Genetic analysis of hyperemesis gravidarum reveals association with intracellular calcium release channel (RYR2)

Marlena Schoenberg Fejzo, a, b, *, Ronny Myhre c, Lucía Colodro-Conde d, Kimber W. MacGibbon e, Janet S. Sinsheimer f, M.V. Prasad Linga Reddy g, Paivi Pajukanta h, Dale R. Nyholt b, Margaret J. Wright i, Nicholas G. Martin j, Stephanie M. Engel k, Sarah E. Medland d, Per Magnus b, Patrick M. Mullin b

a Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
b Department of Maternal-Fetal Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
c Norwegian Institute of Public Health, Oslo Norway
d Psychiatric Genetics Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia
e Hyperemesis Education and Research Foundation, Damascus, OR, USA
f Department of Biostatistics, Biomathematics, & Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
g Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
h Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
i Queensland Brain Institute and Centre for Advanced Imaging, University of Queensland, Brisbane, Australia
j Genetic Epidemiology Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia
k Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

ARTICLE INFO

Article history:
Received 9 July 2016
Received in revised form 19 August 2016
Accepted 19 September 2016
Available online xxx

Keywords:
Hyperemesis gravidarum
Nausea
Vomiting
Pregnancy
RYR2

ABSTRACT

Hyperemesis Gravidarum (HG), severe nausea/vomiting in pregnancy (NVP), can cause poor maternal/fetal outcomes. Genetic predisposition suggests the genetic component is essential in discovering an etiology. We performed whole-exome sequencing of 5 families followed by analysis of variants in 584 cases/431 controls. Variants in RYR2 segregated with disease in 2 families. The novel variant L3277R was not found in any case/control. The rare variant, G1886S was more common in cases (p = 0.046) and extreme cases (p = 0.023). Replication of G1886S using Norwegian/Australian data was supportive. Common variants rs790899 and rs1891246 were significantly associated with HG and weight loss. Copy-number analysis revealed a deletion in a patient. RYR2 encodes an intracellular calcium release channel involved in vomiting, cyclic-vomiting syndrome, and is a thyroid hormone target gene. Additionally, RYR2 is a downstream drug target of Inderal, used to treat HG and CVS. Thus, herein we provide genetic evidence for a pathway and therapy for HG.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Nausea and vomiting of pregnancy is a common symptom affecting 70% of pregnant women (Goodwin, 1998). Clinical intervention is necessary in the severest form, Hyperemesis Gravidarum (HG), which affects up to 2% of pregnancies (Christodoulou-Smith et al., 2011). HG leads to significant weight loss, dehydration, electrolyte imbalance, and ketonuria (Fairweather, 1968; Goodwin et al., 1992a,b; Goodwin, 1998). Although maternal mortality is rare, 6 deaths due to HG have been reported recently (MacGibbon et al., 2015), as well as morbidity including Wernicke’s encephalopathy (Chiòssi et al., 2006), acute renal failure (Hill et al., 2002), liver function abnormalities (Ahmed et al., 2013), splenic avulsion (Nguyen et al., 1995), esophageal rupture (Woolford et al., 1993), pneumothorax (Schwartz and Rosoff, 1994), and post-traumatic stress symptoms (Christodoulou-Smith et al., 2011). HG is also associated with poor fetal/child outcomes including a 4-fold increased risk of preterm birth and a 3-fold increased risk of neurodevelopmental delay in children (Fejzo et al., 2013, 2015).

A variety of potential causative factors have been investigated, but the etiology remains unknown. Evidence for a genetic predisposition is provided by classic twin studies of Norwegian, Spanish,
and Finnish cohorts (Colodro-Conde et al., 2016a; Corey et al., 1992). Family based studies provide evidence that female relatives of patients with HG are more likely to be affected, with a 17-fold increased risk if a sister has HG (Gadsby et al., 1993; Vellacott et al., 1998; Vikanes et al., 2010; Zhang et al., 2011). Recently, mutations in the thyrotropin receptor gene have been linked to hyperemesis gravidarum accompanied by gestational thyrotoxicosis. This suggests a genetic etiology has already been identified in, at minimum, a subgroup of cases (Coulon et al., 2016). Thus, understanding the genetic component is essential in discovering the causal pathway(s).

