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Genetic Study of the Melanin-Concentrating Hormone Receptor 2 (*MCHR2*) in Childhood and Adulthood Severe Obesity.

Short title: Absence of association of *MCHR2* gene with human obesity

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DISCLOSURE STATEMENT: The authors have nothing to declare

Number of words (text) = 3,349, number of words (abstract) = 273, number of tables and figures: 3 Figures + 1 appendix figure, 2 tables.

Abstract

Context: The Melanin Concentrating Hormone Receptor 2 (MCHR2) is a G protein-coupled receptor for MCH, a neuropeptide that plays important role in feeding behaviors. *MCHR2* maps on chromosome 6q16.3, in a susceptibility locus for childhood obesity. Objective: The aim of this study was to investigate the association between MCHR2 variation and human obesity. Design: Case control and family-based studies were performed. Participants: 141 obese children and 24 non-obese adult subjects were sequenced and case-control analyses were conducted using 628 severely obese children and 1,401 controls. Results: Eleven Single Nucleotide Polymorphisms (SNPs) were identified. We showed nominal association between -38,245 ATG A/G SNP (p=0.03, 95%CI=[1.02-1.34], OR=1.17), A76A T/C SNP (p=0.03, 95%CI=[0.58-0.97], OR=0.75) and childhood obesity. Analysis of 645 trios with childhood obesity supported further the A76A T/C association, showing an over-transmission to obese children of the at risk T allele (59.0%, p=0.01), especially in children with most severe forms of obesity (Zscore of BMI>4) (67.0%, p=0.003). The A76A at risk T allele was also associated with overeating during meal (p=0.02) in an additional group of 102 non-obese children. None of *MCHR2* variants, including the A76A SNP, showed association with adult severe obesity, although a trend for association of the T allele of this variant with food disinhibition (p=0.06) and higher hunger (p=0.09) was found. This variant was not associated with childhood obesity in an independent case-control study including 1,573 subjects (p=0.98). Moreover, the A76A SNP did not explain the linkage on the 6q locus. Conclusions: Our results altogether suggest that *MCHR2* is not a major contributor to polygenic obesity and support a modest effect of the A76A SNP on food intake abnormalities in childhood.

If the current epidemic of obesity clearly reflects the environmental and behavior changes during the past half-century, genetic background remains important especially in the severe forms of obesity, as assessed by ethnic (1), familial (2, 3) or linkage (4) studies. We previously identified in the French population, a linkage with childhood obesity on chromosome 6q16.3-q24.2 with a Lod score of 4.06 (5). Recently, we reported that variation in *ENPP1* (Ectonucleotide Pyrophosphatase Phosphodiesterase1) partly contributed to the observed linkage (6). Indeed, if we remove the 15 affected sibling pairs from a total of 135 sibling pairs sharing the *ENPP1* at risk haplotype, the Multipoint Lod Score drops from 4.06 to 1.6 at marker D6S287 and a new maximal score of 2.63 is obtained 16 Mb centromeric to the original linkage peak, at marker D6S301 (6).

MCHR2 is an obvious candidate gene lying under this new peak. The orphan G-coupled protein Melanin-Concentrating Hormone Receptor 2 (MCHR2) consists in 340 amino acids with a coding sequence distributed over 6 exons (7-9) and showing 38% homology with the Melanin-Concentrating Hormone Receptor 1 (MCHR1) (10). MCHR2 displays high-affinity binding to MCH (11), which is known to increase food intake and body weight in rodents after its central administration (12-14). MCH acts as a functional antagonist of the α-MSH (alpha-Melanocyte-Stimulating Hormone), in a complex central network involving the melanocortin pathways (15). *MCH* overexpression leads to obesity and insulin resistance in mice (16). In contrast, mice that lack the *MCH* gene or targeted inactivation of *MCH* gene in neurons cause a phenotype of leanness as a consequence of hypophagia and increased metabolic rate (17). Expression of *MCHR2* is restricted to several regions in the brain, including the arcuate nucleus and the ventral medial nucleus, areas involved in regulation of food intake (18). Consistently, these two nucleus have been recently implicated in mediating the MCH effect via activation or inhibition of feeding circuits (19).

Tan et al. showed that functional expression of the *MCHR2* gene is not conserved during the evolution. In contrast to *MCHR1*, the functional *MCHR2* is only expressed in humans, primates and carnivores but not in rodents (10). Thus, little is known about the physiological role of MCHR2 and human genetics offers unique opportunity to evaluate the contribution of this receptor to appetite regulation and associated diseases in humans. This paper investigates the implication of variation in *MCHR2* in polygenic and monogenic forms of childhood obesity and its related quantitative and eating disorders traits.

