

# Large quantitative effect of melanocortin-4 receptor gene mutations on body mass index

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The melanocortin-4 receptor gene (*MC4R*) is involved in central energy homeostasis and body weight regulation. Both endogenous anorexigenic and orexigenic ligands bind to the receptor.<sup>1</sup> Under normal conditions, the anorexigenic tone prevails as revealed by the fact that *Mc4r* knockout mice<sup>2</sup> develop elevated body weight. *Mc4r*<sup>-/-</sup> mice show higher food intake but a similar metabolic rate and similar decreased physical activity compared to wild type (WT) mice of the same strain.<sup>2–4</sup> In comparison to a standard low fat diet, this deviant regulation of energy homeostasis is even more pronounced upon intake of a moderately fat diet,<sup>5</sup> which leads to an even higher body mass.

In all studies, the effect on body weight is smaller in heterozygous than in homozygous knockout mice, but the exact degree of dominance is not clear. In heterozygous *Mc4r*<sup>+/-</sup> animals, body mass is increased on average by about 7–45% and in homozygous *Mc4r*<sup>-/-</sup> by 50–100% compared to WT<sup>2–5</sup> with substantial overlap between groups. The mutations might have a sex dependent effect, but the results are contradictory. In one study, the effect in males was only about half of that in females.<sup>2</sup> However, two studies did not detect a sex by genotype interaction in this *Mc4r*<sup>-/-</sup> strain.<sup>4, 5</sup> One study in a different knockout line of the same inbred strain found a sex by genotype interaction in the opposite direction, with only a marginal effect in heterozygous females whereas heterozygous males had a body weight intermediate between WT and homozygous knockouts.<sup>3</sup>

The first mutations in the human *MC4R* gene were reported in extremely obese probands.<sup>6–8</sup> Since then, several other studies investigated the association of different *MC4R* mutations with obesity. According to a recent overview,<sup>9</sup> at least 34 putatively functionally relevant variants have been detected in several mutation screens. The variants encompass frameshift, nonsense, and missense mutations, most of which have been shown to lead to total or partial loss of function in appropriate in vitro assays. All mutations are rare, with reported combined frequencies for all functionally relevant mutations typically in the range of 2–3% in extremely obese individuals.<sup>9</sup> In contrast, none of the putatively functionally relevant mutations have been found in controls.<sup>9</sup> The two missense variants V103I and I251L have each been detected with similar frequencies in both cases and controls; I251L is presumed to be a non-functional polymorphism,<sup>10</sup> while V103I shows a negative association with obesity as has recently been shown in a meta-analysis encompassing more than 7500 individuals.<sup>11</sup>

The quantitative effect of human *MC4R* mutations on body weight is currently unknown. Often the study design of screening phenotypically selected probands, for example extremely obese cases and normal weight controls, precludes such an analysis. It is assumed that these mutations more or less completely underlie the obesity of the respective carrier and this therefore is often referred to as a monogenic form of obesity.<sup>12–16</sup> However, in family studies based on obese index

## Key points

- Melanocortin-4 receptor gene (*MC4R*) mutations are known to cause obesity. The usual practice of ascertaining extremely obese probands to enhance the probability of detecting mutation carriers potentially leads to an overestimation of the phenotypic effects of these mutations.
- Our aim is to provide a more valid estimate of the effect of *MC4R* mutations by comparing the body weights of relatives of *MC4R* mutation carriers, comprising 181 phenotypically unselected relatives of extremely obese index patients from 25 pedigrees.
- Carriers of functionally relevant mutations had a significantly higher current body mass index (BMI) than their wild type relatives. The observed effect was about twice as strong in females than in males, with BMI differences between mutation carriers and wild type relatives of approximately 2.5 and 1.3 SD, amounting to 9.5 and 4 kg/m<sup>2</sup> in middle aged women and men, respectively.
- Our findings clearly substantiate that *MC4R* mutations entail a strong predisposition to obesity. Both the high rate and the degree of adiposity among wild type relatives nevertheless suggest that other genetic and/or environmental effects contribute to the obesity seen in mutation carriers.