The objective of this study was to perform whole-exome sequencing on HG families to identify rare variants conferring susceptibility to HG and to validate these findings in a large cohort of affected and unaffected individuals from the United States, followed by replication in cohorts from Australia and Norway.

2. Materials and methods

2.1. Study summary

The size and minimum HG CASE and CONTROL criteria for the 3 populations (US, Norway, Australia) used in this study are summarized in Fig. 1A. The genetic analysis methods used on each population are summarized in Fig. 1B.

2.2. US population

2.2.1. Eligibility criteria

The source population for HG CASES in the US included patients primarily recruited through advertising on the HER Foundation website (www.helpher.org). The stringent study criteria were designed to exclude all cases and controls that would increase phenotypic uncertainty. Briefly, the inclusion criteria for affected individuals were a diagnosis of HG and treatment with intravenous (IV) fluids or total parenteral nutrition/nasogastric feeding tube. Each participant was asked to recruit a non-blood related acquaintance with at least 2 pregnancies that went beyond 27 weeks. Controls were eligible if they experienced normal or no nausea/vomiting in their pregnancy, no weight loss due to nausea/vomiting and no medical attention in their pregnancy due to nausea/vomiting. Participants were enrolled in the family study if an HG CASE had 2 or more additional family members with HG. Additional affected family members were eligible if they reported severe NVP accompanied by >5% weight loss, and medication or hospitalization for HG. Control family members had the same eligibility requirements as Controls.

2.2.2. Description of US families analyzed by whole-exome sequencing and sanger sequencing

The whole-exome sequencing study included 15 affected individuals and 3 unaffected individuals. These 18 individuals came from 5 families (Fig. 1A). Follow-up analysis to confirm segregation in Family 1 included additional family members – 3 unaffected and 1 affected individual from Family 1. Each family submitted saliva samples for a minimum of three affected individuals. We chose to analyze a total of 18 individuals: 3 affected individuals from each of 5 families with HG in addition to 3 unaffected controls from 3 of the 5 families to further limit potential causal variants by dismissing those variants identified in unaffected family members. Pedigrees of two families of Caucasian/European descent analyzed in this whole-exome sequencing study, and whose variants are described herein, are shown in Fig. 2. Family 1 is of mixed Finnish, Swedish, English, and German descent. We collected DNA from 4 CASES (4 sisters) and 4 CONTROLS (2 sisters, mother, and maternal aunt) from Family 1. This family consists of 9 sisters, 5 affected and 4 unaffected, but only those siblings who participated are shown in Fig. 2A. Family 2 is of mixed Scottish, German, Swiss, English, and Italian descent and we collected DNA from 3 affected sisters (Fig. 2B).

2.2.3. Description of US CASE/CONTROL population analyzed by genotyping and copy-number analysis

The follow-up CASE/CONTROL population from the United States included 584 HG CASES and 431 unaffected CONTROLS that were all genotyped. A subgroup analysis was performed comparing the most severe HG CASES requiring total parenteral nutrition/nasogastric feeding tube to Controls who reported no NVP in any pregnancy. The subgroup was also used for a copy-number analysis. All participants gave informed consent. This study was approved by the UCLA Institutional Review Board.

2.3. Norwegian population

2.3.1. Eligibility criteria

Eligibility was determined using data obtained from self-reported questionnaires (Nilsen et al., 2009). HG CASES were eligible if they were admitted to hospital for prolonged nausea and vomiting in pregnancy. Controls were eligible if they were NOT admitted to hospital for prolonged nausea and vomiting of pregnancy.

