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The Spectrum of Genetic Variants Associated with the Development of Monogenic Obesity in Qatar

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Monogenic obesity · Consanguinity · Qatar Biobank · Qatar Genome Programme · Rare variants

Abstract
Introduction: Monogenic obesity (MO) is a rare genetic disease characterized by severe early-onset obesity in affected individuals. Previous genetic studies revealed 8 definitive genes for monogenic non-syndromic obesity; many were discovered in consanguineous populations. Here, we examined MO in the Qatari population, whose population is largely consanguineous (54%) and characterized by extensive obesity (45%). ***Methods:*** Whole genome sequencing data of Qatar Biobank samples from 250 subjects with obesity and 250 subjects with normal weight, obtained in association with the Qatar Genome Programme, were searched for genetic variants in the genes known to be associated with MO (i.e., *LEP, LEPR, POMC, PCSK1, MC3R, MC4R, MRAP2*, and *ADCY3*). The impact of the variants identified was investigated utilizing in silico tools for prediction in combination with protein visualization by PyMOL. ***Results:*** We identified potential MO variants in more than 5% of the cases in our cohort. We revealed 11 rare variants in 6 of the genes targeted, including two disease-causing variants in *MC4R and MRAP2*, all of which were heterozygous. Moreover, enrichment of a heterozygous *ADCY3* variant (c.1658C>T; p.A553V) appeared to cause severe obesity in an autosomal dominant manner. ***Conclusion:*** These findings highlight the importance of implementing routine testing for genetic variants that predispose for MO in Qatar. Clearly, additional studies of this nature on populations not yet examined are required. At the same time, functional investigations, both in vitro and in vivo, are necessary in order to better understand the role of the variants identified in the pathogenesis of obesity.

Introduction

Monogenic obesity (MO) is a rare genetic disease that enhances the risk for severe early-onset obesity considerably. During the past 20 years, several variants in genes involved in the regulation of food intake and energy expenditure, including the genes encoding components of the leptin-melanocortin 4 receptor pathway, have been found to cause MO [1]. Studies on consanguineous families with a history of severe early-onset obesity have pro-

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vided a key tool in this context, especially in the identification of variants inherited in an autosomal recessive manner. For example, the first genetic variant associated with MO in humans (in *LEP*), as well as the most recently identified variants (in *ADCY3*) were discovered in consanguineous families from Pakistan, 60% of whose population is characterized by consanguinity [2]. In addition, there are a number of reports on MO variants in consanguineous Arab populations, such as in Egypt and Saudi Arabia [3, 4].

The current investigation was designed to characterize MO in Qatar, where the extent of consanguinity is estimated to be 54%, with 26.7% of these marriages being between first cousins [5]. In our country 45% of adults [6] and 18.3% of children 5–9 years of age are obese [7]. To this end, we examined 500 genome obtained from Qatar Biobank (QBB) through the Qatar Genome Programme [8] to investigate 8 well-established genes associated with monogenic non-syndromic obesity. Investigations of this nature on understudied populations may reveal novel genetic variants that could be used to improve diagnosis of and/or develop personalized treatment for obesity.

Methodology

Ethical Approval

Ethical approval for this study was obtained from the institutional review boards of the QBB (MOPH-A-QBB-000222) and Qatar University (QU-IRB 952-E/18), with informed consent having been obtained from all participants by the QBB.

Subjects

The 250 subjects with obesity of class II or more (BMI ≥35 kg/m^2) and 250 control individuals (with BMI 18.5–24.9 kg/m^2) were all Qatari nationals at least 18 years of age. Their phenotypic characteristics – including lifestyle, diet, and any history of bariatric surgery, in addition to biochemical test results – were obtained from the QBB, which is described in detail elsewhere [8].

