Iron deficiency responses and integrated compensations in patients according to hereditary haemorrhagic telangiectasia ACVRL1, ENG and SMAD4 genotypes


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Letter to the Editor

Iron deficiency responses and integrated compensations in patients according to hereditary haemorrhagic telangiectasia ACVRL1, ENG and SMAD4 genotypes

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Running Head: HHT genotypes and iron

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Categorized data that do not risk breaching anonymity may be found in a data supplement. Primary data from the 100,000 Genomes Project are held in a secure Research Environment, are available to registered users. Please see https://www.genomicsengland.co.uk/about-gecip/for-gecip-members/data-and-data-access for further information

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LS performed the analyses, generated the Figures and Data Supplement and wrote the first draft. FA examined 100,000 Genomes Project data. HM, JS and HCT acquired postural pulse data. The Genomics England Research Consortium sequenced whole genomes for selected patients. CLS reviewed all patients, generated the database, conceived the study, supervised LS, wrote the manuscript and is the guarantor for the study.

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Severe haemorrhage and anaemia in hereditary haemorrhagic telangiectasia (HHT) is a major focus of drug development and repurposing. HHT is not a single disorder, but molecularly heterogenous, and most commonly caused by a single loss-of-function DNA variant in \textit{ACVRL1}, \textit{ENG} or \textit{SMAD4}. As for all individuals in the general population, HHT patients are at risk of developing iron deficiency, anaemia and sequelae if iron lost through haemorrhage is not adequately replaced, but as we have published, quantitative examination in over two decades of HHT care emphasises that exact responses vary between individuals. We hypothesised that one element of variability may reflect the underlying HHT genotype. Here we demonstrate subtle differences that may be important to recognise when designing randomised controlled trials of new HHT therapies, and also in existing management strategies: Where HHT patients were becoming anaemic and iron deficient, those with a pathogenic variant in \textit{SMAD4} displayed different patterns of compensations compared to those with an \textit{ACVRL1} or \textit{ENG} pathogenic variant. While the study is limited by the rarity of the \textit{SMAD4} genotype (currently, only 2.5% of HHT causal variants on the HHT Mutation Database), we explore reasons that may explain a distinctive phenotypic cluster in \textit{SMAD4} HHT patients. To further stimulate future prospective studies, we outline potential relevance to HHT symptom burden and complications, rationales for differing iron treatment regimes by HHT genotype, and more broadly suggest that the cohorts provide an opportunity to further clarify relationships involved in iron and circulatory homeostasis.

HHT is a complex, heritable vasculopathy that is estimated to affect between 1 in 3,000 – 8,000 people, and is characterised by nosebleeds (epistaxis), mucocutaneous telangiectasia and visceral arteriovenous malformations (AVMs).\textsuperscript{1,2} In haematological circles, the greatest
concern is the management of iron deficiency anaemia secondary to HHT bleeding. This was a focus of recent International HHT Guidelines, and is an active area of pharmaceutical development.\textsuperscript{1} HHT patients develop iron deficiency when dietary iron is inadequate to provide both their usual iron requirements, and to replace additional iron losses due to bleeding from the nose, the gastrointestinal tract, menstruation, blood donation, and other losses as quantified by the haemorrhage adjusted iron requirement (HAIR).\textsuperscript{3} Iron deficiency anaemia (IDA) can have significant consequences for HHT patients and is one of the strongest predictors of mortality in HHT.\textsuperscript{4} As for any anaemia, IDA reduces arterial oxygen content necessitating higher cardiac output to maintain tissue oxygenation, and this is a particular problem in HHT where visceral AVMs lower the systemic vascular resistance, also resulting in higher cardiac outputs.\textsuperscript{2,5} Additional complications associated with iron deficiency and its treatment are reported in HHT, in particular patients with hepatic or pulmonary AVMs.\textsuperscript{1,2} Furthermore, in HHT, ongoing bleeds mean that conventional transfusional support algorithms need to be modified.\textsuperscript{1} Pan-HHT genotyping is identifying more variable expressivity in all genotypes than previously expected, including paucity of clinical signs in patients presenting by less conventional routes (e.g. organ-specific AVMs rather than clinical genetics, haematology or ENT surgery; AVMs/HHT rather than juvenile polyposis gastroenterology).\textsuperscript{6} That said, there is clear evidence that particular AVMs differ between the major HHT genotypes,\textsuperscript{7} and that \textit{SMAD4}\textsuperscript{+/-} patients can have additional phenotypes including juvenile polyposis and aortopathy.\textsuperscript{1,2,7}