2.3.2. Description of Norwegian population analyzed by GWAS and correlation with weight loss

The samples in this HG study included 385 HG CASES and 2280 unaffected Controls. The samples included were singleton pregnancies of Norwegian ancestry. Summary statistics for RYR2 were analyzed for independent replication in the Norwegian Mother and Child Cohort Study (MoBa), a prospective population-based pregnancy cohort conducted by the Norwegian Institute of Public Health recruited from Norway during 1999–2008 (http://www.fhi.no/moba-en; Magnus et al., 2016). In addition, data was collected for each participant on maternal pre-pregnancy weight and weight change until week 18 of gestation and used as a continuous variable in a regression analysis to study genetic associations with weight loss in early pregnancy. Ethical approval for the MoBa study has been approved by the Regional Committee for Medical Research Ethics and all women provided informed written consent.

2.4. Australian population

2.4.1. Eligibility criteria

Eligibility was determined using data collected from health and wellbeing surveys. Women reported their experience in their pregnancy with the most severe NVP using a five-point questionnaire adapted from Zhang et al. (2011). HG CASES (1) were defined as NVP that disrupted daily routine accompanied by weight loss and medication and/or IV fluids and/or feeding tube. A second less stringent CASE criteria included HG CASES (1) in addition to HG CASES (2) that reported NVP that disrupted their daily routine and medication treatment, but did not lose weight. CONTROLS were defined as experiencing no NVP.

Women in between the extreme ends who reported MILD NVP (NVP for more than 7 days, but did not see a doctor or nurse/did not disrupt daily routine very much) and MODERATE NVP (disrupted daily routine but it did not affect my weight and did not need medication, were not included in the CASE: CONTROL study, but were included in a continuous analysis of the GWAS data.

Please cite this article in press as: Fejzo, M.S., et al., Genetic analysis of hyperemesis gravidarum reveals association with intracellular calcium release channel (RYR2), Molecular and Cellular Endocrinology (2016), http://dx.doi.org/10.1016/j.mce.2016.09.017
2.4.2. Description of Australian population GWAS analyzed using CASE:CONTROL and continuous phenotype

The Australian sample is composed of genotyped women unselected for HG who are part of the Australian Endogene Study and the QIMR Mothers of Twins Study, which are two of the cohorts participating in the NVP Genetics Consortium (Colodro-Conde et al., 2016b). As part of health and wellbeing surveys, a total of 1440 women reported on NVP severity. We conducted a CASE:CONTROL analysis using 946 women who were in the extremes of the severity scale. Women reporting no NVP (n = 677) were used as controls and women reporting severe NVP (n = 269) with disruption of their daily routine and medication prescription, including those losing weight and put on a drip or feeding tube, were used as CASES. We also conducted a more stringent CASE/CONTROL analysis that excluded the 139 HG CASES with no weight loss, thus limiting the study to 130 HG CASES and 677 CONTROLS. Finally, we analyzed NVP as a continuous phenotype for all 1440 study participants, which included an additional 163 CASES of MILD NVP and 331 CASES of MODERATE NVP.

