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Research report

A candidate syntenic genetic locus is associated with voluntary exercise levels in mice and humans

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HIGHLIGHTS

- Identification of mouse QTL (Chr2) for voluntary running wheel activity (RWA).
- RWA is considered a model of voluntary exercise (VE) in humans.
- Human region 20q13.2 is syntenic to mouse QTL on Chr2.
- Genetic variants within 20q13.2 are associated with VE.
- This was demonstrated in two independent human cohorts.

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ABSTRACT

Individual levels of physical activity, and especially of voluntary physical exercise, highly contribute to the susceptibility for developing metabolic, cardiovascular diseases, and potentially to psychiatric disorders. Here, we applied a cross-species approach to explore a candidate genetic region for voluntary exercise levels. First, a panel of mouse chromosome substitution strains was used to map a genomic region on mouse chromosome 2 that contributes to voluntary wheel running levels – a behavioral readout considered a model of voluntary exercise in humans. Subsequently, we tested the syntenic region (HSA20: 51,212,545–55,212,986) in a human sample (Saint Thomas Twin Register; $n = 3038$) and found a significant association between voluntary exercise levels (categorized into excessive and non-excessive exercise) and an intergenic SNP *rs459465* (adjusted *P*-value of 0.001). Taking under consideration the methodological challenges embedded in this translational approach in the research of complex phenotypes, we wanted to further test the validity of this finding. Therefore, we repeated the analysis in an independent human population (ALSPAC data set; $n = 2557$). We found a significant association of excessive exercise with two SNPs in the same genomic region (*rs6022999*, adjusted *P*-value of $P = 0.011$ and *rs6092090*, adjusted *P*-value of 0.012). We explored the locus for possible candidate genes by means of literature search and bioinformatics analysis of gene function and of trans-regulatory elements. We propose three potential human candidate genes for voluntary physical exercise levels (*MC3R*, *CYP24A1*, and *GRM8*). To conclude, the identified genetic variance in the human locus 20q13.2 may affect voluntary exercise levels.

1. Introduction

Low level of voluntary exercise (one of types of physical activity) in modern societies is considered to be one of the most profound risk factors for the development of obesity and cardiovascular diseases, among other illnesses [1–3]. Conversely, appropriate level of exercise is believed to have broad beneficial effect on human health. Despite the accumulating evidence and common knowledge regarding benefits of physical activity, only a minority of the Western population engages in sufficient physical activity to experience its benefits [4,5]. Broadened understanding of the mechanisms that influence levels of physical activity could help in improving the existing health programs. However, participation in physical activity is influenced by an array of factors

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including biological, psychological, cultural and environmental ones. Furthermore, many of these factors influence various complex phenotypes which may be correlated (e.g. obesity and levels of physical activity). This complexity hampers efforts to understand biological processes underlying physical activity. One of the possible ways to unravel the biological pathways involved in physical activity is to investigate the genetic basis of this highly heritable phenotype [5–8] under controlled genetic and environmental conditions [9].

Voluntary exercise is a complex phenotype and various definitions [6,10] and operationalizations may be used in studies to examine it, e.g. direct observation, questionnaires (diaries and retrospective recall), surveys, calorimetry, heart rate monitors and motion sensors [10,11]. These assessment methods differ in terms of feasibility and reliability, therefore, the results obtained by the use of various methods do not necessarily correlate [6,10]. Use of animal models may, to some extent, help to standardize the complex conditions influencing the levels of physical activity and methods of measurement. In rodent studies, voluntary running wheel activity (RWA) in a home cage was proposed to be the most appropriate model for voluntary exercise in humans [5,12]. Indeed, if one defines voluntary exercise as locomotor activity "that is not directly required for survival or homeostasis and not directly motivated by any external factor" [10], than voluntary RWA is indeed the most suitable rodent behavior to model human voluntary exercise (although some researchers would not agree [13]). Studying RWA in various mouse lines which differ in expression of this phenotype allows systematic genetic studies on this complex trait.

Previous studies proved that genetic factors have an influence on the levels of physical activity [14]. Heritability of physical activity in humans was assessed repeatedly [6]. Multiple studies pointed to genes associated with physical activity levels in humans [7,15–17], and various linkage and genome wide association studies (GWAS) in humans were able to point to genetic regions associated with physical activity levels [6]. Furthermore, it is possible to selectively breed lines of mice based on their high or low physical activity [18]. Finally, numerous studies using RWA in mice as an animal model for physical activity, pointed to promising candidate genes and genetic regions [19–23]. Nevertheless, despite the relative (in comparison to other complex phenotypes) ease of operationalization of physical activity in humans and translation of this phenotype to an animal model, there is virtually no overlap between the results obtained from rodent and human genetic studies for voluntary activity [6]. Therefore, there is a need for research aiming at the translation of genetic findings from animal to human studies.

