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Melanocortin 4 Receptor Pathway Dysfunction in Obese Patients: Prevalence Estimates of *LEPR*, *POMC*, and *PCSK1* Variants

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Introduction

- The hypothalamic melanocortin 4 receptor (MC4R) pathway plays a critical role in controlling food intake and energy expenditure through brain-periphery signaling networks.[1]
- Mutations in several genes within the MC4R pathway lead to monogenic forms of early-onset obesity, relatively independently of environmental factors.[2]
- Genetic studies of monogenic obesity have revealed the significance of loss of function (LoF) mutations in MC4R pathway genes, including the leptin receptor (*LEPR*), proopiomelanocortin (*POMC*), and proprotein convertase subtilisin/kexin type 1 (*PCSK1*) genes.[3,4]
- Individuals carrying multiple LoF defective alleles in different MC4R pathway genes may exhibit an increased risk for obesity due to the cumulative burden of genetic deficiency in the MC4R pathway.[5]
- The aim of these analyses were to estimate the number of individuals in the United States who are homozygous or compound heterozygous for known and predicted LoF variants in *POMC*, *PCSK1*, and *LEPR*, and to evaluate the association between LoF variants and body mass index (BMI).

Methods

- Known LoF variants in the *POMC*, *PCSK1*, and *LEPR* genes were identified through a comprehensive literature search and through analysis of several genetic databases, including HGMD[6] and ClinVar.[7]
- Computationally predicted LoF variants were identified using the DeepCODE deep learning algorithm (Yang et al 2017, manuscript in preparation).
- The prevalence of individuals with homozygous and compound heterozygous variants of interest were estimated for each gene using Hardy-Weinberg calculations. The probability of recombination between any 2 variants was assumed to be negligible, as most variants are rare with minor allele frequency <0.1%.
  - Prevalence estimates were based on cumulative allele frequencies computed from allele frequencies in gnomAD (http://gnomad.broadinstitute.org).
- In addition to estimating the prevalence of patients with homozygous or compound heterozygous LoF variants, the association between cumulative allelic burden and BMI was investigated utilizing datasets from the UK Biobank (http://www.ukbiobank.ac.uk/), the UK10K (https://www.uk10k.org/), and Mount Sinai Hospital (New York, NY, USA).
  - Odds ratios were computed by dichotomizing cases and controls to severe obesity cases (BMI >40 kg/m^2) and normal controls (BMI <25 kg/m^2). Logistic regression was used to analyze common variants, while Firth regression was used for rare variants.

Results

Identification of LoF Genetic Variants in *POMC*, *PCSK1*, and *LEPR*

- A total of 83 credible LoF variants were identified from the systematic literature review (**Table**).
- An additional 83 nonsense, frame-shift, and splice site variants and 421 computationally predicted LoF missense variants were identified from the gnomAD, UK10K, and UK Biobank databases, and Mount Sinai Hospital internal data (**Table**).
- LoF variants were classified into 2 groups based on the nature of the supporting evidence.
  - Group 1 included LoF variants experimentally validated in the literature or that could be confidently predicted as such based on published protein functional studies.
  - Group 2 included predicted LoF missense variants with a high functional impact based on the DeepCODE algorithm (Yang et al 2017, manuscript in preparation).

Table. Grouping of MC4R Pathway Variants

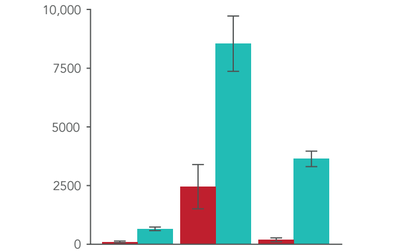
		Gene			
Category	**Source**	***POMC***	***PCSK1***	***LEPR***	**Total**
Group 1	Literature	30	31	22	83
	Novel (nonsense, splice site, frame shift)	20	24	39	83
Group 2	Computationally predicted high-impact missense	85	176	160	421
Total		**135**	**231**	**221**	**587**

LEPR, leptin receptor; MC4R, melanocortin 4 receptor; POMC, proopiomelanocortin; PCSK1, proprotein convertase subtilisin/kexin type 1.