3. Genetic methods

3.1. Genetic methods for US population

3.1.1. Whole-exome sequencing of families

Each study participant was asked to submit a saliva sample for DNA analysis. A saliva collection kit (Oragene, Ottawa, Canada) was self-administered for submitting 2 mL of saliva. DNA was extracted from 75% of the saliva sample according to the manufacturer’s instructions. The size and minimum HG CASE and CONTROL criteria for the 3 populations (US, Norway, Australia) used in this study are summarized in Fig. 1A. The genetic analysis methods used on each population are summarized in Fig. 1B. "MILD NVP was defined as participants who answered: ‘had some NVP for more than 7 days, but did not see a doctor or nurse and did not disrupt daily routine very much’ "MODERATE NVP was defined as ‘disrupted daily routine but did not affect weight and did not need medication’"
We sequenced the entire exomes (~50 Mb) of 15 affected individuals and 3 unaffected individuals from 5 HG families. Paired end reads 100 nucleotides (2 x 100 nucleotides) were generated on an Illumina HiSeq 2000. Each sample was sequenced on 3 different lanes to avoid lane bias. Qseq files were converted into Sanger-formatted FASTQ files and reads were mapped to the reference human genome build hg19 using the Burrows Wheeler Alignment algorithm (BWA) (Li and Durbin, 2009). Duplicated reads were marked by Picard. The Genome Analysis Toolkit (GATK) was used for local realignment around indel sites followed by a base quality recalibration (McKenna et al., 2010). For reliable SNP calling we used genotype quality >10; read QUAL > 30 and a minimum read depth of 4. The combined total variants from all 18 individuals were filtered as shown in Fig. 3. Synonymous variants, which are unlikely to be causal, were discarded. The identified variants were further filtered against variants present in the HapMap, 1000 Genomes Project and dbSNP132 databases, selecting for novel variants and known variants with minor allele frequency <5% (McKenna et al., 2010; International HapMap 3 Consortium et al., 2010). These variants were further filtered by selecting variants predicted to affect protein function using PolyPhen and SIFT (Ramensky et al., 2002; Ng and Henikoff, 2003). Variants were further filtered by deleting variants present in the 3 unaffected family controls. All variants were discarded that were not shared by all 3 whole-exome sequenced affected family members with each family. Finally, we identified a subgroup of genes involved in more than one family, and screened these genes for a functional effect, which included genes 1) functionally relevant to reproduction (ie hormones), 2) nausea and vomiting (ie gastric tract, vomiting center of the brain), and 3) genes expressed in relevant tissues (ie ovary, placenta, vomiting center of the brain).

3.1.2. Sanger Sequencing to analyze segregation in family 1
Sanger Sequencing of the novel variant in Family 1 (RYR2 exon68:c.T9830G:p.Leu3277Arg) was performed to confirm whole-exome sequencing results and to confirm or deny segregation with the disease in the remaining family members who were not included in exome sequencing (1 affected sister and 1 unaffected sister, the unaffected mother, and an unaffected maternal aunt). PCR primer pairs GGAAGTCATACTGCCCATGC and GGGGTA-CATGTTTCTTCTTCCA were designed from genomic DNA to amplify and sequence the variant. PCR amplification and sequencing were carried out using standard methods.

3.1.3. Protein prediction tools used to predict functional effect of L3277R
The SIFT protein prediction tool was used to determine that the novel SNP encoding L3277R resulted in a damaging protein product, and the Provean Prediction tool was used to determine that it was deleterious. (Choi et al., 2012; Ng and Henikoff, 2003).

3.1.4. Genotyping
Taqman primers were designed for both the novel variant L3277R in Family 1 and the rare variant G1886S identified in Family 2, and used to screen individuals from >573 HG CASES and >426 controls using Applied Biosystems PRISM 7900HT Sequence Detection System (TaqMan) for large-scale screening. The call rate was >96%.

3.1.5. Statistical analysis
Statistical significance of association of genotype with HG was determined by calculating the p-values using a 1-tailed Fisher’s exact test (http://graphpad.com/quickcalc/contingency1/) and odds ratios were calculated using the odds ratio calculator (https://

---

**Fig. 3. Whole-exome sequencing filtering steps identifies RYR2 variants.**

---

Please cite this article in press as: Fejzo, M.S., et al., Genetic analysis of hyperemesis gravidarum reveals association with intracellular calcium release channel (RYR2), Molecular and Cellular Endocrinology (2016), http://dx.doi.org/10.1016/j.mce.2016.09.017
levels and calculated using the Number Reference Assay (VIC labeled, TAMRA probe). Melt-curve analysis was applied and all results were normalized to RNaseP copy number reference assay (VIC labeled, TAMRA probe). Melt-curve analysis was performed using a z-score transformed gestational weight gain (GWG) based on maternal pre-pregnancy weight and weight change until week 18 of gestation.

