (University of Cambridge, UK).

GAMES

An international consortium (GAMES) was established to compare allele frequencies between elite endurance athletes and ethnicity-matched controls. GWASs were undertaken on two cohorts of elite endurance athletes (GENATHLETE and Japanese endurance runners) and their respective controls, from which a panel of 45 candidate SNPs was identified. These markers were tested for replication in seven additional cohorts of endurance athletes and controls from Australia, Ethiopia, Japan, Kenya, Poland, Russia, and Spain. The study is based on a total of 1,520 endurance athletes (835 of them had competed in world championships or Olympic Games) and 2,760 controls.

Principal Investigators: Claude Bouchard, Tuomo Rankinen (Pennington Biomedical Research Center, Louisiana State University, USA), Noriyuki Fuku [Juntendo University, Japan (JPN)], Yannis Pitsiladis (University of Brighton, UK), Bernd Wolfarth [Humboldt University, Germany (DEU)], Alejandro Lucia [Universidad Europea de Madrid, Spain (ESP)].

GENATHLETE

The study was launched in 1993 with the aim of identifying DNA variants that are present at different frequencies between elite endurance athletes and sedentary controls. Male endurance athletes and controls were recruited from Canada, Finland, Germany, and the USA. The cohort assembled to date includes 315 elite endurance athletes and 320 matched controls Selection criteria for the all-male endurance athlete sample include that they had to be athletes of national or international caliber with a VO_2max of at least 75 $ml \cdot kg^{-1} \cdot min^{-1}$. The mean value for the

315 athletes is currently 79 ml·kg^{-1}·min^{-1}, while the mean for the 320 control subjects reached 40 ml·kg^{-1}·min^{-1}. Multiple candidate genes have been studied using the resources of GENATHLETE. A genome-wide screen for common variants has been performed on GENATHLETE (see GAMES cohort above) and further studies are focusing on nuclear and mitochondrial DNA sequencing.

Principal Investigators: Claude Bouchard, Tuomo Rankinen (Pennington Biomedical Research Center, Louisiana State University System, USA), Bernd Wolfarth (Department of Sports Medicine, Charité Medical School, Berlin, DEU), Louis Perusse (Laval University, Quebec, Canada), Rainer Rauramaa (University of Eastern Finland, Kuopio, Finland).

GENESIS

The GENetics of Elite Status In Sport (GENESIS) consortium aims to identify molecular genetic characteristics associated with successful sports performance. The cohort (current n > 1,200) is mainly composed of UK athletes. Sports include marathon running and other track-and-field athletics, cycling, and team sports (e.g., soccer). The RugbyGene Study is a major subcomponent of GENESIS and focuses on rugby (both union and league codes). Objectives of GENESIS are: 1) to increase current cohort size substantially, 2) to apply hypothesis-free approaches to identify molecular genomic markers, 3) to expand GENESIS from genomics to other omics, and 4) to combine the omics data with athlete health and performance data to maximize practical impact of GENESIS.

Principal Investigators: Alun G. Williams, Stephen H. Day, Georgina K. Stebbings (Manchester Metropolitan University, UK), Robert M. Erskine (Liverpool John Moores University, UK), Hugh E. Montgomery (University College London, UK).

Gene SMART Study http://www.vu.edu.au/speed-gene

The Gene SMART (Skeletal Muscle Adaptive Response to Training) study aims to identify the gene variants that predict the skeletal muscle response to both a single bout and 4 wk of high-intensity interval training in three different training centers. While the lead training and testing center is located in Victoria University, Melbourne, two other centers have been launched at Bond University, Australia, and the University of Sao Paulo, Brazil. A fourth center (University of Brighton, UK) will focus on the omics analyses. The cohort comprises moderately trained, healthy male participants (aged 20-45 yr, body mass index ≤30 kg/m^2). Participants are undergoing similar exercise testing and exercise training in three different laboratories. Dietary habits are assessed by questionnaire and nutritionist consultation. Activity history is assessed by questionnaire and current activity level is assessed by activity monitoring. A number of muscle and blood analyses are to be performed, including genotyping, mitochondrial respiration, transcriptomics, proteomics, and enzyme activity before, during and after training, where appropriate. Currently —

40 participants have finished the study, and the aim is to train a total of 250 participants. The Gene SMART also includes baseline and posttraining testing and sampling for all participants.

Principal Investigators: David Bishop, Nir Eynon [Victoria University, Australia (AUS)].

GOINg

The recently established Genomics Of INjuries (GOINg) consortium aims to identify DNA variants that modify the risk of anterior cruciate ligament (ACL) injuries. It is the only consortium within the Athlome Project to specifically investigate exercise-associated musculoskeletal injuries. The plan is to screen current known loci for ACL injury susceptibility in larger data sets in an attempt to determine if they remain as susceptibility loci across all populations using the hypothesis-driven candidate gene case-control study design. Care will be taken to use the same criteria to accurately phenotype, widi respect to ancestry, sporting, and occupational details, injury profile and mechanism(s) of injury, other injury history and family history, as well as other appropriate medical history and medication use. The actual functional significance of the identified variants will also be investigated. This initial phase will be followed by sequencing and the research objectives will be eventually expanded to include other omics. Thus far, ACL rupture consortium has collected DNA samples and clinical, as well as physical and occupational activity information from subjects from South Africa, Poland, Australia, Russia, and Italy.

Principal Investigators: Malcolm Collins, Alison September, Michael Posthumus [University of Cape Town, South Africa (ZAF)], Nir Eynon (Victoria University, AUS), Pawel Cieszc-zyk [University of Szczecin, Poland (POL)].

J-HAP

The Japanese Human Athlome Project (J-HAP) focuses on the study of genes associated with physical performance and its related phenotypes (e.g., muscle mass, muscle fiber type, VO_2max). The cohort comprises Japanese athletes (currently > 2,400, mainly international and national levels) and healthy Japanese controls (currently > 1,000). These athletes are mainly track-and-field athletes and swimmers competing in endurance- and sprint/power-oriented events. Multiple omics approaches will be used to determine genes in talent identification in the Japanese population. Among the collected Japanese athletes' and controls' samples, —200 muscle biopsies were obtained from both athletes and controls to investigate genetic variants associated with muscle fiber type distribution.

