GENETIC AND EPIGENETIC PREDISPOSITION MARKERS OF VIRAL AND BACTERIAL INFECTIONS IN ATHLETES AND THEIR PREVENTION

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REVIEW

HIGHLIGHTS

- A link between genetic predisposition and viral infections (HBV, HCV, and HIV) has been established.
- Epigenetic functions such as DNA methylation and RNA silencing (miRNAs) can be triggered by bacterial and viral infections
- Genetic and epigenetic components can influence viral and bacterial infections in athletes involved in high activity exercise
- Epigenetic modifications based on miRNAs make circulating miRNAs suitable as biomarkers in endurance athletes

ABSTRACT

Genetic markers have been established for hepatitis C virus (HCV) and human immunodeficiency virus (HIV). Genome-wide analysis has revealed an association between common polymorphisms and susceptibility to viral infections. Epigenetic modifications have been linked to viral replication and latency, demonstrating aberrant promoter hypermethylation after infection with hepatitis B virus (HBV) and Epstein-Barr virus (EBV). Interestingly epigenetic modifications induced by bacteria have been demonstrated to regulate viral gene expression. Genetic predisposition has been linked to pro-inflammatory responses and dysregulated anti-inflammatory cytokine responses in athletes. Epigenetic hypermethylation can lead to up-regulation of inflammation-associated genes and regular modest exercise has shown reduction in chronic inflammation. Recently, it has been demonstrated that micro-RNA (miRNA)-based epigenetic modifications occurred in endurance athletes, which makes circulating miRNAs suitable as biomarkers. Studies on the association of genetic and epigenetic modifications and infectious diseases in athletes is still in its infancy and additional research is needed.

KEY WORDS *Markers; polymorphism; DNA methylation; micro-RNAs; viral infections; bacterial infections*

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INTRODUCTION

Recent progress in bioinformatics and genomics research has also strongly contributed to accumulated knowledge in the areas of bacterial and viral infections. This has had a substantial effect on therapeutic interventions and prevention of disease by vaccine development, but also demonstrated improvements of diagnostics by the discovery of a variety of markers. Interestingly, recent discoveries have revealed that not only genetic factors contribute to disease development, but epigenetic modifications play an important role in relation to health [1]. In this review, the genetic and epigenetic predispositions of markers for infectious agents are presented with a special emphasis on athletes. The potential of disease prevention is also discussed.

GENETIC PREDISPOSITION

Several genetic markers have been obtained for infectious diseases as described through examples below. In this context, genetic markers have been established for human T-cell leukaemia virus type 1 (HTLV-1), hepatitis C virus (HCV) and human immunodeficiency virus type 1 (HIV-1) [2]. For instance, particularly human leukocyte antigens (HLAs) have been linked to HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). It was demonstrated that genetic markers relate to host immune responses against different viral infections. Moreover, recent genome-wide studies have demonstrated the association between common polymorphisms and susceptibility to infectious diseases such as HIV-1, hepatitis B (HBV) and C viruses (HCV), dengue, malaria, tuberculosis, leprosy, meningococcal disease and prion disease [3]. In parallel, rare mutations have been linked to susceptibility of infectious disease. Related to polymorphisms, IL-28B was investigated for the link to chronic HCV [4]. It was demonstrated that the TBX21 rs4794067 variant genotypes showed a significant correlation with an increased risk of chronic HCV infection. Moreover, no association with susceptibility or spontaneous clearance of HCV was found for rs12979860, rs2227982 and rs36084323 polymorphisms.

Another area affecting the human immune defence against infectious and respiratory diseases is characterized by the microbiome, particularly the part covering viruses, called virome. The virome plays an important role in shaping innate and adaptive host immune defences, which may trigger many acute and chronic diseases [5]. In this context, the recently discovered Anellovirus family is a major component of the virome and acquired early in life in most human beings without causing any apparent disease. However, Anelloviruses modulate the innate and adaptive immune systems, which might induce the development of childhood respiratory diseases.

