Association between gene polymorphisms and obesity and physical fitness in Korean children

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ABSTRACT: Obesity is affected by genetic factors and environmental influences. This research was undertaken to identify single nucleotide polymorphisms (SNPs) related to obesity and physical fitness and then to analyse and compare interactions between physical fitness and obesity-associated genotypes. To investigate relationships between physical fitness and major SNPs previously reported to be related to obesity, 68 SNPs in 32 genes were genotyped in 71 Korean children. Tests were conducted to evaluate five elements of physical fitness (speed, aerobic endurance, muscular endurance, muscular strength, and flexibility). The results obtained showed significant ($P<0.02$) differences in physical fitness scores for the following genotypes: *CNR1* (rs1049353; GG), *LEP* (rs7799039; AA+AG), *HHEX* (rs1111875; TT), *GC* (rs16847015; TG+GG), *LRP5* (rs4988300; GG+GT), *NPY2R* (rs2880415; CT+CC), *PPY* (rs231472; GG), *UCP2* (rs660339; CT+TT), *CDKN2B* (rs10811661; AA+AG), and *ADIPOQ* (rs266729; CG+GG). Ten physical fitness-related genotypes were newly identified during the present study. This study suggests that classification of genotypes by physical fitness level could be used as an index for predicting the risk of obesity and for selecting individuals for intervention programmes. Furthermore, the study shows that even children participating in the same physical fitness improvement programme can exhibit different genotype dependencies.

INTRODUCTION

Obesity and its related chronic diseases profoundly affect physical fitness, and conversely physical fitness significantly prevents and reduces the risk of obesity [1,2]. In addition, people with higher levels of physical activity or physical fitness have lower risk factors for the development of cardiovascular disease, which suggests that a lack of physical fitness is an important cause of chronic diseases [3-5]. Physical fitness is affected by hereditary and acquired factors, and interactions between genes and lifestyle factors, such as physical activity, have been shown to affect obesity [6,7]. Physical fitness improvement programmes can have quite different impacts on the prevention or management of obesity, and these impacts appear to depend on hereditary diversity, which suggests that hereditary factors need to be considered when developing physical fitness programmes for the management of obesity. Recently, obesity-related and physical performance and fitness-related human genes were

identified [8,9]. The authors supposed that obesity-related hereditary factors might be associated with physical fitness, and carried out comparisons to identify overlapping genes related to obesity. One hundred and twelve of 127 candidate genes were found to be related to exercise and physical fitness, and 42 were commonly involved in both phenotypes. Thus, it appears that these genes may be related to degree of obesity and to differences in levels of physical fitness, such as endurance, muscle strength and training response. In another study, the effects of genetic variants of the *FTO* gene (fat mass and obesity associate gene) were found to be suppressed by exercise, which suggested that exercise can reduce an inherited propensity to become obese [7]. Furthermore, these findings indicate that physical fitness programmes might suppress the expressions of genotypes associated with the risk of becoming obese with high levels of physical fitness, and that medical management programmes

should be favoured for those with genotypes associated with the risk of becoming obese and low levels of physical fitness.

Although genetic and environmental factors contribute to the development of obesity and/or physical fitness, gene-environmental interactions remain elusive. Children seem to be more appropriate than adults for evaluations of the effects of hereditary factors on physical fitness, because environmental factors have less influence on children [7]. However, few studies on relations between genes and performance and health-related fitness have been conducted with children [10]. Therefore, we hypothesized that some genes are related to both obesity and physical fitness, and that genotypes of these genes can be classified by degree of obesity and physical fitness in children. In the present study, we attempted to identify single nucleotide polymorphisms (SNPs) related to obesity and physical fitness, and then conducted comparative analysis on interactions between physical fitness and obesity-associated genotypes in children.

MATERIALS AND METHODS

Subjects

This study was performed using a cross-sectional design between March 2013 and November 2013, in a tertiary hospital (Yangsan, South Korea). The study participants were children who volunteered to participate in this study in response to a poster advertisement placed in a primary school (Busan, South Korea). The study was approved by the Institutional Review Board of Kyungnam University (2015-041-HR-03), and complied with the 1964 Declaration of Helsinki. Informed consent was obtained from all parents and participants. Participants received financial reimbursement. The study exclusion criteria were as follows: obesity secondary to hypothyroidism or Cushing's disease, a severe debilitating disease, or treatment with any anti-obesity agent or experience of weight loss during the previous 6 months. The study population was composed of 71 ten-year-old children (31 boys and 40 girls). Children were allocated to a low group or a high group using physical fitness profile cut-off scores as described in the National Survey of Physical Fitness conducted by the Korea Institute of Sport Science in 2004 [Ministry of Culture and Tourism. (2004). A national survey on physical activity participation in Korea. Seoul, South Korea]. The data collected included age, gender, anthropometric measurements, physical fitness profiles, and polymorphism genotypes.