Whole Genome Sequencing

Whole genome sequencing for all participants was obtained through the Qatar Genome Programme (QGP) (https://www.qatargenome.org.qa/). The Illumina HiSeq 10X platform was utilized to achieve an average coverage of 30X and raw sequencing data converted to the paired FASTQ format employing the bcl2fastq software from Illumina (https://github.com/brwnj/bcl2fastq), following which FASTQC [9] was used to assess the quality of these data. The Fastq files were aligned against the reference genome (GRCh37) using the bwakit software (v0.7.11) (https://github.com/lh3/bwa/tree/master/bwakit).

Variant calling was performed using Genome Analysis Toolkit GATK (v3.4) [10] following the GATK best practices and individual g.vcf files were generated. Joint calling was performed on individual gVCF files to generate a joint multi-samples VCF file for all the samples. Only variants which passed through GATK VQSR filtering were considered for further downstream analysis. Thereafter, the VCF files were annotated using the SNPeff software (v4.3t) [11] and further annotated with prediction scores for variant effect, including Sorting Intolerant From Tolerant (SIFT) [12], PolyPhen2 [13], SNPs&GO [14], and Combined Annotation Dependent Depletion (CADD) v1.3 [15]. In addition, variants were assigned allele frequencies on the basis of the Exome Aggregation Consortium (ExAC) [16] and Genome Aggregation Database (gnomAD) v2.1.1 [17], as well as the clinical databases including Human Gene Mutation Database (HGMD, v2018.2) [18] and ClinVar (v20190211) [19]. Furthermore, the variants were classified in accordance with the guidelines of the American College of Medical Genetics/Association for Molecular Pathology (ACMG/AMP) [20].

Selection of Variants for Analysis

Variants in the 8 genes whose association with monogenic non-syndromic obesity has been established in at least two independent studies, i.e., *LEP*, *LEPR*, *POMC*, *PCSK1*, *MC3R*, *MC4R*, *MRAP2*, and *ADCY3*, were examined here. We included only variants indicated by SnpEFF to have a high or moderate impact, since these are more likely to alter protein function, whereas modifier and low-impact variants were excluded [11]. Moreover, only rare variants with a minor allele frequency of <0.01 (based on the QGP frequencies for 6,047 genomes) were considered for subsequent analysis. In the case of missense variants, we focused on those with a CADD score greater than 13, since variants above that threshold are considered to be among the 5% of those within the human genome that are most deleterious [21].

Association with Obesity

The 7 control individuals who had undergone bariatric surgery were reassigned to the obese group, resulting in 257 obese subjects and 243 controls. We then constructed a two-by-two contingency table comparing phenotype (cases/controls) with the genetic predisposition (defined as carrying at least one of the variants analyzed). This contingency table was used subsequently analyzed with Fisher's exact test.

Results

Characteristics of the Study Cohort

The study included 500 participants: 250 subjects with obesity with an average BMI of 40.3 ± 3.7 kg/m^2 and 250 controls with an average BMI of 22.6 ± 1.7 kg/m^2. Among the participants, 340 were women and 160 men, with a combined mean age of 40.5 ± 12.1 years. The average percentage body fat was 45.2 ± 6.3% and 27.6 ± 7.3% for the cases and controls, respectively.

Identification of the Genetic Variants of Interest

The findings concerning the 8 genes analyzed in our 500 subjects are documented in online supplementary Table 1 (see www.karger.com/doi/10.1159/000521851

Table 1. Variants detected within the genes associated with MO and in silico prediction of their pathogenicity