RESEARCH DESIGN AND METHODS

Population used for association studies

Association studies with childhood and adulthood obesity were performed for variants with Minor Allele Frequency (MAF) ≥ 5% using a set of 628 unrelated obese children chosen from the cohort of 849 obese children available (Male/Female=402/447, age=10.7±3.60 years, BMI=28.84±6.56 kg/m^2, Z score of BMI=4.16±1.32), 696 unrelated class III obese adults (Male/Female=176/520, mean age=45.95±12.06, BMI=47.69±7.22 kg/m^2) and 1,401 nonobese normoglycemic adults (Male/Female=564/837, age=41.32±15.07, BMI=22.42±2.31 kg/m^2). The study protocol was approved by all local ethic committees and an informed consent was obtained from each subject before participating in the study.

Obese children cohort

The pool of obese children used for case/control analysis was constituted of a first set of 424 unrelated obese children collected from 424 pedigrees with at least one obese child at the CNRS – UMR8090 Unit in Lille, and at the Jeanne de Flandres Hospital in Lille, a second set of 93 unrelated obese children recruited at the Children's Hospital, Toulouse, a third set of 24

unrelated obese children recruited through the "Fleurbaix-Laventie Ville Santé" study and a fourth set of 87 unrelated children collected from the Trousseau Hospital. We genotyped the A76A T/C SNP in 148 additional obese children collected at the CNRS UMR8090 and in 439 obese children collected at the Saint Vincent de Paul hospital. Children with a BMI greater than the 97th percentile for age and sex reported on the tables of Rolland-Cachera *et al*. (20) (French general population) were defined as obese as recommended by the European Childhood Obesity Group (ECOG) (21).

Obese adults cohort

The class III obese adult subgroup was constituted by 696 class III obese adults collected at the Department of Nutrition of the Hôtel Dieu Hospital in Paris or at the CNRS-Institut Pasteur Unit in Lille. Class III obesity status was defined as BMI $\geq$ 40 kg/m^2 in adults.

Control adults cohort

The same adult control group was used for both association studies in obese children and adults as this group had a longer environmental exposure and still remains non obese. This group consisted in 1,401 nonobese (BMI < 27 kg/m²) normoglycemic (fasting glycemia < 5.56 mmol/l) French Caucasian adults pooled from four separate studies; 360 unrelated nonobese and nondiabetic subjects were recruited at the CNRS- Institut Pasteur Unit in Lille, 235 were recruited by the "Fleurbaix-Laventie Ville Santé" study (22), 396 from the HAGUENEAU study (23) and 410 from the SUVIMAX study (24). Absence of stratification among all the different studied cohorts was verified using 26 neutral polymorphic markers disseminated across the genome (data not shown). We genotyped the A76A T/C SNP in 986 additional lean adult subjects (BMI < 27 kg/m²) issued from the SUVIMAX cohort. The genetic study was approved by the ethical committee's of Hôtel Dieu Hospital in Paris and Centre Hospitalier Régional Universitaire de Lille.

We used 424 pedigrees with childhood obesity (645 childhood obesity trios - two parents and one obese child) and 102 non obese childhood trios (BMI < 97th percentile for gender and age) and 158 pedigrees with adulthood obesity including 514 individuals (303 obese, 72 overweight and 139 lean) for Transmission Disequilibrium Test (TDT) analysis for obesity status and eating behavior traits.

Eating behavior traits

Food behavior in obese adults was assessed by the TFEQ (Three Factor Eating Questionnaire) (25), which evaluates the cognitive restraint of eating, disinhibition, and hunger. Scores for the TFEQ were available for 500 class III obese patients with familial history of obesity. Because the TFEQ is not a validated questionnaire in children, food intake behavior in 102 young non obese children (46 girls and 56 boys; BMI < 97th percentile for gender and age) was assessed by an in-house questionnaire administrated by a trained physician. Seven questions were asked. Two questions were related to food intake behavior during a meal (presence or absence of hyperphagia and rapidity of food ingestion) and between meals (presence or absence of snacking).

Sequencing and genotyping

The screening of the *MCHR2* gene was done using overlapping PCR fragments that cover all exons of the *MCHR2* gene, exon/intron junctions, and a part of the putative promoter and the 3'UTR (UnTranslated Region). Primer details and PCR optimization conditions are available from the authors. PCR amplifications were inspected for single bands of expected sizes on agarose gels before purification with Montage PCR384 Multiscreen® S384PCR (Millipore). Sequencing was performed using the automated ABI Prism 3730 DNA sequencer in combination with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and purification Sequencing reaction with MultiScreen® SEQ384 filter plates (Millipore).