Primary Investigators: Noriyuki Fuku (Juntendo University, JPN), Naoki Kikuchi (Nippon Sport Science University, JPN), Eri Miyamoto-Mikami (The National Institute of Fitness and Sports in Kanoya, JPN).

NTR

The Netherlands Twin Register (NTR) is a population-based cohort recruiting both newborn and adult multiples and their family members with continuous longitudinal data collection. In the past 25+ yr, around 40% of all twins and multiples in the Netherlands have taken part in the NTR research projects. Family members and spouses of twins also took part, leading to a total of over 185,000 participants across multiple research projects. The longitudinal information that has been collected extends from genotype to biomarkers, gene expression to rich behavioral information including biennial reports on (competitive) sports participation and performance level and on injuries related to sports. In its sports research track, NTR aims to understand the interplay between genetic and environmental factors shaping individual differences in sports participation and performance. In the NTR, participants are recruited as newborns and followed into young adulthood, 520 have played competitively at a regional and 189 at a national level. The main sports that Dutch adolescents/young adults engage in are swimming, tennis, bicycling, soccer, and field hockey. The longitudinal data collection of the NTR is ongoing and securely funded for the next 5 yr.

Principal Investigators: Eco de Geus, Meike Battels [Vrije Universiteit (VU University) and VU Medical Centre, the Netherlands (NLD)].

POWERGENE

The POWERGENE consortium aims to characterize the elite sprint/power athlete genotype. The internationally competitive (Olympic/world championship qualifiers) sprint/power athletes are from: Australia, Belgium, Greece, Italy, Jamaica, Japan, Lithuania, Poland, Spain, the USA, Brazil, and Russia. They will be compared with subelite athletes (national qualifiers), endurance athletes, team athletes, and controls. The current cohort consists of female (n = 264) and male (n = 481) specialist power athletes across three major ethnicities (i.e., European, West African, and East Asian ancestries). Sprint/ power athletes include those individuals competing in track (< 800 m) and field (jump, throw) events, cycling (track), swimming (< 200 m), gymnastics (artistic), weightlifting, judo, speed-skating, and power lifting. Endurance athletes (n = 586) include track and road running specialists (> 800 m), rowers, cyclists, swimmers (> 200 m), triathletes and ironmen. Team sports (n = 862) include football (soccer), cricket, hockey, volleyball, and basketball.

Principal Investigators: Yannis Pitsiladis (University of Brighton, UK), Kathryn North (Murdoch Childrens Research Institute, AUS), Nir Eynon (Victoria University, AUS).

Super-athletes: Genes and Sweat

The study aims to 7) identify genetic variants associated with elite athletic perfor-

mance, 2) study potential ethnic differences, and 3) study the functional significance of the identified variants. A GWAS will be carried out in 3,000 consented elite athletes, tested negative for doping substances at the Anti-Doping Laboratories, Federazione Medico Sportiva Itali-ana (FMSI), and Anti-Doping Lab Qatar (ADLQ), using Illu-mina genotyping technologies. Examining genotype frequency distribution of elite athletes from European countries (where most of FMSI samples will be obtained) against those from South Asian and African countries (where most of ADLQ samples are expected to be obtained) would help to identify potential ethnic differences in the genetic predisposition to athletic performance. Subsequently, urine metabolome in a subset of these athletes (1,000 subjects) will be performed and will be related to the athlete's sporting discipline.

Principal Investigators: Mohamed El-Rayess, Costas Geor-gakopoulos, Mohammed Alsayrafi [ADLQ, Qatar (QAT)], Francesco Botre [FMSI, Italy (ITA)], Karsten Suhre (Weill Cornell Medical College in Qatar, QAT), Mike Hubank (University College London, UK).

Epigenetics of Elite Athletic Performance

It is clear from animal and human studies that epigenetic marks play a role in the modulation of gene expression in relevant tissues. There also are indications that epigenetic marks can be altered by acute and chronic exercise in skeletal muscle and adipose tissue where they have been studied. Thus individual differences in any exercise-related traits can potentially be explained not only by the impact of DNA sequence variation on biology and behavior but also by the effects of epigenomic signaling on gene expression. We are formulating the hypothesis that elite athletic performance is influenced by epigenomic alterations, facilitating morphological, physiological, metabolic, cognitive, emotional, and behavioral changes that empower the athlete to push performance beyond existing boundaries. We envisage testing this hypothesis by recruiting twin athletes competing at the Olympic or world championship levels.

Principal Investigators: Vassilis Klissouras [University of Athens, Greece (GRC)], Yannis Pitsiladis (University of Brighton, UK).

Rat Models of Exercise and Health (LCR-HCR rat model)

The purpose of the Low Capacity Rats-High Capacity Rats (LCR-HCR) model is to serve as a resource for the in-depth study of rat models to resolve the extremes of exercise and health. By connecting clinical observation with a theoretical base, the working hypothesis is that: variation in capacity for energy transfer is the central mechanistic determinant between disease and health (energy transfer hypothesis). As an unbiased test of this hypothesis, this study showed that two-way artificial selective breeding of rats for low and high intrinsic endurance exercise capacity

also produced rats that differed for numerous disease risks, including the metabolic syndrome, premature aging, fatty liver disease, obesity, and Alzheimer's disease. Exercise capacity is a result of intrinsic capacity plus adaptation to all aspects of physical activity. To capture this biology, rats for low and high response to 8 wk of treadmill running exercise were selectively bred. Thus, the study has models that represent the four "corners" of exercise capacity. These contrasting animal model systems may prove to be translationally superior relative to more widely used simplistic models for understanding disease conditions. The rat models may be deeply explored to discover causal mechanisms and develop effective therapeutics. These rats are being studied at over 50 institutions in 11 countries.

Principal Investigators: Steven Britton, Lauren Koch (University of Michigan, USA).

