Endocr (2011) 39:62–68
DOI 10.1007/s12020-010-9412-7

ORIGINAL ARTICLE

Melanocortin-4 receptor gene polymorphism and the level of physical activity in men (HALS Study)

Paweł Jóźków · Małgorzata Słowińska-Lisowska · Łukasz Łaczmański · Dorota Jakubiec · Marek Mędraś

Received: 7 July 2010 / Accepted: 5 October 2010 / Published online: 3 November 2010

Abstract Melanocortin plays an important role in the energy balance in humans. Actions of melanocortin are exerted through activation of five receptors among which the melanocortin-4 receptor (MC4R) is especially abundant within the central nervous system (CNS). It has been proved that genetic variations of the MC4R gene are associated with the energy intake. Recent data has suggested that MC4R gene polymorphism might influence physical activity/energy expenditure as well. Our aim was to search for associations between *MC4R* polymorphisms and the level of physical activity. We genotyped *MC4R* in a population-based cohort of 311 men. The level of physical activity was determined with use of the International Physical Activity Questionnaire. We have found that physical effort expressed as log METs-min/week (corrected for age, BMI and educational status) was 6.61 in men declaring low, 7.56—moderate and 8.96—high level of physical activity. We have not found any associations between the C-2745T *MC4R* polymorphism and the level of physical activity ($P = 0.53$). There were no interactions between the level of physical activity and the C-2745T polymorphisms either ($P = 0.47$). We conclude that the C-2745T genetic polymorphism of the MC4R gene does not influence the level of physical activity in healthy, adult men.

Keywords Melanocortin-4 receptor · Genetic polymorphism · Motor activity · Lifestyle

Introduction

Melanocortin and other proopiomelanocortin (POMC) derivatives regulate the intake of food and the energy balance in humans. Actions of melanocortin are exerted through five receptors. Melancortin-4 receptor (MC4R) is most abundant within the central nervous system (CNS) [1]. Diminished locomotor activity and obesity are typical features of MC4R knockout mice and MC4R gene pathogenic mutations are the most important cause of monogenic obesity in humans [2]. What seems intriguing is the fact that the same caloric input results in higher increases of weight in MC4R knockout mice than in wild-type animals [3]. It has been hypothesized that melanocortin system influences physical activity and energy expenditure in humans, too [4]. The impact of physical effort on the overall energy expenditure in humans may reach from 100 up to 800 kcal/24 h [5, 6]. It is tempting to assume that a propensity to be physically active may be at least partially dependent on the functioning of the melanocortin system.

We wanted to verify a hypothesis that MC4R gene polymorphism (C-2745T and Val103Ile) is associated with the level of physical activity in healthy subjects.

P. Jóźków (✉) · M. Słowińska-Lisowska · M. Mędraś
Department of Sports Medicine & Nutrition, University School of Physical Education, ul. Paderewskiego 35, 51-612 Wrocław, Poland
e-mail: jozkow@gmail.com; pjozkow@wp.pl

Ł. Łaczmański · M. Mędraś
Department of Endocrinology, Diabetology & Isotope Treatment, Wrocław Medical University, Wrocław, Poland

D. Jakubiec
Department of Kinesiology, University School of Physical Education, Wrocław, Poland

Results

In the studied population, 59 subjects declared low (L), 95—moderate (M), and 157—high (H) level of physical activity. The mean amount of physical activity expressed as log (METs-min/week) corrected for age, BMI and educational status was, respectively, 6.61 in L, 7.56 in M, and 8.96 in H.

We were not able to analyze associations between the Val103Ile *MC4R* polymorphism and the level of physical activity/sedentarism due to insufficient number of cases. There was only one case of the Ile/Ile and twenty cases of the Val/Ile variant among our study subjects, thus we could not perform Hardy–Weinberg tests.

The variants of the C-2745T *MC4R* genotype showed a deviation from the Hardy–Weinberg equilibrium ($P = 0.03$) in the whole sample; however, no significant differences of the allele distribution were found in groups characterized as L, M, and H (Table 1).

We have not found any associations between the C-2745T *MC4R* polymorphism and the level of physical activity ($P = 0.53$). There were no interactions between the level of physical activity and the C-2745T polymorphisms either ($P = 0.47$) (Tables 2, 3).