EPIGENETIC PREDISPOSITION

Epigenetics has been suggested to play an important role in infectious diseases. For instance, the influence of epigenetic regulation on gene expression related to virus and host interactions has revealed that in the case of viruses causing cancer, viral replication and latency have been demonstrated to be associated with epigenetic changes [6]. Moreover, epigenetic modifications in the form of aberrant promoter hypermethylation has demonstrated an association with human cancers [7]. In this context, viral genes play a crucial role in the regulation of DNA methylation. Therefore, cancers associated with HBV, simian virus 40 (SV40) and Epstein-Barr virus (EBV) trigger hypermethylation and tumor suppressor gene silencing. Interestingly, modern technologies developed for genome-wide detection of epigenetically regulated targets have allowed analysis of DNA hypermethylation, which should improve gene profiling of cancer and utilize epigenetic markers for detection, prognosis and therapeutic applications. In another study, it was demonstrated that epigenetic promoter hypermethylation resulted in the inactivation of the tumor suppressor gene p16INK4A [8]. Moreover, the p16INK4A-methylated tumors were positive for HBV or HCV markers, while no p16INK4A hypermethylation was detected in virus-negative tumors, suggesting that viral infections may be associated with hepatocarcinogenesis. Furthermore, the association between epigenetic input on interferon-λ3 (IFNλ3) promoter DNA methylation and association with response to HCV therapy has been investigated [9]. The study showed that HCV-GT1 infected individuals with the C/C genotype, presented lower methylation levels than both C/T and T/T genotypes, which suggested that the methylation status of the IFNλ3 promoter region might be useful for identification of patients more likely to relapse after HCV therapy.

Related to influenza virus-based activation and suppression of signaling pathways triggering immune responses, gene expression regulation by epigenetic modifications such as DNA methylation has been demonstrated to play an important part [10]. Chicken spleen, thymus and bursa were analyzed after infection with Guangdong (G-H5N1) and Anhui (A-H5N1) H5N1 strains, which revealed no difference in total DNA methylation of spleen genomic DNA compared to control chicken. In contrast, the total DNA methylation levels were significantly higher in the thymus and bursa in A-H5N1 infected chicken compared to the organs from G-H5N1 infected and control animals. The results provide the means for screening

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avian influenza virus resistance genes and methylation markers, analysis of epigenetic regulation mechanisms related to influenza virus infection and selective breeding for disease resistance. In the context of HIV, it was demonstrated that epigenetic modifications affect the cell machinery after viral infections [11]. The epigenetic changes were evaluated in peripheral blood mononuclear cells (PBMCs) and CD4+ cells by global DNA methylation, qPCR assays and western blots. The data showed a trend towards transcriptional repression in cells infected with HIV-1 and by qPCR genes related to epigenetic processes were identified. An interesting finding relates to the interaction of bacteria and viruses for induction of disease [12]. In this context, it has been demonstrated that epigenetic modifications induced by bacteria can regulate gene expression of viruses such as Kaposi's sarcoma-associated herpesvirus (KSHV), EBV and HIV. For instance, the latent infection in host cells can be reactivated by bacteria, which has been suggested to contribute to periodontal disease and AIDS. Moreover, interaction between bacteria and viruses has been indicated to play an important role in various cancers such as Kaposi's sarcoma, gastric cancer and head and neck cancer. The epigenetic modifications associated with viral disease are listed in Table-1.

Target	Action	Outcome	Reference
Hepatocarcinoma	Hypermethylation	HBV, HCV, EBV induce tumors	[8]
	Hypermethylation	Inactivation of p16INK4A tumor suppressor gene	[7]
HCV infection	DNA methylation	Genotype-specific reaction	[9]
Influenza virus infection	DNA methylation	Screening of influenza virus resistance genes	[10]
HIV infection	DNA methylation	Transcriptional repression in HIV-infected cells	[11]
Bacterial infections	Epigenetic modifications	Regulation of KSHV, EBV and HIV gene expression	[12]
Inflammatory diseases	Hypermethylation	Up-regulation of inflammation-associated genes	[22]
Pro-inflammatory cytokines	DNA methylation	Expression regulation of DNA methyltransferases	[22]
Cardiorespiratory fitness	miRNA expression modifications	Enhanced expression of miRNAs in elite athletes	[23]
Cardiovascular disease, cancer	DNA methylation	Lower disease risk in elite athletes	[24]

Table 1. Epigenetic modifications and viral infections leading to disease.

