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REVIEW ARTICLE

# Epigenetics in Sports

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**Abstract** The heritability of specific phenotypical traits relevant for physical performance has been extensively investigated and discussed by experts from various research fields. By deciphering the complete human DNA sequence, the human genome project has provided impressive insights into the genomic landscape. The hope that this information would reveal the origin of phenotypical traits relevant for physical performance or disease risks has proven overly optimistic, and it is still premature to refer to a 'post-genomic' era of biological science. Linking genomic regions with functions, phenotypical traits and variation in disease risk is now a major experimental bottleneck. The recent deluge of genome-wide association studies (GWAS) generates extensive lists of sequence variants and genes potentially linked to phenotypical traits, but functional insight is at best sparse. The focus of this review is on the complex mechanisms that modulate gene expression. A large fraction of these mechanisms is integrated into the field of epigenetics, mainly DNA methylation and histone modifications, which lead to persistent effects on the availability of DNA for transcription. With the exceptions of genomic imprinting and very rare cases of epigenetic inheritance, epigenetic modifications are not inherited transgenerationally. Along with their susceptibility to external influences, epigenetic patterns are highly specific to the individual and may represent pivotal control centers predisposing towards higher or lower physical performance capacities. In that context, we specifically review how epigenetics combined with classical genetics could broaden our knowledge of genotype-phenotype interactions. We discuss some of the shortcomings of GWAS and explain how epigenetic influences can mask the outcome of quantitative genetic studies. We consider epigenetic influences, such as genomic imprinting and epigenetic inheritance, as well as the life-long variability of epigenetic modification patterns and their potential impact on phenotype with special emphasis on traits related to physical performance. We suggest that epigenetic effects may also play a considerable role in the determination of athletic potential and these effects will need to be studied using more sophisticated quantitative genetic models. In the future, epigenetic status and its potential influence on athletic performance will have to be considered, explored and validated using well controlled model systems before we can begin to extrapolate new findings to complex and heterogeneous human populations. A combination of the fields of genomics, epigenomics and transcriptomics along with improved bioinformatics tools and precise phenotyping, as well as a precise classification of the test populations is required for future research to better understand the inter-relations of exercise physiology, performance traits and also susceptibility towards diseases. Only this combined input can provide the overall outlook necessary to decode the molecular foundation of physical performance.

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## 1 Introduction

The extent to which an individual's capacity for physical performance is predetermined has been a long-lasting matter of debate in exercise physiology [1, 2]. Since the human genome was decoded 10 years ago, the challenge is

to create a complete genome catalogue, which can describe individual theoretical abilities based on the linear DNA sequence. At best, this catalogue should comprise all polymorphic regions including single nucleotide polymorphisms (SNPs), insertion-deletions (indels) and copy number variants (CNVs), along with their potential phenotypical and physiological impact, such as, gene-by-gene interactions, gene-by-environment interactions, and gene regulation by epigenetic mechanisms. Given the knowledge of the genetic factors relevant for physical performance traits, it has been proposed that genome sequencing methods could identify genetically gifted individuals as potential top level athletes beginning in early childhood. It also has been hypothesized this could improve the time-consuming and expensive scouting procedures for new talents and potential top level athletes [3]. Therefore, the attention of exercise physiologists has turned to progress in molecular genetics that might provide answers to crucial questions in determination of individual limits of physical capacity.

The first evidence for strong genetic influences on physical capacity has derived from various twin studies, starting in the late 1970s and continuing through the 1990s [4–6]. Furthermore, numerous publications based on results of the HERITAGE family study performed in the 1990s reinforced the finding that a significant fraction of variation in certain performance traits depends on the genotype [7, 8] The inherited proportion that explains variance in performance, capacity for endurance trainability, maximal oxygen consumption $\dot{V}O_2max$, strength and other key traits (physiological and psychological), was estimated to be approximately 50% [9, 10]. It has become a general consensus that these heritable differences are caused by the cumulative effects of large numbers of gene variants that have small individual effects — in other words, that heritable variation in performance is under polygenic control.

There is growing evidence that athletic performance is influenced not only by the mere genetic code but also by numerous processes influencing gene expression transcriptionally and post-transcriptionally. Regulation of transcription by epigenetic modifications, non-coding RNAs (ncRNAs) and a variety of DNA-binding transcription factors has a strong impact on many physiological processes. These factors are known to be affected by internal and external influences [11]. This association could also be interpreted as a form of gene-by-environment interaction. Epigenetics describes the heritable changes in gene expression that occur in the absence of changes to the DNA sequence. For example, "…if the genome is compared to the hardware in a computer, the epigenome is the software that directs the computer's operation…" [12]. On the post-transcriptional level, regulatory ncRNAs such as small interfering RNA (siRNA), natural antisense transcripts (NATs) and microRNA (miRNA) influence protein expression by binding and degrading processed mRNAs. There is growing evidence that ncRNAs and epigenetic modifications interact with each other [13] and are influenced by physiological processes, e.g. induced by physical exercise.

In this review, we summarize the proceedings of molecular genetics and particularly epigenetics and their impact on exercise physiology. We discuss the possible value of current scientific achievements in the search for the 'perfect athlete', focusing on the question of whether a 'perfect sports genome' can be determined at all. In particular, we will address the shortcomings of single gene association studies and extended association studies to reach the level of practical significance for sports. In addition, we will provide an outlook on how the upcoming field of epigenetics will affect our thinking in quantitative genetics. Linking phenotype with genotype more precisely will first require unraveling the extent to which the epigenome influences phenotypical traits. With regard to recent developments, we will argue that implementation of personal diagnostic sports medicine on a classical genetic basis might not be practical.

Ethical objections to genetic testing of athletes are widely discussed elsewhere [14, 15] and even though they are of essential importance they are beyond the scope of this review. Many epigenetic studies presented here describe results deriving from animal or *in vitro* experiments and cannot be directly transferred into humans. However, they demonstrate that the epigenome has greater flexibility than previously thought, with a potentially high impact that extends to humans.

## 2 Heritability of Performance Traits and Related Genetic Associations

Numerous studies have been conducted to investigate the magnitude of genetic determination of the various aspects of physical performance [2, 6]. Physical performance is a complex trait that can be divided into various baseline components such as endurance capacity, (maximal) strength, power or coordination. Apart from that, interaction with environmental influences such as training, sedentariness, injuries and other physiological and/or psychological issues is crucial for individual athletic ability.

To investigate the heritability of aerobic exercise physiology traits, Bouchard et al. tested the effect of a 20 week cycling workout programme on 481 sedentary adults from 98 two-generation families [7]. Based on results from this study, the authors determined 47% to be the 'maximal heritability estimate' of trainability of the

$\dot{V}O_2$max [10]. Supported by similar publications on baseline traits, the inherited proportion of physical performance was estimated to be approximately 50% [16, 17], even though the heritability estimates in strength-related studies tend to be slightly lower [18].

### 2.1 Single Gene Association Studies

Subsequently, many exercise physiologists concluded that at least 50% of physical performance is dependent on external influences such as training and lifestyle. Based on the premise that the immutable influences on performance are encoded completely in the DNA's primary sequence, the individual variance in performance was often summarized by a basic standard equation that divides phenotypical variance into genetic and environmental influences (Table 1).