Anthropometric measurements

Heights and weights were measured using an automatic height-weight scale to the nearest 0.1 cm and 0.1 kg, respectively, and body mass index (BMI) values were calculated by dividing weight (kg) by height squared (m^2).

Measurements of physical fitness

Physical fitness profiles were determined using five measuring instruments: (a) 50-meter sprint, (b) 1000-metre run, (c) one-minute sit-up, (d) standing long jump, and (e) a sit and reach flexibility test,

that is, using measures of speed, aerobic endurance, muscular endurance, muscular strength, and flexibility, respectively. Fifty-metre sprints and 1000-metre runs were recorded in 0.1 and 1 second increments, respectively. One-minute sit-up scores were calculated using the number of sit-ups performed in one minute. For this test, the participant lay supine on the floor with knees bent at a 45-degree angle. A full sit-up started from the supine position, and ended with participants sitting with a vertical trunk and elbows touching the knees. Standing long jump distance was measured from a take-off line to the nearest point of contact on landing (back of the heels). The longest distance jumped for three attempts was recorded. For the sit-and-reach flexibility test, participants, sitting barefoot on the floor with legs out straight ahead, were instructed to lean forward slowly as far as possible, toward a graduated ruler held on the box from -25 to +25 cm, without bending their knees, and to hold maximum stretch for 2 s [11]. Scores were recorded as distances reached before or beyond the toes. The test was repeated twice with a rest period of 10 s [12], and the best results were recorded to the nearest 1.0 cm. All measurements were made by skilled testers.

Genotyping of polymorphisms

Approximately 20 ml of ethylenediaminetetraacetic acid-treated whole blood was obtained for DNA extraction from an antecubital vein in the morning after a 12-h overnight fast, and then centrifuged at 3000 rpm for 10 minutes at 4℃. Supernatants were transferred to Eppendorf tubes and stored at -70℃ until required for DNA extraction, which was conducted using 1-2 ml supernatant samples using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Seoul, Korea) according to the manufacturer's instructions. The final elution volume for DNA extraction was 60 μl and the amount of plasma DNA used for mutation testing was 30 ng. 68 SNPs in 32 genes were selected after carefully reviewing previous studies for gene variants associated with obesity [9]. As a representative example, children with CNR1 genotype (GG) had a significantly higher BMI than children with CNR1 genotype (AG+AA) (17.2 ± 2.2 kg/m^2 vs. 20.5 ± 3.6 kg/m^2, P=0.0001). SNPs were genotyped using SNP-IT assays and the SNPstream 25K System (Orchid Biosciences, Princeton, NJ, USA): *NPY2R* (rs12507396, rs6857715, rs1047214, rs33977152, rs6857530, rs2880415), *GC* (rs16847015, rs17467825, rs3733359, rs705117, rs222003, rs1491711, rs222020, rs222042), *CNR1* (rs1535255, rs806379, rs2938392, rs1175542, rs9353527, rs806370, rs1049353), *ADIPOQ* (rs2241766, rs1501299, rs266729), *LEPR* (rs1137100, rs1137101, rs1805096), *LEP* (rs7799039), *LRP5* (rs4988300), *ADRB2* (rs1042714, rs1042717, rs1042718, rs1042719), *PPY* (rs231471, rs231472, rs162430, rs1058046, T323C), *INSIG2* (rs7566605), *POMC* (rs1866146, rs1009388), *PPARG* (rs3856806, rs1801282), *ENPP1* (rs1044498, rs7754561, rs1799774), *IGF2BP2* (rs4402960, rs1470579), *GAL* (rs3136540, rs1042577), *GHRL* (rs34911341, rs4684677), *SLC30A8* (rs13266634), *FLJ39370* (rs17044137), *BBS2* (rs4784675), *BBS4* (rs7178130),

BBS6 (rs6108572), *UCP1* (rs1800592), *UCP2* (rs660339), *HHEX* (rs1111875), *SCG3* (rs3764220), *MCHR1* (rs133074), *PKN2* (rs6698181), *TCF7L2* (rs7903146), *APOA5* (rs662799), *CDKN2B* (rs10811661), *CDKAL1* (rs7754840), and rs16846971.

