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

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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, dystocia induction of labour, intrauterine pressure, intrauterine pressure catheter, mode of delivery, Montevideo unit, obesity, oxytocin

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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. It is the second leading cause of hospitalisation in pregnancy. 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. Long-term consequences include increased risks of neurodevelopmental delay, autism spectrum disorder and altered brain structure in offspring. Current anti-emetic treatments are largely ineffective in reversing weight loss, so novel approaches are needed.

A genetic origin for HG is supported by familial aggregation studies, twin studies, and a genome-wide associate study (GWAS). The GWAS identified common variants, implicating four genes (GDF15, GFRAL, IGFBP7 and PGR) confirmed in previous replication studies. Among these, GDF15 was found to be the greatest genetic risk factor, and two unlinked variants, rs16982345 and rs1054221, were confirmed in replication studies. 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. 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.

In addition, in maternal serum, GDF15 levels were significantly increased in hospitalised cases, in patients with second-trimester vomiting, and in those prescribed anti-emetics. Lower levels of GDF15 were associated with no NVP, fetal loss and male sex. Variants in GDF15 have been associated with familial and recurrent HG, and with the level of circulating GDF15.

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. 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. 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.

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. 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. The source population for HG cases included patients residing in the USA primarily recruited through advertising on the Hyperemesis Education and Research Foundation website 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. 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 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, 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.

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/GFRA1/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
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 \textit{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 $C \rightarrow G$ 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 (\textit{GFRAL}, \textit{PGR} and \textit{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 \textit{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 \textit{CGB7}), including the top \textit{GDF15} variant already mentioned (allelic $p = 9.98 \times 10^{-11}$) as well as five additional variants in \textit{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 \textit{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

<table>
<thead>
<tr>
<th>Ancestry</th>
<th>rs1058587 C/G (C is risk allele)</th>
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</thead>
<tbody>
<tr>
<td>AFR</td>
<td>HG</td>
<td>C, 60; G, 4</td>
<td>OR 4.41, 95% CI 1.07–18.27</td>
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<tr>
<td>Control</td>
<td>C, 17; G, 5</td>
<td>$p = 0.04$</td>
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<tr>
<td>AMR</td>
<td>HG</td>
<td>C, 96; G, 18</td>
<td>OR 1.92, 95% CI 0.77–4.79</td>
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<tr>
<td>Control</td>
<td>C, 25; G, 9</td>
<td>$p = 0.16$</td>
<td></td>
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<tr>
<td>EAS</td>
<td>HG</td>
<td>C, 12; G, 8</td>
<td>OR 2.27, 95% CI 0.59–8.65</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>C, 17; G, 5</td>
<td>$p = 0.23$</td>
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</tr>
<tr>
<td>OTHER</td>
<td>HG</td>
<td>C, 37; G, 7</td>
<td>OR 5.29, 95% CI 1.32–21.23</td>
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<tr>
<td>Control</td>
<td>C, 6; G, 6</td>
<td>$p = 0.02$</td>
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<tr>
<td>Total (non-European)</td>
<td>HG</td>
<td>C, 205; G, 37</td>
<td>OR 2.13, 95% CI 1.19–3.80</td>
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<tr>
<td>Control</td>
<td>C, 65; G, 25</td>
<td>$p = 0.01$</td>
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<tr>
<td>Total (European)</td>
<td>HG</td>
<td>C, 1338; G, 272</td>
<td>OR 1.64, 95% CI 1.37–1.97</td>
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<tr>
<td>Control</td>
<td>C, 922; G, 308</td>
<td>$p &lt; 0.0001$</td>
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\begin{table}
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\begin{tabular}{|l|l|l|l|l|l|l|}
\hline
Year born & Birth year of firstborn & No. of pregnancies & Live births & Termination (%) & Miscarriage (%) & \\
\hline
C & 1976 (1957–1989) & 2003 (1978–2012) & 2 (1–9) & 2 (1–7) & 3.3 & $p < 0.0001$ & $p < 0.0001$ & \\
\hline
\end{tabular}
\caption{Self-reported demographic characteristics of hyperemesis gravidarum cases (HG) and controls (C).}
\end{table}

\begin{table}
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\begin{tabular}{|l|l|l|l|l|l|}
\hline
Ancestry & rs1058587 C/G (C is risk allele) & | | | |
\hline
AFR    & HG & C, 60; G, 4 | OR 4.41, 95% CI 1.07–18.27 |
& Control & C, 17; G, 5 | $p = 0.04$ |
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& Control & C, 17; G, 5 | $p = 0.23$ |
OTHER  & HG & C, 37; G, 7 | OR 5.29, 95% CI 1.32–21.23 |
& Control & C, 6; G, 6 | $p = 0.02$ |
Total (non-European) & HG & C, 205; G, 37 | OR 2.13, 95% CI 1.19–3.80 |
& Control & C, 65; G, 25 | $p = 0.01$ |
Total (European) & HG & C, 1338; G, 272 | OR 1.64, 95% CI 1.37–1.97 |
& Control & C, 922; G, 308 | $p < 0.0001$ |
\hline
\end{tabular}
\caption{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.}
\end{table}
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 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.24,25 In addition, a search of 4220 GWASs for GDF15 associations did not identify any personality traits, but did identify protein levels, periodontitis and lupus.26 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 × 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. Lower serum levels are associated with lower NVP symptoms, male fetus and pregnancy loss. Conversely, higher serum levels are associated with antinicotinic use, second-trimester vomiting and hospitalisation. GDF15 causes appetite and weight loss, taste aversion, aversion to drinking water, pica and emesis in non-pregnant animal models. Twin studies suggest genes and unique environmental factors contribute almost equally to circulating levels. 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. 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.

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. In addition to regulating appetite, nausea and vomiting, GDF15 may be a T-cell inhibitor, protecting pregnancy from maternal immune attack. Interestingly, GDF15 causes cancer cachexia, a condition with symptoms similar to HG that causes 20% of cancer deaths. This could explain why tumours often overexpress GDF15, hijacking this mechanism to evade host immune attack. 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. 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. Despite this, patients often feel dismissed or not taken seriously, which contributes to suicidal ideation, pregnancy termination and other poor outcomes. An understanding of disease aetiology and more effective treatments are urgently needed to lessen or eliminate these adverse maternal and child outcomes. 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. 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.

ACKNOWLEDGEMENTS

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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.

FUNDING INFORMATION

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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.

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REFERENCES


**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

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