3.1.7. Genetic methods for Norwegian population

Maternal genome-wide data were obtained using Illumina HumanCoreExome genotyping BeadChip v1.1. Imputation was performed with reference panel HapMap phase 3 build 36 using IMPUTE2 (Howie et al., 2009). Standard association analyses were performed in PLINK 1.7 (Purcell et al., 2007). Genotypes were analyzed with allelic and genotypic approach. Regression analysis was performed using a test against variants present in the HapMap database to filter out variants not commonly found in the population.

4. Results

4.1. Whole-exome sequencing identifies RYR2 variants linked to HG in 2 of 5 families

We sequenced the entire exomes (~50 Mb) of 15 affected individuals and 3 unaffected individuals from 5 families with HG. The mean coverage was 54 fold. Reads were mapped to the human genome reference build UCSC hg19 using BWA (Li and Durbin, 2009). On average, 3223 single-nucleotide variants were detected in each individual and a total of 58,006 variants were detected in all 5 families combined (Fig. 3). The synonymous variants were further filtered against variants present in the HapMap, 1000 Genomes Project, and dbSNP132 databases, resulting in 13,509 novel variants and known variants with minor allele frequency <5% (McKenna et al., 2010; International HapMap 3 Consortium et al., 2010). These variants were further filtered by selecting variants predicted to affect protein function using PolyPhen and SIFT (Ramensky et al., 2002; Ng and Henikoff, 2003). Filtering for missense and stop gain or stop loss variants that were shared by any of the 3 whole-exome sequenced unaffected family members resulted in 6481 variants.

As we did not find any single variant that was shared by all the affected members across all of the families, we focused on variants within each family shared by all 3 whole-exome sequenced affected subjects. For example, 94 variants were shared by all 3 affected individuals in Family 1, and 227 variants were shared by all 3 affected individuals in Family 2. We searched for variants and/or genes that were shared by more than one family. 27 genes were identified that carried rare variants in the affected family members in more than one family. These variants were evaluated based on a functional effect, which included variants located in genes functionally relevant to reproduction (ie hormones), nausea and vomiting (ie gastric tract, vomiting center of the brain), and genes expressed in relevant tissues (ie ovary, placenta). This resulted in identification of the gene RYR2 involved in 2 of 5 families as a strong candidate based on its functional potential: RYR2 encodes an intracellular calcium release channel that is part of a signaling pathway for emesis expressed in the vomiting center of the brain (Giannini et al., 1995; Zhong et al., 2014). It is the only gene identified with variants significantly linked to cyclic vomiting syndrome, is a thyroid hormone target gene, and is differentially expressed in cumulus cells of the pre-ovulatory follicle (Grøndahl et al., 2012; Jiang et al., 2000; Lee et al., 2015).

4.2. Genotyping the novel and rare RYR2 variants in the US cohort provides confirmation

In the largest HG family (Family 1), the novel heterozygous variant in the RYR2 gene (R2:NM_001035:exon68:c.T9830G:p.Leu3277Arg) was confirmed by Sanger Sequencing to be shared by four affected sisters and was not shared by either of 2 unaffected sisters, the unaffected mother, nor the unaffected maternal aunt (Fig. 2A). The phenotype and genotype results suggest L3277R is of paternal rather than maternal origin in this family. However, the DNA from the father who is presumed to be a carrier, nor his sister who reportedly did not have HG, was unfortunately not available. We do not have any additional information about phenotype on the father’s side (ie father’s mother). Genotyping via Taqman showed the RYR2 variant to be unique in the sample to Family 1, as it was not identified in 584 HG CASES and 431 unaffected CONTROLS (Table 1). The nucleotide at the location of L3277R is 100% conserved across vertebrates and invertebrates. The mutation changes a hydrophobic amino acid to an electrically charged amino acid, and is predicted to be damaging and deleterious (SIFT Prediction Score = 0; Provean Prediction Score = –5.38).