Similarly to the above, we did not notice any associations between sedentarism (hours spent on sitting corrected for age, BMI and educational status) and the C-2745T polymorphism ($P = 0.92$). In subjects sitting for 0–4, 5–8, and >9 h/day the mean amount of declared physical activity was, respectively, 8.56, 7.97, and 7.55 log METs-min/week. There was no interaction between hours of sitting and C-2745T variants either ($P = 0.79$) (Tables 4, 5).

Exclusion of the obese subjects (BMI $\geq$ 30) from the analysis did not influence associations between C-2745T polymorphism and the level of physical activity (Tables 6, 7).

Discussion

The CNS melanocortin system is implicated as a mediator of the central effects of leptin. Reduced activity of the CNS

Table 1 Chi-square test for compliance of C-2745T *MC4R* polymorphic genotypes with the Hardy–Weinberg equilibrium

Polymorphism	C (%)	C/T (%)	T (%)	PC	PT	*P*
L	54.4	38.7	6.9	0.74	0.26	0.82
M	50.5	41.1	8.4	0.71	0.29	0.13
H	50.0	41.4	8.6	0.71	0.,29	0.22
All	51.0	40.8	8.2	0.71	0.29	0.03

L low, *M* medium, *H* high level of physical activity

Table 2 Distribution of the *MC4R* C-2745T variants in groups characterized by low (L), medium (M) and high (H) level of physical activity

Physical activity			C/C	C/T	T/T
All	*n*	311	168	108	35
	Mean	8.08	8.09	8.06	8.13
	SEM	1.21	1.09	1.34	1.34
L	*n*	59	33	21	5
	Mean	6.61	6.71	6.48	6.44
	SEM	0.99	0.84	1.06	1.64
M	*n*	95	52	31	12
	Mean	7.56	7.64	7.50	7.36
	SEM	0.58	0.61	0.58	0.41
H	*n*	157	83	56	18
	Mean	8.96	8.91	8.97	9.11
	SEM	0.75	0.59	0.96	0.75

n number of subjects, *mean* arithmetic mean, *SEM* standard error of mean

Physical activity is expressed as log (METs-min/week) with adjustments for age, BMI, and educational status

Table 3 Value of *P* in two-way ANOVA

Between L, M, H	0.000
Between C/C, C/T, T/T	0.53
Interaction	0.47

L low, *M* medium, *H* high level of physical activity

Table 4 Distribution of *MC4R* C-2745T variants in subjects sitting for 0–4, 5–8 and >9 h/daily

Hours spent on sitting daily			C/C	C/T	T/T
All	*N*	315	171	112	32
	Mean	8.06	8.07	8.01	8.13
	SEM.	1.20	1.09	1.32	1.35
0–4	*N*	103	59	33	11
	Mean	8.56	8.45	8.69	8.73
	SEM	1.10	1.01	1.22	1.19
5–8	*N*	136	72	52	12
	Mean	7.97	8.02	7.88	8.00
	SEM	1.10	1.06	1,20	1.02
>9	*N*	76	40	27	9
	Mean	7.55	7.60	7.45	7.57
	SEM	1.26	1.11	1.33	1.73

n number of subjects, *mean* arithmetic mean, *SEM* standard error of mean

Mean and SEM refer to the amount of declared physical activity expressed as log (METs-min/week) with adjustments for age, BMI, and educational status

Table 5 Value of *P* in two-way ANOVA

Between 0–4, 5–8, >9 h	0.000
Between C/C, C/T, T/T	0.92
Interaction	0.79

0–4, 5–8, >9 h spent on sitting daily

Table 6 Distribution of the *MC4R* C-2745T variants in groups characterized by low (L), medium (M) and high (H) level of physical activity (only subjects with BMI < 30)

Physical activity			C/C	C/T	T/T
All	*n*	235	135	80	20
	Mean	8.03	8.02	8.03	8.09
	SEM	0.08	0.10	0,14	0.34
L	*n*	47	29	16	2
	Mean	6.61	6.69	6.64	5.15
	SEM	0.15	0.16	0.27	1.43
M	*n*	73	43	23	7
	Mean	7.54	7.60	7.47	7.37
	SEM	0.07	0.10	0.10	0.14
H	*n*	115	63	41	11
	Mean	8.92	8.91	8.89	9.09
	SEM	0.07	0.08	0.16	0.25

n number of subjects, *mean* arithmetic mean, *SEM* standard error of mean

Physical activity is expressed as log (METs-min/week) with adjustments for age, BMI, (<30) and educational status