In this equation, 'genetic variance' not only includes all inherited traits but also all traits that cannot be altered due to external influences, whereas 'variance by environmental influences' represents all acquired traits. In spite of the availability of much more detailed epidemiological approaches to decrypt phenotypical variance [19], such as the quoted second equation by Falconer [20] (Table 1), a considerable amount of the recent literature still relies on this simplistic equation. However, as we will discuss in this review, this equation is outdated and does not sufficiently capture the complex inter-relations between determined and acquired traits.

This shift in the understanding of an athlete's physical potential led to the initiation of numerous association studies. In exercise physiology, these studies aim to link genetic profiles to athletic abilities [21]. Exercise physiology association studies mostly correlate the alleles of single genes with physical performance. In these studies, special groups consist mostly of elite athletes, but also of army recruits or recreational athletes who are genotyped to investigate whether certain alleles are over-represented in the target group and thus likely to be associated with superior athletic performance.

One of the first association studies in exercise physiology was performed in 1998 by Montgomery et al. who could weakly associate a homozygous insertion (II) in the gene coding for the angiotensin I converting enzyme (*ACE*) with endurance traits in mountaineers and soldiers [22]. In another study, the authors found an association between the allele frequency of the *ACE* insertion allele and the trend towards long-distance runner status in Olympic contestants [23]. However, a replication study by Rankinen et al. could not detect any correlation between the indel polymorphism and endurance athlete status [24].

Similar findings have been described for the R577X variant of the actinin-α 3 gene (*ACTN3*). Studies of elite athletes described an association between strength and sprint disciplines and the active *ACTN3* RR variant as well as an association between endurance-related disciplines and the presence of the null alleles [25, 26]. The association of the *ACTN3* XX-variant with endurance performance could not be confirmed by subsequent studies [27, 28]. Likewise, other studies failed to detect an association between the 'strength allele' and the tested 'strength group' [29].

*ACE* and *ACTN3* are the most intensely explored genes for association with athletic performance and there are numerous studies with differing outcomes. Furthermore, a large number of studies detected weak associations with other single gene polymorphisms that could contribute to the collective account of the genetic component of physical performance [30, 31].

### 2.2 Extended Association Studies and Beyond

Based on the results of single gene association studies, further studies expanded the concept to new approaches, associating combinations of gene variants that are postulated to be beneficial with genotyped test groups.

A Spanish study compared the genotypes of seven polymorphisms with a hypothetical impact on endurance performance [32]. All seven polymorphisms were hypothesized to operate additively and to have an equally large influence on the phenotypical trait. Although the highest scores in the combination of observed polymorphisms were achieved by elite athletes, the overlap between the groups was considerable and emphasized the imprecise nature of these scanning procedures. In a second study, top athletes either from endurance (e.g. long-distance running, cycling) or power disciplines (e.g. sprint, jumping or throwing disciplines) were screened for 36 DNA polymorphisms to identify gene variants favoring special disciplines [33]. Only three polymorphisms were considered to be 'predictive' of performance potential. Given that there was no non-athlete control group, these results can be interpreted in two different ways. Either the other polymorphic DNA elements have no detectable influence on individual athletic ability, or

**Table 1** Exemplary equations for phenotypical variance

| | |
|---|---|
| VP = VG + VE | VP = phenotypical variance, VG = genetic variance, VE = variance due to environmental influences |
| VP = VA + VD + VI + VE + VGE | VA = additive genetic variance, VD = dominance genetic variance, VI = gene-gene interactions, VE = environmental variance, VGE = genotype-environment interaction |

there could be general advantages for a sports career that are not predictive of any specific discipline.

Rankinen et al. published an annually updated *Human Gene Map for Performance and Health-Related Fitness Phenotypes* that lists the genes that are likely to influence physical performance [34]. Gene variants are added to this list if at least one study could link the gene with athletic performance. Polymorphisms that failed to be confirmed in further studies remain in the list. Both policies can be regarded as problematic given the many inconsistencies that exist between association studies. Even though the selection criteria for inclusion on the list have been tightened due to the sheer number of new studies [35], there is still a rather uncritical view of the 'established' beneficial genotypes in the scientific community. In 2008, Williams and Folland calculated the individual probability to possess the 'perfect' endurance genotype [36]. Their list of 23 SNPs assumed to be beneficial for endurance performance also contains polymorphisms whose association is still a matter of debate, such as the ACE-I-allele or the R577X variant of *ACTN3*. They calculated a probability of 0.0005% that a single individual in the world possesses all 'beneficial' SNPs. On top of this, the actual impact of such a genotype is unknown. It is still unknown how these SNPs may interact or even counteract each other. Furthermore, most association studies in this field are conducted on athletes of Caucasian descent only. Thereby, an ethnic stratification bias is created neglecting most of the earth's population and, in case of endurance sports, most of the world's elite.

Type 2 diabetes mellitus research could be used as a suitable example to show that even genome-wide association studies with extremely large test groups and a significant research effort do not necessarily lead to the desired results. Similar to physical performance, type 2 diabetes is suggested to have a strong genetic component that is still not completely understood. Furthermore, environmental effects, especially body weight, nutrition and exercise, can be key triggers in the development of the disease [37–39]. Over the past 2 years, nine large-scale studies published by international consortia completed genome-wide scans for over-represented SNPs in thousands of type 2 diabetics from different ethnic populations [40–48]. Even though new locus associations with type 2 diabetes are constantly detected, approximate effect sizes are mostly between 0.9 and 1.2 and the association of only one locus with an effect size over 1.4 could be repeatedly replicated (transcription factor 7-like 2, TCF7L2). Despite the immense scientific effort, it seems that either only a small proportion of the relevant genetic factors for type 2 diabetes has been revealed so far [49], or that the determined component of type 2 diabetes is not only based on genetic variation. These results indicate that the current design of association studies – even when performed genome wide – might not be able uncover the genetic determination for physical performance.

### 2.3 The Limitations of Gene Association Studies in Exercise Physiology

A major problem that occurs in exercise physiology association studies is not rooted in the molecular genetic methodology employed, but rather in the insufficient classification of test groups. It is crucial to use a precise classification of the subjects' phenotypes in order to link genetic variants with athletic abilities (Figure 1). Although an athlete's genotype is not influenced by training, the athletic characteristics that can be associated with this specific genotype are directly dependent on training status. The recruitment of an adequate number of matched athletes is difficult because training status and progress have to be proven by long-term monitoring. Another pitfall in the effort of association studies to identify a genetic component contributing to a perfect sports genotype occurs during the process of phenotyping, as elite physical performance is too often taken as one isolated and independent trait. However, other factors or subphenotypes contribute to elite athletic performance; examples include self-motivation, discipline and low susceptibility to injury, all of which have both genetic and epigenetic components. Additionally, individual variables such as socio-economic status, nutrition, coping with psychological stress and many others, have also been described to have both genetic and epigenetic correlates that might contribute to false positive/negative results in association studies seeking for the 'perfect athletic genotype'. All of these variables should be carefully considered when large cohorts are being tested for any genetic association of physical performance.

The possibility of enhanced performance through doping is another potential confounder when athletes are tested in association studies, especially in elite sports [50, 51]. As a result of doping, the association of an athlete's genotype with unnaturally enhanced performance will be misleading and will distort the study's outcome. This problem is not limited to studies with elite athletes but is also a growing issue when testing recreational athletes [52]. Irrespective of ethical and health concerns, doping could likely distort the outcomes of association studies even long after application of the doping agent.