In Family 2, the heterozygous variant G1886S (RYR2-NM_001035:exon68:c.C2123G:p.Gly1886Ser) was shared by all 3 affected sisters (Fig. 2B). Genotyping via Taqman identified the heterozygous variant G1886S (Family 2) to be twice as common (p = 0.046) in CASES than CONTROLS (in 38 out of 580 additional CASES and 17 out of 431 CONTROLS) and four times more common (p = 0.023) when comparing the extreme ends of the clinical spectrum, 9 out of 106 CASES requiring tube feeding compared to 3 out of 141 controls who reported no nausea/vomiting in pregnancy) (Table 1). The SNP G1886S is already known to have a biological effect in the homozygous state. Homozygous substitution of serine for glycine causes a significant increase in cellular calcium oscillation activity compared to wild-type RYR2 in HEK293 cells (Koop et al., 2008). Interestingly, calcium oscillations are completely abolished by homozygous substitution of a neighboring SNP in the double mutant G1885E/G1886S. The estimated frequency of G1886S in the European_American population (ESP6500) is 0.031
and the estimated frequency of G1885E is 0.023 (The 1000 Genomes Project Consortium, 2015). Therefore the estimated frequency of carrying both mutations is very rare (<0.001), but may be selected for in the extreme control population where the phenotype (no NVP in at least 2 pregnancies) is also rare. The SNP G1886S has also been associated with ventricular arrhythmias and is an independent predictor of sudden cardiac death, while a neighboring SNP rs790896 (G > A) was linked to a decreased risk of sudden cardiac death (Ran et al., 2010). The frequency of the protective A allele rs790896 is predicted to be 0.415 in the European extreme controls. Therefore, it will be interesting to investigate if additional variants in controls carrying RYR2 G1886S (such as G1885E and rs790896) explain why this variant is also present in a subset of controls with no NVP.

4.3. Summary statistics were supportive but not statistically significant for variant G1886S in RYR2 in both a Norwegian and an Australian GWAS

Genotype data for G1886S were imputed in both Norwegian and Australian datasets. Although statistical significance was not achieved probably due to the rarity of G1886S and the small number of affected individuals, there is a supportive trend in both cohorts. In the Norwegian cohort there is a 1.3-fold OR for this SNP (reference allele A), and in the Australian cohort, after removal of the CASES with no weight loss to better reflect the severe end of the clinical spectrum of NVP, there was a 1.2-fold OR for G1886S (Table 1). Common variants in RYR2 (rs790899 and rs1891246) are significantly linked to HG and are highly significant with respect to weight loss in early pregnancy.

In addition to the rare variants, common RYR2 SNPs (rs790899 and rs1891246) were significantly linked to HG in both the Norwegian and Australian GWAS using the CARE/CONTROL phenotypes (Table 1). No other common variants were identified that reached statistical significance in both the Norwegian and Australian datasets.

In the Norwegian dataset, adding the zscore for weight change until gestational week 18 as a covariate increased the odds ratio and significance for the common RYR2 variants, and suggested a strong association with weight loss (p = 2.12E-31 for rs790899 and p = 1.19E-31 for rs1891246, Table 2A). In the smaller Australian dataset, using the continuous severity measure, neither rs790899 nor rs1891246 reached statistical significance.

4.4. Copy-number analysis identifies a deletion in RYR2 in an extreme HG case requiring intravenous feeding (TPN)

We also performed copy-number analysis to search for pathogenic duplications and/or deletions in RYR2. A deletion in exon 16 was identified in RYR2 in DNA from one woman with HG requiring total parenteral nutrition (TPN) among DNA isolated from 101 extreme HG CASE requiring intravenous feeding (TPN).