Gene	Chromosome	Position (GRCh37)	SNP ID	Change in gene	Change in protein	Transcript	QGP allele frequency*	gnomAD allele frequency	CADD	SIFT	PolyPhen 2	Mutation Taster	SNPs&GO	HGMD class	HGMD phenotype	ACMG manual**	ACMG class
ADCY3	2	25,059,790	rs115329263	c.1658C>T	p.A553V	ENST00000260600	0.009343	0.0001704	14.97	Tolerated	Benign	Polymorphism	Neutral	–	–	PM1	Uncertain significance (cool)
	2	25,141,368	rs143034828	c.489C>G	p.H163Q		0.00496	0.000003984	13.48	Tolerated	Probably damaging	Polymorphism	Neutral	–	–	PM1/BP4	Uncertain significance (cool)
MC3R	20	54,824,819	rs1481948431	c.920G>A	p.Arg307His	ENST00000243911	0.00102249	0.000003997	26.7	Deleterious	Probably damaging	Disease causing	–	–	–	PM2/PP3	Uncertain significance (tepid)
MC4R	18	58,039,098	rs1555691402	c.485C>T	p.T162I	ENST00000299766	0.0011576		27.1	Deleterious	Probably damaging	Disease causing	Disease	DM	Early onset obesity	PM1/PM2	Uncertain significance (warm)
MRAP2	6	84,765,081	rs760845706	c.44C>T	p.S15L	ENST00000257776	0.0001654	0.00006373	23.4	Deleterious	Benign	Polymorphism	Neutral	–	–	PM2	Uncertain significance (cool)
	6	84,799,133	rs1054400375	c.551T>C	p.I184T		0.000082686	–	23.2	Deleterious	Benign	Disease causing	Neutral	–	–	PM2	Uncertain significance (cool)
	6	84,798,925	rs368589399	c.343C>G	p.L115V		0.000413428	0.0001034	23	Deleterious	Benign	Disease causing	Neutral	DM	Severe early onset obesity	BP4	Uncertain significance (ice cold)
PCSK1	5	95,761,591	rs748072514	c.329G>A	p.R110H	ENST00000311106	0.000082713	0.00001770	28.3	Deleterious	Probably damaging	Disease causing	Disease	–	–	PM1/PP3	Uncertain significance (tepid)
	5	95,735,682	rs1026383684	c.1405G>A	p.V469I		0.00239868	0.00002122	17.55	Tolerated	Benign	Polymorphism	Neutral	–	–	PM1	Uncertain significance (cool)
	5	95,728,961	rs567748971	c.2006G>T	p.R669L		0.000578991	0.000003983	23.2	Deleterious	Benign	Polymorphism	Neutral	–	–	PM2	Uncertain significance (cool)
POMC	2	25,384,596	rs28932470	c.158A>G	p.D53G	ENST00000264708	0.00124	0.001007	15.53	Tolerated	Benign	Disease causing	Disease	–	–	PM1/BP1	Likely benign

* Based on 6,047 genomes. ** ACMG classification key. Criteria for pathogenicity. PM1 (Moderate): Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation. PM2 (Moderate): Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or ExAC. PP3 (Supporting): Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.). Criteria for a benign nature: BP1 (Supporting): Missense variant in a gene for which primarily truncating variants are known to cause disease. BP4 (Supporting): Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.).

for all online suppl. material). Eleven variants, all missense and heterozygous, were detected in 6 of these genes in 16 of the cases, with no variants being carried by the control individuals (Table 1). Based on HGMD and/or previous reports, two of these variants, i.e., mutations in *MC4R* and *MRAP2,* are known to cause MO. Their ACMG/AMP classifications indicated that most were of unknown significance (Table 1). The clinical characteristics of the subjects carrying these variants are presented in Table 2.

The association between genetic predisposition (as defined in the Methods) and obesity was significant (Fisher's exact test resulting in $p < 0.01$). The corresponding odds ratio indicated 33.3 times increased risk of obesity with the genetic predisposition (significance level $p > 0.05$). Consequently, in light of this high penetrance and probable monogenic nature of each variant, the effect size per variant would appear to be appreciable (the individual odds ratios are listed in online suppl. Table 2). However, due to their rarity, the *p* values associated with individual variants were not significant.