## 3 Influences on Quantitative Trait Analysis in Genetics

Aside from the small influence single alleles may have on performance traits and the above mentioned methodological limitations, there could be other reasons for the

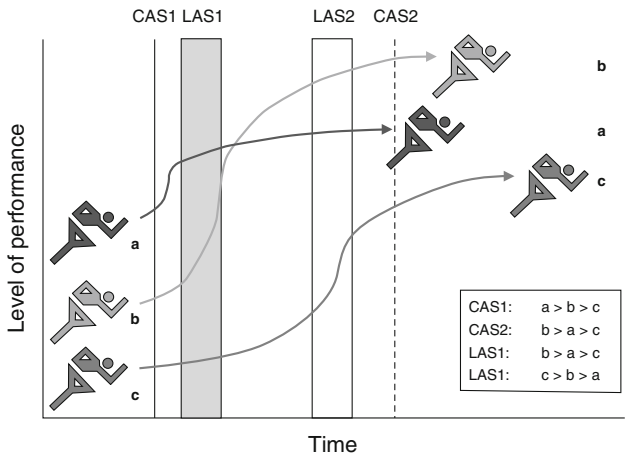


**Fig. 1** The dilemma of cross-sectional and longitudinal genetic association studies: The graph illustrates the hypothetical development of a defined physical performance trait (y-axis) for three individuals with three different genotypes over time (x-axis). All external factors are assumed to be constant, conducive to performance and identical for all individuals. Training response has been simplified. The outcomes of two hypothetical cross-sectional genetic association studies (CAS1 + CAS2) to relate polymorphisms to measured performances at specific points in time and two longitudinal genetic association studies (LAS1 + LAS2) to investigate trainability (Δy, indicating change in performance over the time of the study) vary depending on the time of data collection. All four studies associate different performance levels or performance developments with the same genotypes, only two of the studies present the same ranking of the athletes

difficulties in reproducing genetic associations with testing for performance. When following the phenotype back to its origin, there are many mechanisms regulating gene expression, transcriptionally, post-transcriptionally, translationally and post-translationally [53]. The association between a given genotype and the phenotype can be altered if the expression of genes is influenced by epigenetic modifications and different types of ncRNAs, which potentially explain the problems to confirm data from studies equating heritability with determination.

All known noncoding RNAs are functional and can be crudely divided into three major groups according to their function: the first group consists of those ncRNAs involved in protein synthesis and includes ribosomal RNA and transfer RNA (rRNA and tRNA, respectively); the second group consists of those ncRNAs involved in posttranscriptional RNA processing such as splicing and includes small nuclear RNA (snRNA) and small nucleolar RNA (snoRNA); the third group consists of ncRNAs involved in transcriptional, post-transcriptional and post-translational regulation and includes long-non-coding RNA (lncRNA), natural antisense transcripts (NATs), siRNA, and microRNA (miRNA) [54–57]. However, some ncRNAs (ie. lncRNAs) show involvement in all three processes [57–61]. Antisense RNAs target complementary mRNAs, whereas siRNAs and miRNAs form a functional RNA-protein complex called RNA-induced silencing complex (RISC) that binds with high specificity to complementary mRNAs. These regulatory ncRNAs bind to target mRNAs, mostly preventing translation and stimulating degradation. One type of miRNA can affect many different mRNAs and one mRNA can be targeted by many different miRNAs.

In addition to ncRNA regulation processes, epigenetic influences such as DNA-methylation and histone modifications are potential confounders of the 'genotype–phenotype' assumption on the transcriptional level. They regulate the accessibility of genomic regions for transcription and can alter gene expression, as will be subsequently discussed in detail. In this context, it is noteworthy that SNPs can not only influence the methylation pattern directly at the polymorphic site [62] but can also affect the methylation profile of the entire surrounding genomic region. As described by Gertz et al. [63], a variant genotype affects the DNA-methylation pattern of far more loci than gametic imprinting. This group observed that more than 8% of heterozygous SNPs are associated with differential methylation in the surrounding genomic area. They also showed that SNP dependent alternative methylation results in altered gene expression patterns. This observation highlights the importance of investigating not only the linear DNA sequence but also emphasizes the significance of differential DNA methylation patterns that lead to changes in gene expression.

Epigenetic modifications and expression of ncRNAs are highly tissue specific, with the exception of housekeeping RNAs such as, for instance, rRNA and tRNA. Therefore, experimental results can only be compared if the same tissues have been assayed. Furthermore, the various regulatory mechanisms are influenced by each other [13], and also by internal and external influences such as diet or physical exercise. For example, it has been demonstrated that muscle specific miRNAs, so called myomirs, are upregulated directly after single bouts of endurance exercise and reversibly downregulated by a 12-week endurance training programme [64]. Furthermore, it has been shown that miRNA expression can be regulated differently in training 'responders' and 'non-responders' [65] that might also be influenced by differential epigenetic mechanisms.

Although we will focus on epigenetic processes, it must be noted in the following considerations that the genome, epigenome, transcriptome and proteome are in constant interaction.

## 4 The Influence of Epigenetics on Quantitative Trait Analysis in Genetics

Increasing knowledge in the field of epigenetics during recent years has had crucial consequences for classical

quantitative genetic considerations [66], possibly explaining some of the problems in correlating phenotypical traits with genetic variance [67]. The 'Human Epigenome Project' was founded in 2004 to gain insight into epigenetic regulation of the human genome [68, 69]. Novel techniques such as chromatin immunoprecipitation (ChIP) and next generation sequencing (NGS) are now being employed to explore the epigenome, also in combination with bisulfite genomic sequencing (reduced representation bisulfite sequencing; RRBS), DNA methylation arrays (MeDIP-chip) or DNA methylation sequencing (MeDIP-seq) [70–75].

Understanding of epigenetic processes is becoming more and more important for the understanding of cellular processes, as it is thought that they are a key component in cellular specialization. We are all descendants of fertilized (egg) cells, which have been dividing for billions of cell-generations. All of these cells carry the same DNA information, which is transcribed in a cell type-specific manner, leading to high specialization. Interestingly, recent literature describes these cell-type specific epigenetic signatures to be prone to specific environmental stimuli. In this context the field of epigenetics might contribute to a more detailed molecular understanding of athletic performance including potential influences on physiological and psychological traits.

## 4.1 Epigenetic Modifications

Epigenetics deals with the accessibility of DNA to the transcription machinery [76], association of the epigenetic modifications with gene expression patterns and their potential effects on the phenotype [77]. These processes are regulated by an orchestra of complex mechanisms leading to either heterochromatin (DNA, which is highly condensed and not available for transcription) or euchromatin (open DNA, which is transcriptionally active). Two general mechanisms in this context require clarification, namely: DNA-methylation and chromatin modifications. DNA, on the one hand, consists of a linear nucleotide sequence wherein CpG-dinucleotides are prone to cytosine methylation (CpG in that context indicates that a cytosine is directly followed by a guanosine linked to each other by a phosphodiester on the same strand). In general, DNA methylation leads to reduced transcriptional activity whereas DNA low in methylation is rather prone to transcription.

Chromatin describes the unit of DNA and histones. Histones are basic proteins, which facilitate DNA condensation. In general, histones are positively charged, which leads to high affinity for the negatively charged DNA, resulting in a high level of DNA condensation (heterochromatin). The addition of chemical modifications to histones alters their ability to attract DNA and, thus, the availability of the DNA for transcription.

