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RESEARCH ARTICLE

# Whole-exome sequencing uncovers new variants in *GDF15* associated with hyperemesis gravidarum

**Marlena S. Fejzo**[1] | **Kimber W. MacGibbon**[2] | **Olivia First**[2] | **Courtney Quan**[2] | **Patrick M. Mullin**[1]

[1]Department of Maternal Fetal Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

[2]Hyperemesis Education and Research Foundation, Clackamas, Oregon, USA

**Correspondence**
Marlena S. Fejzo, Department of Obstetrics and Gynecology, Maternal Fetal Medicine Divison, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA.
Email: fejzo@usc.edu

## Abstract

**Objective:** A genome-wide association study (GWAS) linked the placenta and appetite hormone gene *GDF15* to hyperemesis gravidarum (HG). This paradigm-changing finding has shifted the field away from the prevailing hypotheses, but more evidence is needed. This study was performed to identify coding variants in addition to the non-coding variants implicated by GWAS.

**Setting:** Case–control research study performed in a university setting.

**Design:** Case–control study.

**Population:** Hyperemesis gravidarum cases requiring intravenous fluid treatment for disease ($n$ = 926) and controls with normal or no nausea and vomiting of pregnancy ($n$ = 660), from the USA.

**Methods:** Whole exome-wide sequencing and genome informatics were performed using the standard Regeneron pipeline. All variants were compared between cases and controls using dominant, recessive, and allelic models to identify variants with exome-wide significant $p$ values ($p < 10^{-6}$). Odds ratios and associated $p$ values were calculated for exome-wide significant allele(s) in subgroups of genetically predicted ancestries. Variants were filtered to identify rare pathogenic variants occurring in ≥10 cases and in no controls.

**Main outcome measures:** Identification of exome-wide significant and rare genetic variant(s) associated with HG.

**Results:** A common coding variant in *GDF15* was the only exome-wide significant association, and a rare coding variant in *GDF15* was the only predicted disease-causing variant occurring in 10 or more cases.

**Conclusions:** This study confirms the GWAS finding that *GDF15* is the greatest genetic risk factor for HG. The new variants identified may have implications for prediction and diagnosis. The findings provide insight into the cause, and molecular mechanisms for developing therapeutics for HG.

KEYWORDS
body mass index, caesarean section, contraction, dystociainduction of labour, intrauterine pressure, intrauterine pressure catheter, mode of delivery, Montevideo unit, obesity, oxytocin

**Tweetable abstract**: Whole-exome sequencing reveals placenta and vomiting hormone GDF15 most likely cause of Hyperemesis Gravidarum.

## INTRODUCTION

Nausea and vomiting in pregnancy (NVP) is common. The most severe form, hyperemesis gravidarum (HG), is defined as a severe degree of nausea and/or vomiting that strongly impairs daily living activities and leads to an inability to eat and/or drink normally, often resulting in dehydration, weight loss, electrolyte disturbance and nutritional deficiencies.[1] It is the second leading cause of hospitalisation in pregnancy.[1] HG is associated with increased risks of pregnancy termination, preterm birth, maternal suicidal ideation and post-traumatic stress disorder, and can cause maternal and fetal mortality.[1,2] Long-term consequences include increased risks of neurodevelopmental delay, autism spectrum disorder and altered brain structure in offspring.[3] Current anti-emetic treatments are largely ineffective in reversing weight loss, so novel approaches are needed.[4]

A genetic origin for HG is supported by familial aggregation studies, twin studies, and a genome-wide associate study (GWAS).[1] The GWAS identified common variants, implicating four genes (*GDF15*, *GFRAL*, *IGFBP7* and *PGR*) confirmed in previous replication studies.[5–7] Among these, *GDF15* was found to be the greatest genetic risk factor, and two unlinked variants, rs16982345 and rs1054221, were confirmed in replication studies.[5,7] Previously, the prevailing hypothesis identified the hormone human chorionic gonadotropin (hCG) as the likely aetiological factor. However, the GWAS provided no evidence to support an association between HG and hCG, or its receptor.[1,5] Meanwhile, the gene coding for the brainstem-restricted receptor for GDF15, *GFRAL*, was also implicated by GWAS and confirmed in a replication cohort, further supporting this pathway.[5,6] In addition, in maternal serum, GDF15 levels were significantly increased in hospitalized cases, in patients with second-trimester vomiting, and in those prescribed anti-emetics.[8,9] Lower levels of GDF15 were associated with no NVP, fetal loss and male sex.[10,11] Variants in *GDF15* have been associated with familial and recurrent HG, and with the level of circulating GDF15.[12–16]

GDF15 is a hormone highly expressed by the placenta and binds GDNF family receptor α-like (GFRAL) and coreceptor, proto-oncogene tyrosine-protein kinase receptor rearranged during transfection RET.[10,17,18] This pathway causes vomiting in non-human primates, chemotherapy-induced nausea and vomiting and cancer cachexia, a disease similarly characterized by appetite loss, muscle wasting and weight loss.[19–23] Therapeutic agents targeting this pathway are effective in animal models and are under investigation to restore appetite and weight gain or to stimulate weight loss.[20,22,24,25] Herein, we performed whole-exome sequencing of cases affected by HG and unaffected controls to determine whether additional variants can be identified.