5. Discussion

This is the first whole-exome association study of HG and suggests RYR2 may play a role in the biology of HG. We have

Please cite this article in press as: Fejzo, M.S., et al., Genetic analysis of hyperemesis gravidarum reveals association with intracellular calcium release channel (RYR2), Molecular and Cellular Endocrinology (2016), http://dx.doi.org/10.1016/j.mce.2016.09.017

---

**Table 1**

<table>
<thead>
<tr>
<th>RYR2 variant</th>
<th>Source</th>
<th>EXON/INTRON</th>
<th>Method</th>
<th>Screened</th>
<th>OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L3277R*</td>
<td>FAMILY 1, USA</td>
<td>68:c.T9830G</td>
<td>Genotyping</td>
<td>584 HG, 431 C</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>G1886S</td>
<td>FAMILY 2, USA</td>
<td>37:c.G5656A</td>
<td>Genotyping</td>
<td>584 HG, 431 C</td>
<td>1.29</td>
<td>0.046</td>
</tr>
<tr>
<td>G1886S</td>
<td>FAMILY 2, USA2</td>
<td>37:c.G5656A</td>
<td>Genotyping</td>
<td>106 HG, 141 C</td>
<td>4.27</td>
<td>0.023</td>
</tr>
<tr>
<td>G1886S</td>
<td>AUSTRALIAN</td>
<td>37:c.G5656A</td>
<td>GWAS</td>
<td>318 HG, 1823 C</td>
<td>1.32</td>
<td>0.661</td>
</tr>
<tr>
<td>G1886S</td>
<td>AUSTRALIAN2</td>
<td>37:c.G5656A</td>
<td>GWAS</td>
<td>269 HG, 677 C</td>
<td>0.89</td>
<td>0.693</td>
</tr>
<tr>
<td>rs790899</td>
<td>AUSTRALIAN</td>
<td>Intron 95</td>
<td>GWAS</td>
<td>130 HG, 677 C</td>
<td>1.17</td>
<td>0.655</td>
</tr>
<tr>
<td>rs790899</td>
<td>AUSTRALIAN</td>
<td>Intron 95</td>
<td>GWAS</td>
<td>385 HG, 2280 C</td>
<td>1.19</td>
<td>0.033</td>
</tr>
<tr>
<td>rs1891246</td>
<td>AUSTRALIAN</td>
<td>Intron 100</td>
<td>GWAS</td>
<td>269 HG, 677 C</td>
<td>1.23</td>
<td>0.009</td>
</tr>
<tr>
<td>rs1891246</td>
<td>AUSTRALIAN</td>
<td>Intron 100</td>
<td>GWAS</td>
<td>209 HG, 677 C</td>
<td>1.3</td>
<td>0.014</td>
</tr>
<tr>
<td>NOVEL DEL*</td>
<td>USA</td>
<td>16:237619976</td>
<td>Copy Number</td>
<td>101 HG, 139 C</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>CHR</th>
<th>SNP</th>
<th>BP</th>
<th>A1</th>
<th>Test</th>
<th>NMIS</th>
<th>OR</th>
<th>Stat</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs790899</td>
<td>23795768</td>
<td>C</td>
<td>ADD</td>
<td>2499</td>
<td>1.267</td>
<td>2.649</td>
<td>0.00808</td>
</tr>
<tr>
<td>1</td>
<td>rs790899</td>
<td>23795768</td>
<td>C</td>
<td>COVI</td>
<td>2499</td>
<td>0.2447</td>
<td>–11.66</td>
<td>2.12E-31</td>
</tr>
<tr>
<td>1</td>
<td>rs1891246</td>
<td>237981846</td>
<td>C</td>
<td>ADD</td>
<td>2499</td>
<td>1.292</td>
<td>2.968</td>
<td>0.00808</td>
</tr>
<tr>
<td>1</td>
<td>rs1891246</td>
<td>237981846</td>
<td>C</td>
<td>COVI</td>
<td>2499</td>
<td>0.2447</td>
<td>–11.66</td>
<td>2.12E-31</td>
</tr>
</tbody>
</table>