PyMOL Analysis of the Variants Detected

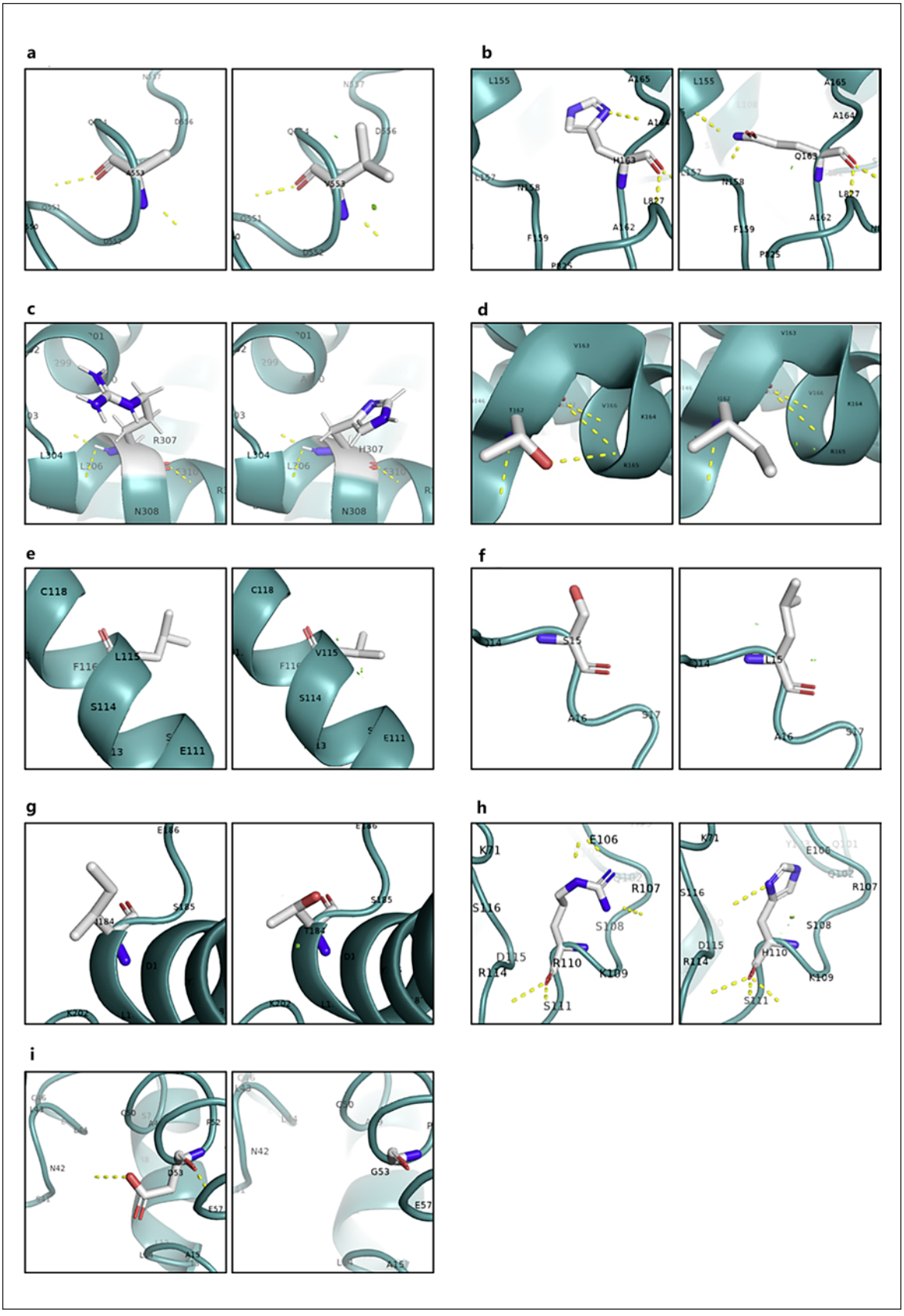
PyMOL was utilized to predict the structure of the protein encoded by the variant. The crystal structure of MC4R was obtained from Protein Data Bank (PDB) (PDB

Table 2. Clinical characteristics of subjects carrying variants in the genes associated with MO