Prevalence of LoF Genetic Variants in *POMC*, *PCSK1*, and *LEPR* in the United States

- Based on an approximate US population of 300 million, the estimated numbers of homozygous and compound heterozygous individuals with rare LoF variants were 3638 individuals for *LEPR*, 656 individuals for α-melanocyte-stimulating hormone (α-*MSH*)/*POMC*, and 8546 individuals for *PCSK1* (**Figure 1**).
- The total combined prevalence for biallelic LoF variants was predicted to be 12,840 individuals in the United States (**Figure 1**).
- We have excluded from these analyses a small subset of less rare variants that have been previously evaluated and where the effects of these variants on severe obesity remain unclear. The estimated prevalence by gene when including these additional variants are: *POMC* with the β-*MSH* variants (n=9914), and *PCSK1* with N221D (n=564,529) where a novel *PCSK1* LoF variant in addition to N221D, T640A, adds ~33,000 to this estimate. To our knowledge, none of these patients have been diagnosed.

Figure 1. Prevalence Estimations of Homozygous and Compound Heterozygous Individuals With LoF Variants in the United States



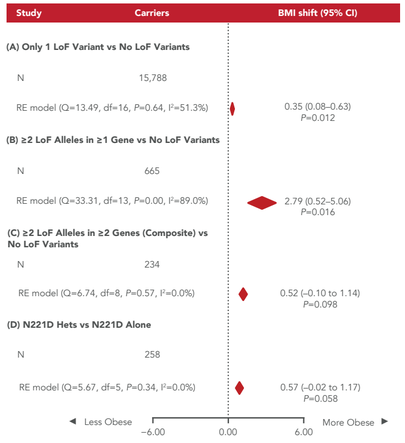
	α-*POMC*	*PCSK1*	*LEPR*	Total
Group 1	99	2452	189	2740
Groups 1 and 2	656	8546	3638	12,840

LEPR, leptin receptor; LoF, loss of function; POMC, proopiomelanocortin; PCSK1, proprotein convertase subtilisin/kexin type 1. Prevalence estimations are based on an approximate US population of 300 million. Error bars represent 95% CIs.

Association of LoF Variants With Increased BMI

- Individuals with ≥1 LoF variant (n=15,788) had significantly higher BMI when compared with individuals with no LoF variants (*P*=0.012; **Figure 2A**).
  - The well-studied *PCSK1* N221D and novel *PCSK1* T640A variants were found to have statistically significant associations with BMI (*P*=0.002 and *P*=0.0002, respectively).
  - No other heterozygous/individual allele variants were found to be statistically significant, possibly due to their low frequencies of occurrence.
- Individuals with ≥2 LoF alleles in ≥1 of the *LEPR*, *POMC*, and/or *PCSK1* genes (n=665) had significantly increased BMI relative to individuals with no LoF variants, indicating a significant cumulative allele burden across these 3 genes (*P*=0.016; **Figure 2B**).
- There was a trend towards an association between variants and increased BMI in a subset of the above population (**Figure 2B**) who have LoF variants in ≥2 of the 3 genes (composite genotype; n=234) compared with individuals with no LoF variants (*P*=0.098; **Figure 2C**).
- To evaluate the significance of variants other than *PCSK1* N221D, we compared individuals with N221D plus another variant (n=258) with individuals with N221D alone (n=11,719).
  - The additional allele on the N221D background resulted in a trend towards an association with a higher BMI in this population (*P*=0.058; **Figure 2D**).
- These results suggest an association between a burden of LoF variants in the MC4R pathway and higher BMI when compared with individuals with no LoF variants.

Figure 2. Effect of LoF Variants on the Risk of Increased BMI (Groups 1 and 2)



BMI, body mass index; LoF, loss of function; RE, random effects. The effect size represents the estimated effect sizes and CIs over all data sets using a RE-weighted model.

Conclusions

- We estimate that ~12,800 individuals in the United States are homozygous or compound heterozygous for LoF variants in *POMC*, *PCSK1*, or *LEPR*.
- This predicted population remains almost entirely undiagnosed because genetic testing is rarely performed in obese patients.
  - Guidelines on pediatric obesity suggest genetic testing in patients with extreme early-onset obesity (<5 years of age), and who have clinical features of genetic obesity syndromes (in particular, extreme hyperphagia) and/or a family history of extreme obesity.[8]
- The cumulative allele burden of ≥2 LoF alleles in ≥1 of the 3 genes studied here (compound or composite genotype) predisposes individuals to a higher BMI.
- Overall, these findings suggest that LoF variants in the MC4R pathway contribute to severe obesity.

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