EFFECTS ON ATHLETES

The intense exercise regimen that athletes are subjected to presents dramatic effects on the immune system [13]. Intense endurance exercise results in an increase in neutrophils, T and B lymphocytes and natural killer cells in the systemic circulation leading to profound leukocytosis [14]. Moreover, several immune cell populations decrease in levels during post-exercise recovery. The most common type of illness in athletes is upper respiratory illnesses (URIs), which has led to investigation of the causes and application of biomarkers for identification of risk factors [15]. Moreover, nutritional modifications have been demonstrated to reduce the incidence of URIs.

The susceptibility of athletes to infectious diseases, multiple outbreaks of vaccine-preventable viral diseases have been monitored in professional athletes [16]. A total of 98 athletes, 62 Major League Baseball (MLB) and 36 National Basketball Association (NBA) players were screened for serologic evidence of immunity against measles, mumps, rubella and varicella designated as adequate (immune) or inadequate (equivocal or nonimmune) responses. The prevalence of inadequate immunity was 35.5% in MLB and 33.3% in NBA players for any virus. Moreover, there was a significantly enhanced risk of inadequate immunity against rubella (risk ratio 6.38; $P < 0.01$) and varicella (risk ratio 4.21; $P < 0.01$) in athletes in comparison to the control group in the study. Additionally, younger players showed a greater risk of inadequate immunity against varicella. Furthermore, as URIs account for 35-65% of non-injury related illnesses in high-performance athletes, it is important to identify the sources and risks of upper respiratory symptoms (URSs) to prescribe preventive clinical training and lifestyle strategies [15]. The majority of URIs are related to common respiratory viruses while bacterial respiratory infections are rare in athletes. Indications from preliminary studies claim a genetic predisposition to a pro-inflammatory response and dysregulated anti-inflammatory cytokine responses. Related to URSs in endurance athletes, the effect of daily ingestion of probiotics, in the form of *Lactobacillus casei* Shirota, was evaluated in a placebo-controlled randomized trial [17]. The study revealed an unexpectedly low incidence of URS episodes in university athletes with no significant differences in daily digestion of probiotics (PRO) and

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placebo (PLA). Moreover, the duration and severity of URS episodes showed no difference between the two groups. Antibody titers in cytomegalovirus (CMV) seropositive individuals decreased over time in the PRO group but remained unchanged in the PLA group. Similar results were found for EBV antibody titers in EBV seropositive athletes. Although the PRO intake did not reduce the incidence of URS episodes, decrease in CMV and EBV antibody titers indicated a benefit to overall immune status.

As the relationship between vitamin D in serum (25[OH]D) and bone health has been established in non-athletic individuals, the association of Vitamin D with extra-skeletal tissues such as muscle and the immune system has been addressed as ways of modulation of recovery from damaging exercise and infection risk [18]. However, no relationship between serum 25(OH)D and bone health could be established. For instance, black athletes were discovered to possess a low serum (25[OH]D) concentration without any physiological consequences [18]. The reason for this relates to genetic differences in vitamin D binding protein based on ethnicity, which generates higher concentrations of bioavailable vitamin D.

GENETIC AND EPIGENETIC FINDINGS IN ATHLETES

There are several studies demonstrating genetic and epigenetic involvement in susceptibility of infectious disease. For instance, it has been demonstrated that athletes involved in high levels of exercise can trigger temporary immunosuppression, which may increase the susceptibility to upper respiratory tract infections [19]. Blood and saliva samples collected from 10 healthy kayakers were analyzed for the levels of secretory immunoglobulin (sIgA) and interleukin-5 (IL-5). No significant correlation between genome expression and sIgA concentration was observed. In contrast, athletes with high IL-5 concentration showed low expression of secretglobin 1C1 (SCGB1C1), a gene involved in protection against the common cold, suggesting that the SCGB1C1 gene could be used as a biomarker for susceptibility of upper respiratory tract infections in athletes.

An interesting finding relates to telomere-regulating genes in endurance athletes [20]. Quantification of leukocyte telomere length and analysis of expression of telomere-regulating genes showed significantly longer leukocyte telomeres and up-regulated telomerase reverse transcriptase (TERT) and tripeptidyl-1 (TPP1) mRNA expression in endurance athletes compared to healthy controls. However, the telomere length and telomere-regulating gene expression profiles were no longer statistically significant after adjustment for resting heart rate and maximum oxygen consumption (VO2 max). Furthermore, individuals in the study were divided into three subgroups subjected to running and cycling activities, which demonstrated that the telomeres were longer in individuals who covered the longest and middle distances. The study supported the importance of cardiorespiratory fitness for prevention of biological aging.