To cover the intronic regions of the *MCHR2* gene, genotypes of nine intronic SNPs have been extracted from a whole-genome association search performed in 325 obese children and 425 control subjects (unpublished results), using DNA pooling strategy, as detailed elsewhere (26). Genotyping was performed by labeling genomic DNA and hybridizing it to Illumina Infinium Human1 and Hap300 BeadArrays, which interrogated 109,365 and 317,503 SNPs, respectively. The nine SNPs were in Hardy-Weinberg equilibrium ($p>0.01$) in both case and control subjects.

The four SNPs with a MAF of >5% were then genotyped in all case and control groups using direct sequencing for -38,245 ATG A/G and -38,244 ATG T/C and Light-Cycler/Typer technology (Roche) for -26,780 ATG C/T and A76A T/C. Genotyping error rates calculated from duplicate genotypes of 250 individuals were 0% for -38,245 ATG A/G, -26,780 ATG C/T, A76A T/C, and 0.9 % for -38,244 ATG T/C. No recurrent mendelian inconsistencies were detected in the 608 pedigrees for the two analyzed SNPs (-38,245 ATG A/G, A76A T/C) using the PEDCHECK 1.1 program.

Statistical analysis

Tests for deviation from Hardy-Weinberg Equilibrium (HWE) and for association were performed with the De Finetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). All SNPs were in HWE. We compared all cases against all control individuals as well as class III obese adults and obese children separately against the control group. These analyses were done by comparing allelic frequencies of the SNPs between cases and controls. Analysis of variance and T-test were performed for the studying of quantitative traits in obese and control adults. Haplotype frequencies were determined and were compared between groups with the UNPHASED software http://www.mrc-bsu.cam.ac.uk/personal/frank/. Familial analysis on binary and quantitative traits were performed by the TDTPHASE and QPDTPHASE methods implemented in the UNPHASED software. In order to evaluate the effect of the T at risk

allele of the A76A variant on linkage, we used the Genotype IBD Sharing Test (GIST) procedure. SPSS 10.1 software was used for general statistical analysis.

Results

Screening of the gene

All exons of the *MCHR2* gene, exon/intron junctions, 870 bp of the putative promoter and 1,095 bp in the 3'UTR were sequenced in 47 obese children from families with evidence for linkage of childhood obesity to 6q, 94 obese children with early obesity onset (Z score of BMI>4.5, obesity onset before 5 years old), and in 24 non obese normoglycemic adults. We identified six SNPs in the promoter region, one non synonymous mutation, two synonymous mutations and two SNPs in the 3' UTR (Figure 1 and 2). Among these SNPs, three were frequent (-38,245 ATG A/G, -38,244 ATG T/C and A76A T/C) with minor allele frequencies > 5% and Linkage Disequilibrium analysis (LD) showed that these SNPs were in incomplete LD (R^2 <0.8). Therefore, the three SNPs were selected to be typed in all our samples (Appendix Figure 1).

The eight remaining rare variants including the non synonymous variant R152Q G/A (minor allele frequencies < 5%, Table1) were found in both obese and control subjects. These results ruled out a potential implication of these variants in monogenic forms of obesity in our studied population.

Association studies of frequent SNPs with childhood obesity

Case/Control analysis was performed for the three SNPs in 2,029 French Caucasians (628 unrelated obese children and 1,401 normoglycemic non-obese control adults). The A/G -38,245 G allele and the A76A T allele showed nominal evidence for association with childhood obesity (p=0.03, 95% CI= [1.02-1.34], OR=1.17; p=0.03, 95% CI= [0.58-0.97],

OR=0.75; Table 2). The remaining SNP -38,244 ATG T/C was not associated with childhood obesity (p=0.50, Table 2).

Because our initial screening did not include intronic regions, we analyzed data concerning nine additional tagged SNPs covering the *MCHR2* introns, that were included in a Whole-Genome Association scan of childhood obesity performed in a subset of of obese children cohort (325 obese children compared to 425 control subjects, unpublished results). The location of the intronic SNPs is shown in Figure 1.

No intronic SNP showed significant ($p<0.05$) association with childhood obesity, except for SNP -26,780 ATG C/T (rs9496085, p=0.035), located in intron 1 of *MCHR2*. We then genotyped rs9496085 in our extended case control set of 2,029 French Caucasians. This SNP showed only a modest trend toward association with childhood obesity (p=0.055, 95% CI= [0.75-1.00], OR=0.87; Table 2).

TDT analysis in trios with childhood obesity

TDT analysis of 424 pedigrees with childhood obesity was then performed for the two frequent SNPs significantly associated ($p<0.05$) with childhood obesity in the case control design. The SNP -38,245 ATG A/G did not show an allelic transmission distortion in obese children ($p>0.05$, data not shown). However we found evidence of an over-transmission of the A76A at risk T allele in obese children (59.0%, p=0.01, 120 transmitted *versus* 85 non transmitted), especially in children with the most severe forms of obesity (Z score of BMI>4) (67.0%, p=0.003, 52 transmitted *versus* 26 non transmitted; Figure 3).