Briefly, genomic DNA regions spanning the polymorphic site of interest were amplified using one phosphorothioate primer and one regular PCR primer. Amplified PCR products were then digested with exonuclease (Amersham Biosciences, Uppsala, Sweden). One primer containing phosphorothioate linkages at its 5′ end was used to protect one strand of PCR products from exonuclease digestion, which resulted in the generation of a single-stranded PCR template. The single-stranded PCR template generated by exonuclease digestion was overlaid on a 384-well plate precoated covalently with primer extension primers (SNP-IT primers), which were designed to hybridize immediately adjacent to polymorphic sites. After hybridizing template strands, SNP-IT primers were extended by a single base using DNA polymerase at polymorphic sites of interest. The extension mixtures contained two labelled terminating nucleotides (FITC, and biotin) and two unlabeled terminating nucleotides. The identities of the incorporated nucleotide were determined using serial colorimetric reactions using anti-FITC-AP (Roche, Basel, Switzerland) and streptavidin-horseradish peroxidase (HRP) (Pierce, Rockford, IL, USA). Development of yellow or blue colour was analysed using an ELISA reader and final genotyping calls were made using QCReview software.

TABLE 1. Demographic and anthropomorphic characteristics of the study participants.

Variables	Mean	SD	Range
Age (years)	10.6	0.5	1.0
Height (cm)	147.1	6.8	27.2
Weight (kg)	44.1	10.7	49.9
Body mass index (kg/m^2)	19.3	3.5	15.3

TABLE 2. Relations between physical fitness and obesity-related genotypes.

Physical Fitness	Gene	SNP	Genotype Frequency (%)		χ^2	P (P)
			LG	**HG**		
Speed	*CNR1*	rs1049353	**GG** 18(66.7)	40(90.9)	6.574	0.010 (0.016)
			AG+AA 9(33.3)	4(9.1)		
Aerobic endurance	*LEP*	rs7799039	**AA+AG** 56(96.6)	10(76.9)	6.250	0.012 (0.016)
			GG 2(3.4)	3(23.1)		
	HHEX	rs1111875	CC+CT 26(44.8)	11(84.6)	6.737	0.009 (0.016)
			TT 32(55.2)	2(15.4)		
Muscular endurance	*GC*	rs16847015	TT 0(0.0)	3(13.0)	6.134	0.013 (0.016)
			TG+GG 47(100)	21(87.0)		
	LRP5	rs4988300	**GG+GT** 47(100)	21(87.0)	6.007	0.014 (0.016)
			TT 0(0.0)	3(13.0)		
	NPY2R	rs2880415	TT 22(46.8)	20(83.3)	8.772	0.003 (0.015)
			CT+CC 25(53.2)	4(16.7)		
Muscular strength	*PPY*	rs231472	**GG** 33(63.5)	6(31.6)	5.714	0.017 (0.017)
			CG+CC 19(36.5)	13(68.4)		
	UCP2	rs660339	CC 13(25.0)	12(63.2)	8.881	0.003 (0.015)
			CT+TT 39(75.0)	7(36.8)		
Flexibility	*CDKN2B*	rs10811661	**AA+AG** 36(92.3)	22(68.8)	6.522	0.011 (0.016)
			GG 3(7.7)	10(31.2)		
	ADIPOQ	rs266729	CC 13(34.2)	**21(67.7)**	7.680	0.006 (0.016)
			CG+GG 25(65.8)	10(32.3)		

LG: low fitness level group, HG: high fitness level group. **Bold characters** indicate significantly high frequency genotypes.
P for raw P values; (P) for false discovery rate (FDR)-adjusted P values

Statistical analyses

Data are presented as means ± SDs or as frequencies (%). The chi-square test was used to test differences between the low and high groups for each SNP genotype and allele. Multivariate multiple logistic regression was used to control for possible confounding by age and gender. SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL) was used for the analysis, and P values of < 0.02 were deemed significant because of the comparatively small size of the study population. Raw P values were adjusted using the Benjamini and Hochberg method (13) to control for the false discovery rate (FDR). All statistical tests were two-sided.

RESULTS

Relations between the five elements of physical fitness and the 68 obesity-related genotypes were analysed. Table 1 summarizes the basic characteristics of the study sample.

Speed

Fifty-meter sprint test scores were used to measure speed and acceleration. Table 2 shows the relation between speed and the obesity-related genotype. In particular, the *CNR1* (rs1049353) genotype (GG, AG+AA) was found to be significantly associated with dash test scores (FDR-adjusted P=0.016). Children with the GG genotype had significantly better speeds and accelerations.

