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

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# **The Athlome Project Consortium: A Concerted Effort to Discover Genomic and other “OMIC” Markers of Athletic Performance.**

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**The Athlome Project Consortium: A Concerted Effort to Discover Genomic and other “OMIC” Markers of Athletic Performance.**

**The Athlome Project Consortium\***

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 (SNPs) 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. Thus, alternative large-scale, collaborative efforts involving well-phenotyped male and female cohorts from which high-resolution genome-wide data is generated and interrogated using advanced bioinformatics approaches are necessary for meaningful progress to be made. Accordingly, a symposium was held in Athens and on the Greek island of Santorini from 14-17th May 2015 (<http://celebratorysymposium.net>) 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., 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*.

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 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; and
4. To conduct functional investigations following replicated findings (e.g., study the replicated 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 predisposition, epigenomic, transcriptomic and proteomic analyses need also be carefully planned to strengthen the understanding of gene functions. Linking these findings with metabolic profiling (the end products of the cellular processes) is also a future aspiration of the Athlome Project. Another challenge is to be able to efficiently integrate the multiple “omics” datasets generated from the different approaches. The ultimate goal of the Athlome Project Consortium is to generate the ethically sound environment, interest and capacity needed to develop the specialist knowledge to inform personalized training and injury prevention, as well as doping detection. The following individual or collaborative studies have agreed to work together in the global partnership that constitutes the Athlome Project Consortium. The participating cohorts and the focus of each are depicted in Figure 1.

#### **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  $n = 8,228$  athletes and  $n = 4,121$  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 ( $\leq 400$  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, RUS), Svitlana B Drozdovska (National University of Physical Education of Ukraine, UKR), Colin N Moran (University of Stirling, GBR), Valentina Ginevičienė (Vilnius University, LTU), Andrei A Gilep (Institute of Bioorganic Chemistry NASB, BLR).

**ELITE** [elite.stanford.edu](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 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 ( $\dot{V}O_2\text{max}$ ). The main inclusion criterion is  $\dot{V}O_2\text{max} > 75$  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 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 gender 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: (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.

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

#### **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 Centre, Louisiana State University, USA), Noriyuki Fuku (Juntendo University, JPN), Yannis Pitsiladis (University of Brighton, GBR), Bernd Wolfarth (Humboldt University, DEU), Alejandro Lucia (Universidad Europea de Madrid, SP).

#### **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  $\dot{V}O_2\text{max}$  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.

*Principal Investigators:* Claude Bouchard, Tuomo Rankinen (Pennington Biomedical Research Centre, Louisiana State University System, USA), Bernd Wolfarth (Department of Sports Medicine, Charite Medical School, Berlin, Germany), 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: (i) to increase 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 practical impact of GENESIS.

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

## **Gene SMART Study** [www.vu.edu.au/speed-gene](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 weeks of High-Intensity Interval Training (HIIT) in three different training centres. While the lead training and testing centre is located in Victoria University, Melbourne, two other centres have been launched at Bond University, Australia and the University of Sao Paulo, Brazil. A fourth centre (University of Brighton, UK) will focus on the omics analyses. The cohort is comprised of moderately-trained, healthy male participants (aged 20-45 years, body mass index  $\leq 30 \text{ kg/m}^2$ ). 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 enzymes 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 post-training testing and sampling for all participants.

*Principal Investigators:* David Bishop, Nir Eynon (Victoria University, 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, 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, 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, ZAF), Nir Eynon (Victoria University, AUS), Pawel Cieszczyk (University of Szczecin, 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,  $\dot{V}O_2\text{max}$ ). The cohort is comprised of 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, approximately 200 muscle biopsies were obtained from both athletes and controls in order to investigate genetic variants associated with muscle fibre 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+ 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 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. 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.

*Principal Investigators:* Eco de Geus, Meike Bartels (VU University and VU medical centre, NLD).

## **POWERGENE**

The POWERGENE consortium aims to characterise 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 U.S.A., Brazil, and Russia. They will be compared with sub-elite 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 ( $\leq 800$  m) and field (jump, throw) events, cycling (track), swimming ( $\leq 200$  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, GBR), Kathryn North (Murdoch Childrens Research Institute, AUS), Nir Eynon (Victoria University, AUS).

## **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 consented 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 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 Georgakopoulos, Mohammed Alsayrafi (ADLQ, QAT), Francesco Botre (FMSI, ITA), Karsten Suhre (Weill Cornell Medical College in Qatar, QAT), Mike Hubank (University College London, GBR).

### **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 by not only 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, GRC), Yannis Pitsiladis (University of Brighton, GBR).

### **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 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 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 Athlome Project**

The 1000 Athlome Project aims to sequence 1000 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 1000 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 Athlome project will serve 1) as a reference panel for future performance studies, and 2) to guide other extreme phenotype studies in medical science.

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

### **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: (i) the consortia are global in reach but there is no universal agreement on the precise nature of ethically justifiable governance for biobanking; (ii) given the globality of the consortia, no single regional (e.g., European, American) framework ought to be adopted; (iii) 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 bio-guardians 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 has been approved by relevant regulatory authorities (e.g., IRBs, RECs 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 und(er)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. In order to preserve the integrity of this process and the principles, rigorous anonymisation 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 re-identifying their data and genomic sequences. The independent institution would also have an oversight of each new proposal for the Athlome project going forward in order 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 it is our intention to show a global view not only 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 which

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, 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 programmes and in the predisposition to injury is certain to provide useful information to prescribe personalised exercise interventions in the context of 21<sup>st</sup> 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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669

Figure Legends:

**Figure 1. The Athlome Project Consortium.** Genomic, epigenomic, transcriptomic, proteomic and metabolomic studies are being conducted by the participating centres to address questions in the three main research areas: elite performance, training response, and injury. Future investigations planned include genetically modified studies.