## METHODS

This study includes 926 unrelated participants with HG requiring treatment with intravenous fluids, and 660 unaffected controls with at least two pregnancies that went beyond 27 weeks of gestation and did not require any treatment for symptoms of NVP in any pregnancy. This cohort is a different cohort than the 23andMe customers genotyped in our previous GWAS.[5] Controls were eligible if they experienced normal or no nausea/vomiting in their pregnancy, no weight loss as a result of nausea/vomiting and no medical attention in their pregnancy for nausea/vomiting. All participants gave informed consent. Patients were not involved in the development of the research and a core outcome set was not used. This study was approved by the Institutional Review Board.

### Recruitment

Recruitment for the study was performed as published previously.[5] The source population for HG cases included patients residing in the USA primarily recruited through advertising on the Hyperemesis Education and Research Foundation website (www.hyperemesis.org) from 2007 to 2017. Minors (under 18 years of age) were not included in the study because few teens were expected to fit the study criteria for the controls of having had two pregnancies, and it would be difficult to justify the risks/benefits to normal control minors. As multiple gestations or chromosome abnormalities may be associated with HG as a result of unique physiological pathways, women with these types of pregnancies were also excluded. Each case was asked to provide medical records including intravenous fluid treatment for HG and recruit a non-blood-related acquaintance with at least two pregnancies that went beyond 20 weeks of gestation to participate as a control. Acquaintance controls are increasingly used in genetic studies and have advantages that include higher response rates and closer matching for race/ethnicity, education and age, compared with alternative methods.[26] All cases and controls were required to go over an information sheet by phone and return a signed information sheet with all elements of consent in order to enrol in the study.

### Sample and demographic data collection

Each study participant was asked to submit a saliva sample for DNA analysis and participate in an online survey. The survey asked participants the year of the participant's birth, the year of birth of their firstborn child, the number of pregnancies, number of live births, number of terminations and number of miscarriages. DNA Genotek saliva kits (Oragene, Ottawa, ON, Canada) were mailed to all cases and controls. The saliva

collection kit was self-administered and came with directions for submitting 2 ml of saliva into a collection vial and returning the sample to the study site via an addressed and postage-paid return envelope provided with the collection kit.

## DNA extraction

DNA was extracted from the saliva samples according to manufacturer's instructions (Oragene). Using the kit, we have successfully isolated, on average, 197 μg of DNA of high quality (260/280 = 1.84) from 2 ml of saliva. The low end of expected DNA quantity reported by the manufacturer is 30 μg/ml of saliva, or 60 μg/sample. After the exaction, the DNA was stored at −20°C.

## Whole-exome sequencing

Whole-exome sequencing (WES) was performed by the Regeneron Genetics Center (RGC, Tarrytown, NY, USA), as described previously.[27] The exome was captured using a slightly modified version of the xGen design available from Integrated DNA Technologies (IDT, Coralville, IA, USA). The captured libraries were sequenced on the Illumina NovaSeq platform using paired-end 75-bp reads (Illumina, San Diego, CA, USA). The captured bases were sequenced so that greater than 95% of samples passing initial quality control had at least 90% of the targeted bases covered at 20× or greater. Paired-end reads and genetic variants were called using the RGC DNAseq analysis pipeline.

## Genome informatics

RGC used cloud-based pipeline standard tools for sample-level data production and analysis. Raw sequence data from the Illumina sequencers was uploaded to DNAnexus, which triggered the automated production analysis pipeline. Key steps were sample de-multiplexing using Illumina software, alignment to the GRCh38 reference, post-alignment binary alignment map (BAM, the compressed binary version of a sequence alignment map (SAM) file that is used to represent aligned sequences up to 128 Mb) processing, and single-nucleotide polymorphism (SNP) and intra-read insertion and deletion (INDEL) calling with genotyping software. Sequencing and data quality metric statistics were captured for each sample to evaluate capture performance, alignment performance and variant calling. Sample-level files including BAMs in reference-compressed columnar file (CRAM) format and variant call formats (VCFs) were completed.

## Filter steps

Variants were annotated using OpenCravat on the DNAnexus platform and further filtered to identify all rare (global allele frequency < 0.05), pathogenic (rare exome variant ensemble learner (REVEL) score > 0.75), missense variants that occurred in ≥10 cases and in no controls.[28] The cut-off of ≥10 cases was chosen based on the use of this cut-off in a previous WES study.[29]

All variants were compared between cases and controls using dominant, recessive, and allelic models (code deposited on GitHub). The standard cut-off $p < 10^{-6}$ was considered exome-wide significant.