To take a first look at whether there were distinctive iron deficiency indices and responses between the HHT genotypes, with ethical approval (LREC 2000/5764), serial anonymised data were analysed retrospectively from all genotyped patients with clinical HHT reviewed at a single institution between May 1999-August 2021 where serum iron, transferrin saturation index (Tf/Si) and supine/erect pulse were measured as standard of care. The 426 patients had
a median age of 50 (IQR 39, 62) years at the time of their measurements, and 264 (62%) were female (Table S1). They provided 686 measurements in 246 ENG+/− patients, 166 measurements in 102 ACVRL1+/− patients, 32 measurements in 11 SMAD4+/− patients, and 118 measurements in patients who tested negative for variants in known HHT causal genes (Table S1). Analyses were conducted using all available measurements, and also in the smaller dataset of first measurements only per patient, where the small number of SMAD4 cases (N=11) impeded statistical comparisons.

First, we examined iron indices across the three genotypes. Overall serum iron ranged from 6-18 (median 11)µmol/L, transferrin saturation index (TfSI) from 9-28 (median 18)% and ferritin from 14-67(median 28) µg/L. As shown in Figure 1A, serum ferritin was similar in ACVRL1+/−, ENG+/− and SMAD4+/− patients (median values 31, 25 and 26ug/L respectively).

Despite the similar serum ferritin, there were differences examining serum iron and TfSI. Serum iron was lower in SMAD4+/− patients than in ACVRL1+/− or ENG+/− patients (median values 5, 12 and 11 µmol/L respectively, Figure 1B). Similarly, TfSI was lower in SMAD4+/− than in ACVRL1+/− or ENG+/− patients (median values 7, 19 and 16% respectively, Figure 1C) with similar trends in first-visit measurements (Figure S2).

Next, we examined distributions of red cell indices. As shown in Figure 2A, there were similar patterns in the relationship between haemoglobin and serum iron levels between the different HHT molecular genotypes. However, red blood cell mean corpuscular volume (MCV) was lower in SMAD4+/− than ACVRL1+/− or ENG+/− (medians 75.1; 89.7; 89.0fl respectively (S Table1) and visual comparison suggested this was not fully explained by iron status (Figure 2B). Similar patterns were seen for mean corpuscular haemoglobin
concentration (MCHC) overall (Table S1) and in relationship to serum iron (Figure 2C). Haemoglobin was maintained in the SMAD4+/− patients despite lower haemoglobin content per red cell, by a higher total red cell count compared to ACVRL1+/− and ENG+/− patients (median 5.4; 4.7 and 4.8x10^{12}/L respectively (Table S1, visual comparisons in Figure 2D).

Uniquely, our institution has measured postural changes in the heart rate, alongside SaO2, for more than three decades7. A higher pulse rate is seen in response to acute blood volume loss through haemorrhage, and in barometric responses to preserve cerebral perfusion on standing with such autonomic response stronger in younger individuals 8,9 We therefore examined whether the resting pulse, a crude measure of circulation adjustment relevant to anaemia, differed between the genotypes. As expected for HHT patients8, the pulse rate increased when patients moved from a supine to an erect position (median values 71.9 and 76.3 beats per minute (bpm), Figure 3), and a higher pulse rate on standing was seen in all three molecular genotypes. We had expected the magnitude of the increase to be marginally greater in ENG+/− patients with lower SaO2 due to pulmonary AVMs, as we have previously shown with pan-HHT genotype analyses.8 Instead, the magnitude of pulse increase was almost twice as high in SMAD4+/− than ENG+/− (Figure 3), beyond any increment predicted by their marginally younger age, lower body mass index, or pulmonary AVM status8,9 (Table S1).