In an attempt to gain statistical power in our analysis, we then took into account the phenotypes and genotypes of three available generations (children, parents and grand parents) for the obesity threshold (97^{th} percentile) for gender and age. We analyzed together the over-transmission of the at risk allele from the heterozygous grand parents to the obese parents and

the over-transmission of the at risk allele from the heterozygous parents to the obese children. An over-transmission of the "at risk allele" to obese offspring's was found (61.0%, p=0.002,). To investigate if the A76A variant could affect primarily eating behaviors and thus induce obesity, we restricted the TDT analysis of eating behavior phenotypes to a subgroup of 102 non-obese children , given that the T allele was over-transmitted to obese children. These non-obese children were issued from the initial 424 pedigrees with childhood obesity and showed no distortion of segregation for the T allele (53.8%, p=0.69). Interestingly, the analysis of non-obese children with overeating during meal showed a systematic transmission of the T allele to these subjects (100%, p=0.004, 6 transmitted *versus* 0 non transmitted). Accordingly, 25% of the TT genotype carriers of the A76A variant showed overeating during meal, whereas none of the TC and CC genotype carriers harbored this disorder (p=0.02).

Association studies with adulthood obesity

No association with adult class III obesity was found for any of the four SNPs (Table 2). However, pooled data from obese children and adults showed nominal evidence of association with obesity for the -38,245 A/G SNP (p=0.02, 95% CI=[1.02-1.28], OR=1.15) and a trend towards association for the A76A T/C SNP (p=0.07, 95% CI=[0.67-1.02], OR=0.83) (Table 2). In adults there was also a trend toward association of the T allele of the A76A variant with higher hunger (p=0.09) and with disinhibition for food (p=0.06, data not shown).

Haplotype analysis

To estimate the potential effect of the combination of the *MCHR2* SNPs, we performed haplotype analysis in obese subjects and controls using the UNPHASED software. The haplotype including the two associated SNPs or the four frequent SNPs did not provide stronger evidence for association than SNPs analyzed independently (data not shown).

Linkage analysis

After exclusion of subjects carrying at least one risk haplotype of the *ENPP1* gene, we tested the contribution of the -38,245 A/G and A76A T/C SNPs to the maximum of the linkage peak at marker D6S301 using GIST procedure. Our results did not provide any evidence for participation of this SNP to the linkage observed on the 6q locus (dominant model, p=0.16).

Replication study between A76A T/C and childhood obesity

In an attempt to replicate the association of the A76A T/C in an independent French cohort, we genotyped this polymorphism in two additional sets including 986 lean adult subjects issued from the SUVIMAX cohort, and 587 obese children collected at the CNRS UMR8090 (N=148) and the Saint Vincent de Paul hospital (N=439). We did not replicate the association between the A76A T allele and childhood obesity (p=0.98, 95% CI=[0.77-1.29], OR=0.99, data not shown).

Discussion

This study is the first to investigate a possible role of *MCHR2* SNPs in human polygenic severe obesity in a large population of French Caucasians. The initial case/control analysis gave nominal evidence for association between the two variants -38,245 A/G and A76A T/C and childhood obesity but this result does not resist to multiple testing correction. Even if the TDT analysis supports the contribution of the A76A T/C SNP to childhood obesity, it is noteworthy to indicate that none of these two variants showed association with severe forms of obesity in adults. We were not able to replicate the association between A76A T/C SNP and childhood obesity in an independent case control design including 1,573 subjects. Moreover, our results did not provide any evidence for participation of these variants to the linkage observed on the 6q locus. This underlines the need to carry on the search for other

genetic variants contributing to the observed linkage with childhood obesity on chromosome 6q.

Our data suggests that the A76A T/C *MCHR2* SNP may mediate modest eating behavior disorders in childhood such as overeating during meals. However, this result should be interpreted with caution because we used a non-validated in-house questionnaire in children. We also found a trend for an effect of this SNP on food intake parameters in adults. The impact of genetic variants of the *MCHR2* gene on food intake, if any, seems to be attenuated in adulthood, which could explain the lack of association of the A76A variant in severe adult obesity. Similar observations have been found for other key components of the central regulation of food intake. Farooqi *et al.* showed an age-related decrease in hyperphagia in obese subjects with *MC4R* mutations, that seems to occur with adulthood (27).