Table 7 Value of *P* in two-way ANOVA

Between L, M, H	0.000
Between C/C, C/T, T/T	0.077
Interaction	0.097

melanocortin system promotes reduced locomotor activity and obesity in both rodents and humans. Activation of the CNS MC4R has direct effects on autonomic outflow and metabolism. Proopiomelanocortin is the precursor of α-melanocyte-stimulating hormone (α-MSH). α-MSH and agouti-related protein (AgRP) are, respectively, an agonist and an antagonist of the brain melanocortin receptors [7, 8].

Expression of the MC4R is especially high in the hypothalamus and the spinal cord, where it regulates food intake and energy balance. Interruption of the melanocortin signaling in the hypothalamus may lead to reduced physical activity and obesity in mice. Some authors reported that male nonobese MC4R knockout mice are less physically active than wild-type controls [3], though these results were not confirmed in other observations [9]. It has been also shown that administration of an antagonist of MC4R reduces spontaneous locomotor activity of rats [7].

Many mutations in the MC4R gene result in complete or partial function loss of the MC4R. The melanocortin-4 receptor deficiency is the most common genetic cause of obesity [10]. It is especially interesting that carriers of the *MC4R* mutations do not present a typical negative association between BMI and the educational status [10]. Asians with heterozygous or homozygous mutations of the *MC4R* (but no Caucasians with heterozygous mutations) reveal a tendency toward lower basal metabolic rate [11]. It has been also shown that stimulation of MC4R increases physical activity/energy expenditure and leads to weight loss [12].

In the framework of Quebec Family Study, it was found that T/T variant of C-2745T polymorphism of *MC4R* gene correlated with lower levels of moderate/high physical activity and sedentary lifestyle (1 h/week less physical activity during the past year). Surprisingly, offsprings who were homozygotes for the T-allele were less physically active and had lower BMI. In parents' homozygotes, there was no such an association and they were slightly heavier than other genotypes. Authors of the report hypothesized that an influence of the T/T variant could lead to a weight gain in later life. They also observed that the least physically active subjects were T/T homozygotes for the *MC4R* C-2745T and concomitantly A/A homozygotes for the CART-A1475G variant, while they were most physically active if they carried the G-allele of CART-A1475G variant [13].

In our material, there was no statistically significant relationship between the *MC4R* C-2745T polymorphism and the level of physical activity. Contrary to the above mentioned results T/T homozygotes seemed to be more active than C/C or C/T variants (8.13 vs. 8.09 or 8.06 log METs-min/week, ns). However, it is worth mentioning that within subgroups characterized by low or moderate level of physical activity carriers of the T/T genotype had less physical exercise than others (ns). T/T homozygotes spent also slightly more time on sitting (ns).

Among reasons that might have been the source of the discrepancies with the study by Loos et al. was a high number of highly active subjects in our cohort, their lower BMI, differences of the mean age in the samples and different methods used to assess physical activity.

Half of the men in our investigation presented high level of physical activity. To the contrary, in the study by Loos et al. moderate-to-strenuous physical activity score prevailed over physical inactivity score in both male parents and male offspring (respectively, 238 vs. 461 and 233 vs. 471). T/T homozygotes in the study by Loos et al. had higher inactivity score and lower BMI. In our study T/T, variant had higher level of physical activity and greater BMI (ns).

In both the studies, there was no association between *MC4R* C-2745T polymorphism and BMI. Mean BMI of

subjects in our study was 27 ± 4, whereas in the study by Loos et al. it was 28.6 ± 6.2 in male parents and 25.7 ± 6.3 in male offspring. Excluding the obese subjects (with BMI ≥ 30) from the analysis did not influence our results (Tables 6, 7). The association between physical activity (log METs-min/week) and the C-2745T polymorphism remained weak ($P = 0.077$) and the interaction between the levels of physical activity and the C-2745T polymorphism was statistically insignificant ($P = 0.097$). BMI of C/C, C/T, and T/T variants was, respectively, 26.71 ± 0.25, 27.50 ± 0.35, and 29.56 ± 0.71 ($P < 0.001$). The mean age of subjects in our study was 47 ± 12, while in the study by Loos et al., it was 53.7 ± 7.2 in male parents and 27.8 ± 8.2 in male offspring. In the study by Loos et al., there was used a 3-day physical activity diary, while we used last 7 days recall. One must keep in mind that different physical activity measurements provide significantly different results (e.g., between European Union countries) [14].