1000 Athlomes Project

The 1000 Athlomes Project aims to sequence 1000 genomes of sprinters and distance runners of West and East African descent. Phase 1 of the project has already commended and involves the sequencing of 12 sprinters and 12 distance runners of the highest level (i.e., world record holders, Olympians, and world champions). Phase 2 (2016-2018) will involve increasing the sample size for sequencing to 100 genomes. The pool of the runners to be sequenced will be expanded to 1,000 by 2020 (phase 3). An important aim of this sequencing project is to document the genotype distribution of elite East and West African athletes. The large amount of genotype data to be generated from the 1000 Athlomes Project will serve as 7) a reference panel for future performance studies and 2) a guide for other extreme phenotype studies in medical science.

Principal Investigators: Masashi Tanaka (Tokyo Metropolitan Institute of Gerontology, JPN), Yannis Pitsiladis (University of Brighton, UK).

Ethical Principles for Athlome Biobanking

The rise of biobanking has brought about a whole range of issues that are not all wholly relevant to the Athlome Project. Nevertheless, certain key principles must be noted here that will inform the governance framework for Athlome: 1) the consortia are global in reach, but there is no universal agreement on the precise nature of ethically justifiable governance for biobanking; 2) given the globality of the consortia, no single regional (e.g., European, American) framework ought to be adopted; 3) a general framework drawing on widely shared principles should be discussed and adopted. Chief among the concerns, but only one among several, is the problem of consent.

Each of the projects that comprise Athlome are existing bioguardians with a duty to protect the rights of participants who have contributed their samples to the

individual projects noted above. The collection, storage, access to, and use by researchers of those samples have been approved by relevant regulatory authorities (e.g., institutional review boards, research ethics committees, national health services research ethics services) appropriate to the lead institution of the individual projects/consortia. Existing procedures do not currently extend to the sharing of samples beyond the study, since consent models are prospective (i.e., they guide future actions of researchers) and typically entail a form of specificity and the specific consent obtained varies between project partners. No retrospective consent is feasible, and this is a widely shared problem for biobank development. Since the form of collaboration Athlome envisages was not laid out before participants gave their consent, it might be concluded that the sharing of data beyond the original research group and its stated purposes invalidates that consent. The problem for Athlome is not an uncommon one for biobank collaborations since it seeks retrospective extension of the consent model.

An ethical solution to this problem and related consent problems for new participants is to consider the use of a technique such as "broad consent." The nomenclature here is important since this notion is variously described as "broad consent," "blanket consent," "future consent," "hypothetical consent," "passive/tacit/silent consent," or "waived consent" (4, 5). This would entail asking participants to agree to future unspecified uses of their data that are un(der)determined in the consent process and relevant forms (6). Without sufficient grasp of the uses of the data or with whom it might be shared, this process fails the test of "comprehension": a user must understand sufficiently what they are agreeing to (3). Another possibility going forward would be "meta-consent," where consent is sought for broad categories of unspecified future research (7, 8). Others have argued with respect to biobanking that the ethical issues entailed (e.g., privacy, confidentiality, ownership of access to the data) may be sufficiently assuaged by rigorous anonymization (1) and associated practices of data storage, though this is far from universally agreed upon (2).

The Athlome project will develop principles and protocols for safeguarding participants rights to access, confidentiality, privacy of data, and assurances that there is no significant mission drift of the kind of which is permitted under some conceptions of broad consent (or its similes). This would, for example, prohibit commercialization of participants' data. To preserve the integrity of this process and the principles, rigorous anonymization processes will be developed by a partner institution that does not have any direct role in data collection, storage, or analysis. This will assure independence and integrity to the process. This is especially important in this case since some of the research participants are public figures, which increases the likelihood that someone might be interested in reidentifying their data and genomic sequences. The independent institution would also have an oversight

of each new proposal for the Athlome Project going forward to ensure compliance with those principles and protocols.

In conclusion, by presenting the main study cohorts and projects that are currently included in the Athlome consortium not only do we intend to show a global view of the main studies and initiatives that will be performed in the foreseeable future in the field of sports genomics (and that are likely to provide new exciting findings), we also wish to motivate potential collaboration initiatives with other research groups worldwide. International collaborations are likely to go well beyond the study of sports performance per se. Indeed, the Athlome consortium presents a unique chance to study the biology of the best elite athletes across most ethnicities, which is profoundly interesting from a medical point of view. World-class athletes represent the actual end-point of the human continuum of fitness-related phenotypes. In this regard, there is growing evidence (coming from both human and rodent study approaches, such as those included in the consortium) that not only physical activity levels but also individual fitness levels (a trait that has a strong genetic component independent of activity levels) are inversely associated with the risk of major cardiometabolic diseases of Western civilization, several cancer types, and Alzheimer's disease. Thus, studying the genes of elite athletes offers a unique chance to gain insight into important medical conditions, including genetic predisposition (or resilience) to chronic disease. Indeed, the "rare-common" strategy, underpinned by ethically sound research governance, is a valuable approach model to examine general mechanisms of disease pathophysiology, with world-class athletes representing the "rare" ("super-fit") human phenotype. Finally, identifying genetic markers of exercise capacity, adaptation to exercise programs, and the predisposition to injury is certain to provide useful information to prescribe personalized exercise interventions in the context of 21st-century medicine, which should not be based only on identifying new drug targets but also on implementing lifestyle interventions for disease prevention at the individual level.

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Athlome Symposium: Genetics & Genome
FIMS Congress, Ljubljana, October 1, 2016
Invited Lecture

Epigenetics: A Path to Elite Sport Performance

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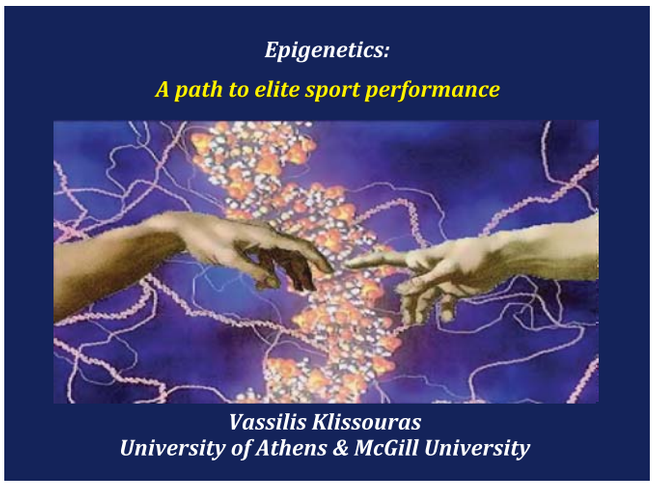
University of Athens & McGill University

Preamble



In recent years, progress in Genomics and Neuroimaging techniques has helped to bring together Molecular Neuroscience and Cognitive Psychology and hence opened a window in our quest to understand better human performance.