Aerobic endurance

Endurance test scores were used to measure aerobic endurance and fitness. Table 2 shows relations between aerobic endurance and obesity-related genotypes. In particular, the *LEP* (rs7799039) (AA+AG, GG) and *HHEX* (rs1111875) (CC+CT, TT) genotypes were found to be significantly associated with endurance scores (FDR-adjusted P=0.016 for both). Children with the AA+AG genotype of *LEP* (rs7799039) or the TT genotype of *HHEX* (rs1111875) had poorer aerobic endurances and fitness.

Muscular endurance

One-minute sit-up scores were used to measure the strength and endurances of abdominal and hip-flexor muscles. Table 2 shows relations between muscular endurance and obesity-related genotypes. The *GC* (rs16847015) genotype (TT, TG+GG), the *LRP5* (rs4988300) genotype (GG+GT, TT), and the *NPY2R* (rs2880415) genotype (TT, CT+CC) were found to be associated with sit-up scores (P<0.02). Children with the TG+GG genotype of *GC* (rs16847015), the GG+GT genotype of *LRP5* (rs4988300), or the CT+CC genotype of *NPY2R* (rs2880415) showed poorer abdominal and hip flexor strength and endurance.

Muscular strength

The standing-long-jump test was used as a measure of leg muscle muscular strength and explosive power. Table 2 summarizes relations between standing-long-jump scores and obesity-related genotypes. In particular, the *PPY* (rsrs231472) (GG, CG+CC) and the *UCP2* (rs660339) genotypes (CC, CT+TT) were found to be significantly associated with standing-long-jump scores (P<0.02). Children with the GG genotype of *PPY* (rs231472) or the CT+TT genotype of *UCP2* (rs660339) had poorer performances than those with the CG+CC genotype of *PPY* or the CC genotype of *UCP*.

Flexibility

Sit-and-reach flexibility test scores were used as a measure of lower back and hamstring muscle flexibilities. Table 2 shows relations between sit-and-reach scores and obesity-related genotypes. The *CDKN2B* (rs10811661) (AA+AG, GG) and *ADIPOQ* (rs266729) (CC, CG+GG) genotypes were associated with test scores (P<0.02). Children with the GG genotype of *CDKN2B* (rs10811661) or the CC genotype of *ADIPOQ* (rs266729) had significantly better scores than those with the AA+AG genotype of *CDKN2B* or the CG+GG genotype of *ADIPOQ*.

TABLE 3. Classification of genotypes by obesity risk and physical fitness levels.

Physical fitness	Risk of Obesity*	
	Low	**High**
High	*CNR1* (rs1049353; GG)	
Low	*GC* (rs16847015; TG+GG) *PPY* (rs231472; GG) *UCP2* (rs660339; CT+TT) *ADIPOQ* (rs266729; CG+GG)	*LEP* (rs7799039; AA+AG) *HHEX* (rs1111875; TT) *LRP5* (rs4988300; GG+GT) *NPY2R* (rs2880415; CT+CC) *CDKN2B* (rs10811661; AA+AG)

*Gene variants were divided into low and high risk of obesity groups as previously described [6].

Classification of genotype by obesity and physical fitness

Table 3 provides a classification of genotypes by obesity and physical fitness. To summarize, *CNR1* (rs1049353; GG) was found to be associated with a low risk of obesity and high physical fitness, whereas *LEP* (rs7799039; AA+AG), *HHEX* (rs1111875; TT), *LRP5* (rs4988300; GG+GT), *NPY2R* (rs2880415; CT+CC), and *CDKN2B* (rs10811661; AA+AG) were associated with a high risk of obesity and low physical fitness. *GC* (rs16847015; TG+GG), *PPY* (rs231472; GG), *UCP2* (rs660339; CT+TT) and *ADIPOQ* (rs266729; CG+GG) were related to a low risk of obesity and low physical fitness.

DISCUSSION

The present study was undertaken to identify physical fitness-related SNPs and to analyse the relationship between physical fitness and major obesity-related genotypes in Korean children. The study revealed significant (P<0.02) differences between the scores of the five physical fitness elements for genotypes of *CNR1* (GG, P=0.010), *LEP* (AA+AG, P=0.012), *HHEX* (TT, P=0.009), *GC* (TG+GG, P=0.013), *LRP5* (GG+GT, P=0.014), *NPY2R* (CT+CC, P=0.003), *PPY* (GG, P=0.017), *UCP2* (CT+TT, P=0.003), *CDKN2B* (AA+AG, P=0.011) and *ADIPOQ* (CG+GG, P=0.006).