Gene	Position (GRCh37)	SNP ID	Change in DNA	Change in protein	Case control ratio	Age, years	Gender	BMI, kg/m^2	% body fat	Diabetes	History of surgery
ADCY3	25,059,790	rs115329263	c.1658C>T	p.A553V	4:1	28	F	40.8	44	No	No
						50	M	40.3	36.1	Yes	No
						57	F	37.1	47.7	No	No
						46	M	38.3	36.6	No	No
						30	M	23.8	21.5	No	Gastric restriction
ADCY3	25,141,368	rs143034828	c.489C>G	p.H163Q	1:0	50	M	36.5	36.9	No	No
MC3R	54,824,819	rs1481948431	c.920G>A	p.Arg307His	1:0	60	F	39	47.3	No	No
MC4R	58,039,098	rs1555691402	c.485C>T	p.T162I	3:00	39	F	39.3	47.5	No	No
						33	F	42.8	51.2	No	No
						57	F	39	47.1	Yes	No
MRAP2	84,765,081	rs760845706	c.44C>T	p.S15L	1:00	39	M	40.6	40.1	No	No
MRAP2	84,799,133	rs1054400375	c.551T>C	p.I184T	1:00	35	M	40.8	36.2	No	No
MRAP2	84,798,925	rs368589399	c.343C>G	p.L115V	1:00	39	M	23.9	16.8	Yes	Gastric restriction
*PCSK1**	95,761,591	rs567748971	c.329G>A	p.R110H	1:00	33	F	38.3	47.4	Yes	No
*PCSK1**	95,735,682	rs1026383684	c.1405G>A	p.V469I	2:00						
PCSK1	95,728,961	rs748072514	c.2006G>T	p.R669L	1:00	32	M	41.1	36.9	No	No
POMC	25,384,596	rs28932470	c.158A>G	p.D53G	1:00	39	M	37.2	33.4	No	No

* Compound heterozygous for two *PCSK1* variants.

Fig. 1. Molecular visualization of variants found. Minor clash points between variants and neighboring residues are shown in small green disks, while larger red disks show more significant clash points. **a** ADCY3; p.A553V showing minor clash points. **b** ADCY3; p.H163Q showing minor clash points and gain of polar contacts with L155 and L157. **c** MC3R; p.R307H showing no clash points or loss/gain of polar contacts. **d** MC4R; p.T162I showing minor clash points and loss of polar contacts with A165. **e** MRAP2; p.L115V showing minor clash points. **f** MRAP2; p.S15L showing minor clash points. **g** MRAP2; p.I184T showing minor clash points. **h** PCSK1; p.R110H showing minor clash points and loss of polar contacts with E106 and R107 as well as a gain of contacts with S116 and S111. **i** POMC; p.D53G showing loss of polar contacts with N42 and E57. *(For figure see next page.)*



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ID6W25) [22], with the I-TASSER server [23] being employed to predict the structures of those proteins whose regions of interest were not available in PDB.

The vast majority of variant rotamers were predicted to interfere with neighboring residues, which might result in steric hindrance and subsequent destabilization of the protein structure. As shown in Figure 1a, b, d–h, this was the case for ADCY3; p.A553V, ADCY3; p.H163Q, MC4R; p.T162I, MRAP2; p.L115V, MRAP2; p.S15L, MRAP2; p.I184T, and PCSK1; p.R110H. Moreover, the loss of polar contacts predicted to occur in MC4R; p.T162I and POMC; p.D53G (Fig. 1d, i) could also be destabilizing; while the gain of polar contacts predicted to occur in ADCY3; p.H163Q (Fig. 1b) could render the protein more rigid. PCSK1; p.R110H was the only variant for which both loss and gain of polar contacts were predicted (Fig. 1h). Finally, in POMC; p.D53G, the native amino acid was predicted to be replaced by a residue quite different in size (Fig. 1i). There was no gain or loss of polar contacts predicted or clash in neighboring residues in MC3R; p.R307H (Fig. 1c).

Discussion

Here, we identified in our cases 11 rare variants within 6 genes associated with MO, including variants in *MC4R and MRAP2* known to cause this disease. In addition, we considered the impact of other variants on the development of obesity for the first time.