In this new era, we need to go beyond traditional reductionist thinking and employ integrative systems, linking biological phenomena with cognitive processes and behavior. I would like to suggest that epigenetics may offer a platform or such an integrative approach.

The prevailing view to day is that understanding sport performance will require the deciphering two major sources of individual differences: Genes & Environment. It is believed that superior performers are endowed with a high genetic potential actualized through hard and prodigious effort. Yet, growing evidence suggests that epigenetic factors may influence the traditional dyad of genes and environment and may play an important role in elite sport performance.

In this presentation, I will briefly review the evidence on the importance of **Genetic influence** and point out that sport performance is under strong genetic control.

Then, I will postulate on the potential role of **Epigenetic influence** and will argue that the ceiling of an athlete's performance may lie on his brain, on his capacity for mental excitement, which is particularly susceptible to epigenetic influence.

Genetic influence

Most phenotypes related to high peak performance are normally distributed in the population. The relative power of genes and environment in the development of

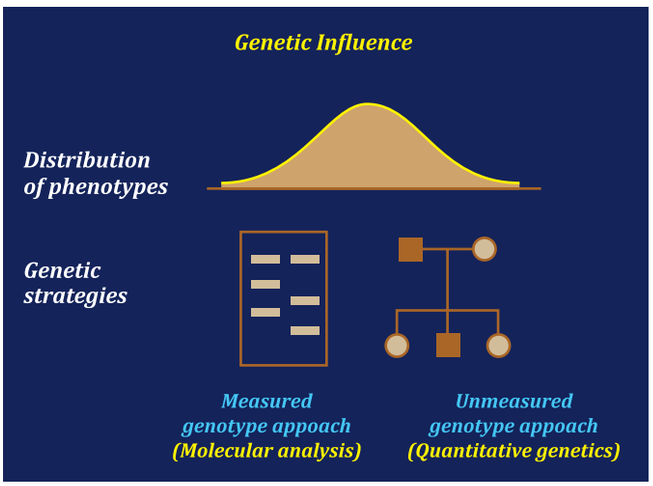


FIG. 1.

these performance phenotypes, can be disentangled by using two genetic strategies: The measured genotype approach of Molecular Genetics and the unmeasured genotype approach of Quantitative Genetics (Fig. 1).

A very powerful method in Quantitative Genetics is the **twin model,** based on phenotypic similarities between monozygotic (MZ) and dizygotic (DZ) twins. MZ share all of their nuclear DNA, while DZ only 50% of DNA sequence variation. From such comparisons, we derive heritability estimates. **Heritability (Hest)** is an expression of the amount of genetic contribution. The closer the heritability to unity the stronger the genetic influence.

The concept of Hest is often misinterpreted. Hest has no etiologic role in the phenotype. Nor has it sensible meaning with reference to measurement in an individual. It refers only to the population, and describes the extent to which heredity affects the variation of a given attribute in a given population exposed to common environmental influences at a given time. A high heritable attribute does not mean that it is predetermined and the environment has no effect. It only indicates that observed individual differences in the given attribute are due to genetic differences and are highly predictable.

The early twin studies. Personally I was confronted by the question of heritability at the onset of my academic life, more than half a century ago, in an unexpected way. As a young professor at McGill University, I was lucky enough to have in my Physiology class an inquisitive student who was a twin athlete. It was then that the idea of using the twin model to explore the genetic basis of adaptive variation struck me.

We put in use this model in the late 60s, to determine the **Heritability of adaptive variation.** In our studies we rigorously controlled for the main assumption on which the twin model is based, namely that environmental influences are comparable for both types of twins. Figure 2 shows the first study published in the Journal of Applied Physiology, where a high heritability (93%) of VO_2max was reported. VO_2max is considered the most important criterion of cardiorespiratory endurance and reflects during maximal effort the mobilization in the body of all systems, organs, processes and functions.

In a subsequent cross-age study we confirmed that intrapair differences in VO_2max were minimal across age, and within the experimental error, for identical

twins and quite large for non-identical. On the grounds of the evidence obtained in these early studies, we concluded that: **Heredity alone accounts** almost entirely for existing differences in VO_2max.

Claude Bouchard and co-workers (1986), a little later, in a comprehensive study using both twin and non-twin brothers, established that heredity is a major determinant of aerobic power, but reported a lower heritability, accounting for about one-half of the variation observed in VO_2max. A similar conclusion he reached in the seminal HERITAGE family study (1999).

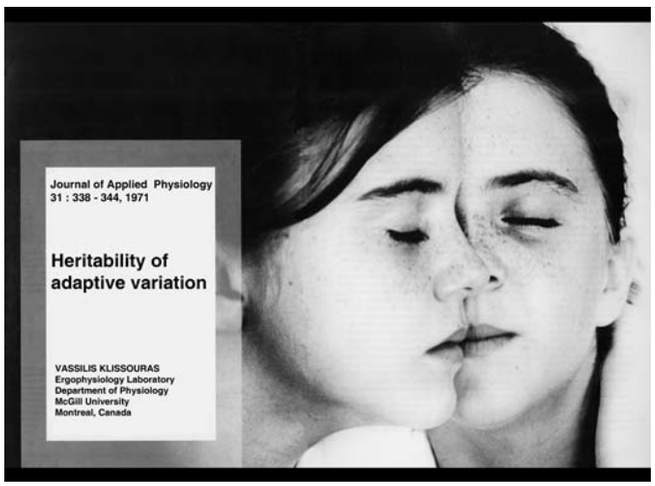


FIG. 2.