In the current study, we aimed at identifying a narrow candidate genetic region contributing to physical activity levels. For this purpose we used a cross-species approach. First, we used a panel of mouse chromosome substitution strains (CSS, also called consomic strains or lines) [24] that enable identification of candidate genetic regions for complex traits, such as voluntary RWA. Later, we tested this discovered candidate region for mouse RWA in two independent human populations. Based on the integrated mouse-human approach, we propose new candidate genes potentially contributing to the individual levels of physical activity.

2. Methods

2.1. Ethical statement

All animal experiments were approved by Animal Experiments Committee of the Academic Biomedical Centre, Utrecht-The Netherlands. The Animal Experiments Committee based its decision on 'De Wet op de Dierproeven' (The Dutch 'Experiments on Animals Act'; 1996) and on the Dierproevenbesluit' (The Dutch 'Experiments on Animals Decision'; 1996). Every effort was made to minimize animal suffering.

The relevant institutional review boards or ethics committees approved the research protocol of the individual population based studies used in the current analysis. The study involving participants enrolled to TwinsUK was approved by the St Thomas' Hospital research ethics committee. Ethical approval for the study involving ALSPAC dataset was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. All human participants and also, in case of the minors, their first kin gave written informed consent.

2.2. Animal studies

2.2.1. Mice

Initial breeding pairs for CSS and their progenitors A/J (A) and C57BL/6J (B6) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). The advantages of using CSS strains as well as the steps required to generate a CSS panel has been described previously [25]. All mice were bred in the Rudolf Magnus Institute of Neuroscience animal facility and were 11–13 weeks old at the start of the experiment (lights on at 2:00 a.m. off at 2:00 p.m., temperature $22.0 \pm 2.0\,^{\circ}C$, fed ad libitum). In total, 384 female mice were tested in the experimental procedure; B6 strain ($n = 36$), A strain ($n = 23$) and of the 19 tested CS strains ($n = 257$; median of 12 mice per CS strain), CSS2F2 (F_2-intercross between C57BL/6J-Chr 2^A/NaJ (CSS2) and B6) ($n = 68$). Mice from CSS16 were not tested due to low availability. The low number of mice in the F_2 population was adequate for proper analysis due to the used methodology. The consomic F_2 cross approach is a specifically designed to identify single QTLs on a single chromosome as there is a strong reduction in the amount of epistatic interactions with loci from the other 20 chromosomes. Because of this sensitivity, significant less F_2 mice are necessary to identify these QTLs when compared to the traditional whole genome intercross mapping approach (this has been indicated elegantly by the laboratory that generated the consomic mice [24]. In addition QTL analysis was performed by MQM-mapping (multiple-QTL-model or marker-QTL-marker) which is more powerful than the traditional interval mapping approach [26]. The levels for physical activity, such as voluntary RWA, are known to be very different in males and females. As we are interested in the genetics of physical activity levels in relation to eating disorders with a gender pre-dominance in females (such as in anorexia nervosa) [27], we decided to perform this genetic screen in females.

2.2.2. Running wheel activity measurement in mice

Mice were maintained in running wheel cages (wheel circumference: 43.96 cm; surface made of metal rods) for a week. We did not observe coasting of mice in the running wheels. Individual wheel running revolutions were automatically registered. The average of the RWA for the days 6 and 7 was used in the QTL analysis. This was done because mice require time to adapt to the running wheel cages and to develop stable RWA pattern. Patterns of the RWA for CSS2, A, B6 mice as well as for mice from the F_2 populations are present in Figure A1.

2.2.3. DNA samples, genetic marker analysis and map construction

Genomic DNA was isolated from spleen and/or tail from F_1-hybrids, F_2-intercross mice and CSS2 and B6 mice, using a phenol/chlorophorm/iso-amylalcohol protocol [28]. A total of 14 microsatellite markers (D2Mit117, D2Mit417, D2Mit370, D2Mit458, D2Mit156, D2Mit380, D2Mit94, D2Mit66, D2Mit206, D2Mit525, D2Mit493, D2Mit51, D2Mit113, D2Mit148), dispersed throughout mouse chromosome 2, was used to map a region on chromosome 2 associated with RWA in the F_2 population.