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Age of obesity onset in *MC4R* mutation carriers

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Objectives. The mutations in gene for the melanocortin-4 receptor (*MC4R*) are the most common etiology factors of monogenic obesity development. Recently, it has been shown that current life style has a significant impact on the phenotype of *MC4R* mutation carriers – increases the penetrance of the mutations. We aimed to study the impact of the current age on the time of obesity onset among *MC4R* mutation carriers.

Methods. DNA analysis of the *MC4R* gene was performed in 268 unrelated Slovak and Moravian obese children and adolescents <18 years old. The control group included 174 unrelated obese individuals aged >18 years and 28 blood relatives >18 years of the probands with a mutation.

Results. Three different previously described heterozygous loss of function *MC4R* mutations (p.Ser19Alafs*34, p.Ser127Leu, and p.Gly181Asp) were found in 3 <18 years probands, 3 adult probands, and 6 adult obese/overweight family relatives. The age of obesity onset in mutation carriers was 1 year in all probands in the children group and 1-35 years (median 11 years) in adults. The age of the obesity onset significantly correlated (R=0.809, p=0.028) with the current age in all of the *MC4R* mutation carriers.

Conclusions. The age of obesity onset in the present child generation of *MC4R* mutation carriers is decreasing compared to the age of onset in their parents' generation. This is in agreement with similarly increasing penetrance of obesity in *MC4R* mutation carriers and it points out to escalation of obesogenic potential of environment.

Key words: *MC4R* mutation carriers, obesity, children, adolescents

Non-syndromic monogenic obesity is the result of a mutation in one of the genes encoding enzymes or receptors of the leptin-melanocortin pathway, which plays a key role in the regulation of satiety and maintaining energy homeostasis in the body. The most common of them are mutations in the gene coding melanocortin-4 receptor (*MC4R*) (Farooqi et al. 2003; Farooqi and O'Rahilly 2008). *MC4R* mutation carriers develop obesity due to the non-selective hyperphagia, particularly during childhood (Farooqi et al. 2003). In 2008, it has been shown that obesogenic environment has an impact on the phenotype of *MC4R* mutation carriers increasing the penetrance of the mutations (Stutzmann et al. 2008).

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If the penetrance increases, the age of obesity onset should be logically decreased. Therefore, we searched, whether a relationship between the current age of *MC4R* mutation carriers and the time of obesity onset in *MC4R* mutation carriers might exist.

Materials and Methods

Study design and participants. Together, 470 individuals from Slovakia and Moravia (Czech Republic) belonging to three cohorts were included into the study: 1) 268 unrelated obese children with body mass index (BMI) above the 97th percentile born later than in year 1996 (i.e. <18 years old); 2) 174 unrelated obese adults with BMI above 30 kg/m^2 born in or earlier than in year 1996 (de Pontual et al. 2008) (18-73 years); and 3) 28 blood relatives of probands with a mutation (both overweight, obese or lean), all aged more than 18 years. All patients with syndromic causes of obesity were primarily excluded. Anthropometric data were taken by specialized nurses in pediatric endocrinology outpatient clinics. The BMI percentiles and standard deviation score (SDS) in children and adolescents were calculated using IOTF standards (Cole et al. 2000). During regular health check-up, information on genetic testing was given and Informed Consent was signed. Samples of 8 ml venous blood were collected into EDTA tubes (Sarstedt, Numbrecht, Germany) for DNA analysis.

Molecular genetics analyses. Genomic DNA was extracted from the whole blood using standard procedures, and the coding exons and intron/exon boundaries of the *MC4R* gene were amplified by polymerase chain reaction (PCR) using previously described primers (Yeo et al. 2003). PCR products were sequenced using standard methods on ABI/Hitachi 3500 (Applied Biosystems, Warrington, UK) and were compared with the reference sequence NM_005912.2 using SeqScape software (version 2.1.1; Applied Biosystems, Warrington, UK).

Statistical analyses. Student t-test and Pearson correlation (SPSS, IBM) were used for data analysis.

Ethics committee approval. The present study was approved by the institutional Ethics Committees (University Hospital of Bratislava and National Institute of Endocrinology and Diabetology in Lubochna, Slovakia) and all of the participants signed an informed consent for the genotype and phenotype analyses.

Results

The mean current age in the children group was 11.8±3.8 years, mean BMI SDS was 3.1±0.8 SD, and mean age at obesity onset was 4.2±2.6 years. In the adult group, the mean BMI was 42.0±7.8 kg/m^2, current age 39.1±14.3 years, and mean age at obesity onset was 14.2±12.6 years.