Shared & non-shared environmental effects. One of the criticisms of the classic twin study method is that it fails to separate the variance attributable to non-shared and shared environmental effects. To get over this limitation more recent studies, applied to twin and nuclear family data, the elaborate model of **path genetic analysis** (Fig. 3). Phenotypes of the twins are modeled, as being determined by additive genetic effects, common environmental effects, and specific environmental effects, whille interaction effects between co-twins could also be detected.

Using this research design Fagard and coworkers (1991) demonstrated that there is a high heritability for VO_2max – in the range of 80% - reduced to 74% when adjusted for body weight, skinfold thickness and sport participation. Using the same analysis Maes et al (1996) reported heritabilities as high as 87%.

Moreover, in these studies the remaining variance was attributable to non-shared environmental factors. In other words, the source of environmental variation in VO_2max is **specific** to the individual and not the shared, or common environment.

This finding may just be as important in other domains and may have far-reaching implications for understanding how the environment works in human performance. For example, reviews of the genetics of body mass index find that nearly all environmental influences are specific or non-shared (Stunkard et al 1990). In Behavioral Genetics, Plomin et al (2004) brought together evidence

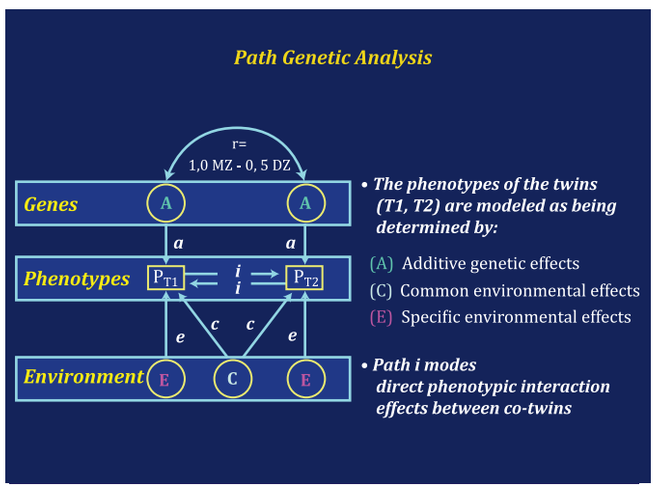


FIG. 3.

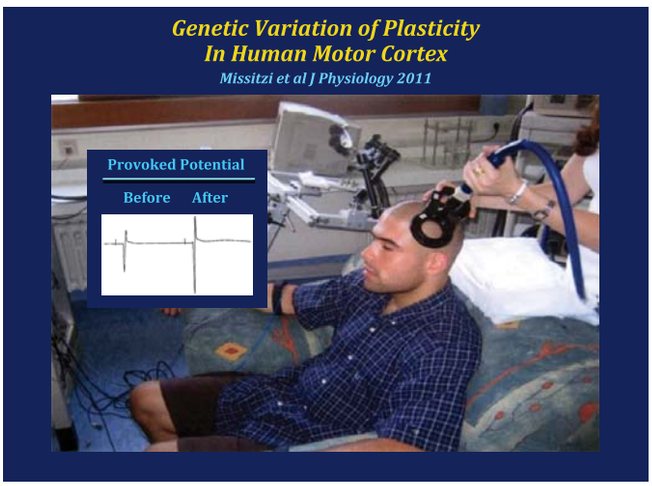


FIG. 4.

for the importance of no shared environment in the development of personality traits and cognitive abilities, while in another study Plominand Daniels (1987) observed the fundamental phenomenon that children growing up in the same family are very different and noted that perceptions of environment can be an important source of non-shared experience.

In addition to maximal aerobic power a significant genetic variance has been obtained in numerous investigations for functional abilities, morphological characteristics, muscle composition, motor attributes and behavioral traits, all related to elite sport performance.

Recently, in our laboratory Julia Missitzi using transcranial magnetic stimulation in MZ & DZ twin pairs, determined the Genetic variation of plasticity in human motor cortex and found a heritability estimate of 68% (Fig. 4). In another study Maria Pelliciari and coworkers (2009), using the same technique, provided clear evidence of heritable individual differences in motor cortex excitability. The heritability estimate was 82% for intracortical facilitation and 92% for intracortical inhibition.

Taken together, heritability studies converge on the conclusion that not only genetic influence is significant, but it is also substantial, accounting for individual differences in most phenotypes related to sport performance.

Genetic influence is so ubiquitous and persuasive in most determinants of sport performance that, as Plomin put it, we ask: *not what is heritable, but what is not heritable.*

Yet the heritability for most phenotypic traits apparently is well bellow 100%, meaning that environment is important. This assertion raises the question of epigenic influence.

Epigenetic influence

Could epigenetics, which is susceptible to environmental conditions, contribute to phenotypic variance in sport performance? And if so, could individual differences in performance related traits be explained not only by the impact of DNA sequence variation on biology and behavior, but also by the effects of epigenomic signaling on gene expression?

Olympic Co-Twin Athletes. In this respect a co-twin study of Olympic athletes is revealing. Molecular analysis showed the twins to be identical. Both participated

in 20-km race-walking, had undergone the same strenuous training and had been exposed to virtually identical life-style influences from the time of their birth. However, their achievement was distinctly different. One being an Olympic Winner (gold, silver, bronze) in three consecutive Olympiads, while his brother was also an Olympic athlete, but an inferior performer and managed to win when his co-twin was not competing.

This fairly unique example of performance difference, in otherwise identical twins, reveals that genes as well as external conditions alone are not sufficient to make an Olympic winner.

I argue that epigenetics is the ultimate enabler of achieving a seemingly impossible athletic feat, provided that one processes the endowment and abides by the appropriate external conditions, such as training, nutrition and modus vivendi.

Inspite of the unknowns in this area we could postulate that epigenetic alterations facilitate cognitive, emotional and behavioral changes that empower man to push performance beyond existing boundaries.

While there is a scarcity of information on epigenetics related directly to human performance, a growing body of evidence suggests that internal and external signals activate intracellular pathways leading to changes in gene expression that modulate neural function and behavior.