**HG**—Hyperemesis Gravidarum, **C**—Unaffected Control, **TPN**—Severe HG requiring tube feeding. L3277R*—novel deleterious SNP (c.9830T > G, p.Leu3277Arg).
are several intriguing avenues to explore further. Firstly, pathway underlying emesis in an animal model (Zhong et al., 2014). Functional mutations/deletions not identiﬁed in this study, although some clues. The novel variant L3277R is predicted to be deleterious and map between a putative phosphorylation site (AA2947) and a CALM interacting site (AA3581). And exon 16, the location of the deletion, contains, an RIH Domain (CDD:250561) which may form a binding site for IP3. The common variants identiﬁed in this study (rs790899 and rs1891246) both map in introns toward the end of the gene in sites with no predicted regulatory signiﬁcance (Kent et al., 2002), and therefore, are not likely to have a phenotype on their own, but may be linked to functional mutations/deletions not identiﬁed in this study.

The role RYR2 variants play in HG etiology is unknown, but there are several intriguing avenues to explore further. Firstly, RYR2 encodes an intracellular calcium release channel that is the only ryanodine receptor expressed in the vomiting center of the brain (Giannini et al., 1995) and has been implicated in a signaling pathway underlying emesis in an animal model (Zhong et al., 2014). Secondly, the thyroid hormone has been shown to induce RYR2 overexpression (Jiang et al., 2000), while the drug Inderal (Propranolol, used to treat hyperthyroidism) blocks RYR2 phosphorylation and lowers its expression (Yoshida et al., 1992). Hyperthyroidism accompanies HG in as many as 60% of pregnancies (Goodwin et al., 1992a,b) and mutations in the thyrotropin receptor-signaling pathway may account for a substantial amount of the attributable risk of HG, although just how much must be deferred to a follow-up study as causality has not been deﬁnitively established. Additional studies are required, such as functional analysis of the deleterious RYR2 variant L3277R, complete deletion analysis of RYR2, and a larger GWAS. However, this novel discovery may provide the ﬁrst step in understanding the etiology of HG. The identiﬁcation of genes linking HG to RYR2 provides an intriguing new avenue for diagnosis, research, and therapy.

Conflict of interest

The authors report no competing ﬁnancial interests or conﬂicts of interest.

Disclosure of interests

The authors declare no competing ﬁnancial interests.
Contribution to authorship
All authors fulfill authorship criteria as defined in the instructions for authors.

Details of ethics approval
This study was approved by the UCLA Institutional Review Board on 5/20/2011 as IRB#I11-001681 and the Queensland Institute of Medical Research Human Research Ethics Committee.

Funding
The research was supported, in part, by the Hyperemesis Education and Research Foundation (MSF), the Paul and Janis Potkin Family Foundation, the UCLA Clinical and Translational Science Institute (CTSI) UL1TR000124 (MSF), and NIH Grant GM053275 (JSS). NICHD Grant R01HD058008. The Norwegian Mother and Child Cohort Study was also supported by the Norwegian Ministry of Health and the Ministry of Education and Research, NIH/NIEHS (contract no N01-ES-75558), NIH/NINDS (grant no.1 U01 NS 047537-01 and grant no.2 U01 NS 047537-06A1). Australian Research Council (ARC) Grants A7960334, A79906588, A79801419, DP0212016, DP0334921 and the National Health and Medical Research Council Project Grant (NHMRC) Grants 249144, 389875, 552485, 552471, 1031119, 1049894, 1084325. This work was also supported by Fundación Séneca-Regional Agency for Science and Technology, Murcia, Spain [19151/PD/13 to L.C.C.]. SEM is expressed in murine brain and peripheral tissues. J. Cell. Biol. 128 (5 Pt 2), 245–248.


Please cite this article in press as: Fejzo, M.S., et al., Genetic analysis of hyperemesis gravidarum reveals association with intracellular calcium release channel (RYR2). Molecular and Cellular Endocrinology 387, 1–7 (2016).