carriers, single relatives harbouring the same mutation have been identified who were only moderately overweight or even lean.<sup>10, 17</sup> Whereas this can be explained by an underlying medical condition in single cases,<sup>17</sup> the lean carriers identified by Vaisse *et al*<sup>10</sup> were healthy. Recently, three missense mutations (which have not yet been functionally characterised) have also been found in a group of 48 controls with a body mass index (BMI) below 30 kg/m<sup>2</sup>.<sup>18</sup> Assessment of the effect size of *MC4R* mutations is further complicated by the fact that WT relatives of extremely obese mutation carriers are often also obese,<sup>15, 17, 19, 20</sup> indicating that other genetic and/or environmental factors are operative in these families, which accordingly could also contribute to the obesity of the index cases. It is well known that body weight is influenced by many genetic and environmental factors. Heritability estimates for BMI derived from family and twin studies range

**Abbreviations:**  $\alpha$ -MSH,  $\alpha$ -melanocyte stimulation hormone; BMI, body mass index; LOF, loss of function; *MC4R*, melanocortin-4 receptor gene; QTDT, Quantitative Transmission Disequilibrium Test; RF, reduced function; SDS, standard deviation score; SSCP, single strand conformation polymorphism; WT, wild type

from 0.3–0.7 to 0.6–0.8, respectively.<sup>21</sup> Evaluation of the phenotypic effects of *MC4R* mutations has to take this polygenic, multifactorial context into account and cannot simply assume that mutation carriers identified in samples of extremely obese probands are obese only because of their mutations.

In this study, we estimate the quantitative effect of *MC4R* mutations on BMI in a sample of 25 pedigrees (three of which have been reported previously<sup>17</sup>) with segregating mutations using only relatives of extremely obese index patients. These relatives have the advantage of being enriched for mutations without being ascertained for severe obesity.

## METHODS

### Study sample

The sample is based on an *MC4R* mutation screen in 887 extremely obese children (BMI $\geq$ 90th age and sex specific percentile<sup>22</sup>) and both parents of 520 of these (slightly extended sample of Hinney *et al*<sup>23</sup>). In 24 of the young index patients and in three parents of WT index patients, 17 different nonsense, missense, and frameshift mutations with potential functional relevance were detected (table 1). The two previously reported polymorphisms (V103I and I251L<sup>8 10</sup>) are not considered further here.

Written informed consent was given by participants, or in case of minors by their parents; the study was approved by the Ethics Committee of the University of Marburg. Because all 27 mutation carriers (24 index patients, three parents) had indicated that they wanted to be recontacted in case of a positive molecular finding upon the initial assessment, we were able to get in touch with all of them and obtained permission to also contact additional family members. Two families declined to participate in an extended family study. One of these families was included as the originally ascertained trio only; the other had to be excluded as we had no phenotypical information pertaining to mutation carriers other than the index patient. A total of 24 extended pedigrees were recruited through the mutation carriers (one family was recruited independently via two index cases who are second cousins and carriers of the same mutation; see Sina *et al*<sup>17</sup>) and all participating relatives were also screened

by single strand conformation polymorphism (SSCP) analysis and independent confirmatory mutation analyses (PCR-RFLP and allele-specific PCR; see Hinney *et al*<sup>23</sup>). SSCP is known to have a sensitivity of approximately 85%<sup>24</sup>; PCR-RFLP and allele-specific PCR are very sensitive mutation-detection tools.<sup>25 26</sup> We have performed both the SSCP and the independent mutation-specific analyses (PCR-RFLP or PCR with allele-specific primers) in duplicate, thus virtually eliminating the risk of undetected or wrongly classified mutations.

The final sample consisted of 284 subjects in 25 families comprising 3–30 individuals. A total of 207 subjects were both phenotyped and genotyped (98 males), the others define relationships between genotyped individuals. The 207 genotyped subjects included the 26 (23 with mutations) extremely obese index patients, 43 parents, 22 siblings, and 116 second or higher degree relatives. The mean (SD) age of the 207 genotyped individuals was 37.8 (19.4) years, ranging from 6 to 81 years.

The phenotype we considered was the standard deviation score (SDS) of both the current BMI and the recalled maximum lifetime BMI (using recalled maximum weight and currently measured height for adults; for children and adolescents the current weight was designated the maximum weight). SDS were derived from a large German population sample (German National Nutrition Survey<sup>22 27</sup>) and have the advantage that they are independent of sex and age (in contrast to BMI itself), thus enabling comparisons across this sample of relatives in different generations. As in our previous family study,<sup>17</sup> obesity was defined as a BMI $\geq$ 85th age and sex specific percentile.<sup>28</sup>

