Microcytic anaemias in childhood and iron-refractory iron deficiency anaemia

Pippard, Martin J.

Published in:
British Journal of Haematology

DOI:
10.1111/bjh.14558

Publication date:
2017

Document Version
Peer reviewed version

British Journal of Haematology editorial comment

Microcytic anaemias in childhood and iron-refractory iron deficiency anaemia

Martin J. Pippard

University of Dundee, Dundee, UK

Keywords: microcytic anaemia, iron deficiency, TMPRSS6, hepcidin, IRIDA

In this issue, Bhatia *et al* (2017) report a study from India in which iron deficiency anaemia was found to be resistant to oral iron therapy in over 10% of 550 young children: those resistant were subsequently investigated for the phenotype of Iron Refractory Iron Deficiency Anaemia (IRIDA) and, where this was present, whether *TMPRSS6* gene variations might explain this finding. Their work draws attention to the approach to diagnosis of microcytic anaemias, and how new understanding of the molecular pathways involved in systemic and cellular iron metabolism (comprehensively reviewed by Hentze *et al*, 2010) may change this.

Microcytic anaemias accompany reduced haemoglobin production in developing red cells. This in turn results from failure of either haem synthesis (most commonly from lack of availability of iron but rarely from failure of protoporphyrin synthesis or of iron incorporation into the porphyrin ring in sideroblastic anaemias), or of globin synthesis in the thalassaemia disorders. Many young children have minimal or no iron stores as assessed by low serum ferritin concentrations and are at particular risk of developing iron deficiency anaemia, by far the commonest cause of childhood anaemia: in the UK National Diet and Nutrition Surveys, up to a third of children aged 1.5 -4.5 years had serum ferritin values below WHO cut off values (Scientific Advisory Committee on Nutrition, 2010). Iron deficiency in such children is usually the result of a combination of the increased iron requirements of growth and limited availability of iron in the diet, particularly if the latter is predominantly vegetarian, though chronic blood loss (e.g. from intestinal hookworm infestation) and exposure to cow's milk may be important in many parts of the world. Chronic disease may also give rise to microcytic red cells as a result of iron malutilisation rather than any reduction in total body iron and may impair the response to iron therapy. Contributions from multiple causes in a single patient may sometimes cause diagnostic difficulty, and interpretations of individual laboratory measures of iron status need to consider whether they are appropriate to the overall clinical context. For example, a "normal" plasma ferritin in the presence of anaemia may be inappropriate in the sense that together the measures imply an overall reduction of total body iron, as may a "normal" plasma hepcidin in the presence of iron deficiency (Girelli *et al*, 2016).

In most cases iron deficiency anaemia responds rapidly to oral iron, but less common alternative diagnoses must be considered, especially if there is an inadequate response. Such refractoriness may result from failure of absorption: mucosal damage in coeliac disease has long been recognised as a potential cause for this, and more recently has been joined by *Helicobacter pylori* infection, at least in adults, where it is most probably the result of reduction in the gastric acid secretion needed to solubilise dietary iron (Hershko and Camaschella, 2014). However, rarer inherited defects may lead to impaired iron availability or utilisation and inappropriately low amounts of iron absorption (Donker *et al*, 2014). Foremost among inherited defects leading to impaired iron absorption are those resulting in loss of function of the *TMPRSS6* gene and of the ferroportin gene, *SLC40A1*, though the latter leads to macrophage iron retention and loading rather than a microcytic anaemia.

TMPRSS6 codes for the serine protease, matriptase-2, which inhibits the transcription of hepcidin within hepatocytes. Hepcidin is the major systemic regulator of internal iron exchange, downregulating iron release via the membrane transporter, ferroportin, from cells (enterocytes, macrophages and hepatocytes) that donate iron to circulating transferrin and thus make it available for incorporation into haemoglobin by developing erythroblasts (Hentze *et al*, 2010). Matriptase-2

modulates hepcidin synthesis by interrupting the main iron-responsive signal transduction pathway. It cleaves the membrane protein haemojuvelin (HJV), one of several co-receptors, including transferrin receptor 2(TfR2) and HFE protein, for the bone morphogenetic protein (BMP) receptor. The BMP receptor complex initiates signal transduction after interaction with BMPs, particularly BMP-6: the production of BMP-6 is regulated at mRNA level by iron (Meynard *et al*, 2009) and is therefore related to intracellular iron levels, particularly in liver non-parenchymal cells (Rausa *et al*, 2015). Diferric (iron saturated) transferrin stabilises TfR2 (Johnson & Enns, 2004), and by displacing of HFE from binding to transferrin receptor 1 makes HFE available to interact with the BMP receptor complex (Goswami & Andrews, 2006) , allowing hepcidin synthesis to be sensitive to circulating transferrin saturation. While defects in HFE, HJV and TfR-2 underlie impaired hepcidin production and the development of haemochromatosis types 1,2 and 3, homozygous or double heterozygous defects in the *TMPRSS6* gene lead to an enhanced hepcidin production that is inappropriate with respect to circulating transferrin saturation and to intracellular iron (as reflected by serum ferritin concentration). The resulting reduction in iron absorption and increased retention of iron within macrophages leads to the picture of IRIDA (Fineberg *et al* 2008).