To come back to the co-twin study, an assessment of their bio-behavioral profile, revealed that intrapair differences were negligible in physiological attributes related to endurance, such as VO_2max, running economy and anaerobic threshold, but were divergent in personality traits related to performance, such as anger control and reaction to anger (Fig. 5). Apparently, the twins although identical were very different in their non-shared experiences. The perceptions of the environment were specific to the individual, which clearly alludes to epigenetic influence.

Minnesota Study of Twins. We also know that exposure to different external conditions do not significantly increase the degree of phenotypic variation in personality traits. One of the landmark studies in human twin research that challenges the importance of shared or common environment is the Minnesota Study of Twins Reared Apart, conducted by Thomas Bouchard and colleagues (1990).

Identical twins raised together (MZT) were compared with identical twins reared apart (MZA), since early childhood. The intraclass correlations (R) within MZ twins raised

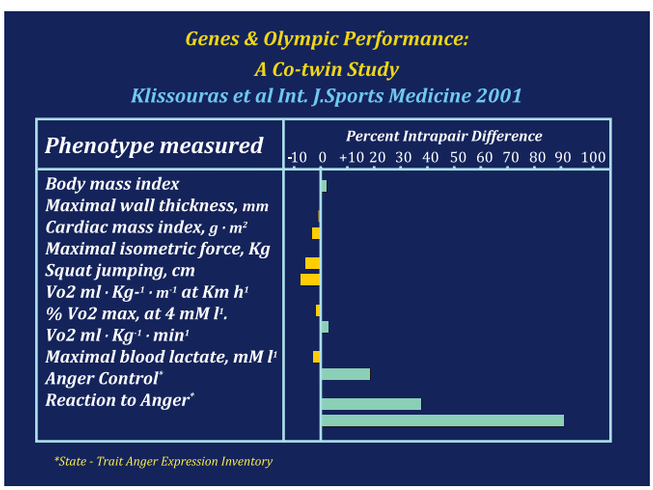


FIG. 5.

together (MZT) and MZ twins reared apart (MZA) on personality measurements were almost identical, 0.49 and 0.50 respectively, while the intraclass ratio between them (R_{MZA}/R_{MZA}) was almost unity (1.02). This means that differences in external environment have no effect on phenotypic variation in personality traits. What then can account for the discordance in identical twin pairs?

Apparently, identical twins are different for some traits, not because of their genes, or because they are exposed to different environment. Epigenetics may be an attractive hypothesis and may open a path for finding an answer to these paradoxical findings.

Genetic & Epigenetic process. There are essential differences between genetic and epigenetic process. The **Genetic process** is fixed at conception and refers to potentially heritable changes in DNA, the giant molecule that encodes the genetic message for proteins responsible for function and which passes from one generation to the next. The **Epigenetic process** is interactive in nature and the information flow is not unidirectional. It involves open networks of genes, proteins, and environmental signals. *It is a mechanism by which DNA is regulated to produce patterns of gene expression, in the face of environmental signals* (Strohman 1997).

Epigenetics is concerned with heritable changes in gene function not determined by the genetic code, but by such processes as DNA methylation & Histone modification (Fig. 6).

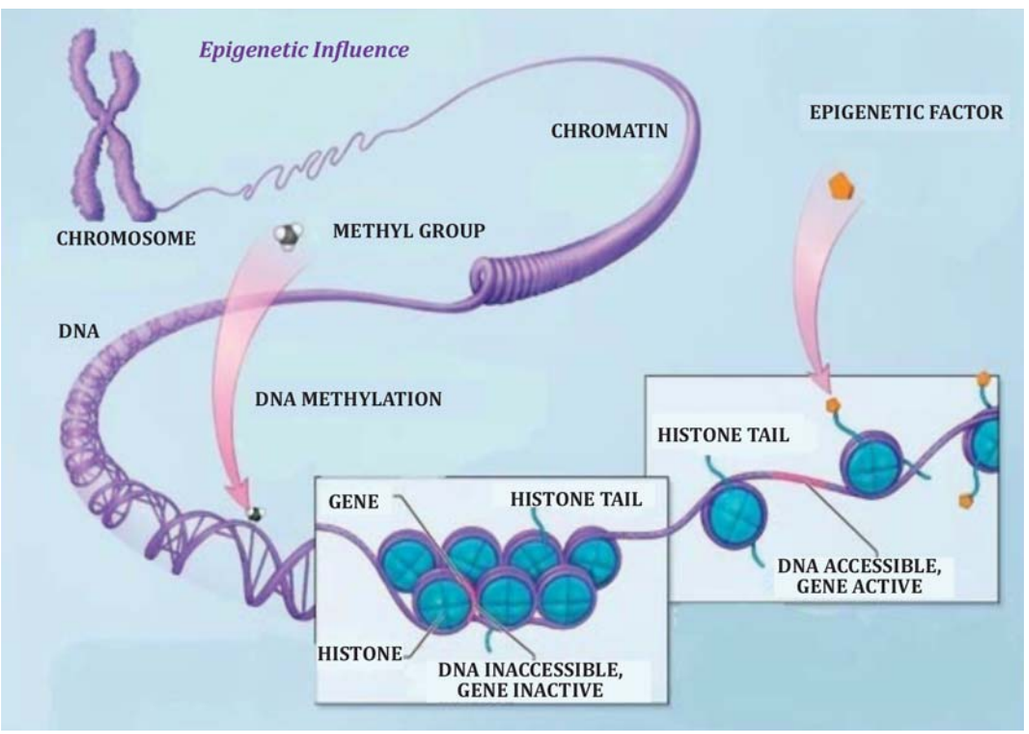


FIG. 6.

In the process of **DNA methylation** Methyl group, an epigenetic factor, can tag DNA and change the pattern of gene expression, making it weaker or stronger.

In the process of **Histone modification,** the binding of an epigenetic factor to histone "tails" alters the extent to which DNA is wrapped around histones AND the availability of genes in DNA to be activated. If DNA is inaccessible, Gene is inactive. If DNA is accessible, Gene is active.

Epigenetic differences in MZ twins. Epigenetic differences in genetically identical humans have been demonstrated repeatedly. Fraga et al (2005) in a landmark study measured the amount and pattern of DNA methylation and histone acetylation in different tissue types of MZ twins (Fig. 7). In 65% of twins, epigenetic markers were similar within pairs. However, 35% of them exhibited epigenetic differences.