The observation that genetic variation in *MCHR2* could modulate food intake is consistent with the proposed role of MCH/MCH receptor pathway in the literature (11). As MCHR1, MCHR2 is specifically activated by nanomolar concentrations of MCH, but signals through Gq proteins to induce an increase in intracellular [Ca^{2+}] and inositol phosphate 3 (7, 18). As A76A is a synonymous coding variation, it seems unlikely that this variant could act by increasing the affinity and the binding of MCHR2 to its ligand and thereby induce an enhancement in the orexigenic effect of MCH. A more plausible hypothesis could be that this variant confers increased RNA stability to *MCHR2*, which could affect the MCHR2 receptor density and the orexigenic effect of MCH. Several reports have highlighted the significance of synonymous mutations that affect mRNA secondary structure, which in some cases induce diseases (28, 29). In addition, it remains possible that A76A genetic variant could affect exon skipping or disrupt splicing process as previously documented in abundant examples of synonymous mutations (30, 31). Finally, we cannot exclude the possibility that this variant is in linkage disequilibrium with the true functional variant located elsewhere in the *MCHR2*

gene. Unfortunately, this SNP was not genotyped in the Hapmap II avoiding us to study the extensive LD with A76A in the 6q16 region. A detailed analysis of linkage disequilibrium in the *MCHR2* region by sequencing of large size population-based cohorts could be useful. If LD analysis and association studies revealed that the A76A variant is itself a primary variant determining obesity susceptibility, then functional analysis should be undertaken.

In our study, the eight rare SNPs have been found in both obese and control individuals. This lack of implication of rare variants of the *MCHR2* gene in monogenic forms of obesity in our studied population is consistent with the findings of Bell et al. for the *MCHR1* gene (32). A previous study performed in the United Kingdom population found two non coding SNPs in the *MCHR2* gene, which were not analyzed as they were rare (33). Screening of this gene in white and African-American individuals identified four non coding SNPs and four coding mutations (34) that include the three coding mutations identified in our study. A fourth non synonymous mutation R63K was identified and was not detected in our initial sequenced set. Functional analysis of *MCHR2*-carrying each of the non synonymous mutations G152Q or R63Q demonstrated that this receptor binds MCH and couples to intracellular signalling pathways in a similar way to wild type *MCHR2* (34). The absence of functional effect particularly of the *MCHR2*-carrying G152Q mutation is consistent with our findings. This SNP was found in obese and control subjects at equal frequencies.

In conclusion, our results suggest that *MCHR2* gene is not a major contributor to polygenic and monogenic forms of childhood and adulthood obesity. However, the A76A T/C SNP of *MCHR2* might have a modest effect on food intake abnormalities. These preliminary results need to be confirmed in additional populations.

Acknowledgments

We are indebted to all families who participated to this study. We also thank “le Conseil National de la Recherche Scientifique Libanais (CNRS-L)”, “le Conseil Régional Nord Pas de Calais / FEDER”, “200 Familles pour Vaincre le Diabète et l'Obésite”, “Association Française des Diabétiques” for their financial support, C. G. Bell for the helpful figure of MCHR1, Christian Dina and Sophie Gallina for the statistical help and Kirsten .J. Ward for the improvement of the paper. We also gratefully acknowledge Olfert Landt at Tib-Molbiol (www.tib-molbiol.com <http://www.tib-molbiol.com>) for their technical assistance.

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Table 1: Positions, base or amino acid changes, RS number (if known) and frequencies of the eleven SNPs identified within the *MCHR2* gene in the initial screened set. SNPs positions in the first column are indicated in relation to the initiation codon ATG or the stop codon TGA (in the UCSC genome browser)

Table 2: Association (p≤0.05) of genotypes and alleles of *MCHR2* gene SNPs with obesity. Cases Set 1 = 628 French Caucasian children with BMI > 97th percentile. Cases Set 2 = 696 obese French Caucasian adults with BMI ≥ 40. Control Set = 1,401 non obese and normoglycemic French Caucasian adults. N: number of subjects, OR: Odd Ratio, CI: 95% Confidence Interval, HW: Hardy Weinberg. Significant p-values are indicated in bold.

Figure 1: Location of the 20 SNPs in the *MCHR2* gene. Exons one to six are represented in grey. The eleven SNPs shown below the figure are those identified after the screening of the MCHR2 gene: Three variants -38,245 ATG A/G, -38,244 ATG T/C and A76A T/C had a MAF>5% and were studied in case/control analysis. The eight remaining SNPs had a MAF<5% and were found in both obese and control subjects ruling out a contribution in monogenic form of obesity. The nine additional SNPs shown above the figure are the intronic tagged SNPs that were included from the whole genome scan study of childhood obesity to cover the *MCHR2* genetic variation in introns.