Ancestry was not based on self-report but from standard genetic prediction methods based on the intersection of SNPs between HapMap3 and the non-filtered project level VCF. SNPs were filtered to include common, high-quality SNPs and merged for the HapMap3 data set.

The principal components (PCs) were calculated for the HapMap3 samples and all non-HapMap3 samples were projected onto those PCs. A kernel density estimation (KDE) was trained for each ancestral superclass with the PCs of the HapMap3 samples. The likelihood of each sample being from the different ancestral superclasses based on the KDEs was calculated. For each sample, the ancestral superclass based on the likelihoods was reported. If no superclass had a high enough likelihood, then the ancestry was recorded as OTHER (unknown). If one superclass had a high enough likelihood, then that superclass was recorded. If two superclasses had a high enough likelihood (borderline samples), then African (AFR) over European (EUR), AMR (admixed American) over EUR, AMR over East Asian (EAS), South Asian (SAS) over EUR and AMR over AFR were recorded; otherwise OTHER (unknown) was recorded (this was done to provide stringent estimates of the EUR and EAS populations and inclusive estimates for the more admixed populations in the data sets. If more than two superclasses had a high enough likelihood, then OTHER (unknown) was recorded.

Odds ratios and corresponding *p* values were calculated for termination rates and miscarriage rates, compared with live birth rates, in cases and controls using standard methods.[30,31] Odds ratios and corresponding *p* values were calculated for rs1058587 in cases and controls of AFR, AMR, EAS, EUR, OTHER and TOTAL (all non-European ancestries combined), using standard methods.[30,31]

Ancestries were assigned for each variant identified. Functional consequences predicted by REVEL were included. In addition, functional consequences were predicted based on analyses of the cryogenic electron microscopic (cryo-EM) structure of the GDF15/GFRAL/RET complex, as described previously.[32] The potential effects of the risk alleles on the stability of the individual proteins and the formation of the complex were assessed by mapping the mutations to the corresponding positions in the structure of the wild-type complex.

# RESULTS

The basic demographic characteristics of cases and controls are presented in Table 1. Cases had significantly higher

**TABLE 1** Self-reported demographic characteristics of hyperemesis gravidarum cases (HG) and controls (C)

| | Year born | Birth year of firstborn | No. of pregnancies | Live births | | | | |
|---|---|---|---|---|---|---|---|---|
| | Median (range) | Median (range) | Median (range) | Median (range) | Termination (%) | Termination | Miscarriage (%) | Miscarriage |
| HG | 1978 (1950–1999) | 2005 (1979–2016) | 2 (1–10) | 2 (0–9) | 8.7 | OR 3.03, 95% CI 2.22–4.15 | 20.5 | OR 1.66, 95% CI 1.39–1.99 |
| C | 1976 (1957–1989) | 2003 (1978–2012) | 2 (1–9) | 2 (1–7) | 3.3 | $p < 0.0001$ | 14.3 | $p < 0.0001$ |

**TABLE 2** GDF15 variant rs1058587, found in this study to be of genome-wide significance (allelic $p = 9.98 \times 10^{-11}$, dominant $p = 1.14 \times 10^{-8}$) in the whole data set (European and non-European combined), shown here in the minority subpopulations of cases affected by hyperemesis gravidarum (HG) and controls separated by African (AFR), admixed American (AMR) and East Asian (EAS) ancestry, as well as OTHER (unknown ancestry), and the majority population of European ancestry

| Ancestry | rs1058587 C/G (C is risk allele) | | |
|---|---|---|---|
| AFR | HG | C, 60; G, 4 | OR 4.41, 95% CI 1.07–18.27 $p = 0.04$ |
| | Control | C, 17; G, 5 | |
| AMR | HG | C, 96; G, 18 | OR 1.92, 95% CI 0.77–4.79 $p = 0.16$ |
| | Control | C, 25; G, 9 | |
| EAS | HG | C, 12; G, 8 | OR 2.27, 95% CI 0.59–8.65 $p = 0.23$ |
| | Control | C, 17; G, 5 | |
| OTHER | HG | C, 37; G, 7 | OR 5.29, 95% CI 1.32–21.23 $p = 0.02$ |
| | Control | C, 6; G, 6 | |
| Total (non-European) | HG | C, 205; G, 37 | OR 2.13, 95% CI 1.19–3.80 $p = 0.01$ |
| | Control | C, 65; G, 25 | |
| Total (European) | HG | C, 1338; G, 272 | OR 1.64, 95% CI 1.37–1.97 $p < 0.0001$ |
| | Control | C, 922; G, 308 | |

