

## **GDF15, Nausea and Vomiting in Pregnancy (NVP) and its most severe form, Hyperemesis Gravidarum (HG). What have we discovered?**

1. We discovered that the elevated levels of GDF15 known to be present in maternal blood from the early stages of pregnancy are derived predominantly, if not exclusively, from the fetal trophoblast (and later in pregnancy by the fetal component of the placenta) and not from the mother's own tissues.
2. We have securely established, using well-validated immunoassays and large sample sizes, that GDF15 levels in maternal blood are significantly higher in women who report vomiting in pregnancy, or have a diagnosis of hyperemesis gravidarum (HG), compared to women who report no or low levels of nausea or vomiting.
3. However, there is considerable overlap between symptomatic and asymptomatic women so GDF15 levels alone cannot be responsible for determining who is and who is not affected. Nor can it be used as a diagnostic test for HG.
4. It has previously been reported that heterozygous female carriers of a rare coding mutation in GDF15 (C211G) have a ~10-fold increased risk of developing HG in pregnancy. We now report that this mutation a) prevents the secretion of GDF15 including the secretion of wild type GDF15 monomers that form part of the dimeric hormone b) because of this, non-pregnant carriers of this mutation have very low levels of GDF15. Thus, **low** levels of GDF15 pre-pregnancy appear to **increase** the risk of HG in a subsequent pregnancy.
5. Consistent with this observation, we found that common genetic variants in the vicinity of the *GDF15* gene that are associated with **lower** levels of GDF15 in the non-pregnant state are associated with **higher** risk of HG.
6. We conducted a questionnaire-based study of NVP symptoms in women with Thalassemia Major who had recently given birth and matched controls without thalassemia. Thalassemia is a condition where GDF15 levels derived from the stressed abnormal erythroblasts, are exceptionally high life long. Women with thalassemia reported negligible nausea or vomiting in pregnancy in contrast to the high prevalence reported by the controls.
7. These results suggested that the aversive response to a sudden increase in GDF15, such as occurs in pregnancy, is likely to be significantly influenced by levels of prior exposure to GDF15. Although mice cannot vomit, they do show aversive responses to sudden increases in GDF15 and this is accompanied by an acute reduction in food intake and body weight. We treated wild-type mice with low levels of long acting GDF15 for 3 days prior to injecting a bolus dose of the native hormone. Mice pre-treated with the low dose GDF15 showed a considerably blunted response to the bolus of native GDF15. In orthogonal experiments we showed that mice lacking GDF15 from conception were hypersensitive to very low doses of GDF15. We conclude that the brain's response to GDF15, mediated by neurons expressing GFRAL-Ret and projecting to other areas of the brain is a system that exhibits features of ligand induced desensitisation, though we do not yet understand at what level this occurs.
8. Our findings have obvious therapeutic implications. They firmly place GDF15 action at the centre of the causation of HG, a serious disorder affecting 1-2% of pregnancies and one

for which there is no specific treatment. Blocking the action of GDF15 at its receptor in the mother is very likely to be highly effective in reducing symptoms. Even though transplacental transfer of antibodies only occurs to a small extent during the first trimester, using a therapeutic antibody engineered not to cross the placenta would provide considerable reassurance about reducing the risk of teratogenesis. Even if there was some transplacental passage of a blocking antibody, this would have a low risk of teratogenesis. GFRAL mRNA is not detectable in first trimester human brain. Several adult humans exist who totally lack either GDF15 or GFRAL due to homozygous nonsense mutations and have developed normally (as do mice lacking GDF15 or GFRAL from conception).