The melanocortin 4 receptor (MC4R) plays a key role in energy homeostasis, food intake, and maintenance of body weight [24] and variants in the gene encoding this protein, which are usually inherited in an autosomal dominant manner, are the most common cause of MO [24, 25]. In HEK293 cells, Collet et al. [26] (2017) found that among the various *MC4R* variants, c.485C>T; p.T162I leads to partial loss of the signaling activity of this receptor. Moreover, Jelin et al. [27] (2016) detected this same variant in a homozygous state in four children aged 4.5–14 years who underwent laparoscopic sleeve for severe obesity (BMIs of 71.2, 44, 45, and 53 kg/m^2), the first living in Kuwait and the other three in the United Arab Emirates [27]. As expected, the three heterozygous carriers in whom we detected this variant here exhibited a lower BMI (average 40.4 + 2.1 kg/m^2) than those homozygous carriers. The occurrence of this rare variant in individuals living in several different Arab countries is likely to reflect a common ancestry.

Like MC4R, MC3R is expressed in the hypothalamus [28]. Mutations in *MC3R*, which are rare, have also been linked to obesity [29]. For example, a recent meta-analysis of 2,969 individuals with obesity and 2,572 with normal weight confirmed that loss-of-function mutations in this gene increase the risk for obesity [30]. In our present study we identified one *MC3R* variant (c.920C>A; p.R307H) in an obese subject (BMI 39 kg/m^2). We were unable to find any previous reports on this variant in the literature.

The melanocortin-receptor accessory protein 2 (MRAP2) is primarily responsible for regulating the activities of MC4R and MC3R [31–33]. Here, we identified three rare missense variants in the *MRAP2* gene: c.44C>T; p.S15L and c.551T>C; p.I184T were present in individuals with severe obesity (BMI >40 kg/m^2), in agreement with the expected phenotype and autosomal dominant inheritance of *MRAP2* variants [31, 33]. Interestingly, the third variant c.343C>G; p.L115V, detected in a subject with a normal BMI who had undergone gastric restriction, is known to cause MO. This finding provides further evidence for the involvement of this specific variant in the development of obesity and highlights the necessity of obtaining comprehensive information concerning the medical history of an individual when looking for potential correlations between genotype and phenotype.

Adenylyl cyclase 3 (ADCY3) plays essential roles in the regulation of adiposity and glucose homeostasis [34]. Saeed et al. [35] (2018) reported three homozygous variants in the gene encoding this protein in children with severe obesity from consanguineous Pakistani families, as well as a compound heterozygous *ADCY3* variant in a severely obese European-American child. A later study in Greenland identified an additional *ADCY3* variant that was associated with a marked increase in the risk for obesity and type 2 diabetes, particularly in homozygous carriers [36]. In the current investigation, we identified two additional rare heterozygous *ADCY3* variants in six Qatari individuals with obesity. Although this finding may indicate that *ADCY3* variants may exert a dominant impact on the development of obesity; such a conclusion cannot be drawn at present because of the genetic and biological overlap between monogenic and the common form of obesity [35].

Although the two *ADCY3* variants detected here can be found in public databases, they have not previously been discussed in the context of obesity. The variant c.1658C>T; p.A553V was detected in four individuals with obesity (one of whom was also diabetic), as well as in a subject of normal weight who had undergone bariat-

ric surgery, suggesting severe obesity prior to surgery. Interestingly, this *ADCY3* variant was enriched in the Qatari population, with a frequency (0.009343 in the 6,047 genomes covered by the QGP) that was significantly higher than in gnomAD (0.0001704; Fisher's exact test resulting in a p value of 0.0085). These results highlight the important role played by *ADCY3* variants in the pathogenesis of MO in this population, especially since these appear to be relatively common and may, like variants in *MC4R,* exert a dominant effect.