The Val103Ile is the most frequent polymorphism of the *MC4R* and it is negatively associated with obesity [15, 16]. It was confirmed, e.g., in a population-based study on 7,937 participants in which a relatively infrequent G/A genotype of the Val103Ile *MC4R* polymorphism was negatively associated with average weight and obesity [17]. Recently, it has been provided evidence for an association between the Val103Ile polymorphism and physical activity/energy expenditure as well as features of the metabolic syndrome. Carriers of the *MC4R* 103I had decreased waist circumference, reduced glycosylated hemoglobin and increased HDL-cholesterol [18].

The association between the Val103Ile polymorphism of the *MC4R* gene and energy expenditure (measured by indirect calorimetry) was investigated in 229 Finnish subjects. Their age was 51 ± 10 years and BMI 26.8 ± 4. It was found that subjects with Val103Ile genotype had higher energy expenditure than subjects with Val103Val genotype. During a 3.5-year follow up of a subgroup aged 70 ± 3 years and with BMI 27.4 ± 4, subjects with 103Ile genotype gained weight whereas subjects with Val103Val genotype lost weight; however, information on energy intake was lacking. Authors hypothesized that 103Ile genotype could contribute primarily to food intake and secondarily to energy expenditure [4]. Because of too few Val/Ile and Ile/Ile variants in our sample, we were not able to perform proper analysis.

We keep in mind that genetic association studies using single nucleotide polymorphisms have certain limitations. It always appears a question on the proper description of the phenotypic effect of a given genotype—in our case, the assessment of physical activity. So far there is no standard method for evaluation of the physical activity in a scale of population. The questionnaire used in our study (IPAQ) was validated in healthy, free-living adults from 12-countries [19], and it was compared against doubly labeled water [20]. Application of the IPAQ questionnaire has been also assessed in Poland [21]. We wanted to use an instrument that would be accepted by participants (ease of use), validated and comparable nationally and internationally. An advantage of IPAQ is that it comprises physical activity at work, at home, during transportation and leisure-time. Previous studies of the Polish population suggested rather high incidence of sedentary lifestyle and relatively low level of occupational physical activity [22]. Unlike above, in our investigation about 50% of men declared high level of daily physical activity. Similar outcomes were reported by authors who showed that the amount of physical activity was higher when IPAQ questionnaires were filled by subjects themselves rather than by experienced interviewers [21]. We also have to mention a suggestion that IPAQ may overestimate the level of physical activity [23]. Alike other authors, we observed a decline of physical activity with age; however, we could not confirm a common association between physical activity and body mass index [24].

The results of our investigation do not help to elucidate the role of genes for maintaining a healthy behavior and a physically active lifestyle. There is no doubt that locomotor activity of humans is multifactorial. Nevertheless, limited external stimulation emphasizes the role of genetic/metabolic factors in shaping the daily amount of physical effort. It is especially intriguing in the context of a high rate of subjects whose activity level is insufficient to provide health benefits. This number is estimated to be between 36–51% in the USA [25] and 50–70% in Europe [26].

We conclude that the C-2745T melanocortin-4 receptor gene polymorphism does not influence the level of physical activity and the sedentary lifestyle in healthy, adult men.

Materials and methods

Our investigation was carried out within a project named Health of Adults in Lower Silesia (HALS). The study was approved by the Bioethics Committee at the University School of Physical Education, Wrocław.

We sent invitations to participate in the study to 900 adult men. They were randomly chosen by the Local Data Bank (Regional Statistical Office). As an operator the personal identification number was used. After receiving written consents, 387 men were enrolled for further investigation (43% of the target group). All of them were Polish and lived within the borders of one administrative region of Poland (Lower Silesia). The region's population is homogenous, 100% Caucasian and counts around 2.9 million. This fact unifies comparisons of physical activity among studied subjects.

The researchers performed a standard medical interview and a full physical/anthropological examination in each of the recruited subjects. Blood samples were acquired after 12 h fasting by a venipuncture of the cubital vein. The plasma was stored in temperature of -80^{o} C until the analysis.