9. An obvious concern is that there might be some biological actions of GDF15 in pregnancy which are essential to its normal progress. The high expression of GDF15 in placenta appears to be a phenomenon restricted to certain higher species including primates. We hypothesise that this evolved to send a signal to the mother's brain in early pregnancy that made her averse to ingestion of foodstuffs that had a higher risk of containing teratogens or infectious organisms. >90% of women describe specific food and beverage avoidance in early pregnancy (most frequently for meat and alkaloid containing beverages) and while we have not proven this it seems likely that GDF15 is involved in signalling such aversions. While this would have been critical to the survival chances of the offspring (and also the mother, who is immunosuppressed in early pregnancy and therefore more susceptible to infection) during most of our evolution during which we were largely hunter-gatherers, in the modern era of safe food production (in developed countries at least) such a signalling system may be seen as redundant. Thus, placental production of GDF15 may be the endocrine equivalent of the appendix, a piece of human biology that has outlived its necessity and now causes us more trouble than it is worth.
10. Our findings also suggest a way in which women at high risk of HG could have that risk reduced. If such women could be given an agent that gradually increased GDF15 levels prior to any planned pregnancy, this should reduce the risk of developing HG in that pregnancy. Our data tentatively suggest that a doubling of pre-pregnancy GDF15 levels would reduce HG risk by 50%. Metformin, a widely used drug which has been given to people with diabetes for over 50 years, increases GDF15 levels by 2 to 3-fold and is widely used in pregnancy in many countries. Clinical trials of such an intervention are currently being planned, in partnership with patient-led groups.

## The story behind the paper (from a highly personal perspective)

### Stephen O'Rahilly

GDF15 was discovered by Sam Breit in 1997 as a product of activated macrophages ([PMID: 9326641](#)).

A member of the TGF beta superfamily, this homodimeric protein can be synthesised by most cells in the body. Cellular stresses of various kinds increase its expression so, unsurprisingly, it was found to be high in the blood in a number of diseases including infections and certain cancers. It is markedly elevated in renal failure.

Breit showed that it acted to reduce food intake and body weight ([PMID: 17982462](#)) and he localised the site of action of GDF15 on food intake to the brain stem using lesioning experiments [PMID: 24971956](#). In 2000, he reported high levels in pregnant women with the placenta being the likely source ([PMID: 11134143](#)).



As its receptor remained unknown GDF15, for almost 20 years GDF15 attracted most scientific attention as a biomarker of various disease states. This changed dramatically in 2017 when four different industrial teams isolated its receptor, and found it to be a heterodimer of GFRAL and Ret ([PMID: 28846097](#), [PMID: 28846098](#), [PMID: 28846099](#), [PMID: 28953886](#)). Remarkably the expression of GFRAL was exquisitely specific to the hindbrain, specifically to area postrema (which is open to the general circulation) and an adjacent portion of the nucleus of the solitary tract.

I had attended a scientific advisory board at Novo Nordisk in October 2016 where the nature of the receptor was confidentially revealed. This immediately grabbed my attention. Having worked for many years on leptin, a hormone made in the periphery but acting predominantly in the brain, it was exciting to see the birth of another such hormone, albeit one that was secreted from more widespread anatomical sites than leptin.

As the receptor is expressed in a region of the brain classically associated with the induction of aversive responses (including nausea and vomiting) it seemed likely that GDF15 would be a stimulus animals would wish to avoid (something which was first demonstrated by Danna Breen at Pfizer in a collaboration with my lab and that of David Savage (*Patel et al Cell Metabolism* 2019 [PMID: 30639358](#)) and further reinforced by work from the De Jonghe, Borner and Grill labs who formally demonstrated conditioned aversive responses and vomiting in species, like shrews, that can vomit.

On reading about the high expression of GDF15 in human trophoblast and placenta it became clear to me that it was a good candidate to be the mediator of the nausea and vomiting of pregnancy (NVP) including its most severe form, **Hyperemesis Gravidarum** or **HG**. GDF15 levels were known to go up rapidly in maternal blood in the first trimester and

