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

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GDF 15 was discovered by Sam Breit in 1997 as a product of activated macrophages. 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.

Sam Breit showed that it acted to reduce food intake and body weight and he localised the site of action of GDF15 on food intake to the brainstem using lesioning experiments. In 2000, he reported high levels in pregnant women with the placenta being the likely source.

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 founds it to be a heterodimer of GFEAL and Ret. 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 early 2017 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. Luckily, I worked in Cambridge where my colleagues Ieuan Hughes, David Dunger (sadly deceased) and Ken Ong had 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. Clive Petry quickly got hold of those
samples and (using an assay that we now know to be very susceptible to interference by a common amino acid variant) we found that levels of GDF15 were modestly but significantly higher in women who reported vomiting than those who did not and published this as a BioRxv preprint in November 2017 (doi: https://doi.org/10.1101/221267 published in 2018 Petry et al Wellcome Open Research PMID: 30345390).

In December 2017, I wrote a Cell Metabolism commentary PMID: 29195860 in response to the publications announcing the discovery of the receptor in which I predicted that GDF15 would have aversive properties and would be an excellent candidate for the causation of HG.

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).

A few months later I first became aware of the amazing Marlena Fejzo (see recent profile in New York Times 14th March 2023) and that the GWAS she was about to publish with data generated by 23 and Me had highlighted polymorphisms in and around the GDF15 gene as the top predisposing hit for HG! (Fejzo et al Nat Comms 2018 PMID: 29563502)

We were both invited to the international HG conference in Windsor in the summer of 2018, Marlena to present her genetic work, me to talk about GDF15, and we met up and chatted. We agreed to keep each other informed about our respective progress in the area and to collaborate when it seemed a sensible thing to do.

My lab continued to work on various aspects of the biology of GDF15 (PMID 30639358, 31853550, 31875646, 32310257, 34819667, 34187898).

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 sent 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 with levels being further increased in women with HG. But where was the GDF15 coming from?

The gene is ubiquitously expressed so even though it is high in the placenta it is possible that maternal circulating GDF5 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 spec 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.
Meanwhile, Marlena’s second HG genetics paper appeared ([Fejzo et al BJOG 2022 PMID 35218128](https://doi.org/10.1111/1471-0528.16289)). She had worked with Regeneron, who exome sequenced 926 HG cases and 660 NVP-free controls. 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 ~1/1000 in Caucasians. Thus, its carriage looks to increase the risk of HG by ~10 fold, though the numbers are small and confidence interval large.

I had been discussing the structure function relationship of GDF15 both with Vladimir Saudek, a great bioinformatician associated with the IMS, and with Marko Hyvonen (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 HDF15 (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 23 and Me data from [Fejzo et al 2018 PMID: 29563502](https://www.ncbi.nlm.nih.gov/pubmed/29563502) which unfortunately was not publicly accessible. To do this we approached Marlena and initiated a collaboration with her and her genetic epidemiological colleague Nick Mancuso. 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 fetoplacentally derived GDF15 in pregnancy, were more susceptible than those women who had habituated to higher levels? Marlena
had been studying the pregnancies of women with Hg who carry the C211G variant. Intriguingly when foetuses were homozygous wild type, the women always developed severe HG. But in 4/7 cases where the fetus was a heterozygote, the N and V symptoms were much milder. Although the small numbers meant this was not statistically significant it 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.

Finally, we wanted to find a situation where women are naturally exposed to high concentrations of GDF15 prior to pregnancy and see whether the rates of NVP and HG were different in that group. 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 patents 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 recent 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 vanishingly low prevalence if 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.
A shorter summary is provided below along with some further discussion of the potential for safe and effective treatment and prevention:

**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, for the first time, 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 GDF15.

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. 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. Doing so with a therapeutic antibody engineered not to cross the placenta would provide considerable
reassurance about reducing the risk of teratogenesis. Even some transplacental passage of a blocking antibody 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 hypothesize 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 5 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.