ACTN3, a speed gene, is known to be associated with athletic status and muscle phenotypes [14]. However, ACTN3 genotype was not included in the present study, because the genotype has not been reported to be related to obesity or BMI. On the other hand, we found a relation between speed and the *CNR1* (rs1049353) genotype in children; that is, the children with the GG genotype of *CNR1* had better speeds and accelerations. This association could be partially explained by the ameliorative effect of cannabinoid agonists on motor symptoms of multiple sclerosis in both humans and animal models [15]. For aerobic endurance, obesity is associated with imbalances in fatty acid trafficking between and within tissues and cells [16]. Also, obese individuals have more type II muscle fibres than type I muscle fibres, which may reduce oxygen uptake. De Araujo et al. [17] reported that children with a higher BMI have lower maximal oxygen consumption levels. In the present study, we observed that *LEP* and *HHEX* are related to aerobic endurance and fitness, which is consistent with the findings of the previous studies [18, 20, 21]. Walsh et al. [18] found that leptin genetic variants are associated with habitual vigorous or light intensity physical activity. Lakka et al. [19] found that polymorphic variations in the *LEP* gene are associated with the magnitudes of the effects of regular exercise on glucose homeostasis, and Zhao et al. [20] showed that endurance exercise activates the signalling pathways induced by leptin in the rat hypothalamus. Furthermore, it has been reported that the HHEX gene exhibits differential DNA methylation and mRNA expression in human adipose tissue in response to exercise [21]. Obesity has also been shown to be correlated with greater absolute maximum muscle strength, which may be caused by chronic fat mass overload on antigravity muscles. On the other hand, if maximum muscular strength is normalized with respect to body mass, obese

people appear to be weaker [22]. In fact, in a Brazilian study, more than 40% of obese children had a poor muscular strength/endurance [23]. Tomlinson et al. [24] reported that high BMI and high percentage of body fat are associated with lower skeletal muscle contractile capacity in whole muscle and fascicular levels. Tomlinson et al. [22] suggested that this relative weakness may be caused by reduced mobility, neural adaptations, and changes in muscle morphology. Kiel et al. [25] reported that polymorphisms in the low-density lipoprotein receptor-related protein (*LRP5*) gene modulated the relationship between physical activity (as determined using a self-administered questionnaire) and bone mineral density. Neuropeptide Y (NPY) regulates appetite, and thus influences the development of obesity. NPY was shown to be released after intense physical exercise [26], and neuropeptide Y mRNA expression was related to recovery times after moderate-intensity and high-intensity exercise [27]. Buemann et al. [28] found that a polymorphism in the *UCP2* gene was related to energy consumption-related exercise efficiency during acute exercise, and suggested that the *UCP2* gene not only affects basal metabolic rate but also influence energy consumption during exercise. It was also reported that UCP2 affects human physical performance by negatively regulating mitochondrial ATP synthesis [29]. Interestingly, we also found an association between flexibility measurement and obesity-related physical fitness genotypes. Our results parallel those of previous studies, which reported that obese or overweight children show physically and functionally lower flexibilities [30,31]. These studies demonstrate that the different genotypes have unique functions with respect to the control of insulin, bone mineral density, and energy consumption during exercise, and our findings support the results of these previous studies.

A comparison between the physical fitness-related SNPs identified in the present study and the genes previously reported to be associated with performance and health-related fitness phenotypes, by Rankinen et al. [9] and Bray et al. [8], revealed no common factors. In the present study, we identified ten novel SNPs associated with physical fitness from among 68 genes previously identified by the systematic genome-wide functional screening of genes related to physical fitness. In these previous studies, it was suggested that the expression of *CNR1* (GG), *GC* (TG+GG), *PPY* (GG), *UCP2* (CT+TT) and *ADIPOQ* (CG+GG) genotypes might lower the risk of obesity by reducing fat mass [31-35], whereas the expression of *LEP* (AA+AG), *HHEX* (TT), *LRP5* (GG+GT), *NPY2R* (CT+CC), and *CDKN2B* (AA+AG) genotypes was suggested to increase the risk of obesity by reducing energy consumption [36-40]. The present study suggests that children with the *CNR1* genotype (GG) might achieve a high level of physical fitness by steady exercise, and thus long-term exercise programmes might be expected to benefit children with this genotype. On the other hand, physical fitness was low in children with the *GC* (TG+GG), *PPY* (GG), *UCP2* (CT+TT), *ADIPOQ* (CG+GG), *LEP* (AA+AG), *HHEX* (TT), *LRP5* (GG+GT), *NPY2R* (CT+CC), or *CDKN2B* (AA+AG) genotypes, which suggests children with these

genotypes might not achieve satisfactory improvements in physical fitness from long-term participation in exercise programmes.