remain high throughout pregnancy ([PMID: 11134143](#)). Luckily, my colleagues from the Dept of Paediatrics, Ieuan Hughes, David Dunger (sadly deceased) and Ken Ong had previously set up the Cambridge Baby Growth Study (CBGS). I discovered that they had stored plasma samples from pregnant volunteers (not actually selected for NV symptoms) who had filled in a questionnaire about nausea and vomiting in pregnancy. So, we planned to study those as soon as possible. In addition, I collaborated with Claire Meek, one of my colleagues in the Dept of Clinical Biochemistry. She had a generic ethics committee-approved study that allowed analytes of any kind to be measured in residual serum or plasma from clinical samples from patients attending Addenbrooke's or the Rosie Maternity Hospitals that went through our NHS Department of Clinical Biochemistry in Cambridge. Data were pseudonymised and while researchers could relate analyte levels to clinical diagnosis there was no ability to go back and get more data. In a small pilot study, we couldn't find any significant differences in levels between women diagnosed with HG and those of similar gestational age without HG. I contacted the UK charity for HG, Pregnancy Sickness Support to ask if there were any studies going on more widely in UK. I spoke to Margaret O'Hara who told me that while there was no study at that time collecting blood samples from UK women with HG, there was an upcoming Windsor meeting at which a US researcher, Marlena Fejzo would be presenting new and exciting genetic data. Margaret kindly arranged for me to meet Marlena in Windsor.

Marlena had been working on HG since the early 2000s, following her own tragic experience of the condition and had more recently collaborated with 23andMe to undertake a genome wide association study. I went to see Marlena at Windsor and she shared her genetic data with me. It was very exciting to see that the top genetic hits in her GWAS were in or around the *GDF15* gene. She also showed immunoassay (Origene GDF15 Elisa kit EA100484) data from a small number of women hospitalised with HG which suggested that levels were higher than those found in a similar modestly sized group of controls. This data was later published in Fejzo et al *Geburtshilfe Frauenheilkd* 2019 [PMID: 31000883](#).

Very shortly afterwards (thanks to Clive Petry and the staff at the CBAL lab) the GDF15 immunoassay data from the CBGS emerged. We found that levels of GDF15 were modestly but significantly higher in women who reported vomiting than those who did not. In November 2017 we publicly disclosed our Cambridge Baby Growth Study data as a Biorxiv preprint (<https://doi.org/10.1101/221267>). This was formally published in 2018 Petry et al *Wellcome Open Research* [PMID: 30345390](#). We now know that we were using an assay that was very susceptible to serious confounding by a common amino acid variant; however later reanalysis using an appropriate assay that we had validated in-house, confirmed the validity of our results and indeed resulted in the detection of a much more significant difference.

Later in November of that year the four papers appeared from four different pharmaceutical companies all identifying GFRAL-Ret heterodimers as the receptor for GDF15. All agreed that the only site of expression of GFRAL was in the area postrema and nucleus tractus solitarius of the hindbrain.

In December 2017, I wrote a *Cell Metabolism* commentary [PMID: 29195860](#) in response to those publications and suggested that the aversive properties of GDF15 should be explored.

Somewhat cheekily, I suggested that the hormone might be more appositely called “aversin”!

In March 2018, Marlena published her seminal GWAS paper in Nature Communications ([Fejzo et al Nat Comms 2018 PMID: 29563502](#)) and my lab continued to work on various aspects of the biology of GDF15 ([PMID 30639358](#), [31853550](#), [31875646](#), [32310257](#), [34819667](#), [34187898](#)). We also collaborated with my old friend Ashley Moffett in studying human trophoblast organoids and with the wonderful peptidomic expert in the Institute of Metabolic Science, Richard Kay, were able to demonstrate the very high secretion of GDF15 protein by human trophoblast organoids in vitro ([Turco et al, Nature 2018 PMID: 30487605](#)).

Meanwhile, frustrated by the fact that some of the most widely used assays for GDF15 seemed to be seriously interfered with by the common variant at H6D of the mature peptide we spent a lot of time validating other immunoassays and proving their superior performance ([Karusheva et al J App Lab Med PMID: 35796717](#)) before looking again at samples from women with NVP and HG vs controls.

With immunoassays we could now trust and a new study of HG sufferers and controls that we had been undertaking in the Rosie Maternity Hospital in Cambridge with Claire Meek and Miriam Baumgarten, we were finally able to show that women with NVP had robustly higher levels than control women without NVP and that levels were further increased in women with HG. Two questions presented themselves to us at that stage i) Where was the GDF15 coming from in pregnancy? and ii) As there is considerable overlap in GDF15 levels in pregnancy between affected and unaffected women, what other explanations were there for the symptomatic difference between cases and controls?