Taken together, the study findings indicate that in the setting of iron deficiency to which SMAD4 patients may be more susceptible through gastrointestinal bleeding and polyps,2,10 physiological compensation mechanisms appeared to differ between HHT molecular genotypes.
The study has strengths, including a unique dataset that allowed for the comparison of multiple indices between the molecular genotypes, and a notably large sample size of genotyped patients given the rarity of the condition, particularly the \( SMAD4^{+/+} \) genotype. As described further in the Data Supplement, previous studies have predominantly compared \( ENG^{+/-} \) and \( ACVRL1^{+/-} \) patients, whereas this current study also assessed differences in \( SMAD4^{+/-} \) patients. Limitations stem from its observational and retrospective nature, as the results may reflect other demographic differences or confounders between the HHT molecular genotype cohorts. Furthermore, the small \( SMAD4 \) numbers prevented two-way adjustments, thus it is not possible to assign causation to possession of the \( SMAD4 \) variant.

That said, the evidence presented begins to point to physiologically different iron homeostasis in \( SMAD4^{+/-} \) patients, highlighting new concepts to be considered as HHT management recommendations and clinical trials proceed. The pattern resembles functioning as though the patient is in a more iron-deficient state: despite similar serum ferritin values, \( SMAD4^{+/-} \) patients displayed lower serum iron and TF/Sl than the other HHT molecular genotypes. Furthermore, similar haemoglobin was achieved by a greater number of smaller red cells. \( SMAD4 \) regulates hepcidin\(^{11,12} \), the key regulator of iron homeostasis\(^{11} \), which is reduced in individuals with active bleeding (via erythroferrone\(^{13} \)) and iron deficiency\(^{11} \). Our previously reported hepcidin dataset\(^3 \) included only one individual with \( SMAD4^{+/-} \). Their hepcidin:ferritin ratio (1.2ng/ml/µg/L) was higher than the other HHT patients (0.2-0.5 [mean 0.3]ng/ml/µg/L), and controls (0.1-1.0 [mean 0.5]ng/ml/µg/L), but the \( SMAD4 \) patient was iron replete, limiting interpretations. Whole genome sequencing of \( ACVRL1^{+/-}, ENG^{+/-} \) and \( SMAD4^{+/-} \) patients was performed through the 100,000 Genomes Project\(^4 \), but no hepcidin (\( HAMP \)) DNA variants were identified in these individuals, precluding similar analyses to those we have recently performed for haemorrhage
susceptibility in HHT. Published murine data is of only limited help—while basic
erthropoiesis was normal in SMAD4-deficient mice, it was not examined during iron-
restricted erythropoiesis, nor in the setting of secondary erythrocytosis that compensates for
pulmonary AVM-induced low oxygen saturation in order to maintain arterial oxygen
content, and we speculate may be employed differently in the maintenance of haemoglobin
in iron-deficient SMAD4+/− patients. Future prospective studies will be enhanced by
incorporating measurements at the time of iron deficiency.

Importantly, and unexpectedly, there were differing magnitudes of response to acute changes
to the circulation on standing. Given the separate arterial pathologies observed in SMAD4
patients (e.g. aortic dilatation), possession of a SMAD4 causal variant is a plausible
differential to why the orthostatic pulse discrepancies were observed and further examination
is warranted, particularly as this may highlight arterial pathology beyond the expected HHT-
specific variables.

In conclusion, this study contributes to the growing body of literature that indicate
phenotypic differences between the HHT molecular genotypes, by demonstrating differences
in iron, red cell and haemodynamic indices that SMAD4+/− patients may use to compensate
for iron deficiency complications. Future studies should aim to confirm with larger numbers,
requiring introduction of routine measurements of iron indices and postural pulse into
assessment, and likely multicentre analyses given the sparsity of SMAD4+/− HHT patients
and importance for adjusting for confounders in multivariable analyses. Future studies
should also evaluate if differences identified in SMAD4+/− patients are associated with a
modified symptom burden, for instance, tolerance of standing; elucidate better mechanistic
understanding of the relationship between SMAD4 and hepcidin in the setting of iron
deficiency to which all HHT patients are prone, and whether there should be changes to the type of iron deficiency treatment these patients receive.
References:


FIGURE LEGENDS

Figure 1: Comparison of iron indices between HHT molecular genotypes

All dataset comparisons of values for HHT patients by molecular genotype. **1A** Serum ferritin. **1B** Serum iron. **1C** Serum transferrin saturation index (TfSI). Error bars indicate median and interquartile range. Non independent datasets unequal in number mean absolute p values for the indicated comparisons should be viewed with caution, though for relative comparisons, for ferritin, iron and TfSI, p-values were 0.08, 0.02 and 0.007 by Spearman’s rank correlation and Mann Whitney comparing SMAD4 to ENG and ACVRL1 combined.