Previous studies have reported that physical fitness reduces the risk of chronic disease development, and that physical fitness-related genomic factors should be considered in the management of obesity [1,2]. In the present study, it was also found that physical fitness related SNPs were associated with obesity. In other words, our findings suggest that physical fitness is an important consideration for the prevention and treatment of obesity, and that it is possible to predict the efficacy of physical fitness using physical fitness-related genotypes.

Table 3 provides a classification of SNPs with respect to physical fitness and obesity risk. No genotype was found to be associated with a high level of physical fitness and a high risk of obesity. However, associations were observed between *CNR1* genotype (GG) and a low risk of obesity and a high level of physical fitness. This finding indicates that children with *CNR1* genotype (GG) are at low risk of becoming obese because they have high exercise efficiencies. Some SNPs, such as the *LEP* (AA+AG), *HHEX* (TT), *LRP5* (GG+GT), *NPY2R* (CT+CC), and *CDKN2B* (AA+AG) genotypes, were detected in the low physical fitness/high obesity risk subgroup. Exercise programmes are usually recommended for the prevention and management of obesity in children, but this finding suggests that children with these genotypes are predisposed to obesity despite exercise.

Our study is limited by its cross-sectional study design, and thus a further cohort study is warranted. The present study was undertaken on children based on the presumption that they are less affected by their environments than adults. However, even if a child carries a particular gene, the gene may unavailable, as this depends on the timing of its expression, and thus determined relations between physical fitness and genotype may not fully reflect the effects of a gene. The study was also limited by the relatively small sample size. We attempted to overcome this and to avoid selection bias by accepting significance for P values of < 0.02 rather than < 0.05 and using the method devised by Benjamini and Hochberg (13) to control for FDRs. Furthermore, the present study was not designed to analyse relationships between genotypes and physical fitness improvements after exercise, and thus we are not in a position to predict changes in physical fitness according to the presences of physical fitness-related genes.

CONCLUSIONS

In conclusion, 10 new physical fitness-related genotypes were identified among the 68 genotypes examined, that is, *CNR1* (rs1049353; GG), *LEP* (rs7799039; AA+AG), *HHEX* (rs1111875; TT), *GC* (rs16847015; TG+GG), *LRP5* (rs4988300; GG+GT), *NPY2R* (rs2880415; CT+CC), *PPY* (rs231472; GG), *UCP2* (rs660339; CT+TT), *CDKN2B* (rs10811661; AA+AG), and *ADIPOQ* (rs266729; CG+GG), which suggests that these genotypes influence the development of physical fitness. We suggest that classification of genotypes according to the achievement of physical fitness by exercise could be used to predict the risk of obesity and to select appropriate intervention programmes. These findings suggest that children participating in the same physical fitness improvement programme achieve results that are dependent on genotypes. Further research is needed to confirm our findings.