### Classification of *MC4R* mutations

In order to decide whether or not to consider different groups of mutations separately in the analysis, we evaluated all published information on putative functional relevance based on in vitro assays of the 17 mutations.<sup>10 23 29–34</sup> Assays that measured different properties of the receptor, and in some cases even the same assay performed by different groups, led to different conclusions about the functional relevance of mutations (table 1). Of the 17 mutations 16 have been shown to lead to a significant reduction of the receptor function as

**Table 1** Putative function of the different mutations in the coding region of the *MC4R* gene segregating in the 26 families according to different assays

Amino acid position and change	Cell surface expression	Ligand binding		Signalling properties as measured by cAMP ( $\alpha$ -MSH) assay
		IC <sub>50</sub>	Specific binding (relative to WT)	
S30F	About 120% <sup>31</sup>			Like WT, <sup>23</sup> RF <sup>31</sup>
[Y35X, D37V]*				LOF <sup>31</sup>
P78L	31%, <sup>31</sup> 1%, <sup>32</sup> 0% <sup>34</sup>		About 10% <sup>32</sup>	RF, <sup>23</sup> LOF, <sup>31</sup> LOF <sup>34</sup>
S94R				LOF <sup>23</sup>
V95I				LOF <sup>23</sup>
T112M	41%, <sup>32</sup> like WT <sup>35</sup>	Sign. reduced <sup>32</sup>		Like WT, <sup>29</sup> like WT <sup>35</sup>
I121T				RF <sup>23</sup>
S127L	101%, <sup>31</sup> like WT <sup>35</sup>			Const. active, <sup>23</sup> RF, <sup>31</sup> RF <sup>35</sup>
R165W	26%, <sup>31</sup> 9% <sup>32</sup>		About 0% <sup>10</sup>	RF <sup>10</sup>
G181D†				LOF <sup>23</sup>
L211fsX216	0% <sup>30</sup>		0% <sup>30</sup>	LOF, <sup>23</sup> LOF, <sup>31</sup> LOF <sup>30</sup>
P230L				Const. active <sup>23</sup>
A244E	90% <sup>31</sup>			RF, <sup>23</sup> RF <sup>31</sup>
L250fsX284				LOF <sup>23</sup>
G252S	About 100% <sup>31</sup>			Like WT, <sup>23</sup> RF <sup>31</sup>
I317T	66%, <sup>31</sup> 42%, <sup>32</sup> reduced <sup>33</sup>		About 42% <sup>32</sup>	Like WT, <sup>23</sup> RF, <sup>31</sup> RF <sup>33</sup>
Y320fsX354				RF <sup>23</sup>

\*The Y35X and D37V mutations only occur together on a haplotype and thus are not analysed separately; †The G181D mutation only occurred in an extremely obese index patient but not in his two available relatives and could therefore not be included in the statistical analysis. LOF, loss of function; RF, reduced function.

measured by cell surface expression, ligand binding (IC<sub>50</sub> values and specific binding relative to WT), and/or signal transduction ( $\alpha$ -melanocyte stimulation hormone ( $\alpha$ -MSH) stimulated cAMP signalling). One mutation (P230L) has been shown to be constitutively active in the cAMP assay,<sup>23</sup> as characterised by increased basal cAMP levels, but also showed a clear impairment in EC<sub>50</sub> values. Additionally, such mutations often have decreased cell surface expression,<sup>32</sup> and therefore we classified this mutation as also resulting in functional impairment. Results and interpretation of the published functional assays are not consistent enough to group mutations by different levels of functional impairment such as total loss of function and merely reduced function. In conclusion, all 17 identified mutations were classified as leading to an impaired receptor function. Accordingly, for the main statistical analyses we regarded them as equivalent and included a descriptive analysis of all mutations separately.

The two polymorphisms (V103I and I251L) were coded as WT and therefore not included in the effect estimation: V103I has been shown to be negatively associated with obesity<sup>11</sup>; I251L occurs with a similar frequency of 0.5–2% in obese and normal subjects in different populations<sup>8 10 15 18 23 35</sup> and behaves like WT in the cAMP assay.<sup>10</sup>

The distribution of genotypes in index patients and relatives in the study sample is shown in table 2. Two of the index patients (one male, one female) were compound heterozygous mutation carriers, whereas none of the relatives carried two mutations. The female compound heterozygous mutation carrier (with mutations S30F and S127L) was the most obese in our study group with a current BMI of 65.6 kg/m<sup>2</sup> (BMI SDS of 15.9), while the male compound heterozygous mutation carrier (mutations R165W and G252S) had a current BMI of 41.6 kg/m<sup>2</sup> (BMI SDS of 7.07).