Figure 2: Schematic representation of the structure of the seven-helix transmembrane protein *MCHR2*. Positions of the three coding variations (two synonymous and one non synonymous), identified after the *MCHR2* screening, are indicated as grey circles. These three mutations were also identified in the study of Hawes *et al.* (34). The bold circles indicate the amino acid delimiting the transmembrane domain from both sides of the protein. The dotted

circles indicate the non-synonymous mutation R63K that was found in the study of Hawes *et al.* but was not found through our initial screened set.

Figure 3: Familial association of the A76A T/C in pedigrees with childhood obesity. TDT analysis of 424 pedigrees (645 trios) with childhood obesity showed an over-transmission of the A76A at risk T allele in obese children (59.0%, p=0.01) and in children with the more severe forms of obesity (Z score of BMI >4) (67.0%, p=0.003).

Table 1: Positions, base or amino acid changes, RS number (if known) and frequencies of the eleven SNPs identified within the *MCHR2* gene in the initial screened set. SNPs positions in the first column are indicated in relation to the initiation codon ATG or the stop codon TGA (in the UCSC genome browser).

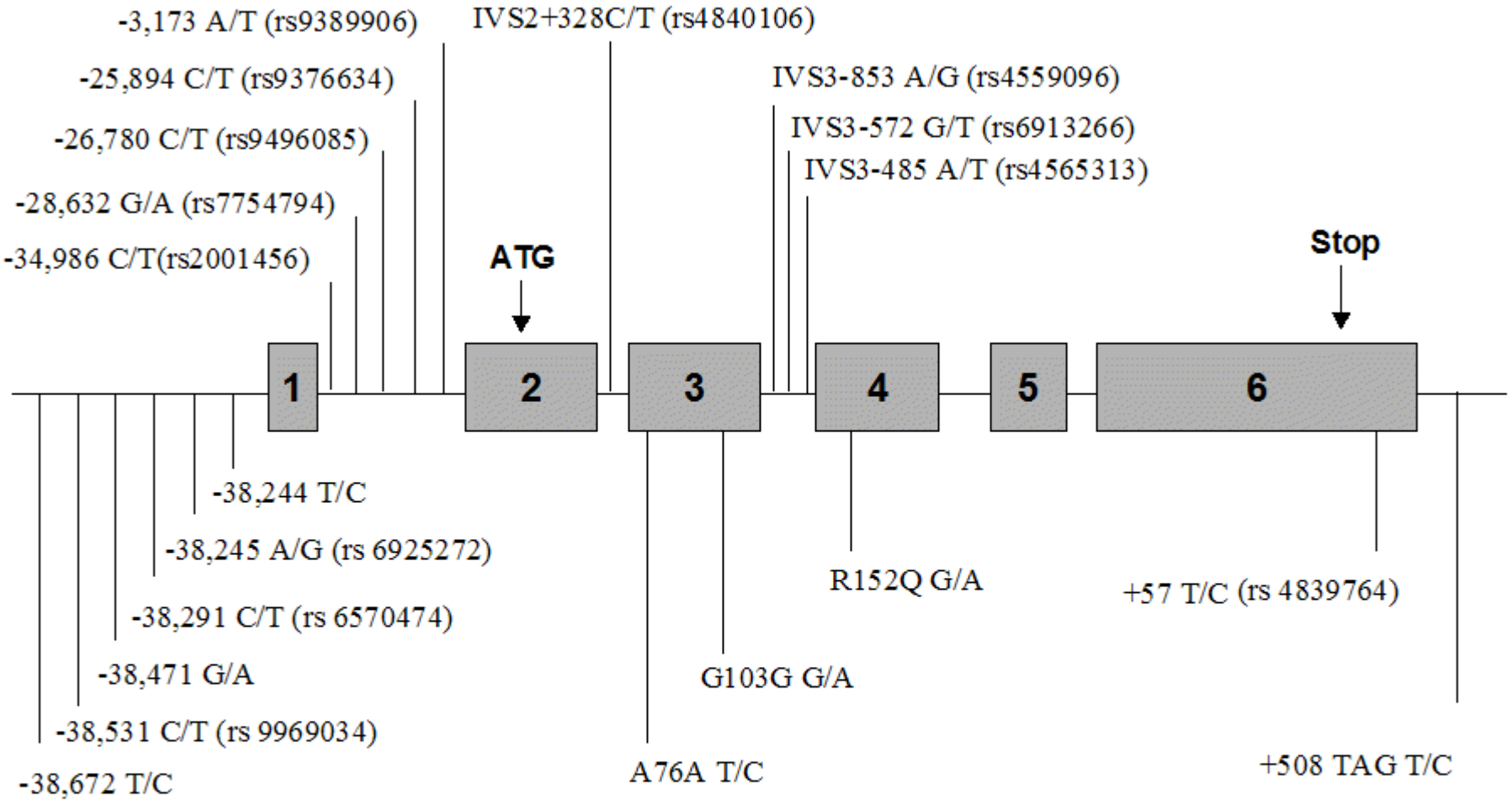
SNPs	Location	Nucleotide change	rs correspondence	Frequency
-38,672 ATG	Promoter	T/C	-	0.7
-38,531 ATG	Promoter	C/T	rs 9969034	1.4
-38,471 ATG	Promoter	G/A	-	0.7
-38,291 ATG	Promoter	C/T	rs 6570474	1.4
-38,245 ATG	Promoter	A/G	rs 6925272	41.8
-38,244 ATG	Promoter	T/C	-	19.4
A76A (Synonymous)	Exon 3	T/C	-	8.6
G103G (Synonymous)	Exon 3	G/A	-	0.7
R152Q (Non synonymous)	Exon 4	G/A	-	1.4
+57 TGA	Exon 6 (3'UTR)	T/C	rs 4839764	0.7
+508 TGA	3'UTR	T/C	-	0.7