REFERENCES

1. Sallis JF, Prochaska JJ, Taylor WC. A review of correlates of physical activity of children and adolescents. Med Sci Sports Exerc 2000; 32(5):963-975.
2. Trost SG, Kerr LM, Ward DS, Pate RR. Physical activity and determinants of physical activity in obese and non-obese children. Int J Obes Relat Metab Disord 2001;25(6):822-829.
3. Jae SY, Fernhall B, Heffernan KS, Jeong M, Chun EM, Sung J, Lee SH, Lim YJ, Park WH. Effects of lifestyle modifications on C-reactive protein: Contribution of weight loss and improved aerobic capacity. Metabolism 2006;55(6):825-831.
4. LaMonte MJ, Blair SN. Physical activity, cardiorespiratory fitness, and adiposity: Contributions to disease risk. Curr Opin Clin Nutr Metab Care 2006; 9(5):540-546.
5. Telford RD. Low physical activity and obesity: Causes of chronic disease or simply predictors? Med Sci Sports Exerc 2007;39(8):1233-1240.
6. Marti A, Martinez-Gonzalez MA, Martinez JA. Interaction between genes and lifestyle factors on obesity. Proc Nutr Soc 2008;67(1):1-8.
7. Rampersaud E, Mitchell BD, Pollin TI, Fu M, Shen H, O'Connell JR, Ducharme JL, Hines S, Sack P, Naglieri R, Shuldiner AR, Snitker S. Physical activity and the association of common FTO gene variants with body mass index and obesity. Arch Intern Med 2008; 168(16):1791-1797.
8. Bray MS, Hagberg JM, Perusse L, Rankinen T, Roth SM, Wolfarth B, Bouchard C. The human gene map for performance and health-related fitness phenotypes: The 2006-2007 update. Med Sci Sports Exerc 2009; 41(1):35-73.
9. Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Pérusse L, Bouchard C. The human obesity gene map: The 2005 update. Obesity (Silver Spring) 2006; 14(4):529-644.
10. Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, Lank E, Bottomley B, Lopez-Fernandez J, Ferraz-Amaro I, Dattani MT, Ercan O, Myhre AG, Retterstol L, Stanhope R, Edge JA, McKenzie S, Lessan N, Ghodsi M, De Rosa V, Perna F, Fontana S, Barroso I, Undlien DE, O'Rahilly S. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. N Engl J Med 2007; 356(3):237-247.
11. Fagnani F, Giombini A, Di Cesare A, Pigozzi F, Di Salvo V. The effects of a whole-body vibration program on muscle performance and flexibility in female athletes. Am J Phys Med Rehabil 2006;85(12):956-962.
12. Cochrane DJ, Stannard SR. Acute whole body vibration training increases vertical jump and flexibility performance in elite female field hockey players. Br J Sports Med 2005;39(11):860-865.
13. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. Stat Med. 199;9(7):811-818.

14. Moran CN, Yang N, Bailey ME, Tsiokanos A, Jamurtas A, MacArthur DG, North K, Pitsiladis YP, Wilson RH. Association analysis of the ACTN3 R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks. Eur J Hum Genet. 2007;15(1):88-93.

15. Clifford DB. Tetrahydrocannabinol for tremor in multiple sclerosis. Ann Neurol. 1983;13(6):669-71. Jabusch HC, Schneider U, Altenmüller E. Delta9-tetrahydrocannabinol improves motor control in a patient with musician's dystonia. Mov Disord. 2004; 19(8):990-1.

16. Mittendorfer B. Origins of metabolic complications in obesity: adipose tissue and free fatty acid trafficking. Curr Opin Clin Nutr Metab Care. 2011; 14(6):535-541.

17. De Araujo SS, Miguel-dos-Santos R, Silva RJS, Cabral-de-Oliveira AC. Association between body mass index and cardiorespiratory fitness as predictor of health status in school children. Rev Andal Med Deporte. 2015;8(2):73–78.

18. Walsh S, Haddad CJ, Kostek MA, Angelopoulos TJ, Clarkson PM, Gordon PM, Moyna NM, Visich PS, Zoeller RF, Seip RL, Bilbie S, Thompson PD, Devaney J, Gordish-Dressman H, Hoffman EP, Price TB, Pescatello LS. Leptin and leptin receptor genetic variants associate with habitual physical activity and the arm body composition response to resistance training. Gene. 2012;510(1):66-70.

19. Lakka TA, Rankinen T, Weisnagel SJ, Chagnon YC, Lakka HM, Ukkola O, Boulé N, Rice T, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bergman R, Bouchard C. Leptin and leptin receptor gene polymorphisms and changes in glucose homeostasis in response to regular exercise in nondiabetic individuals: The HERITAGE family study. Diabetes 2004;53(6):1603-1608.

20. Zhao J, Tian Y, Xu J, Liu D, Wang X, Zhao B. Endurance exercise is a leptin signaling mimetic in hypothalamus of Wistar rats. Lipids Health Dis. 2011; 10:225.

21. Rönn T, Volkov P, Davegårdh C, Dayeh T, Hall E, Olsson AH, Nilsson E, Tornberg A, Dekker Nitert M, Eriksson KF, Jones HA, Groop L, Ling C. A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. PLoS Genet. 2013;9(6):e1003572.

22. Tomlinson DJ, Erskine RM, Morse CI, Winwood K, Onambélé-Pearson G. The impact of obesity on skeletal muscle strength and structure through adolescence to old age. Biogerontology. 2016;17(3):467-483.

23. Casonatto J, Fernandes RA, Batista MB, Cyrino ES, Coelho-E-Silva MJ, de Arruda M, Vaz Ronque ER. Association between health-related physical fitness and body mass index status in children. J Child Health Care. 2016;20(3):294-303.

24. Tomlinson DJ, Erskine RM, Winwood K, Morse CI, Onambélé GL. Obesity decreases both whole muscle and fascicle strength in young females but only exacerbates the aging-related whole muscle level asthenia. Physiol Rep. 2014;2(6). pii: e12030.