### Statistical analyses

All statistical analyses were done without the index patients, because their inclusion would have led to biased estimates due to their ascertainment on the basis of their extreme obesity.

We tested for differences in current and maximum BMI SDS with the Quantitative Transmission Disequilibrium Test (QTDT),<sup>36</sup> a generalisation of the transmission/disequilibrium test<sup>37</sup> for quantitative phenotypes in general pedigrees which gives a valid test for association while accounting for correlations between relatives. Here, major gene effects through linkage and association are modelled separately in a variance components framework. The model takes into account the specific family structure of each pedigree and resulting correlations caused by polygenic and/or common environmental factors. Because the phenotypes are not normally distributed in this selected sample, significance was tested by a randomisation test (with 50 000 permutations), where transmitted and non-transmitted alleles in each meiosis are permuted. Finally, the expected mean BMI

SDS was estimated for WT and mutation carriers by inserting the obtained regression parameters into the respective equations. The difference between these means is the quantitative displacement attributable to the mutation. A one-sided 95% confidence interval (CI) for this displacement estimate was calculated as the difference which results in a p value of 0.05 in the randomisation test.<sup>38</sup> To investigate a possible sex by genotype interaction, we tested separately for differences between female mutation carriers and females with the WT genotype (by setting the phenotypes of all males to missing, while retaining their genotypes) and analogously between male mutation carriers and males with the WT genotype.

QTDT effectively compares the BMI SDS of individuals with a heterozygous parent from whom the mutation was transmitted with those who did not inherit the mutation. The family structure is also included in the QTDT model so that basically a comparison within sibships is performed to separate effects of the gene of interest from other polygenic and/or environmental effects.

On a descriptive basis, we also considered all mutations separately and calculated the mean per-family difference in BMI SDS between all WT and mutation carriers (again excluding index patients). For mutations which occurred in more than one family, relatives were weighted so each family contributed equally to the respective estimate.

### RESULTS

The permutation QTDT revealed a significant difference in current BMI SDS between WT individuals and mutation carriers within a family of approximately 1.8 (with a lower bound of a one-sided confidence interval of 0.6; table 3). For maximum lifetime BMI, the difference between carriers and non-carriers is even slightly larger than the difference in current BMI SDS. This effect of *MC4R* mutations is stronger in females than in males (no p value for the interaction can be given by QTDT). The average current SDS of male and female mutation carriers correspond to BMIs of 31.9 and 36.2 kg/m<sup>2</sup>, respectively, for subjects in the age range 30–40 years, whereas the average male and female WT carriers in our sample have corresponding BMIs of 27.9 and 26.7 kg/m<sup>2</sup>. Therefore the mutations account for respective BMI elevations of 14% and 36% above the average WT BMI in these families. In contrast to the apparent difference among mutation carriers, WT males and females have very similar BMI SDS. The estimated mean current and maximum lifetime BMI SDS for both WT and mutation carriers are clearly higher than the population means of 0 (table 3).

A descriptive analysis of the effects of each single mutation is shown in table 4, giving the mean per-family difference in current BMI SDS between mutation carriers and WT carriers. The effects range from –2.02 to 2.70 SDS.

On a descriptive basis, the rates for current and lifetime obesity decrease more strongly among WT than mutation carriers with respect to degree of relationship to the proband (table 5). Because of the mentioned ascertainment effect, these rates are much higher than in a population sample and only comparisons between mutation carriers and WT carriers within families are valid.

### DISCUSSION

We have, for the first time, systematically estimated the quantitative effect of *MC4R* mutations on BMI. Morton<sup>39</sup> used the expression “major gene” for a quantitative phenotype with a displacement greater than 1 SD. Thus, the observed difference of almost 2 SD for current BMI suggests that relevant mutations in the *MC4R* gene indicate it is a major gene for the development of obesity. The observed effect was about twice as strong in females than in males and

**Table 2** Number of index patients and relatives with different *MC4R* genotypes

	Homozygous WT	Heterozygous mutation carriers	Compound heterozygous mutation carriers
Index patients,	3	21	2
First degree relatives,	29	36	–
Second and higher degree relatives,	86	30	–