termination and miscarriage rates than controls. WES was performed on 926 unrelated participants with HG and 660 unaffected controls. All variants were compared between cases and controls using dominant, recessive, and allelic models. The only variant reaching exome-wide significance ($p < 10^{-6}$) was rs1058587 in *GDF15* (allelic $p = 9.98 \times 10^{-11}$, dominant $p = 1.14 \times 10^{-8}$). The GG genotype was identified in 25 of 926 (2.7%) cases and 50 of 660 (7.6%) controls. The CG genotype was identified in 277 of 926 (29.9%) cases and 254 of 660 (38.5%) controls. The C → G polymorphism in rs1058587 results in the substitution of a histidine residue containing a positively charged side chain by aspartic acid containing a polar uncharged side chain. It is often termed *H6D* because of the substitution at residue 6 of the mature GDF15 protein. The wild-type variant (H) is associated with HG in this study, and has been associated with increased serum GDF15, and with a reduction in body weight, abdominal fat, body mass index and obesity.[16,33] Cryo-EM predicted H6D is an interface residue that may partially alter GDF15–GFRAL interaction.

Variants in the genes previously identified by GWAS (*GFRAL*, *PGR* and *IGFBP7*) did not reach exome-wide significance in this study, nor did any other gene in the exome. Of note, none of the genes encoding the hCG hormone, widely hypothesized to be the most likely cause of HG, were found to be of exome-wide significance using the allelic, dominant or recessive models. For example, the most significant variant coding for hCG, which, like *GDF15*, maps to chromosome 19, was not even close to exome-wide significance (allelic $p = 0.0028$). Two hundred and eighty-nine variants on chromosome 19 were of greater significance than this variant (in *CGB7*), including the top *GDF15* variant already mentioned (allelic $p = 9.98 \times 10^{-11}$) as well as five additional variants in *GDF15* that did not reach exome-wide significance.

Participants of the WES were primarily of European ancestry, but subpopulations of African (32 cases, 11 controls), Latino or admixed American (57 cases, 17 controls) and East Asian ancestry (10 cases, 11 controls) also participated. To determine whether the exome-wide significant *GDF15* variant rs1058587 may also be associated with HG in these populations, we compared rs1058587-C with rs1058587-G in cases and controls separated by ancestry (Table 2). The C-His variant, which is associated with an increased risk for HG in the whole data set (European and non-European combined), showed a similar trend (odds ratios in the same direction) in participants of European ancestry (OR = 1.64, $p < 0.0001$) and participants of non-European ancestry (OR = 2.13, $p = 0.01$) as a whole, as well as subpopulations classified as unknown (OR = 5.29, $p = 0.02$), African (OR = 4.41, $p = 0.04$), admixed

American (OR = 1.92, $p = 0.16$) and East Asian (OR = 2.27, $p = 0.23$) ancestries.

Next, we identified all rare (allele frequency < 0.05), pathogenic (REVEL score > 0.75), missense variants occurring in ≥10 cases and no controls. Only one variant fit the criteria, again in *GDF15* (rs372120002). All cases with this variant were predicted to be of European ancestry: eight sharing ancestry with those from the Tuscany region of Italy (TSI) and two who shared ancestry with those from the Tuscan region of Italy and those with Northern and Western European ancestry from Utah (TSI/CEU). Among the 745 cases predicted to share European (TSI or TSI/CEU) descent in this study, 10 (1.3%) carried the rare variant. None of the other participants sharing non-Tuscan descent nor any of the 574 controls sharing European (TSI or TSI/CEU) descent carried this variant. Cryo-EM predicted that this variant abolishes a disulfide bond between C211 and C274. No rare pathogenic missense variants in the genes previously identified by GWAS (*GFRAL*, *PGR* and *IGFBP7*), nor any other gene, were identified in ≥10 cases and in no controls. Of note, even when lowering the criteria to in two or more cases and no controls, no rare pathogenic variants coding for the hCG hormone were identified.

# DISCUSSION

## Main findings

The paradigm-changing finding that *GDF15* is the greatest genetic risk factor for HG is now supported by a second genetic technique, WES, in an independent cohort. This study provides mounting genetic evidence that variants in *GDF15* are associated with HG. This study is the first to identify missense variants within *GDF15* (rs1058587 and rs372120002) associated with HG. As no other exome-wide significant nor causal variants in ≥10 cases were identified, this study does not support the two predominant historical theories that HG has a psychological origin or is caused by the pregnancy hormone hCG. Indeed, this study did not identify any rare pathogenic variants coding for hCG occurring in two or more cases and in no controls, nor any that were even close to reaching exome-wide significance. Focus on these unsupported theories has limited progress in identifying causal factors for one of the most common pregnancy conditions.[1] Future work on aetiology should focus on GDF15.