Moreover, young identical twin pairs had similar amounts of DNA methylation, whereas, older identical twin pairs differed considerably in the amounts and patterns of this modification. Differences in gene expression among older twin pairs were some 4x greater than those observed in young twin pairs.

Maternal nurturing. Substances and physical agents are not the only sources of epigenetic change. Several environmental events including social experience may induce epigenetic changes. A high-profile study conducted by Weaver and co-wor-

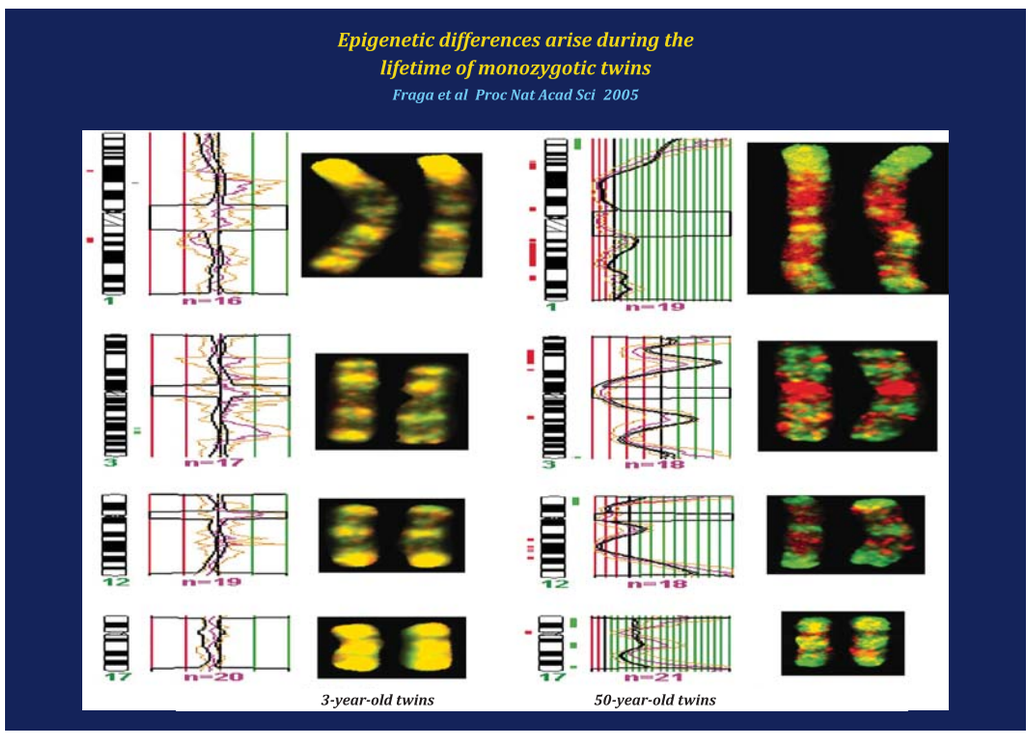


FIG. 7.

FIG. 8.

kers (2004) at McGill University, has raised the possibility that a mother's behavior can affect the chemistry of DNA in her offspring (Fig. 8). Maternal nurturing in rats alters DNA methylation at the gene encoding the glucocorticoid receptor in hippocampus. And thus affecting, hypothalamic- pituitary-adrenal (HPA) responses to stress.

How all these observations could have relevance to elite sport performance? While there is a scarcity of information on epigenetics related directly to human performance, a growing body of evidence suggests that internal and external signals activate intracellular pathways leading to changes in gene expression that modulate neural function and behavior. Epigenetic markers appear to be at the interface between environmental stimuli and long-lasting molecular, cellular and behavioral phenotypes (Petronis 2010).

In spite of the unknowns in this area we could postulate that epigenetic alterations facilitate cognitive, emotional and behavioral changes that empower the athlete to push performance beyond existing boundaries

The Brain is susceptible to Epigenetics. The brain is particularly susceptible to epigenetic alterations. Noninvasive electrophysiological and neuroimaging techniques, are revealing the role of the brain in sport performance. Using functional MRI Chambers et al (2009) had shown that the brain could command performance before muscle, heart and lungs. It has been repeatedly demonstrated that by simply mouth-rinsing a carbohydrate solution improves endurance performance by 2 - 3%.

These brain regions in particular the dopaminergic pathways within the striatum are the reward centers of the brain and are susceptible to epigenetic influence (Fig. 9).

Moreover, studies using fMRI revealed that elite athletes and non athletes use their brains differently. Novices activate the limbic regions, while experts activate the supplementary motor region (Milton et al 2007).

Input from the limbic structures can disturb activation of cortical motor programs, leading to catastrophic deterioration of skill and performance. Here again epigenetic influences on motor planning could be expected.