Homozygous, compound heterozygous, and heterozygous loss-of-function variants in *PCSK1* have all been reported to cause MO [37–39]. In our two subjects with obesity carrying *PCSK1* variants, one was compound heterozygous for c.329G>A; p.R110H and c.1405G>A; p.V469I, while the second was heterozygous for c.2006G>T; p.R669L. Additional functional analyses and case-control studies involving larger cohorts are required to better understand the role of these variants in the pathogenesis of obesity.

It is worth noting that we also investigated whether our diabetic subjects were carriers of variants in known monogenic diabetes genes [40], using the same variant filtration approach as for MO. The absence of any potential variants of this nature indicates that obesity in these cases was unlikely to be due to diabetes.

Deficiency in pro-opiomelanocortin (POMC), a precursor of a number of hormones and neuropeptides, has been associated with obesity, as well as with adrenal insufficiency and red hair color [41]. One of our subjects with a BMI of 37.2 kg/m^2 was a heterozygous carrier of the *POMC* variant c.158A>G; p.D53G, classified as likely benign. This same heterozygous variant was detected in four severely obese children and one control in France [42], as well as in a Brazilian control group [31], suggesting that it may be benign or confers risk for obesity with variable penetrance.

Despite the extensive consanguinity in the Qatari population, we did not detect any homozygous variants within the 8 genes associated with MO that we examined, which might be due to our relatively small study population, as well as the pilot nature of this analysis. Another limitation here is the lack of functional characterization of the variants, both in vitro and in vivo. Furthermore, information concerning the onset of obesity in the cases studied might have provided additional insight into the role of the MO variants identified in the development of obesity. Clearly, more extensive genetic and/or functional studies are required in order to draw definitive conclusions concerning the contribution of the variants identified to obesity, especially since, on the basis of available evidence, these variants are classified to varying degrees by the ACMG/AMP as of unknown significance being.

Here, we identified MO variants in more than 5% of our subjects with obesity subjects. This highlights the value of routine diagnostic testing for these and perhaps other variants as well, possibly employing targeted gene panels already available, especially in individuals with severe early-onset obesity. Facilitating proper diagnosis of MO has important clinical consequences, since the Federal Drug Administration in the United States has approved treatments for certain forms of MO, e.g., IMCIVREE™ (setmelanotide) for patients with a deficiency in POMC, PCSK1 [43] or LEPR, and Myalep™ (recombinant leptin) in cases of leptin deficiency [44, 45]. Reliable diagnosis could also help decide which patients could undergo bariatric surgery with minimal complications and maximal chance of success [46, 47]. In addition to aiding both diagnosis and treatment, such testing could provide more conclusive evidence concerning the potential of the variants we focused on to cause obesity.

Conclusion

In the current investigation, we identify genetic variants associated with MO in the Qatari population and discuss some of these in relationship to obesity for the first time. Our results also indicate that in this population, an autosomal dominant form of MO may occur as a result of *ADCY3* variants. Further functional studies are required to improve our understanding of the potential role of some of these variants in the development of MO. Finally, our observations highlight the importance of implementing routine diagnostic genetic testing for MO in Qatar, as well as of investigating such populations more thoroughly in order to identify the entire spectrum of genes and variants thereof that influence the development of MO.

Acknowledgments

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Statement of Ethics

The study protocol was reviewed and approved by the institutional review boards of the QBB (approval number: MOPH-A-QBB-000222) and Qatar University (approval number: QU-IRB 952-E/18) in accordance with the World Medical Association Declaration of Helsinki. Written informed consent to participate was obtained from all individuals included by the QBB.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.A. originally posed the research question and designed the study. N.A.H. and R.Z. analyzed the data under the supervision of M.A. K.A., N.S., and M.N. and also contributed to the data analysis. N.A.H., R.Z., M.A., and K.A. wrote the manuscript. All authors have read and approved the final version of the manuscript.

Data Availability Statement

The informed consent given by the study participants does not cover posting of participant-level phenotype and genotype data of QBB/QGP in public databases. However, access to QBB/QGP data can be obtained through an established ISO-certified process by submitting a project request at https://www.qatarbiobank.org.qa/research/how-to-apply which is subject to approval by the QBB IRB committee.

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