Table 2 : Association (p≤0.05) of genotypes and alleles of *MCHR2* gene SNPs with obesity. Cases Set 1 = 628 French Caucasian children with BMI > 97th percentile. Cases Set 2 = 696 obese French Caucasian adults with BMI ≥ 40. Control Set = 1,401 non obese and normoglycemic French Caucasian adults. N: number of subjects, OR: Odd Ratio, CI: 95% Confidence Interval, HW: Hardy Weinberg. Significant p-values are indicated in bold.

	Cohorts	Genotypes (frequency)					Allele freq. OR (p-val.)[CI]	HW (p-val.)
	-38,245 ATG A/G	**AA**	**AG**	**GG**	**A**	**G**		
Set 1	**Obese children (N=606)**	229 (0.38)	280 (0.46)	97 (0.16)	0.61	0.39	1.17 (**0.03**)	0.46
	Control (N=1363)	565 (0.41)	630 (0.46)	168 (0.13)	0.65	0.35	[1.02-1.34]	0.72
Set 2	**Morbidly obese (N=623)**	234 (0.38)	302 (0.48)	87 (0.14)	0.62	0.38	1.13 (0.09)	0.51
	Control (N=1363)	565 (0.41)	630 (0.46)	168 (0.13)	0.65	0.35	[0.98-1.29]	0.72
Set 1+2	**Obese (N=1229)**	463 (0.38)	582 (0.47)	184 (0.15)	0.61	0.39	1.15 (**0.02**)	0.96
	Control (N=1363)	565 (0.41)	630 (0.46)	168 (0.13)	0.65	0.35	[1.02-1.28]	0.72
	-38,244 ATG T/C	**TT**	**TC**	**CC**	**T**	**C**		
Set 1	**Obese children (N=591)**	323 (0.55)	226(0.38)	42 (0.07)	0.74	0.26	1.06 (0.49)	0.75
	Control (N=1362)	760 (0.56)	518 (0.38)	84 (0.06)	0.75	0.25	[0.90-1.23]	0.77
Set 2	**Morbidly obese (N=627)**	362 (0.58)	228 (0.36)	37 (0.06)	0.76	0.24	0.92 (0.33)	0.89
	Control (N=1362)	760 (0.56)	518 (0.38)	84 (0.06)	0.75	0.25	[0.79-1.08]	0.77
Set 1+2	**Obese (N=1218)**	685 (0.56)	454 (0.37)	79 (0.06)	0.75	0.25	1.00 (0.96)	0.76
	Control (N=1362)	760 (0.56)	518 (0.38)	84 (0.06)	0.75	0.25	[0.88-1.13]	0.77

	-26,780 ATG C/T	CC	CT	TT	C	T		
Set 1	**Obese children (N=587)**	263 (0.45)	249 (0.42)	75 (0.13)	0.66	0.34	0.87 (0.055)	0.19
	Control (N=1401)	559 (0.40)	642 (0.46)	200 (0.14)	0.63	0.37	[0.75-1.00]	0.49
Set 2	**Morbidly obese (N=634)**	252 (0.40)	297 (0.47)	85 (0.13)	0.63	0.37	0.98 (0.83)	0.93
	Control (N=1401)	559 (0.40)	642 (0.46)	200 (0.14)	0.63	0.37	[0.86-1.13]	0.49
Set 1+2	**Obese (N=1221)**	515 (0.42)	546 (0.45)	160 (0.13)	0.65	0.35	0.93 (0.19)	0.42
	Control (N=1401)	559 (0.40)	642 (0.46)	200 (0.14)	0.63	0.37	[0.83-1.04]	0.49