Therefore, epigenetic processes determine which genes are expressed [78]. We will not discuss the molecular mechanisms of epigenetic processes in detail in order to concentrate on the effects of these modifications on exercise physiology. However, relevant literature will be provided in the respective sections of our review for further information.

### *4.1.1 DNA Methylation*

DNA methylation results from the addition of a methyl group to a cytosine by DNA methyltransferases [79], influencing transcription. In vertebrates, the primary sites for DNA methylation are cytosines in the symmetric CpG context but also non-CpGs (CpA; CpC; CpT) were found to be methylated in low amounts, predominantly in pluripotent cell-types (Ziller et al. [80]). Methylated sections of DNA are less accessible to for DNA-binding proteins and RNA-polymerases and therefore downstream genes are generally attenuated in their expression [81]. However, in some rare cases, methylation enhances transcription, e.g. by methylation and thus blocking of a silencing element [82]. Interestingly, DNA-methylation rarely takes place in CpG islands, where large numbers of CpG-dinucleotides are clustered together, but occurs mostly in regions outside of core promoters, e.g. enhancer regions of genes [83] and in large proportions of intragenic gene bodies [84]. Generally, CpG-poor promoters tend to be methylated to higher extent than promoters with greater CpG abundance [85].

It is still unclear whether loss of DNA methylation is an active or passive event, even though there is growing evidence for active demethylation [86, 87]. Recently, two studies proposed an iterative process, oxidating 5-methylcytosine (mC) to intermediate products [88, 89]. He et al. have shown that a subsequent step resulting in unmethylated cytosine is mediated by thymine-DNA-glycosylase (TDG) [88]. However, future research is warranted to evaluate whether this is a physiologically relevant pathway for active DNA demethylation.

### *4.1.2 Histone Modifications*

Histone modifications affect the accessibility of DNA through changes in chromatin structure by regulating histone binding to DNA. DNA is wrapped around complexes of eight structural proteins called histones, forming nucleosomes. DNA that is packed into nucleosomes (heterochromatin) is poorly accessible to transcription factors and RNA polymerases [90]. Histone proteins are targets for manifold covalent modifications, such as methylation, acetylation, phosphorylation and ubiquitination. Histone

acetylation exposes DNA for transcription, whereas the different types of histone methylation can have activating or inactivating effects. H3K4 tri-methylations activate the expression of nucleosomal DNA, whereas H3K9 and H3K27 methylations tend to repress transcription [90].

The effects of histone methylation also depend on the CpG content of the promoter and its DNA-methylation status. Whereas high CpG-content promoters (HCPs) show a high expression activity and are mostly unmethylated on the DNA level [84], low CpG-content promoters (LCPs) are often DNA methylated and inactive. Signatures of active promoters are mostly H3K4me3 [91], whereas inactivation is characterized by H3K27me3 and is mainly regulated by polycomb repressive complexes (polycomb group ring finger 1 and 2, PCGF1, PCGF2) in HCPs. Generally, activating H3K4me3 and the inactivating DNA-methylation are mutually exclusive [92]. LCPs can be re-activated by histone methylation or acetylation and transcription factor binding [85].

Recent studies have shown that histone methylation in non-methylated CpG-rich promoter regions is mediated by DNA-binding proteins [93, 94]. Therefore, histone methylation processes might be directly dependent on the methylation status of the promoter. Histone modifications in enhancers or silencing elements likely alter the expression of target genes in a more graded way [95] and exhibit a relatively higher cell-type specificity compared with promoter sites [96]. Aside from effects of epigenetic modifications on transcription, various studies indicate that there are epigenetic mechanisms that assist in the regulation of splicing [97] and alternative splicing [98]. Therefore, the influence of epigenetic modifications may persist post-transcriptionally.

### 4.2 Influences on Epigenetics

As the epigenome is a key regulator of gene activity, the question arises of how and to what extent it contributes to phenotypical variance. Epigenetic processes are partly reversible and can occur during varying developmental phases [99]. Histone modifications are in a constant state of alteration, whereas DNA methylation is generally assumed to be more stable, possibly with lifelong effects on gene expression. Intra-individually, the epigenetic makeup is highly cell-type specific [100] and is passed from cell to cell (generation) through mitosis [101]. In ontogenesis, the majority of DNA methylation in the zygote is erased directly after fertilization and is *de novo* methylated afterwards [102]. All methylation patterns are erased in a second genome-wide demethylation process that takes place in the primordial germ cells of the embryo during early development. Sperm cells become *de novo* methylated a few days afterwards, whereas re-methylation in the ovules does not take place until birth [103]. Thus, in contrast to classical mendelian inheritance of genetic information (Figure 2a), a non-inherited determination of epigenetic information takes place during this step in ontogenesis (Figure 2b), which is in contrast to classical mendelian inheritance of genetic information (Figure 2a). The *de novo* methylation processes are assumed to play an essential role in the specialization of pluripotent cells [104].

As reviewed by Zwijnenburg et al., the investigation of monozygotic twins discordant for a specific trait is a promising tool to identify post-zygotic genetic and epigenetic events [105]. It has been suggested that unequal splitting of the inner cell mass or the lack of methylation maintenance in the early zygote may result in differences in DNA methylation in monozygotic twins, potentially accounting for phenotypical discordance. Even though DNA-methylation is determined primarily in prenatal and early postnatal development, it still underlies life-long modification. A study by Fraga et al. provided evidence that the epigenomes of monozygotic twins differ with increasing age and the more their lifestyles vary [106]. Determinations that have already taken place are often difficult to classify with regard to their reversibility. It seems that many cannot be influenced after a particular point in time in development. Therefore, long-term determinations mediated by the environment might take place.

Furthermore, recent studies suggest that DNA-methylation patterns may be more dynamic than assumed. Kangaspeska et al. discovered rapid cell cycle dependent methylation and active demethylation of different promoters, particularly of an estrogen-responsive gene promoter [107]. If cyclical regulation of transcription via DNA methylation should also be confirmed for other genes, this might enforce a new understanding of DNA methylation processes. In the field of physical performance, these outcomes might be especially interesting as, in this case, the effect is hormone mediated. Furthermore, if methylation patterns vary cyclically, future studies investigating methylation status will have to focus on time curves. Epimutations are more frequent than spontaneous somatic or germline mutations and they can have a profound impact on physiological traits [106]. Therefore, if phenotypical variance is measured by quantitative approaches, epigenetic modifications might further amplify or suppress genetic variance. To summarize, from an epidemiological point of view, environmental factors interact with the epigenome, which may contribute to phenotypical differences [108].

### 4.3 Epigenetic Influences on the Phenotype

To evaluate the relevance of epigenetics to physical performance, it is necessary to examine a variety of physiological traits and their potential epigenetic regulation.