Studied men aged from 24 to 72 (mean 47 ± 12). Majority of them (55%) accomplished 8–12 years of education, while 43% had university degree. The most frequently reported diseases were: arterial hypertension (21% of subjects), chronic low back pain (19%), benign prostatic hyperplasia (5%) and diabetes (4%). Characteristics of the study group are summarized in Table 8.

Our goal was to assess the overall, everyday physical activity of the investigated men including occupational, transport, housework and leisure-time activities. Studied subjects did not participate in any imposed by us training programmes. We evaluated the level of physical activity with the International Physical Activity Questionnaire (last 7-day recall). The Polish version of the questionnaire has been checked by the IPAQ scientific group and assessed in the Polish population [21]. The questionnaires were filled by the participants themselves in presence of the investigators.

The amount of physical exercise was counted as multiples of resting metabolic rate by minutes of performance during a week (METs-minute/week). Upon the IPAQ categorical scoring we distinguished three groups of subjects. Their level of physical activity was classified as: low (L), medium (M), and high (H). According to IPAQ criteria, persons classified as highly active (H) moved at least 12,500 steps a day or the equivalent in moderate or vigorous activities. This is at least an hour more moderate-intensity activity over and above the basal level of activity or half an hour of vigorous-intensity activity over and above basal levels daily (basal is 5,000 steps/day). Group M had on average half an hour of at least moderate-intensity physical activity on most days. Group L did not meet any of the above criteria. Details of the scoring protocol are available on IPAQ web site [http://www.ipaq.ki.se/ipaq.htm]. The application of IPAQ has been discussed in detail elsewhere [14, 27, 28].

Table 8 Characteristics of the study group

Parameter	Mean ± SD
Age (years)	47 ± 12
Weight (kg)	85 ± 13
Height (cm)	176 ± 6
Body mass index (kg/(m)^2)	27 ± 4
Waist circumference (cm)	98 ± 12
Hip circumference (cm)	102 ± 9

Genotyping procedures were uniform for all studied individuals. Both phenotyping and genotyping were blinded to each other.

Common

Whole genomic DNA was obtained from blood leucocytes using standard isolating methods. Melanocortin receptor genotyping was performed by the polymerase chain reaction (PCR) and the minisequencing. Genotyping was elaborated and validated in the Laboratory of the Molecular Endocrinology, Department of Endocrinology, Diabetology and Isotope Treatment, Wroclaw Medical University.

MC4R-Val103Ile (dbSNP database: 2229616)

In order to amplify 146-bp fragment of the melanocortin receptor gene it was used a mix containing: forward primer (5′-GAATCTGCATTCACCCATGTACT-3′), reverse primer (5′-ACCTTGCTAATTTTTTGTAT-3′), 1× PCR buffer, 1.5 mM MgCl2, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, 2 polymerase units (ROCHE), 200 ng genomic DNA, water up to 20 μl.

The DNA was denatured at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 30 s.

The amplified 146-bp fragment was purified from free primers and free dNTPs by SAP and ExoI treatment (Fermentas).

The minisequencing method was based on the incorporation of single fluorescence-labeled dideoxynucleotides to the 3′ end of the oligonuclotide which was correctly paired to the specific template DNA fragment using SNaPshot kit (Applied Biosystems). SNaPshot reaction was carried out using the oligonucleotide: 5′-TGCTGGTGAGCGTTT CAAATGGATCAGAAACCATT-3′ designed to end right before the polymorphic site. The SNaPshot reaction consisted of 25 cycles: denaturation at 96°C for 10 s, annealing at 50°C for 5 s, extension at 60°C for 30 s.

The product (36 bp) was analyzed by the ABI 310 sequencer (Applied Biosystems) and allels were calculated by the dedicated software (GeneScan 3.1.2).

MC4R-C-2745T (dbSNP database: rs7242169)

In order to amplify 570-bp fragment of the melanocortin receptor gene it was used a mix containing: forward primer (5′-GGCATTTCTCCAAAGATTATTAC-3′), reverse primer (5′-ACCTTGCTAATTTTTTGTAT-3′), 1× PCR buffer, 1.5 mM MgCl2, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, 2 polymerase units (Roche), 200 ng genomic DNA, water up to 20 μl.