In another approach, footballers were subjected to nasal swabs for the isolation of *Staphylococcus aureus* strains [21]. In comparison to the control group of healthy volunteers, the footballers showed a statistically significant difference between the frequency of mecA and slime genes, which facilitated the identification of coagulase-positive and -negative staphylococci and early and accurate diagnosis.

Related to epigenetics, the association between exercise and inflammation-related epigenetic modifications has been demonstrated for DNA methylation [22]. However, on one hand, hypermethylation can cause up-regulation of inflammation-associated genes, while on the other hand, pro-inflammatory cytokines can regulate the expression of DNA methyltransferases. In the context of exercise, it needs to be investigated whether intense exercise induces the inflammatory state and potentially predispose athletes to disease. In contrast, as regular moderate exercise has been associated with a reduction in chronic inflammation, it may be due in part to favorable epigenetic modifications.

As an example of epigenetic modifications, it has been demonstrated that micro-RNAs (miRNAs) influence exercise-induced health and performance adaptations [23]. Five muscle-enriched miRNAs (miR-1, miR-133a, miR-181a, miR-486 and miR-494) were evaluated in a long-term strenuous aerobic exercise training, which demonstrated that endurance athletes exhibited enhanced expression of mi-R1, miR-486 and miR-494 in comparison to individuals in the control group consisting of 19 healthy young men. Moreover, the levels of miR-1, miR-133a and miR-486 were decreasing immediately after the maximal aerobic exercise. The study also showed a positive correlation between miRNA abundance and maximum oxygen uptake, but an inverse correlation between miR-486 and resting heart rate. In summary, circulating miRNAs can serve as biomarkers for cardiorespiratory fitness and disease.

FUTURE ASPECTS OF GENETICS AND EPIGENETICS IN RELATION TO ATHLETES

The recent progress in genomics and epigenomics research has provided tools to analyze the effects of genetic and epigenetic functions on disease, which was thought not to be possible only a decade ago. This

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has provided new approaches to investigate the effects of genetics and epigenetics on athletes and how biomarkers can support future risk estimation and prevention of infectious and other diseases. In this context, prediction systems have been developed for accurate age estimation based on DNA methylation data [24]. When age-correlated DNA methylation markers were analyzed, it was demonstrated that elite athletes showed a pattern of several years of aging compared to control individuals. Particularly, DNA hypermethylation in athletes involved in power sports for two CpG sites in the TRIM59 and KLF14 genes was substantial. Due to the known anti-tumor and anti-inflammatory activities associated with TRIM59 and KLF14, indicated that intense physical training presents a complex influence on aging and can also contribute to a lower risk of cardiovascular disease and cancer in elite athletes.

An important point to address relates to the misuse of genetics and epigenetics for doping purposes. In this context, more than 200 fitness genes [25, 26] have been identified, which can be the targets for manipulations of genes, genetic elements and genetically engineered cells to enhance athletic performance [27].

Finally, affordable high throughput sequencing in combination with efficient data handling and bioinformatics will support genetic and epigenetic studies on athletes and will allow detailed analysis of the risk of infectious bacterial and viral diseases and the means of prevention. It will also allow the foundation of athleticogenomics as an area of research.

CONCLUSIONS

Several studies have confirmed the link between genetic predisposition and viral infections such as HBV [3], HCV [2, 3], and HIV [2]. Similarly, epigenetic functions including DNA methylation [7, 9, 10] and miRNA-based gene silencing [23] have been demonstrated to be triggered by viral and bacterial infections. Several studies have investigated the occurrence of infectious diseases in athletes in comparison to control groups and the effect of exercise on resistance to infections [13-15]. Genetic and epigenetic components have been analyzed and demonstrated to be subjected to influence viral and bacterial infections in athletes involved in high activity exercise. Unfortunately, there is only a limited amount of information available in this area today. However, the establishment of atheletogenomics bodes well for acceleration of future studies in the area, which will support the establishment of both genetic and epigenetic markers to monitor the health of elite athletes and also aid in the well-being of the general population involved in regular exercise.

CONFLICT OF INTEREST
The author declares no competing interests.

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