Figure 2: Comparison of the relationship between haemoglobin and red cell indices

Data in patients with confirmed HHT molecular genotypes. 865 values are plotted on each graph, with quadratic regression lines for the 3 HHT genotypes illustrated for ACVRL1 (blue), ENG (yellow) and SMAD4 (purple). **2A** Haemoglobin concentration (Hb). **2B** Mean corpuscular volume (MCV). **2C** Mean corpuscular haemoglobin concentration (MCHC). **2D** Red cell count (RBC).

Figure 3: Comparison of orthostatic changes in pulse.

Data are shown for all patients in the database (N=286), and separated by confirmed HHT molecular genotypes, showing the change in pulse (in beats per minute) between a patient in a supine position and the same patient in an erect position. Data were recorded at 1 minute intervals over a 20 minute period for 10 minutes supine and 10 minutes erect, with the pulse rates recorded as the mean recorded across minutes 7, 8, 9 and 10 in each posture. Error bars indicate
median and interquartile range. For the indicated comparisons using first measurements only, the p value was 0.04 by Mann Whitney comparing \textit{SMAD4} to \textit{ENG} and \textit{ACVRL1} combined.
Iron deficiency responses and integrated compensations in patients according to hereditary haemorrhagic telangiectasia ACVRL1, ENG and SMAD4 genotypes

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² NIHR Imperial Biomedical Research Centre, London, UK
³ Specialist Medicine, Imperial College Healthcare NHS Trust, London, UK

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SUPPLEMENTARY DATA

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### Table S1  Population demographic data for all measurements by HHT genotype

<table>
<thead>
<tr>
<th></th>
<th>Total (Patients, N=426)</th>
<th>ENG+/- (Patients, N=246)</th>
<th>ACVRL1+/- (Patients, N=102)</th>
<th>SMAD4+/- (Patients, N=11)</th>
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<td>N=57</td>
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<td>[76.8%]</td>
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<tr>
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<tr>
<td>C reactive protein (mg/L)</td>
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<td>2.0</td>
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<td>95.0</td>
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<tr>
<td>Supine SaO2 (%)</td>
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<td>96.0</td>
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<td>[91.1,96.6]</td>
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<td>Erect pulse/minute</td>
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<td>87.4</td>
<td>108</td>
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<td>Supine pulse/minute</td>
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<td>[15.0,19.0]</td>
<td>[14.4,17.4]</td>
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</table>

Measurements available for all patients in the database including patients with variants in ENG, ACVRL1, SMAD4, GDF2 and those who tested negative for variants in known HHT causal genes. Binary variables (sex and female and presence of pulmonary AVMs) are reported as number of patients (N) and percentage (%). Continuous variables reported as median and interquartile range. Data on ‘gene negative’ patients not presented separately. Trends were still apparent in the smaller dataset of first measurements only per patient (S2).

**Study Cohort and Assessment:** For the purposes of the current manuscript, all patients who had been genotyped through clinical or research programmes were included. As described, full blood count, serum iron and transferrin saturation index (TfSI) have been measured in all patients since 1999, and serum ferritin in all patients since 2005. Additionally, since 1986, at each assessment, postural oxygen saturation (SaO2) and pulse is measured by pulse oximetry (Ohmeda Biox 3900, Boulder, Colorado) for 10 minutes in supine and erect postures, recorded at one minute intervals, with the mean values from minutes 7-10 reported, and arterial oxygen content (CaO2, mls/dL) calculated by 1.34 x haemoglobin x SaO2, as discussed elsewhere.1-10

Figure S1 First-visit measurement analyses

Distributions and means of first measurements only per patient, reference ranges shaded for A) Ferritin 15-150 mg/L (log transformed: 1.18-2.18); B Serum iron 7-27 µmol/L (log transformed: 0.85-1.43); C) Transferrin saturation index (T/SI, 15-40%) (log transformed: 1.18-1.60). P values calculated by Mann Whitney between SMAD4 and non SMAD4 genotypes as indicated. We considered it preferable to include all datapoints to promote further research and advance clinical care, rather than excluding the smaller number of SMAD4 cases as is usually the case in ‘HHT genotype phenotype’ studies.11-21

Table S2: The Genomics England Research Consortium Members 8th May 2022

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