## Strengths and limitations

A strength of this study is that, to the best of our knowledge, it is the first large WES study of HG. A weakness of this study is that the majority of participants are of European descent, with cases recruited from the Hyperemesis Education and Research Foundation website, so the findings may not be generalisable to other populations that may have been less likely to have had internet access, for example. However, data on participants with other ancestries were included for the exome-wide significant *GDF15* variant rs1058587, so future studies can combine results for analysis in under-represented populations. In addition, the finding that rs1058587-C was also more common in cases of non-European descent suggests that GDF15 may play a role in other populations, although the confidence intervals were wide, particularly for African and unknown ancestries, and larger sample sizes are needed. Finally, the use of self-selection of acquaintance controls may introduce some bias. Although acquaintance controls tend to be highly concordant for demographic factors, there may be unknown genetic factors that may influence a person's desire to serve as a control. For example, it has been noted that extroverts may be more likely to become friend controls.[26] That being said, to date, none of the genetic studies of extraversion have identified genes that overlap with those associated with HG.[34,35] In addition, a search of 4220 GWASs for *GDF15* associations did not identify any personality traits, but did identify protein levels, periodontitis and lupus.[36] We can think of no reason for any of these associated factors to be biased by the selection of acquaintance controls. Although there may be other unknown confounding factors, it is highly unlikely that they would nullify the strong association (rs1058587, $p = 9.98 \times 10^{-11}$) with *GDF15*. Moreover, the rare *GDF15* variant rs372120002 found in 10 cases has a global allele frequency of 0.0007, making it unlikely to be an artefactual result.

This study provides strong evidence of a role for the GDF15 pathway, but other genes may be involved. The original GWAS identified additional risk loci, including the GDF15 receptor gene *GFRAL* and placenta genes *IGFBP7* and *PGR*.[5] Although these loci were not of exome-wide significance, and no rare causal variants were identified in ≥10 cases, common variants were validated previously in an independent cohort, suggesting that these genes also play a role.[5,6] WES has the caveat of excluding variants mapping outside the exome and thus may miss some common non-coding variants that are captured by GWAS. This limitation and/or the sample size may explain why other associations were not identified in this study. Additionally, medical records were requested for all cases, but controls were classified based on recall, which may result in misclassification. In the future, larger sample sizes that combine the results of this study with other WES, as well as additional GWAS studies in meta-analyses of HG using well-classified controls, may lead to the identification of additional associations. Regardless of whether other genes are involved, it is important to emphasise that now there are two separate genetic approaches (GWAS and WES), on two separate populations (23andMe customers and HG study participants), that have both identified *GDF15* to be the greatest genetic risk factor for HG.

## Interpretation

In addition to the genetic findings, there is now a large body of research supporting a causal role for the GDF15/GFRAL/RET pathway. GDF15 is most highly expressed by the placenta,

increases significantly in the first trimester and activates the vomiting centre of the brainstem through binding GFRAL and its co-receptor RET.[1] Lower serum levels are associated with lower NVP symptoms, male fetus and pregnancy loss.[10,11] Conversely, higher serum levels are associated with antiemetic use, second-trimester vomiting and hospitalisation.[8,9] GDF15 causes appetite and weight loss, taste aversion, aversion to drinking water, pica and emesis in non-pregnant animal models.[19,23,37–43] Twin studies suggest genes and unique environmental factors contribute almost equally to circulating levels.[44] Therefore, it is notable that several non-genetic factors are associated with increasing GDF15: in addition to placental production, GDF15 is a cellular stress-response hormone upregulated in tissues in response to nutrient deficiencies, long-term fasting, hyperthyroidism and infection.[45–48] All of these factors are associated with HG pregnancies and may explain why a genetic predisposition to increased GDF15/GFRAL/RET signalling can evolve from NVP to HG.[1]

Nausea and vomiting in pregnancy (NVP) is hypothesized to have evolved as a mechanism to avoid the consumption of teratogenic foods that could disrupt fetal organogenesis.[49,50] In addition to regulating appetite, nausea and vomiting, GDF15 may be a T-cell inhibitor, protecting pregnancy from maternal immune attack.[51] Interestingly, GDF15 causes cancer cachexia, a condition with symptoms similar to HG that causes 20% of cancer deaths.[1,52] This could explain why tumours often overexpress GDF15, hijacking this mechanism to evade host immune attack.[51] Screening methods and therapeutics targeting the GDF15 pathway are currently under investigation for cancer cachexia and chemotherapy-induced nausea and vomiting. Future studies should determine whether the variants identified in this study correlate with GDF15 levels or signalling in pregnancy. Of note, one study recently concluded that the *H6D* variant does not alter bioactivity and may result in inaccurate measurements of circulating GDF15, so more research is needed to determine whether there is a direct causal relationship between this variant and activity of the vomiting pathway, or whether it may be linked to another biologically active variant.[53] Once this is resolved, the findings, in addition to the potentially applicable work on GDF15 in cancer, may have clinical utility for the prediction, diagnosis and treatment of HG, which is urgently needed.