25. Kiel DP, Ferrari SL, Cupples LA, Karasik D, Manen D, Imamovic A, Herbert AG, Dupuis J. Genetic variation at the low-density lipoprotein receptor-related protein 5 (LRP5) locus modulates wnt signaling and the relationship of physical activity with bone mineral density in men. Bone 2007; 40(3):587-596.

26. Zajadacz B, Skarpańska – Stejnborn A, Brzenczek-Owczarzak W, Juszkiewicz A, Naczk M, Adach Z. The influence of physical exercise on alterations in concentration of neuropeptide Y, leptin and other selected hormonal and metabolic parameters in sportspeople. Biol Sport. 2009;26(4):310-324.

27. Han D, Kim S, Cho B. Neuropeptide Y(NPY) mRNA Expression to Exercise Intensity and Recovery times. J Phys Ther Sci. 2011;23:781-784.

28. Buemann B, Schierning B, Toubro S, Bibby BM, Sørensen T, Dalgaard L, Pedersen O, Astrup A. The association between the val/ala-55 polymorphism of the uncoupling protein 2 gene and exercise efficiency. Int J Obes Relat Metab Disord 2001;25(4):467-471.

29. Dhamrait SS, Williams AG, Day SH, Skipworth J, Payne JR, World M, Humphries SE, Montgomery HE. Variation in the uncoupling protein 2 and 3 genes and human performance. J Appl Physiol (1985). 2012; 112(7):1122-1127.

30. Casonatto J, Fernandes RA, Batista MB, Cyrino ES, Coelho-E-Silva MJ, de Arruda M, Vaz Ronque ER. Association between health-related physical fitness and body mass index status in children. J Child Health Care. 2016; 20(3):294-303.

31. Andreasi V, Michelin E, Rinaldi AE, Burini RC. Physical fitness and associations with anthropometric measurements in 7 to 15-year-old school children. J Pediatr (Rio J). 2010;86(6):497-502.

32. Batterham RL, Le Roux CW, Cohen MA, Park AJ, Ellis SM, Patterson M, Frost GS, Ghatei MA, Bloom SR. Pancreatic polypeptide reduces appetite and food intake in humans. J Clin Endocrinol Metab 2003;88(8):3989-3992.

33. Baye TM, Zhang Y, Smith E, Gunnell J, Myklebust J, James R, James R, Kissebah AH, Olivier M, Wilke RA. Genetic variation in cannabinoid receptor 1 (CNR1) is associated with derangements in lipid homeostasis, independent of body mass index. Pharmacogenomics 2008; 9(11):1647-1656.

34. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996;271(18):10697-10703.

35. Jiang H, Xiong DH, Guo YF, Shen H, Xiao P, Yang F, Chen Y, Zhang F, Recker RR, Deng HW. Association analysis of vitamin D-binding protein gene polymorphisms with variations of obesity-related traits in caucasian nuclear families. Int J Obes (Lond) 2007;31(8):1319-1324.

36. Jun HS, Kim IK, Lee HJ, Lee HJ, Kang JH, Kim JR, et al. Effects of UCP2 and UCP3 variants on the manifestation of overweight in Korean children. Obesity (Silver Spring) 2009;17:355-362.

37. Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Shin HD, Song J. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 1998;392(2):398-401.

38. Guo YF, Xiong DH, Shen H, Zhao LJ, Xiao P, Guo Y, Wang W, Yang TL, Recker RR, Deng HW. Polymorphisms of the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with obesity phenotypes in a large family-based association study. J Med Genet 2006; 43(10):798-803.

39. Horikoshi M, Hara K, Ito C, Shojima N, Nagai R, Ueki K, Froguel P, Kadowaki T. Variations in the HHEX gene are associated with increased risk of type 2 diabetes in the Japanese population. Diabetologia 2007; 50(12):2461-2466.

40. Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, Weedon MN, Mari A, Hattersley AT, McCarthy MI, Frayling TM, Walker M; RISC Consortium; U.K. Type 2 Diabetes Genetics Consortium. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. Diabetes 2007;56(2):3101-3104.

41. Torekov SS, Larsen LH, Andersen G, Albrechtsen A, Glümer C, Borch-Johnsen K, Jørgensen T, Hansen T, Pedersen O. Variants in the 5' region of the neuropeptide Y receptor Y2 gene (NPY2R) are associated with obesity in 5,971 white subjects. Diabetologia 2006;49(11):2653-2658.