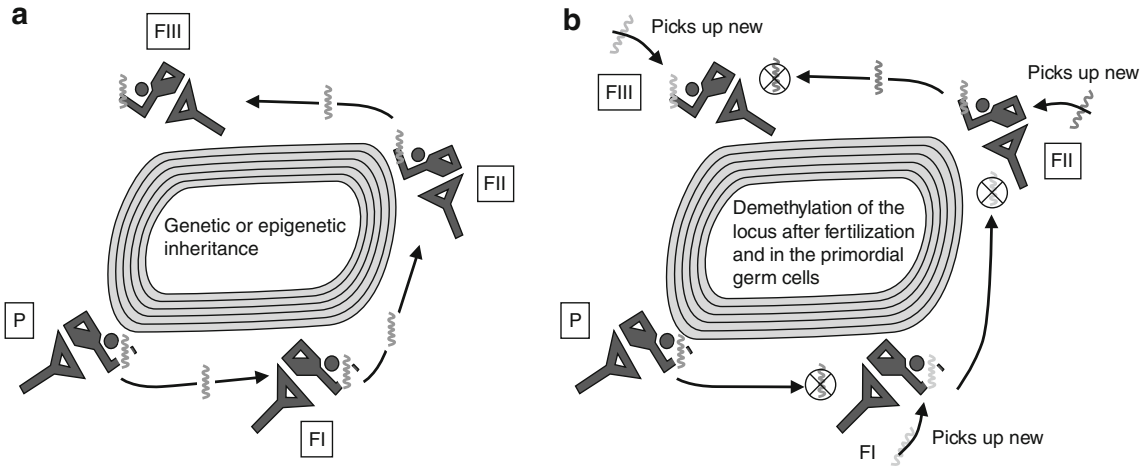


**Fig. 2** Genetic and epigenetic development over four generations: (**a**) Genetic or epigenetic inheritance. The schematic analogy of transgenerational genetic or epigenetic transmission displayed as a relay run. Each runner represents one generation. The relay baton symbolizes one specific transmitted allele or, in case of epigenetic inheritance, methylation status. The allele is passed on unchanged over generations, starting with the parental generation. Of course, the probability of transmitting this allele is only 50% due to the haploid germ cells. In cases of non-mendelian epigenetic inheritance, the possibility that an inherited epigenetic mark is phenotypically expressed is further limited, although in case of our relay run analogy, the runners still may transmit the baton. (**b**) Loss of methylation: DNA-*de novo* methylation displayed as a relay run. The baton, symbolizing a single DNA-methylation status, is not passed on to the next generation, but is erased after fertilization and in the primordial germ cells of the embryo. Every generation picks a new baton, symbolizing *de novo* methylation throughout ontogenesis. *FI–FIII* = filial (offspring) generations one to three, *P* parental generation)

During the past decade, epigenetic signatures have been associated with various diseases [109–111] and immune processes [112]. Studies have suggested a profound epigenetic impact on type I diabetes [113] and insulin resistance [114], metabolic and cardiovascular diseases [115], asthma [116], and psychiatric conditions like schizophrenia [117]. It will be a crucial challenge in the future to investigate, the extent to which the fraction of phenotypical variance that arises from environmental influences is actually mediated by epigenetic modifications [118].

In this context, it is interesting that the epigenome was shown to be influenced by maternal behaviour. Weaver et al. detected an impairment of the hormonal stress response in rat offspring based on DNA methylation and histone acetylation dependent on the intensity of maternal nursing behaviour [119, 120]. This change in stress response persisted into adulthood and was reversible after application of the histone de-acetylation inhibitor trichostatin A (TSA). This was the first study to show how early life environment can permanently influence endogenous abilities through epigenetic modifications like DNA methylation and histone acetylation. Both results, especially the pharmaceutically induced reversibility, necessitate further investigation to better understand the complex nature of epigenome-by-environment interactions.

Inspired by these results, various studies have demonstrated the distinct effects of maternal care and nutrition on phenotype, mediated by epigenetic modifications [121], In 2007, Burdge et al. observed differential methylation in the FI (first filial generation) and the FII (second) filial generation of rats whose F0 female ancestors (parental generation) had been fed a protein restricted diet [122]. Further studies in rats have demonstrated that a high-fibre diet fed to the dams during the gestation and nursing periods was significantly linked to a lifelong vulnerability towards overweight and a lower life expectancy in the offspring [123]. Another study suggested that this determination can be distinctly diminished through high physical activity in the early infantile period of the offspring [124].

A study by Shelnutt et al. demonstrated that DNA methylation can also be modulated in humans by application of a special diet containing high doses of folic acid [125]. Furthermore, it has been shown that hunger periods during pregnancy can alter the DNA-methylation profile of the offspring also in humans [126]. These results indicate transgenerational, possibly epigenetic effects that directly affect performance-related traits. In addition, regardless of the transgenerational aspect, long-term diet-induced epigenetic effects that are not induced by methylation agents are of special interest to exercise physiology given that most athletes follow strict diets to support their training efforts and to optimize performance. Thus, the epigenome is likely to be susceptible to direct dietary effects along with effects due to external factors such as training. Therefore, it can be assumed that at least some of the determinations that take place during the gestational period might be influenced by environmental factors in a lifelong manner.

## 5 Epigenetic Inheritance and Early-Life Influences

Epigenetic methylation marks are erased in the zygote and in primordial germ cells. However, several studies indicate that there are single genes whose methylation patterns are not erased but are passed on across generations with phenotypical consequences [127, 128]. In such cases, epigenetics potentially combine the factor of heritability, which we know from classical genetics, with the factor of inducibility by environmental influences.

The best investigated case of epigenetic inheritance in mammals is the agouti locus in mice [129, 130]. If expressed, the agouti viable yellow allele ($A^{vy}$) leads to offspring exhibiting a yellow coat phenotype along with predisposition towards obesity, diabetes and tumour development. The stable insertion of a transposable DNA element (called intracisternal A particle [IAP] retrotransposon) activates transcription of the naturally silenced gene, but only in case the IAP is hypomethylated. This so-called ectopic expression is graded, such that resultant phenotypes are dependent on the degree of methylation, resulting in different phenotypes [131]. Offspring carrying a methylated promoter are agouti coated, called pseudoagouti. This methylation pattern is passed from the dam to the offspring in a non-mendelian way (Figure 2a). This means that a significantly higher proportion of a yellow-coated dam's progeny is also yellow, when compared with pseudoagouti coloured dams.

Another well investigated epigenetically inherited trait in mice is the $Axin^{Fu}$ allele, a gain-of-function allele leading to a kinked tail through differential methylation [132]. This allele also contains an IAP retrotransposon but, in contrast to the $A^{vy}$ allele, it can be passed on transgenerationally by both parents. Epigenetic inheritance seems to accumulate in IAPs, which seem to evade loss of methylation during gametogenesis [133].

Epigenetic inheritance of the $A^{vy}$ phenotype can be diminished through changes in DNA-methylation status if the dams are fed a special methyl donor diet containing folic acid, vitamin $B_{12}$, choline, and betaine [134–136]. Interestingly, suppression of the $A^{vy}$ phenotype is only observed in the FI generation and is not inherited further [137]. Therefore, the diet-induced changes seem to affect the offspring's somatic cells, not the germ cells. In another study, ethanol consumption by the dam during gestation was shown to silence the $A^{vy}$-allele [138]. Accordingly, the expression of epigenetically inherited traits appears to be much more prone to environmental influences during ontogenesis than genetic inheritance. One of the most interesting considerations is that acquired features might be inherited under distinct circumstances, even though the establishment of epigenetic inheritance is difficult to verify *in vivo*.

In a qualitative study, Suter et al. described two subjects with epigenetic malfunction leading to multiple cancers [139], initiating a vivid discussion of whether this might be the first observed case of epigenetic inheritance in humans [140–144]. However, the irregular methylation pattern leading to cancer susceptibility was caused by an epigenetic germline mutation and not inherited transgenerationally.