Table 1
General description of the *MC4R* mutation carriers

Group	No of probands with *MC4R* mutation	Proband ID	Mutation	Current age of proband [years]	Age at obesity onset in probands [years]	BMI of proband [kg/m^2]/ BMI SDS in <18 years	Normal weight/ overweight/ obese family relatives with mutation	BMI of family relatives with mutation [kg/m^2]	Current age of relatives with mutation	Age at obesity onset of relatives with mutation
< 18 years	3	MO 56	p.Ser19 Alafs*34	14	1	36.2/3.5	0/0/1	34.5	54	N/A
		MO 310	p.Ser 127Leu	17	1	34.6/2.88	0/1/1	28.6, 31.3	42, 62	1, 35
		MO 359	p.Gly 181Asp	5	1	30.5/6.0	0/0/1	43.8	32	3
> 18 years	3	MO 111	p.Ser 127Leu	19	11	32.7	1/0/0	24.2	48	N/A
		MO 154	p.Ser 127Leu	48	30	41.9	0/0/2	N/A	51, 74	N/A
		MO 251	p.Ser 127Leu	33	N/A	N/A	0/0/0	N/A	N/A	N/A

N/A – not available

All 442 unrelated probands were tested for presence of mutations in the *MC4R* gene. Among them, three different previously described heterozygous loss of function *MC4R* mutations (p.Ser19Alafs*34, p.Ser127Leu, and p. Gly181Asp) were found together in 6 individuals - 3 cases from 268 in the children group (1.1%) and 3 from 174 in the group of adults (1.7%) (Table 1) (Hainerova et al. 2007; Stanikova et al. 2015). Consecutively, 28 relatives of mutation carriers were tested and 7 of them were positive for *MC4R* mutation.

Together, we have identified 13 mutation carriers, where 12 of them were obese or overweight at the time of DNA analysis. Three of them were younger than 18 years (probands only) with the age of obesity onset ≤1 year; 9 of them (3 probands and 6 relatives) were more than 18 years old with the age of obesity/overweight onset 1-35 years (median 11 years).

To establish a relation between current age and age of obesity onset, data from 12 obese mutation carriers were used. The age of obesity onset in the < 18 years group was not significantly lower compared to the adult *MC4R* mutation carriers (p=0.164). However, there was a significant positive correlation between these two variables (R=0.809, p=0.028), meaning that the younger generation of mutation carriers tends to become obese sooner than that of their parents.

Discussion

In this study, we investigated whether the trend of increased obesogenic potential of environment in present time may influence the time of obesity onset in carriers of *MC4R* mutations. The largest study focused on the impact of the age on the phenotype of the *MC4R* mutation carriers has been published by Stutzmann et al. (2008). Although this study was not primarily aimed to investigate the age of obesity onset of the mutation carriers, the above mentioned authors have shown a trend of decrease in age at obesity onset in younger *MC4R* mutation carriers.

However, there are no other studies available dealing with the correlation of current age and the age at obesity onset. Therefore, it is difficult to extract this information from the publications, since as usually, only the age at DNA analysis is reported. With this limitation, we searched in the most recent studies for the age of obesity onset among *MC4R* mutation carriers, who should be < 18 years of age, at present. We were able to identify one individual in Czech Republic (obesity onset at the age of 3 years) (Hainerova et al. 2007), 10 in the Netherlands (age at obesity onset ranged from < 1 to 3 years with median at 1 year) (van den Berg et al. 2011), one in Austria (obesity onset during the first year of life) (Rettenbacher et al. 2007), and 5 in Italy (age at obesity onset ranged from 1 to 7 years with median at 3 years) (Santoro et al. 2009). Thus, among *MC4R* mutation carriers supposedly aged < 18 years at present, only one from 20 (5%) exceeded 5 years at obesity onset (Santoro et al. 2009). Nevertheless, it is also apparent from other studies (Stutzmann et al. 2008; van den Berg et al. 2011) that there exists a much larger heterogeneity of age at obesity onset in actual adult population.

Despite the small number of mutation carriers analyzed in our study, this trend is, after all, noticeable from our data. All three MC4R positive children became obese around 1 year of age, whereas older MC4R positive patients had mean age of obesity onset of 16 years. As this trend was statistically significant, our results may confirm the hypothesis that the age of obesity onset is still decreasing.

Conclusion

The data of the present study as well as the recent clinical reports strongly indicate that a need of using different cut-off values for the age of obesity onset for children and for adults is emerging. However, before these cut-off values could be established for the *MC4R* gene DNA analysis, higher number of patients has to be analyzed.

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