Regarding the source of GDF15 in pregnancy, the gene is ubiquitously expressed so even though it is high in the placenta it is possible that maternal circulating GDF15 came from a mixture of the fetal and the maternal genes. To solve this, we turned again to Richard Kay and to our colleagues in the Department of Obstetrics and Gynaecology, Gordon Smith and Steve Charnock-Jones, who have for many years been running a large prospective study of pregnant women and their offspring from whom stored plasma at timepoints throughout pregnancy was available, as well as RNA sequence data from term placenta. Using the RNAseq data we could identify the genotypes of foetus at the site of the common missense variant (H6D) and, in DNA from the mother, identify fetus/mother pairs where only one of the two contributed a particular allele. Richard could then use his mass spectrometry tools to quantitate the amount of H or D containing GDF15 in maternal plasma. The results were stunning. At all-time points >95% (in most cases >99%) of the GDF15 in the mother's blood had originated from the fetal, not the maternal, genome.

We then turned to the second question. Marlena Fejzo and I communicated in early 2022 and she let me know of her exome sequencing study which was published later that year ([Fejzo et al BJOG 2022 PMID: 35218128](#)). She recruited 926 HG cases and 660 unaffected controls primarily with the help of the [HER Foundation](#), which were subsequently exome-sequenced in partnership with Regeneron. The only rare non-synonymous variant which was found in 10 or more cases and no controls was a rare missense mutation C211G found

in 10 HG cases and no controls. Looking at its frequency in GNOMAD the carriage of this allele appears to have a frequency of  $\sim 1/1000$  in Caucasians. Thus, its carriage looks to increase the risk of HG by  $\sim 10$ -fold, though the numbers are small and confidence interval large. She also had some unpublished data on mother offspring pairs where the mother carried the C211G. Intriguingly, when fetuses were homozygous wild type, the women always developed severe HG. But in 3/7 cases where the fetus was a heterozygote, the N and V symptoms were much milder.

I had been discussing the structure function relationship of GDF15 both with Vladimir Saudek, a great bioinformatician associated with the IMS, and with Marko Hyvönen (Biochemistry Dept in Cambridge) and it seemed pretty clear that this mutation would remove a cysteine critical for its folding. If GDF15 levels were what made you sick and this mutation was likely to cause low GDF15 production how could we square these ideas?

Firstly, we needed to test our hunch that C211G reduced GDF15 protein expression. Nuno Rocha did beautiful cellular work and showed very clearly that the G variant completely blocked secretion of GDF15. Importantly it also reduced the secretion of co-expressed wild type GDF15, indicating that it also interfered with the secretion of heterodimers between mutant and wild type monomers. We needed to find a population of people where this variant had been genotyped and in which plasma was available to measure GDF15.

Fortunately, Caroline Hayward in Edinburgh University came up trumps through her involvement in a population study from the Dalmatian region of Croatia. In that study, exome sequencing had found that 11 out of 2872 participants were heterozygous carriers of that variant. We measured GDF15 levels in these 11 participants and a larger number of matched controls from that population and found that the C211G carriers had markedly reduced levels of circulating GDF15 (in the non-pregnant state). We then turned to see whether the common variants in and around the GDF15 gene which, if present in the maternal genome influence HG risk, are associated with circulating GDF15 levels in the non-pregnant state and, if so, in which direction. For these analyses Sam Lockhart worked with Caroline Hayward, this time on the Generation Scotland dataset of 18,184 participants, in whom Naveed Sattar and team at the University of Glasgow had measured GDF15 using the high-quality Roche assay. Our initial analyses looked exciting and consistent with the rare variant work. The common variants that had been reported to increase HG risk all looked like they were associated with lower, not higher, GDF15 levels in the non-pregnant state. To really firm up on this we needed to access the original *23andMe* data from *Fejzo et al 2018* [PMID: 29563502](https://pubmed.ncbi.nlm.nih.gov/29563502/) which unfortunately was not publicly accessible. Marlena and her genetic epidemiologist colleague at USC, Nick Mancuso, were able to access this data and they agreed to collaborate. Using state of the art Mendelian Randomisation approaches we were able to firmly establish that risk variants for HG were indeed strongly and highly significantly associated with lower levels of GDF15 in the non-pregnant state and that GDF15 levels were likely causally related to HG.