Interestingly, epidemiological studies investigating heritable epigenetic influences on type 2 diabetes suggested that epigenetic determination for diabetes due to overeating during the children's slow growing period (girls 8–10 years of age, boys 9–12 years of age) might be transmitted transgenerationally through the male germline [145, 146]

### 5.1 Genomic Imprinting

Genomic imprinting characterizes a distinct number of genes that remain DNA methylated during the demethylation process in the zygote after fertilization [147, 148]. In contrast to epigenetic inheritance, imprinted DNA methylation is only stable over one generation and is erased in primordial germ cells during early development. Therefore, genomic imprinting must be separated from (epigenetic) inheritance, for the methylation pattern that is transmitted from the parents to the offspring is not necessarily the same pattern that the parents exhibit themselves. A further difference between parental genomes is that the paternal genome is demethylated actively after fertilization, whereas maternal genes are protected from this process [149].

Genes carrying imprinting marks are concentrated in imprinting centres whose special architecture leads to resistance to the demethylation process [150]. By now, there are 64 genes shown to be imprinted in humans and another 112 genes that are predicted to be imprinted [151]. Genomic imprinting leads to sex-specific determination of given alleles through DNA-methylation of differentially methylated regions (DMR). Maternal or paternal selection largely leads to the silencing of one allele in the zygote, although there are imprinted alleles that are also active in the methylated state [152]. Maternal and paternal imprinting vary due to different demethylation mechanisms in sperm cells and ovules [152]. Maternally imprinted genes tend to slow down fetal growth whereas paternally imprinted genes enhance it [153].

The importance of genomic imprinting for embryonic development can be deduced from the physiological and anatomical defects that emerge in cases of irregular methylation of imprinting regions. The consequences reach from lethality due to developmental disorders such as Silver-Russell-Syndrome or Beckwith-Wiedemann-Syndrome to metabolic diseases and cancer [154, 155]. Furthermore, there are investigations suggesting behavioral and cognitive effects of imprinting disorders in mice and humans [156, 157].

### 5.2 Epigenetics and Physical Performance

In order to effectively classify the genetic determination of performance traits, it is crucial to acknowledge that the epigenome may be associated inseparably with physical performance traits. Even though it is difficult to explore the direct impact of epigenetic modifications on physical performance, there are many phenotypical traits that are intrinsically tied to exercise physiology.

In this context, a recent study by Potthoff et al. demonstrated that class II histone deacetylase (HDAC) suppressed the formation of slow-twitch muscle fibres through repression of the myocyte enhancer factor 2 (*Mef2*) in a mouse model [158], revealing direct epigenetic impact on skeletal muscle development. A further study detected a downregulation of the slow-twitching type I myosin heavy chain gene via histone deacetylation in rats and confirmed the association between histone methylation, acetylation, and muscle fibre type (slow vs. fast twitching) [159]. An additional exercise-induced regulatory mechanism could be transient export of the transcription repressing HDACs 4 and 5 from the nucleus into the cytoplasm [160], a process induced by HDAC-phosphorylation [161]. Furthermore, the recruitment of muscle stem cells for muscle regeneration seems to be regulated by histone modifications [162]. These results suggest a direct impact of histone modifications on exercise adaption and emphasize the important mediatory role of epigenetic modifications in physiological processes.

While we hypothesize interplay between physical exercise, DNA methylation and chromatin modifications, there is sparse literature on this topic so far. In a recent study performed in a cohort of elite athletes and a cellular model of differentially methylated C2C12 myoblasts, Terruzzi et al. investigated SNPs located in five genes that are thought to be involved in DNA methylation [163]. The investigated polymorphisms and their potential effects on athletic performance show statistical significance, suggesting that athletic performance may be mediated by an altered epigenotype. However, the study does not show any direct impact of the investigated SNPs on the epigenotype nor does it demonstrate any altered gene expression as a consequence of differential DNA-methylation. Barrès et al. demonstrated that exercise induced gene expression in sedentary men and women is associated with transient alterations in muscle cell DNA methylation [164]. The study discovered that several genes previously reported to be differentially methylated in type 2 diabetes, to show reduced promoter-DNA methylation after acute exercise, whereas muscle-specific transcription factors, as well as the housekeeping gene glycerine-aldehyde 3 phosphate dehydrogenase (*GAPDH*), remained unchanged. They could further show that the intensity of aerobic exercise drives gene expression in a dose-dependent manner accompanied by reduced promoter methylation levels. *Ex vivo* muscle contraction performed in isolated mouse soleus muscle confirmed these findings. Addition of caffeine to L6 myotubes elevated cytoplasmic $Ca^{2+}$ levels, mimicking exercise-induced expression of genes related to mitochondrial function. Again, increased gene expression was associated with decreased promoter methylation, an effect which could be inhibited by co-incubation with the $Ca^{2+}$ blocker, dantrolene. This study nicely describes how physical exercise could influence muscle DNA methylation patterns and muscle gene expression, and consequently muscle activity.

Alterations in epigenetic modification patterns have been demonstrated to be dependent on exercise and growth hormone (GH), insulin-like growth factor 1 (IGF-1), and steroid administration. In 2009, Collins et al. described improved cognitive responses to psychological stress after exercise in a rodent model [165]. Testing exercised versus non-exercised rats exposed to a novel environment, the authors observed improved stress coping in exercised subjects. Investigating the dentate gyrus, a brain region which is involved in learning and coping with stressful and traumatic events, they could show that this effect is mediated by increased phosphorylation of serine 10 combined with H3K14 acetylation, which is associated with local opening of condensed chromatin. Consequently, they found increased immediate early gene expression as shown for *c-FOS* (FBJ murine osteosarcoma viral oncogene homologue).

Performing quantitative ChIP, Chia et al. investigated the *Igf-1* promoter in Gh deficient rats and controls before and after Gh administration [166]. Within 60 minutes following Gh treatment, they observed a 6-fold increase in acetylation of H3K4/14- and H4K5/8/12/19 in the entire *Igf-1* promoter region and a 2-fold increase in H3K4me3 in intron 3, flanking a potential Stat5b binding sequence. Altogether, they could show that chromatin re-organization in the *Igf-1*-gene was concurrent with increased *Igf*-1 transcription mediated by Gh injection. Based on this study, the long-term influences of hormone application, possibly passed on mitotically, could also be of specific interest.

Therefore, investigation into the effects of additional hormones and messenger substances on the epigenotype, especially in an exercise physiology context, might be warranted. The impact of doping on epigenetic mechanisms must not be underestimated. For instance, it is likely that substance abuse influences epigenetic processes [167]. Even though the direct effect of doping substances on the body is mostly transient, epigenetic consequences might be persistent. Since influences on hormonal pathways are important to exercise physiology and doping detection,

these results could indicate new approaches to further investigate the inter-relation between epigenetics and physical performance.

Furthermore, the possible effects of epigenetic modifications on various medical conditions and injuries, de- or overtraining periods, application of legal drugs and psychological stress, just to name a few, on epigenetic modifications should be investigated in more detail to gain a more comprehensive understanding of the dynamics and functional consequences of the epigenome. To categorize all non-genetic influences on phenotypical variation from an epidemiological point of view, Christopher P. Wild has introduced the term 'exposome' that can be further divided into general and specific external domains, as well as an internal domain [168].