## CONCLUSION

Hyperemesis gravidarum (HG) can cause prolonged pregnancy starvation.[1] Despite this, patients often feel dismissed or not taken seriously, which contributes to suicidal ideation, pregnancy termination and other poor outcomes.[1,2,54] An understanding of disease aetiology and more effective treatments are urgently needed to lessen or eliminate these adverse maternal and child outcomes.[1] The involvement of the GDF15/GFRAL/RET pathway in cancer cachexia and chemotherapy-induced nausea and vomiting has motivated the development of novel treatments blocking this pathway, which show great promise in resolving symptoms in animal models.[20,22] If safe, they may be therapeutic for HG. Finally, in addition to supporting a paradigm-changing treatment pathway, this study may provide tools for the prediction and diagnosis of HG and advance molecular understanding and therapeutic design for HG and other appetite disorders. In the meantime, providing clinicians and patients with an evidence-based cause of HG may lessen the historical stigma and allow patients to be taken more seriously, resulting in improved care and healthier mothers and babies.

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### CONFLICT OF INTERESTS

MSF is a paid consultant for Materna Biosciences, Inc. Completed disclosure of interests form available to view online as supporting information.

### AUTHOR CONTRIBUTIONS

MSF jointly supervised the research, conceived, designed and performed the experiments, performed the statistical analysis, analysed data, contributed reagents, materials and analysis, and wrote the article. KWM aided in recruitment and manuscript preparation. OF analysed data and aided in manuscript preparation. CQ performed experiments and contributed reagents and materials. PMM jointly supervised the research and aided in manuscript preparation.

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### ETHICAL APPROVAL

Ethical approval was obtained for this study from the review board of the University of Southern California.

### CODE AVAILABILITY STATEMENT

The code generated during this study is available at GitHub (https://github.com/KarchinLab/open-cravat).