**Table 3** Mean current and maximum lifetime BMI SDS by genotype for informative families as revealed by QTD analysis

Sample	Number of informative individuals	Mean current BMI SDS			p Value	Mean maximum lifetime BMI SDS			
		WT carriers	Mutation carriers	Lower bound of one-sided 95% CI of mean difference between WT and mutation carriers		WT carriers	Mutation carriers	Lower bound of one-sided 95% CI of mean difference between WT and mutation carriers	p Value
All relatives	82	1.00*	2.82	0.57	0.0112	1.60	3.57	0.9	0.0024
Females only	43	0.95	3.41	0.77	0.0094	1.58	4.14	0.98	0.0052
Males only	39	0.99	2.27	0.14	0.0284	1.52	3.12	0.46	0.0015

\*All estimators were derived by inserting the obtained QTD regression parameters in the respective linear model equations.

corresponds to a difference in current BMI of approximately 9.5 kg/m<sup>2</sup> for females and 4.0 kg/m<sup>2</sup> for males in the age range 30–40 years. This represents BMI elevations of 14% and 36% above the average WT BMI in these families. These results are consistent with animal studies, in which heterozygous *Mc4r*<sup>+/-</sup> mice also showed an average increase in body weight of 7–45% compared to WT littermates. Our observed sex effect is also similar to the results of the original *Mc4r*<sup>+/-</sup> model.<sup>2</sup>

The obesity of *MC4R* mutation carriers has previously been termed monogenic.<sup>12–16</sup> Although our results indeed point to a strong quantitative effect, it should be noted that the differences in current BMI between WT and mutation carriers of 4 and 9.5 kg/m<sup>2</sup> for males and females, respectively, do not necessarily imply the development of obesity. Indeed, lean mutation carriers have been observed<sup>10</sup> who may not have crossed the threshold to severe obesity due to other, as yet unidentified, modifier genes and/or environmental factors. The rates of obesity in our WT carriers (table 5) illustrate that in these families, including the mutation carriers, other influences predisposing to an elevated body weight are operative. As expected for a multifactorial disorder, rates of obesity drop substantially between the first and second degree WT relatives of the index patients. In contrast, this decline is considerably less pronounced among the mutation carrying relatives, again underscoring the major effect of *MC4R* mutations (table 5). In the light of these considerations we prefer the term major gene effect instead of monogenic for *MC4R* mutations. Like Morton,<sup>39</sup> we use this expression in the sense of a high individual risk for

a complex, quantitative trait without implying a high population attributable risk (due to the rareness of these mutations).

Because of our sampling scheme we are unable to draw definite conclusions for mutation carriers with a genetic and/or environmental predisposition to leanness; in principle our results are only generalisable to relatives of obese probands living in our current (German) obesigenic environment. If *MC4R* mutations have an additive effect independent of other genetic and/or environmental factors, the observed difference in BMI should be approximately constant over the whole BMI range. The effect of ascertainment on parameter estimates was previously shown in the penetrance estimation of the breast cancer 1 gene (*BRCA1*) on breast cancer<sup>40</sup>: the highest penetrance estimates were obtained in studies of “high-risk” families with at least four affecteds.<sup>41</sup> Lower estimates were derived from studies based on cases ascertained independently of family history.<sup>42</sup> Even lower estimates were obtained in a population based study.<sup>43</sup> The explanation for this systematic trend results from the overrepresentation of all risk factors in cases and even more so in “high-risk” families. Our displacement estimate would be similarly inflated if we had compared the BMI of the mutation carrying relatives of our extremely obese index patients with those of the general population (for example, by testing whether the BMI SDS of these relatives have a mean of 0). The effect of ascertainment can also be seen in the high rate of obesity among these relatives (table 5) and has to be taken into account properly in the analysis. However, we evaluated the difference between mutation carriers and WT carriers within families upon exclusion of index patients, which reduces the ascertainment bias substantially. Exclusion of index patients is important because they were recruited based on their extreme phenotypes and are almost all mutation carriers (23 out of 26). A completely unbiased estimation of phenotypic effects of *MC4R* mutations is only possible with a population based sample, which would have to be prohibitively large due to the very low frequency of these mutations in the general population.