	A76A T/C	TT	TC	CC	T	C		
Set 1	**Obese children (N=625)**	545 (0.87)	77 (0.13)	3 (-)	0.93	0.07	0.75 (**0.03**)	0.87
	Control (N=1365)	1139 (0.83)	215 (0.16)	11 (0.01)	0.91	0.09	[0.58-0.97]	0.73
Set 2	**Morbidly obese (N=519)**	436 (0.84)	82 (0.16)	1 (-)	0.92	0.08	0.93 (0.56)	0.16
	Control (N=1365)	1139 (0.83)	215 (0.16)	11 (0.01)	0.91	0.09	[0.71-1.20]	0.73
Set 1+2	**Obese (N=1144)**	981 (0.86)	159 (0.14)	4 (-)	0.93	0.07	0.83 (0.07)	0.36
	Control (N=1365)	1139 (0.83)	215 (0.16)	11 (0.01)	0.91	0.09	[0.71-1.20]	0.73



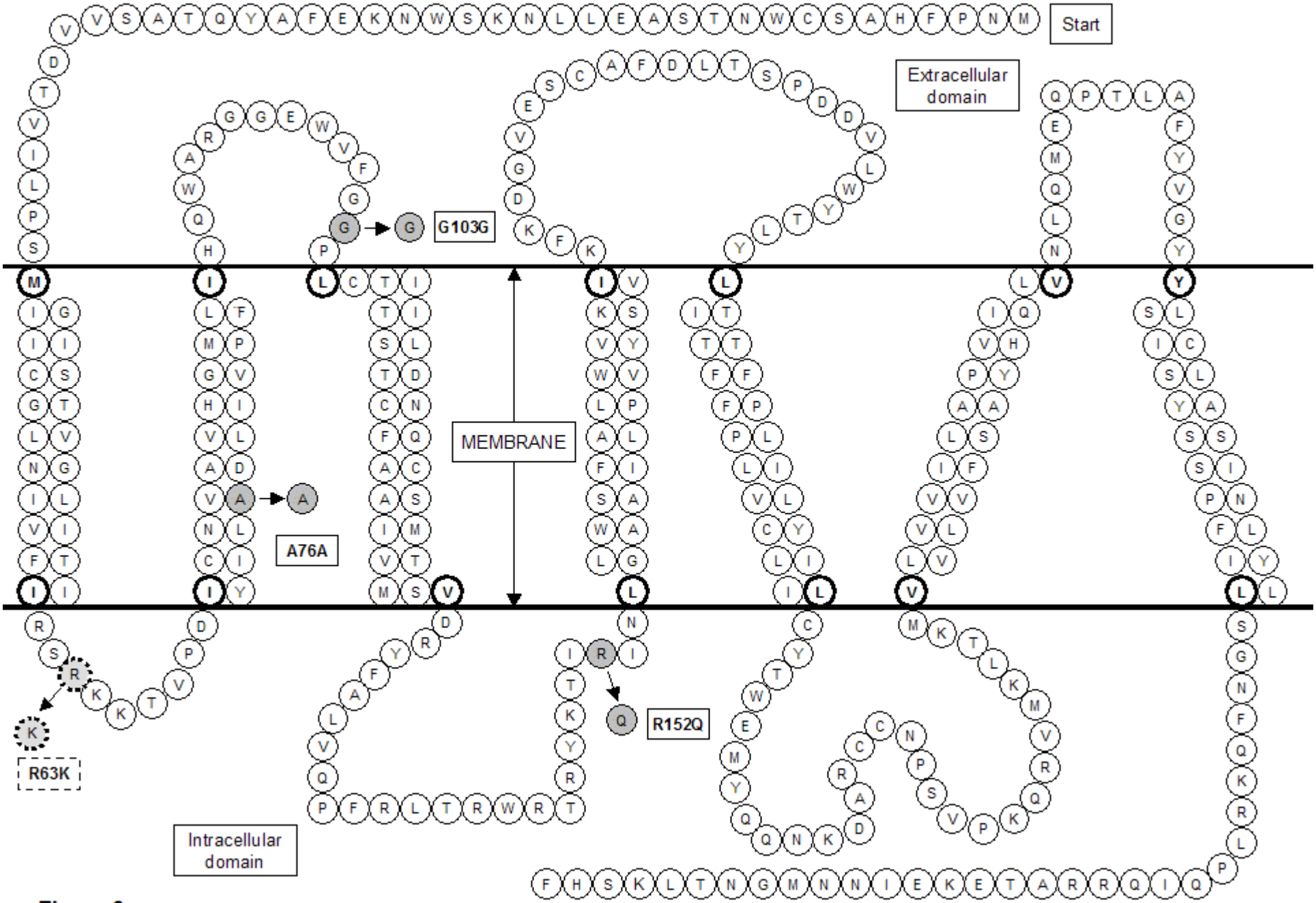


Figure 2