How could we account for these apparently paradoxical observations? Risk alleles for a condition associated with (and likely caused by) increased GDF15 levels in pregnancy were

associated with lower, not higher, circulating levels of GDF15 in non-pregnant women (and men).

Ligand induced desensitisation is a feature of many hormonal systems and we wondered if this could provide an explanation. What if women who had constitutively low levels of GDF15, when suddenly exposed to a surge in feto-placentally derived GDF15 in pregnancy, were more susceptible than those women who had habituated to higher levels? Although the small numbers meant the data were not statistically significant, the results from Marlina's mother-child pairs with C211G was consistent with our emerging idea that HG might result from a combination of enhanced maternal sensitivity (possibly due to low prior exposure to GDF15 and higher levels of GDF15 coming from the FP unit).

We turned to the mouse to see if we could find any evidence of ligand-induced desensitisation. Tony Coll, Irene Cimino and Deb Rimmington all contributed to the design and/or execution of these studies. In brief, if we gave a low dose injection of a long-acting version of GDF15 (kindly provided by Danna Breen from Pfizer); a dose which itself had little effect on food intake or body weight after three days, and then, at three days gave an acute bolus of GDF15. Mice who had received a control pre-injection showed a marked reduction in food intake and body weight, whereas those who had been pre-treated with low dose GDF15 had almost no response. In orthogonal experiments we showed that mice that lacked GDF15 from conception were hypersensitive to ultra-low doses of GDF15. This provides compelling data that ligand induced desensitisation is a feature of the central response to GDF15.

Beta Thalassemia is a genetic disorder of haemoglobin production by red blood cells. As a result of their abnormal haemoglobin patients with this disorder have a massively expanded pool of red cell precursors undergoing an enormous amount of cellular stress due to persistent activation of the unfolded protein response. Circulating levels of GDF15 in Thalassemia patients are among the highest recorded. We worked with Prof. Sachith Mettananda at the University of Kelaniya, Sri Lanka, to identify women with Beta Thalassemia who had recently had a successful pregnancy. These women and matched healthy controls agreed to fill in a questionnaire about nausea and vomiting symptoms in pregnancy. The control women reported NVP and HG-like symptoms at just as high a rate as has been reported in Western women. In contrast the mothers with thalassemia reported a very low prevalence of any symptoms of nausea or vomiting in their pregnancies. It looked like they were almost completely protected. It is possible, of course, that other features of the thalassaemic state contributed to this but it seems likely that the very high pre-pregnancy GDF15 is a contributory factor.

So, we have assembled a compelling body of data which indicates that the occurrence and severity of nausea and vomiting in pregnancy are determined by a combination of a) levels of GDF15 produced by the feto-placental unit and b) maternal sensitivity, which itself is highly influenced by pre-pregnancy levels of exposure. The high degree of protection from NVP/HG provided by Beta Thalassemia where pre-pregnancy GDF15 is very high suggests that GDF15 is likely to be a major, if not the major factor in causing NVP. The corollary of this is that blocking GDF15 action at the maternal hindbrain is very likely to be efficacious in

reducing symptoms of NVP and HG. The data from thalassemia also suggest a way forward for the prophylaxis of HG in women at high risk. The administration of an agent that increases circulating GDF15 before pregnancy, particularly if it does so in a graded manner seems a promising approach to prophylaxis. One such drug, metformin, is widely used and has an excellent safety record. Formulations of metformin which largely restrict it to the gut epithelium (a key site at which metformin increases GDF15) may be particularly attractive in women planning pregnancies.

This paper was the culmination of the collaborative work of many excellent scientists in Cambridge, Los Angeles, Edinburgh, Glasgow and Colombo, Sri Lanka without whom this clear picture would not have emerged. Particular thanks go to Sam Lockhart for taking on the Herculean post submission/pre-publication tasks now required to get through the process of having a paper published in Nature.