The DNA was denatured at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 30 s.

The amplified 570-bp fragment was purified from oligonucleotides and free dNTPs by SAP and ExoI treatment (Fermentas).

The minisequencing method was based on the incorporation of single fluorescence-labeled dideoxynucleotides to the 3′ end of oligonuclotide which was correctly paired to the specific template DNA fragment using SNaPshot kit (Applied Biosystems). SNaPshot reaction was carried out with use of the oligonucleotide: 5′-TCATGAGGTCAG-GAGATCGAGACCA-3′ designed to end right before the polymorphic site. The SNaPshot reaction consisted of 25 cycles: denaturation at 96°C for 10 s, annealing at 50°C for 5 s, extension at 60°C for 30 s.

The product (26 bp) was analyzed by the ABI 310 sequencer (Applied Biosystems) and allels were calculated by the dedicated software (GeneScan 3.1.2).

Statistical analysis

Due to insufficient amount of blood, presence of PCR inhibitors or missing information in the IPAQ questionnaires we were able to analyse data from 311 subjects (315 in regard to sedentarism). Physical activity was evaluated as log transformed METs-min/week. The latter value was corrected for age, BMI and educational status according to the equation:

$$\begin{aligned}\text{MET\_LOGcor} = {} & \text{MET\_LOG} - 0.51 + 0.020 * \text{age} \\ & - 0.020 * \text{BMI} + 0.23 \\ & * \text{higher education}\end{aligned}$$

For the analysis of the subgroup with BMI < 30 we used the equation:

$$\begin{aligned}\text{MET\_LOG30} = {} & \text{MET\_LOG} - 1.033 + 0.018 * \text{age} \\ & + 0.0034 * \text{BMI} < 30 + 0.274 \\ & * \text{higher education}.\end{aligned}$$

In addition, we evaluated associations between studied polymorphisms and the amount of hours spent on sitting in each of the groups (sedentarism). The differences between the groups were tested with the χ^2 test and, if appropriate, the analysis of variance (ANOVA). A P value less than 0.05 was considered as significant in all analyses. All calculations were done with the StatSoft, Inc. (2008). STATISTICA (data analysis software system), version 8.0. www.statsoft.com.

Acknowledgments This work was supported by the Ministry of Science and Higher Education of Poland (grant code: 2PO5D 0730).