## 6 Genotype-Epigenotype-Phenotype Associations and Quantitative Genetics

To gain a better understanding of the nature of DNA methylation, Herman et al. transferred imprinted methylation patterns from the inactive maternal allele to the active paternal allele, which was thereby inactivated [169]. Another study exposed female mice during the period of gonadal sex determination to vinclozolin, a strong endocrine disruptor, and demonstrated that the male offspring from filial generation I to IV were impaired in fertility due to methylation processes [170]. These are indicators of the flexible nature of epigenetic functionalities. Along with the insights gained from studies of epigenetic inheritance, one can speculate about potential transgenerational epigenetic determinations [171], as illustrated in Figure 3.

The various considerations that document the importance of epigenetics for the genotype-phenotype relationship need to be incorporated into quantitative genetics (Figure 4). A quantitative genetic model that incorporates epigenetic and other transcriptional and post-transcriptional regulations could be divided into several levels of influences on phenotypic variance. Environmental influences, transient and stable epigenetic modifications, genomic imprinting, as well as inherited epigenetic modifications along with the underlying DNA sequence all determine the phenotype through constant interaction (Figure 4b). Interpreting Bouchard et al., genetic and inherited epigenetic variance add up to the presumed 50% of inherited variance. The resulting phenotypical consequences are expressed transgenerationally.

The pivotal challenge is to classify the dimension of interaction between environment, epigenetic modifications and other functions that regulate transcription. Based on that knowledge, models for gene expression can be combined with genotypes to diagnose their impact on phenotype. It is important to keep in mind that epigenetic determinations are not as stable as genetic determinations. Epigenetic marks can be modified under special circumstances throughout ontogenesis. Introduction of various instances of genetic and epigenetic influences on the phenotype will additionally raise the question of how these instances interact.

Furthermore, the phenotype is influenced by ncRNAs, transcription factors, hormones, etc. which are also determined by genetic predispositions and influenced by epigenetic and environmental impact. In 2008, Johannes et al. introduced a bioinformatics approach to include epigenetic processes and epigenetic inheritance into quantitative genetics in plants [172], based on a division of epigenetic traits into three categories according to their interaction with the DNA [173]. However, in contrast to mammals, plants exhibit stable epigenetic inheritance, and therefore such a model is not transferable to humans. Nevertheless, this model could serve as a prototype for generation of a suitable model for humans [174, 175] as soon as sufficient knowledge about the nature of epigenetic determination is available.

## 7 Discussion

Physical performance is a complex trait that is in large part predetermined by the genetic and epigenetic background. Family studies have suggested that approximately 50% of the physical performance potential is inherited. Genetic association studies have attempted to link either single SNPs or combinations of SNPs to athletic phenotypes. However, the most promising candidate genes often could not be confirmed by follow-up studies [176] and the SNPs that have been investigated cannot explain the high inter-individual differences observed. Furthermore, calculating 'genotype scores' from combinations of SNPs did not show the desired prognostic practicality [32].

Potential confounders in the design of association studies further complicate research. Examples include the problem of recruiting sufficiently sized test groups of comparable athletes, insufficient monitoring capabilities and performance enhancement through doping. As a further potential confounder, especially in extended association studies, gene-by-gene interactions have to be considered and investigated. The potentially interactive nature of 'performance polymorphisms' would distort the results of a study that merely sums up 'beneficial' alleles. Therefore, association studies have either provided only a small fraction of the genes responsible for physical performance, or the conventional design of association studies is not powerful enough to detect all aspects of complex traits and therefore inadequate in predicting physical performance.

A hint for the latter can be found in the research field of type 2 diabetes, where in spite of great research effort, only

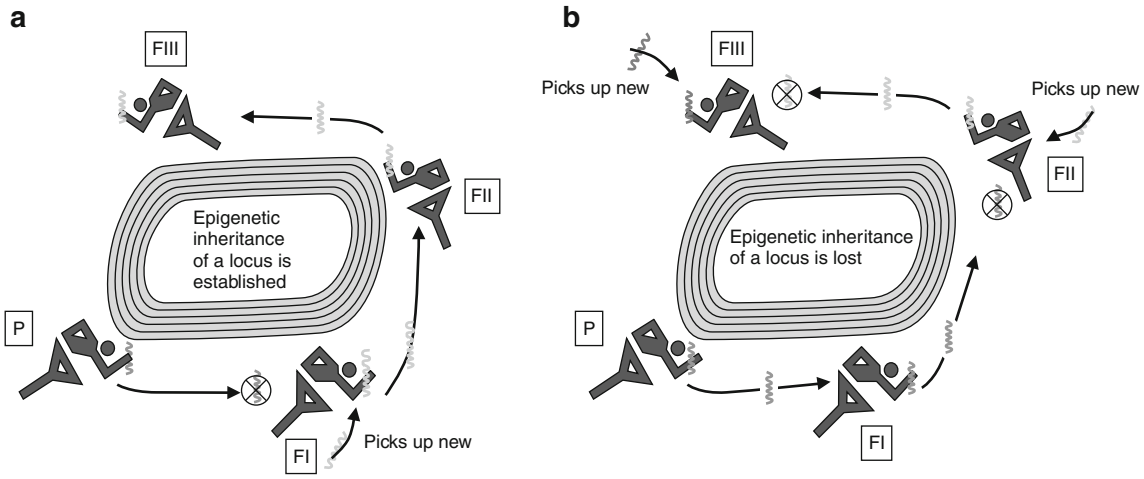


**Fig. 3** Hypothetical establishment or loss of epigenetic inheritance: The schematic analogy of the hypothetical establishment (**a**) or loss (**b**) of transgenerational epigenetic inheritance displayed as a relay run. The baton, symbolizing methylation status, is lost from the parental to the filial-one (offspring) generation. In this generation, the methylation status could become fixed by environmental factors and is neither demethylated following fertilization, nor in the primordial germ cells of the filial-two generation. *FI–FIII* filial (offspring) generations one to three, *P* parental generation)

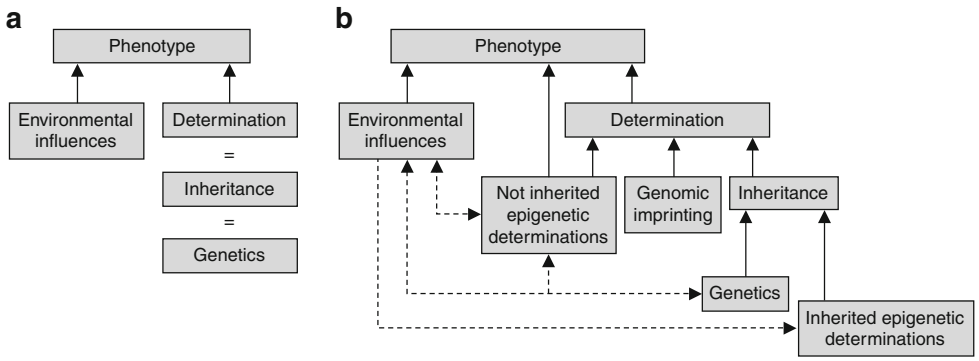


**Fig. 4** The changing conception of genotype phenotype interactions: (**a**) The classic design of genotype-phenotype interaction. The phenotype is determined by environmental influences and inherited genetic determinations. Although this design has been obsolete for a long time, the conception is still employed in recent studies because of its simplicity. (**b**) The revised design of the genotype-phenotype interaction. The phenotype is directly influenced by environmental factors, transient non-inherited epigenetic regulations (e.g. histone modifications) and long-term determinations. The latter consists of long-term epigenetic regulations (e.g. DNA methylation), genomic imprinting and inherited determinations that can be subdivided into genetic and epigenetic inheritance. Environmental and epigenetic influences interact constantly (*dotted lines*) = indicates assumed to be exchangeable

a small proportion of the responsible gene loci have been discovered [49]. Even if the complexity of physical performance is reduced to a technical parameter with high reliability such as $\dot{V}O_2max$, it is still an obstacle to find an experimental setting that sufficiently controls for potential bias (see also Figure 1). Existing difficulties in revealing a robust genotype-phenotype interaction suggest that inter-individual variance cannot be exhaustively explained by our genotype.