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

### ORCID

*Marlena S. Fejzo* https://orcid.org/0000-0001-6800-687X

## REFERENCES


1. Fejzo MS, Trovik J, Grooten IJ, Sridharan K, Roseboom TJ, Vikanes A, et al. Nausea and vomiting of pregnancy and hyperemesis gravidarum. Nat Rev Dis Primers. 2019;5(1):62.
2. Nana M, Tydeman F, Bevan G, Boulding H, Kavanagh K, Dean C, et al. Hyperemesis gravidarum is associated with increased rates of termination of pregnancy and suicidal ideation: results from a survey completed by >5000 participants. Am J Obstet Gynecol. 2021;224(6):629–31.
3. Martin C, Preedy V, Rajendram R. The neuroscience of normal and pathological development. 1st ed. Cambridge, MA: Academic Press; 2021.
4. First O, MacGibbon KW, Cahill CM, Cooper ZD, Gelberg L, Cortessis V, Mullin PM, et al. Trends in cannabis use for hyperemesis gravidarum. Jerusalem: International Cannabinoid Research Society; 2021.
5. Fejzo MS, Sazonova OV, Sathirapongsasuti JF, Hallgrimsdottir IB, Vacic V, MacGibbon KW, et al. Placenta and appetite genes GDF15 and IGFBP7 are associated with hyperemesis gravidarum. Nat Commun. 2018;9(1):1178.
6. Fejzo M, MacGibbon K, Mullin P. Hormone receptor genes PGR and GFRAL linked to hyperemesis gravidarum. Am J Obstet Gynecol. 2019;220(1):S585–S6.
7. Mullin PMK, Morgan Z, Fejzo M. Additional risk variant in GDF15 and a stronger maternal genetic influence linked to hyperemesis gravidarum. AJOG. 2020;222(1):S68.
8. Fejzo MS, Fasching PA, Schneider MO, Schwitulla J, Beckmann MW, Schwenke E, et al. Analysis of GDF15 and IGFBP7 in hyperemesis gravidarum support causality. Geburtshilfe Frauenheilkd. 2019;79(4):382–8.
9. Petry CJ, Ong KK, Burling KA, Barker P, Goodburn SF, Perry JRB, et al. Associations of vomiting and antiemetic use in pregnancy with levels of circulating GDF15 early in the second trimester: a nested case-control study. Wellcome Open Res. 2018;3:123.
10. Tong S, Marjono B, Brown DA, Mulvey S, Breit SN, Manuelpillai U, et al. Serum concentrations of macrophage inhibitory cytokine 1 (MIC 1) as a predictor of miscarriage. Lancet. 2004;363(9403):129–30.
11. Andersson-Hall U, Joelsson L, Svedin P, Mallard C, Holmang A. Growth-differentiation-factor 15 levels in obese and healthy pregnancies: relation to insulin resistance and insulin secretory function. Clin Endocrinol (Oxf). 2021;95(1):92–100.
12. Ho JE, Mahajan A, Chen MH, Larson MG, McCabe EL, Ghorbani A, et al. Clinical and genetic correlates of growth differentiation factor 15 in the community. Clin Chem. 2012;58(11):1582–91.
13. Jiang J, Thalamuthu A, Ho JE, Mahajan A, Ek WE, Brown DA, et al. A meta-analysis of genome-wide association studies of growth differentiation factor-15 concentration in blood. Front Genet. 2018;9:97.
14. Fejzo MS, Arzy D, Tian R, MacGibbon KW, Mullin PM. Evidence GDF15 plays a role in familial and recurrent hyperemesis gravidarum. Geburtshilfe Frauenheilkd. 2018;78(9):866–70.
15. Linhares ND, Pereira DA, Conceicao IM, Franco GR, Eckalbar WL, Ahituv N, et al. Noncoding SNPs associated with increased GDF15 levels located in a metformin-activated enhancer region upstream of GDF15. Pharmacogenomics. 2020;21(8):509–20.
16. Wang X, Chrysovergis K, Bienstock RJ, Shim M, Eling TE. The H6D variant of NAG-1/GDF15 inhibits prostate xenograft growth in vivo. Prostate. 2012;72(6):677–89.
17. Marjono AB, Brown DA, Horton KE, Wallace EM, Breit SN, Manuelpillai U. Macrophage inhibitory cytokine-1 in gestational tissues and maternal serum in normal and pre-eclamptic pregnancy. Placenta. 2003;24(1):100–6.
18. Breit SN, Brown DA, Tsai VW. The GDF15-GFRAL pathway in health and metabolic disease: friend or foe? Annu Rev Physiol. 2021;83:127–51.
19. Johnen H, Lin S, Kuffner T, Brown DA, Tsai VW, Bauskin AR, et al. Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. Nat Med. 2007;13(11):1333–40.
20. Lerner L, Tao J, Liu Q, Nicoletti R, Feng B, Krieger B, et al. MAP3K11/GDF15 axis is a critical driver of cancer cachexia. J Cachexia Sarcopenia Muscle. 2016;7(4):467–82.
21. Breen DM, Kim H, Bennett D, Calle RA, Collins S, Esquejo RM, et al. GDF-15 neutralization alleviates platinum-based chemotherapy-induced emesis, anorexia, and weight loss in mice and nonhuman primates. Cell Metab. 2020;32(6):938–50 e6.
22. Suriben R, Chen M, Higbee J, Oeffinger J, Ventura R, Li B, et al. Antibody-mediated inhibition of GDF15-GFRAL activity reverses cancer cachexia in mice. Nat Med. 2020;26(8):1264–70.
23. Borner T, Shaulson ED, Ghidewon MY, Barnett AB, Horn CC, Doyle RP, et al. GDF15 induces anorexia through nausea and emesis. Cell Metab. 2020;31(2):351–62 e5.
24. Fung E, Kang L, Sapashnik D, Benard S, Sievers A, Liu Y, et al. Fc-GDF15 glyco-engineering and receptor binding affinity optimization for body weight regulation. Sci Rep. 2021;11(1):8921.
25. Hale C, Veniant MM. Growth differentiation factor 15 as a potential therapeutic for treating obesity. Mol Metab. 2021;46:101117.
26. Bunin GR, Vardhanabhuti S, Lin A, Anschuetz GL, Mitra N. Practical and analytical aspects of using friend controls in case-control studies: experience from a case-control study of childhood cancer. Paediatr Perinat Epidemiol. 2011;25(5):402–12.
27. Williams RC, Knowler WC, Shuldiner AR, Gosalia N, Van Hout C, Regeneron Genetics C, et al. Next generation sequencing and the classical HLA loci in full heritage Pima Indians of Arizona: defining the core HLA variation for north American paleo-Indians. Hum Immunol. 2019;80(12):955–65.
28. Pagel KA, Kim R, Moad K, Busby B, Zheng L, Tokheim C, et al. Integrated informatics analysis of cancer-related variants. JCO Clin Cancer Inform. 2020;4:310–7.
29. Day SE, Muller YL, Koroglu C, Kobes S, Wiedrich K, Mahkee D, et al. Exome sequencing of 21 Bardet-Biedl syndrome (BBS) genes to identify obesity variants in 6,851 American Indians. Obesity (Silver Spring). 2021;29(4):748–54.
30. Altman DG. Practical statistics for medical research. 1st ed. London; New York, NY: Chapman and Hall; 1991. p. 611, xii.
31. Sheskin D. Handbook of parametric and nonparametric statistical procedures. 3rd ed. Boca Raton, FL: Chapman & Hall/CRC; 2004. p. 1193, xxxiii.
32. Li J, Shang G, Chen YJ, Brautigam CA, Liou J, Zhang X, et al. Cryo-EM analyses reveal the common mechanism and diversification in the activation of RET by different ligands. Elife. 2019;8:e47650.
33. Amaya-Amaya J, Rojas-Villarraga A, Molano-Gonzalez N, Montoya-Sanchez L, Nath SK, Anaya JM. GDF15(MIC1) H6D polymorphism does not influence cardiovascular disease in a Latin American population with rheumatoid arthritis. J Immunol Res. 2015;2015:270763.
34. Terracciano A, Esko T, Sutin AR, de Moor MH, Meirelles O, Zhu G, et al. Meta-analysis of genome-wide association studies identifies common variants in CTNNA2 associated with excitement-seeking. Transl Psychiatry. 2011;1:e49.
35. Heilbronner U, Papiol S, Budde M, Andlauer TFM, Strohmaier J, Streit F, et al. "The Heidelberg five" personality dimensions: genome-wide associations, polygenic risk for neuroticism, and psychopathology 20 years after assessment. Am J Med Genet B Neuropsychiatr Genet. 2021;186(2):77–89.
36. Fejzo MS, Burch K, Mullin P, KW MG, Pasaniuc B. Nausea and vomiting of pregnancy and HG risk genes associated with blood levels, sleep, stomach pain, and other traits. Obstet Gynecol. 2020;135:151S–2S.
37. Hsu JY, Crawley S, Chen M, Ayupova DA, Lindhout DA, Higbee J, et al. Non-homeostatic body weight regulation through a brainstem-restricted receptor for GDF15. Nature. 2017;550(7675):255–9.
38. Emmerson PJ, Wang F, Du Y, Liu Q, Pickard RT, Gonciarz MD, et al. The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. Nat Med. 2017;23(10):1215–9.
39. Mullican SE, Lin-Schmidt X, Chin CN, Chavez JA, Furman JL, Armstrong AA, et al. GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. Nat Med. 2017;23(10):1150–7.