In six other human studies,<sup>7 10 19 20 31 44</sup> the body weight in SDS of genotyped family members of index patients has

**Table 4** Mean current BMI SDS difference between WT and mutation carrying relatives within families for single mutations

Amino acid position and change	Number of families segregating this mutation	Number of WT individuals	Number of mutation carriers	Mean BMI SDS difference
S30F	2	18	4	1.2
[Y35X, D37V]	8	23	22	1.05
P78L	1	10	2	0.43
S94R	1	1	2	1.04
V95I	1	3	2	-1.5
T112M	1	2	2	0.56
I121T	1	3	2	-0.57
S127L	3	12	6	2.18
R165W	1	7	4	2.08
L211fsX216	2	19	10	2.70
P230L	1	10	2	1.84
A244E	1	5	4	0.50
L250fsX284	1	1	1	-0.21
G252S	1	2	16	2.13
I317T	1	2	1	-2.02
Y320fsX354	1	5	2	0.14

**Table 5** Rates of obesity among WT and mutation carriers, percentage currently and ever above 85th sex and age specific BMI percentile

	Currently obese		Ever obese	
	WT	Mutation carriers	WT	Mutation carriers
Index patients	100%	100%	100%	100%
First degree relatives	68%	92%	81%	97%
Second and higher degree relatives	50%	80%	58%	97%

previously been presented. In 14 families, data on 46 first degree relatives with different genotypes were reported, and data on 17 second and higher degree relatives for six of these families. Based on these data, we calculated that the mean difference between mutation carriers and WT with the same degree of relationship to the proband within a family is 2.1 SDS and thus similar to the results obtained in our systematic analysis.

For our statistical analyses, mutations had to be grouped since most are individually too rare to draw meaningful conclusions.<sup>9</sup> After careful consideration of all available functional data, we decided to consider only one group despite the fact that the different mutations might exert different quantitative effects in vivo; all those mutations were included for which in vitro characterisations have indicated or suggested an impaired function.<sup>10 23 29–34</sup> Our decision to form only one group was based on the limited number of available families and the fact that different functional assays have rendered divergent and sometimes inconsistent results as to the degree of functional impairment of specific mutations. Furthermore, most mutations have not yet been fully tested for all experimentally accessible functions (for example, signalling properties, binding of endogenous ligands, cell surface expression; see table 1), so that a final classification based on such detailed in vitro results is not yet possible. Ultimately, a classification of MC4R mutations should be based on both functional and quantitative phenotypic data.

As expected, a separate consideration of single mutations showed large variation in effect estimates (table 4). In some families, mutation carriers had even smaller BMI SDS than WT carriers, again underlining that among individual members of a particular family other factors, such as polygenic background, other major genes, and environmental factors also play an important role in body weight regulation. Clearly, random variation in other factors adds to the quantitative differences in BMI SDS of carriers of the same mutation. The mean effect of each single mutation on the phenotype can only be estimated reliably from a large enough population or family based sample. Because most of our detected mutations segregated in only one single family with a limited number of mutation carriers and WT individuals, respectively, this expected random variation also contributes to the interfamilial differences in observed effects of different mutations.

However, the observed interfamilial differences in effect sizes are also due to the fact that different mutations likely imply a quantitative range of functional impairment theoretically ranging from enhanced function (as suggested for V103I or an associated variant<sup>11</sup>), no functional effect—even if in vitro results indicate a reduced function, this does not necessarily apply in vivo—to a more or less complete loss of MC4R function due to a dominant negative effect. In this context, it is of interest that the most commonly detected MC4R mutation (Y35X), which was detected in eight families with a total of 44 relatives plus eight index patients, resulted in a difference of only 1.05 SD between mutation and WT carriers. The respective mutation most likely leads to haplo-insufficiency for MC4R<sup>45</sup> because the short N-terminal sequence is presumably rapidly degraded upon translation. In contrast, other mutations could entail a dominant negative effect,<sup>45</sup> thus rendering the quantitative effect of such mutations even stronger than haplo-insufficiency. This could be the case for the mutation with the highest observed BMI increase (2.70 SDS; L211fsX216). To what extent dominant negativity plays a role in MC4R signalling is not yet fully elucidated.<sup>30 45 46</sup> It seems that the mutation for which the least functional evidence of an impaired receptor function is available (the constitutively active mutation P230L) shows a

similar effect on BMI as the combined sample of all mutations. Additional family studies offer a powerful approach to indeed clarify if such constitutively active mutations entail a strong quantitative effect on BMI in vivo.

In conclusion, our approach of recruiting relatives of known mutation carriers is a solid strategy to estimate the quantitative effect of genetic variants in a sample enriched for mutation carriers. Such family studies should complement functional in vitro studies as these cannot readily be related to the in vivo effect of a particular mutation, and pedigree analyses represent the only way to estimate the quantitative effect on BMI in humans for such rare mutations. Our results indicate that adult male and female MC4R mutation carriers have an increased risk of obesity, which amounts to a BMI elevated by approximately 4 and 9.5 kg/m<sup>2</sup>, respectively, in comparison to their WT relatives in families of obese index patients.

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