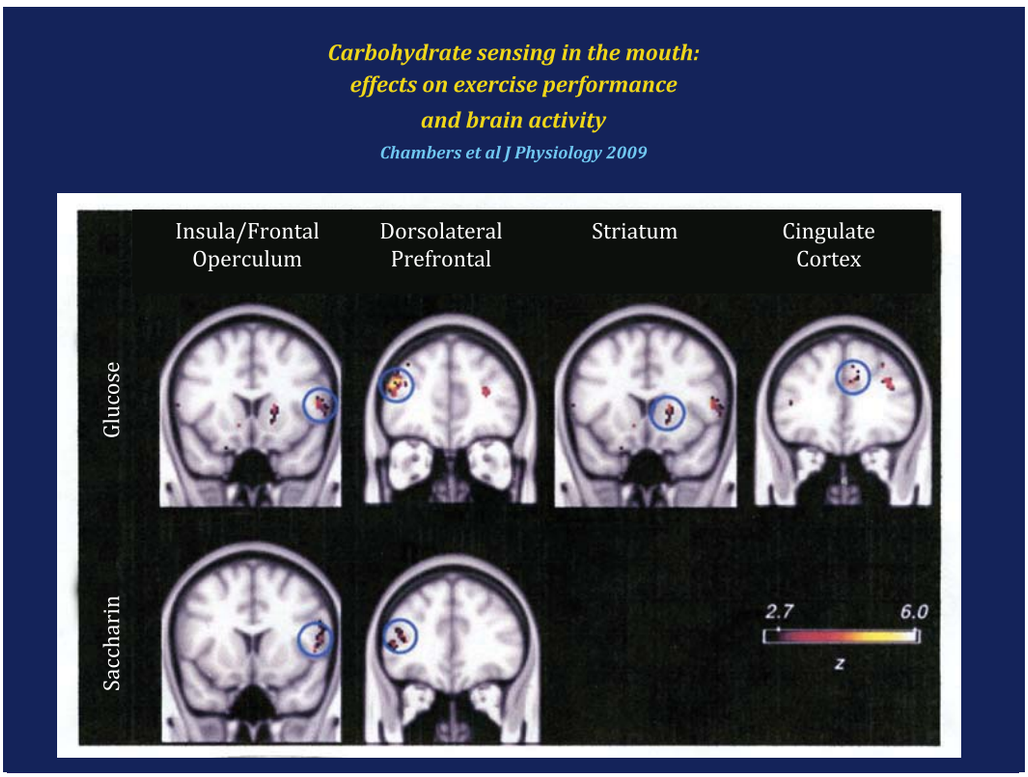


FIG. 9.

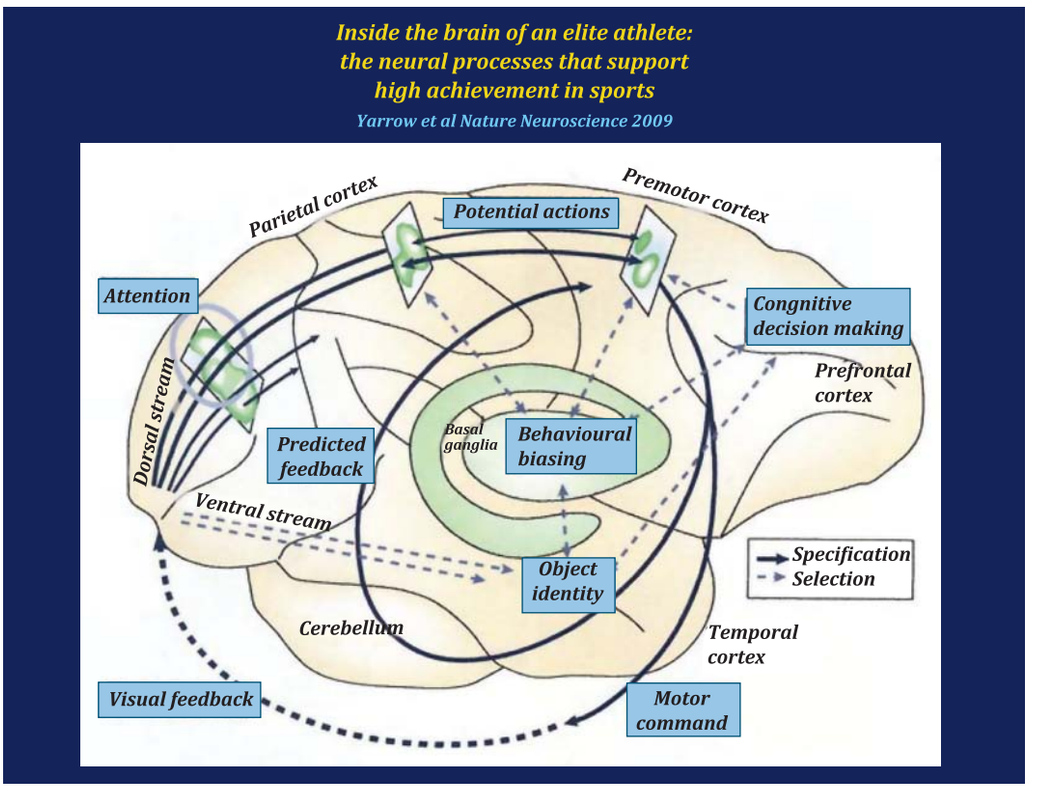
The picture emerging is that although fatigue can develop within muscle fibers, motor unit activity is centrally modulated. The descending neural drive from the brain to the exercising muscle determines the strategy of motor unit recruitment. The origins ot peak performance are to be found in the interactions of neural networks in the brain and the modulation of corticospinal excitability.

Studies in neuroplasticity using PET scans and functional MRI's reveal that the brain can adapt and generate new neurons. Neurons are able to convert a variety of stimuli into high order functions, such as storing memories, controlling behavior and governing consciousness. All these unique properties are based on the highly flexible nature of neurons and it appears that *epigenetics lies in the heart of neural plasticity* (Sassone-Corsi and Christen 2012).

Man has the potential to change how the brain functions by the way he thinks. This new evidence confirms what the Greek philosopher Epictetus noted 2,000 years ago: **"We are disturbed not by things, but by the views we take of things."** So it is not reality itself but the perception we take of it. Environmental signals - internal and external - activate intracellular pathways that directly remodel the "epigenome", leading to changes in gene expression that modulate neural function.

An athlete's high achievement depends on what is happening inside his brain

FIG. 10.



(Fig. 10). It appears that the ultimate limit of his performance is set by his mind. The human mind can go *"beyond the information given",* which clearly implies going beyond genes and genome and the need for some epigenetic function, having to do with a behavior of surpassing the of performance.

At one time breaking the 4-minute mile barrier was viewed as an impossible feat, until Bannister overcame it. And then, shortly after a lot of runners did the same. The genetic make-up of runners and external conditions did not change. What changed was the internal condition, the perception of the barrier, the vision of the seemingly impossible feat; *What changed was the belief in what was possible.* A belief that frees the body & the mind to perform your best, push aside the barrier, stretch and extend the limits with every fiber of your being. In the human mind lies the power to dream, to transcend reality to make the impossible possible.

Bannister phrased this notion in a more eloquent way. He wrote more than half century ago, after breaking the 4-minute mile barrier:

"the inner urge to set sights on an unreachable goal, which stretches the very nature of the living processes and stirs with excitement the soul and the body, determines the ultimate limits of human performance".