40. Xiong Y, Walker K, Min X, Hale C, Tran T, Komorowski R, et al. Long-acting MIC-1/GDF15 molecules to treat obesity: evidence from mice to monkeys. Sci Transl Med. 2017;9(412):eaan8732.
41. Yang L, Chang CC, Sun Z, Madsen D, Zhu H, Padkjaer SB, et al. GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. Nat Med. 2017;23(10):1158–66.
42. Borner T, Wald HS, Ghidewon MY, Zhang B, Wu Z, De Jonghe BC, et al. GDF15 induces an aversive visceral malaise state that drives anorexia and weight loss. Cell Rep. 2020;31(3):107543.
43. Sabatini PV, Frikke-Schmidt H, Arthurs J, Gordian D, Patel A, Rupp AC, et al. GFRAL-expressing neurons suppress food intake via aversive pathways. Proc Natl Acad Sci USA. 2021;118(8):e2021357118.
44. Wiklund FE, Bennet AM, Magnusson PK, Eriksson UK, Lindmark F, Wu L, et al. Macrophage inhibitory cytokine-1 (MIC-1/GDF15): a new marker of all-cause mortality. Aging Cell. 2010;9(6):1057–64.
45. Zhao J, Li M, Chen Y, Zhang S, Ying H, Song Z, et al. Elevated serum growth differentiation factor 15 levels in hyperthyroid patients. Front Endocrinol (Lausanne). 2018;9:793.
46. Zhang M, Sun W, Qian J, Tang Y. Fasting exacerbates hepatic growth differentiation factor 15 to promote fatty acid beta-oxidation and ketogenesis via activating XBP1 signaling in liver. Redox Biol. 2018;16:87–96.
47. Lockhart SM, O'Rahilly S. The wasting hormone GDF15 frees up fat to fight infection. Nat Metab. 2019;1(10):935–6.
48. Lockhart SM, Saudek V, O'Rahilly S. GDF15: a hormone conveying somatic distress to the brain. Endocr Rev. 2020;41(4):bnaa007.
49. Sherman PW, Flaxman SM. Nausea and vomiting of pregnancy in an evolutionary perspective. Am J Obstet Gynecol. 2002;186(5 Suppl Understanding):S190–7.
50. Fessler DM. Reproductive immunosupression and diet. An evolutionary perspective on pregnancy sickness and meat consumption. Curr Anthropol. 2002;43(1):19–61.
51. Wischhusen J, Melero I, Fridman WH. Growth/differentiation factor-15 (GDF-15): from biomarker to novel targetable immune checkpoint. Front Immunol. 2020;11:951.
52. Sadeghi M, Keshavarz-Fathi M, Baracos V, Arends J, Mahmoudi M, Rezaei N. Cancer cachexia: diagnosis, assessment, and treatment. Crit Rev Oncol Hematol. 2018;127:91–104.
53. Karusheva Y, Ratcliff M, Melvin A, Mörseburg A, Sattar N, Barker P, et al. The common H202D variant in GDF-15 does not affect its bioactivity but can significantly interfere with measurement of its circulating levels. medRxiv preprint. 2022. https://doi.org/10.1101/2022.01.03.22268655
54. Dean C. Does the historical stigma of hyperemesis gravidarum impact healthcare professional's attitudes and treatment towards women with the condition today? A review of recent literature. MIDIRS Midwifery Digest. 2016;26(2):186–93.

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