We argued that epigenetic modifications are a potential reason for the limited practicability of association studies to reveal strong genotype-phenotype associations. The epigenetic status might in its nature lie midway between the classical genotype and the transcriptome/metabolome of the athlete, reflecting a temporary rather than inert condition [177]. These determinations are established individually, in part during gestation and in the early infantile period. As indicated, little is known about the possible epigenetic influences on physical performance in humans. However, first studies have shown a distinct impact of epigenetic marks on physiological pathways, including traits of high relevance for locomotor activity and fitness [158, 160]. A recent study demonstrated higher methylation of the *ASC* gene (apoptosis-associated speck-like protein containing a caspase recruitment domain) after a high-intensity walking exercise programme in elderly probands, likely resulting in a lower level of the inflammation markers interleukin (IL)-1ß and IL-18 [178].

Studies in mice have shown the existence of epigenetic traits that are inherited over generations [129], and it has been suggested that such inheritance plays a role in humans as well [146]. Furthermore, many genes are controlled by genomic imprinting, another epigenetic regulation that is passed on to the following generation [148].

Consequently, epigenetics could be an important confounder in association studies in general. Because of individual bias [179], cell-type specificity [180] and especially alterability during ontogenesis [82], epigenetic regulation cannot be integrated into the concepts of quantitative genetics unless we have generated further knowledge of epigenetics and its physiological impact. Therefore, a method for simple and suitable classification of measurable athletic traits cannot be provided at this time. The impact of epigenetics and gene-by-environment interactions on phenotypical variance will need to be considered for a serious diagnosis of an athlete's theoretical physical capacity in future studies.

## 8 Conclusion

After the human genome project concluded in 2003 with deciphering of the human DNA sequence, most expectations to directly link genes [181], distinct SNPs and/or alleles with phenotypes were not fulfilled. With technological advances, the once simple idea of one-gene-encodes-one-phene has been adapted several times. Originating from the Mendelian concept [182] describing the gene as a heritable unit predicting a phenotype, the one-gene-encodes-one-phene hypothesis has been expanded into the 'one gene one enzyme' [183] and to the 'one-gene-one-polypeptide definition'. In an effort to incorporate perpetual scientific progress deriving from various disciplines, the most recent gene definition has been conceptualized as "The gene is a union of genomic sequences encoding a coherent set of potentially overlapping functional products" [184]. In the simplest case, a DNA sequence codes for one protein or one RNA. But in the most general case, genes consist of sequence modules that combine in multiple ways to generate products with complex function leading to even more complex cellular and multicellular organisms and phenotypes. Consequently, research that is still aiming to correlate single genes or SNPs with phenotypes is no longer contemporary.

Time has shown that it is insufficient to link protein encoding regions with traits and diseases while simultaneously ignoring non-protein-coding DNA sequences. For future studies, it will become increasingly essential to investigate non-protein-coding regions such as those that encode miRNAs or other non-coding RNAs along with their regulatory patterns and functions. An additional, crucial challenge will also lie in the investigation of cell-type-specific gene regulatory mechanisms.

Surprisingly, the sequencing of the human genome has determined only $\sim$20,000 protein-coding genes, representing $<$2% of the total genomic sequence, which is a similar number to that of the nematode *Caenorhabditis elegans*. Recent research on the 98% of the human genome that is not protein coding has demonstrated a complex network of transcripts that includes tens of thousands of RNAs with little or no protein coding capacity, such as long non-coding RNAs and miRNAs [61]. Other studies using new highly sophisticated methods such as genome tiling arrays revealed that the abundance of cellular non-coding RNAs is four times greater than that of protein coding sequences [185]. High cellular abundance of non-coding RNAs underlying complex gene regulatory mechanisms underlines their potential importance in cellular regulation and suggests a role in cellular specialization [59, 60], and in the development of complex phenotypes. As excellently reviewed by Mercer et al. [57], long non-coding RNAs are gaining increasing interest in the field of epigenetics due to their widespread functionality in chromatin remodelling [58, 186, 187], as well as transcriptional [188–190] and post-transcriptional regulation [191, 192]. In the context of determining an individual's theoretical physical abilities these examples should demonstrate that linkage of genomic variants with any complex trait might represent an oversimplification of the complex cellular orchestra.

In the meantime, technical progress using next generation sequencing (NGS) has enabled scientists to uncover genome-wide RNA expression specific to various cell types [193, 194]. Similar technologies are used to investigate epigenetic signatures, such as DNA-methylation and chromatin modification. Thus, for the very first time in research history, scientists are able to investigate cell-type-specific regulatory mechanisms genome wide. In line with biotechnological standards, the demands on the sports scientific study protocol must be strict. An approach using clear phenotypization of the trait of interest and its investigation on a cell-type-specific basis comparing trait-specific individuals versus controls is inevitable. For sports medicine, this means that we likely cannot investigate the epigenetic characteristics of endurance in a single study but will have to further divide the phenotype into 'sub-phenotypes'. In addition, adequate sample sizes with comparable backgrounds and analogy in the variants of more than three or four polymorphisms of interest must be the basis for effective studies evaluating the potentially epistatic impact of SNPs and epigenetic modifications in elite athlete populations. Without extensive knowledge of the interactions between these mechanisms, an integration of epigenetic factors into quantitative genetics is precipitate. On the other hand, all these techniques could also be used

to explain whether the individual's capabilities are predetermined *in utero* [195] or to what extent they are modifiable ontogenetically. Furthermore, we must clarify how much of what we consider to be 'long-term environmental influence' is operated epigenetically [12]. For sports medicine, this aspect could be further supplemented by data comparing epigenetic profiles of participants before, during and after intake of stimulants that are meant to enhance physical performance.

All in all, newly discovered information about interactions between genetic and epigenetic factors will force us to adjust our expectations of the extent to which an individual athlete's physical potential is predetermined [196]. It appears to be hardly possible to make a precise prediction of physical performance potential based upon present molecular genetic and epigenetic analyses. For future epigenetic studies, it is important to learn from the difficulties that occurred in genetic association studies. The effect of single epigenetic modifications in most cases only serves to fine tune gene expression; furthermore, in a cell-type-specific manner. Therefore, the investigation of associations between single DNA methylations or histone modifications will likely have even less impact than single-gene association studies in quantitative genetics. Currently, it is still a long way to a complete understanding of the processes that determine the differences in physical performance. A combination of the fields of genomics, epigenomics and transcriptomics along with improved bioinformatics tools and precise phenotyping is required for future research that will lead to an improved understanding of the inter-relations between exercise physiology, performance traits and disease. In that context, the field of systems biology, focusing on the dynamics of cellular networks through an integrated approach of mathematical models coupled to biological experiments, might represent a promising way in the future. A combined approach may provide an overall outlook that can potentially decode the molecular foundation of cellular processes and, finally, physical performance.

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