References

1. V.J. Hruby, M. Cai, J.P. Cain et al., Design, synthesis and biological evaluation of ligands selective for the melanocortin-3 receptor. Curr. Top. Med. Chem. **7**(11), 1107–1119 (2007)
2. F. Stutzmann, V. Vatin, S. Cauchi et al., Non-synonymous polymorphisms in melanocortin-4 receptor protect against obesity: the two facets of a Janus obesity gene. Hum. Mol. Genet. **16**(15), 1837–1844 (2007)
3. L. Ste Marie, G.I. Miura, D.J. Marsh, K. Yagaloff, R.D. Palmiter, A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. Proc. Natl. Acad. Sci. USA **97**(22), 12339–12344 (2000)
4. J. Rutanen, J. Pihlajamaki, P. Karhapaa et al., The Val103Ile polymorphism of melanocortin-4 receptor regulates energy expenditure and weight gain. Obes. Res. **12**(7), 1060–1066 (2004)
5. J.C. Tou, C.E. Wade, Determinants affecting physical activity levels in animal models. Exp. Biol. Med. (Maywood) **227**(8), 587–600 (2002)
6. A.W. Thorburn, J. Proietto, Biological determinants of spontaneous physical activity. Obes. Rev. **1**(2), 87–94 (2000)
7. T. Adage, A.J. Scheurink, S.F. de Boer et al., Hypothalamic, metabolic, and behavioral responses to pharmacological inhibition of CNS melanocortin signaling in rats. J. Neurosci. **21**(10), 3639–3645 (2001)
8. Z. Xiang, S.A. Litherland, N.B. Sorensen et al., Pharmacological characterization of 40 human melanocortin-4 receptor polymorphisms with the endogenous proopiomelanocortin-derived agonists and the agouti-related protein (AGRP) antagonist. Biochemistry **45**(23), 7277–7288 (2006)
9. A.A. Butler, D.L. Marks, W. Fan et al., Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat. Neurosci. **4**(6), 605–611 (2001)
10. F. Stutzmann, K. Tan, V. Vatin et al., Prevalence of melanocortin-4 receptor deficiency in Europeans and their age-dependent penetrance in multigenerational pedigrees. Diabetes **57**(9), 2511–2518 (2008)
11. I.S. Farooqi, G.S. Yeo, J.M. Keogh et al., Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. J. Clin. Investig. **106**(2), 271–279 (2000)
12. D.L. Marks, N. Ling, R.D. Cone, Role of the central melanocortin system in cachexia. Cancer. Res. **61**(4), 1432–1438 (2001)
13. R.J. Loos, T. Rankinen, A. Tremblay et al., Melanocortin-4 receptor gene and physical activity in the Quebec Family Study. Int. J. Obes. (Lond.) **29**(4), 420–428 (2005)
14. A. Rutten, H. Ziemainz, F. Schena et al., Using different physical activity measurements in eight European countries. Results of the European Physical Activity Surveillance System (EUPASS) time series survey. Public Health Nutr. **6**(4), 371–376 (2003)
15. F. Geller, K. Reichwald, A. Dempfle et al., Melanocortin-4 receptor gene variant I103 is negatively associated with obesity. Am. J. Hum. Genet. **74**(3), 572–581 (2004)
16. E.H. Young, N.J. Wareham, S. Farooqi et al., The V103I polymorphism of the MC4R gene and obesity: population based studies and meta-analysis of 29563 individuals. Int. J. Obes. (Lond.) **31**(9), 1437–1441 (2007)
17. I.M. Heid, C. Vollmert, A. Hinney et al., Association of the 103I MC4R allele with decreased body mass in 7937 participants of two population based surveys. J. Med. Genet. **42**(4), e21 (2005)
18. I.M. Heid, C. Vollmert, F. Kronenberg et al., Association of the MC4R V103I polymorphism with the metabolic syndrome: the KORA Study. Obesity (Silver Spring) **16**(2), 369–376 (2008)

19. C.L. Craig, A.L. Marshall, M. Sjostrom et al., International physical activity questionnaire: 12-country reliability and validity. Med. Sci. Sports. Exerc. **35**(8), 1381–1395 (2003)
20. R. Maddison, C. Ni Mhurchu, Y. Jiang et al., International Physical Activity Questionnaire (IPAQ) and New Zealand Physical Activity Questionnaire (NZPAQ): a doubly labelled water validation. Int. J. Behav. Nutr. Phys. Act. **4**, 62 (2007)
21. E. Biernat, R. Stupnicki, B. Lebiedziński, L. Janczewska, Assessment of physical activity by applying IPAQ questionnaire. Phys. Educ. Sport. **52**(2), 83–89 (2008)
22. D. Kaleta, T. Makowiec-Dabrowska, A. Jegier, Occupational and leisure-time energy expenditure and body mass index. Int. J. Occup. Med. Environ. Health. **20**(1), 9–16 (2007)
23. R. Rzewnicki, Y. Vanden Auweele, I. De Bourdeaudhuij, Addressing overreporting on the International Physical Activity Questionnaire (IPAQ) telephone survey with a population sample. Public Health Nutr. **6**(3), 299–305 (2003)
24. R.P. Troiano, D. Berrigan, K.W. Dodd et al., Physical activity in the United States measured by accelerometer. Med. Sci. Sports Exerc. **40**(1), 181–188 (2008)
25. CDC, Prevalence of self-reported physically active adults, United States, 2007. MMWR Morb. Mortal Wkly. Rep. **57**(48), 1297–1300 (2008)
26. H. Besson, U. Ekelund, J. Luan et al., A cross-sectional analysis of physical activity and obesity indicators in European participants of the EPIC-PANACEA study. Int. J. Obes. (Lond) **33**(4), 497–506 (2009)
27. N. Kurtze, V. Rangul, B.E. Hustvedt, Reliability and validity of the international physical activity questionnaire in the Nord-Trondelag health study (HUNT) population of men. BMC Med. Res. Methodol. **8**, 63 (2008)
28. U. Ekelund, H. Sepp, S. Brage et al., Criterion-related validity of the last 7-day, short form of the International Physical Activity Questionnaire in Swedish adults. Public Health Nutr. **9**(2), 258–265 (2006)