Bhatia *et al* (2017) restricted their analyses of *TMPRSS6* to children whose phenotype included not only a sub-optimal response to oral iron (found in 60 of their patients), but also "normal" plasma ferritin concentrations, and plasma hepcidin concentrations that were inappropriately normal or high (a combination found in 23 of their patients). As in several previous studies (e.g. Delbini *et al*, 2010), they found many single nucleotide variations of *TMPRSS6* including, in over half the children with this phenotype, multiple intronic variants (some known polymorphisms and some novel) that were potentially deleterious to mRNA synthesis. These were sometimes also combined with exonic polymorphisms, including the common variant V737A associated with mildly reduced iron status (Nai *et al*, 2011). They suggest that such variations may cause or contribute to causation in rather more patients than might be expected from the relatively limited number of literature reports of IRIDA due to *TMPRSS6* mutations. However, their identification of the IRIDA phenotype depends on measuring an inappropriate hepcidin response and there is still much to do in developing a reproducible standardised assay that can be used in routine practice (Girelli *et al* 2016). Furthermore, many of the remaining children who were refractory to oral iron therapy and who did not meet the authors' phenotypic criteria for IRIDA had unexplained inappropriate normal, or occasionally high, plasma hepcidin concentrations in the presence of a low plasma ferritin – it would be of considerable interest to study their *TMPRSS6* genotypes, as well as those from control iron-responsive patients, in order to better understand the clinical significance of the authors' genetic findings. The question of possible non-compliance with oral iron treatment in a paediatric population looms large, as well as whether hepcidin synthesis may have been stimulated by intercurrent childhood infection or more chronic diseases, which are not always associated with raised plasma C-reactive protein, e.g. in only some patients with intestinal hookworm (Le *et al*, 2007).

Clearly, these findings are preliminary, but they add weight to the emerging picture of a complex interaction of *TMPRSS6* variants with other physiological, dietary, and perhaps pathological factors, contributing to variably reduced iron absorption in what has been described as the "genotypically and phenotypically heterogenous" disease of IRIDA (Donker *et al*, 2016). For the moment, however, the authors' caution that investigation of other causes for failure to respond to oral iron should be undertaken before hepcidin assay or *TMPRSS6* analysis, seems well-founded.

Bhatia, P., Singh, A., Hegde, A., Jain, R. & Bansal, D. 2017. Systematic evaluation of paediatric cohort with iron refractory iron deficiency anaemia (IRIDA) phenotype reveals multiple *TMPRSS6* gene variations. *British Journal of Haematology*,

Delbini, P., Vaja, V., Graziadei, G., Duca, L., Nava, I., Refaldi, C. & Cappellini, M.D. 2010. Genetic variability of TMPRSS6 and its association with iron deficiency anaemia. *British Journal of Haematology*, **151**, 281-4.

Donker, A.E., Raymakers, R.A.P., Vlasveld, L.T., van Barneveld, T., Terink, R., Dors, N., Brons, P.P.T., Knoers, N.V.A.M. & Swinkels, D.W. 2014. Practice guidelines for the diagnosis and management of microcytic anemias due to genetic disorders of iron metabolism or heme synthesis. *Blood*, **123**, 3873-86.

Donker, A.F., Schapp, C.C.M., Novotny, V.M.J., Smeets, R., Peters, T.M.A., van den Heuvel, B.L.P., Raphael, M.F., Rijneveld, A.W., Appel, I.M., Vlot, A.J., Versluijs, A.B., van Gelder, M., Granzen, B., Janssen, M.C.H., Rennings, A.J.M., van de Veerdonk, F.L., Brons, P.P.T., Bakkeren, D.L., Nijziel, M.R., Vlasveld, L.t. & Swinkels, D.W. 2016. Iron refractory iron deficiency anemia: a heterogeneous disease that is not always iron refractory. *American Journal of Hematology*, **91**, E482-90

Fineberg, K.E., Heeney, M.H., Campagna, D.R., Aydinok, Y., Pearson, H.A., Hartman, K.R., Mayo, M.M., Samuel, S.M., Strouse, J.J., Markianos, K., Andrews, N.C. & Fleming, M.D. 2008. Mutations in *TMPRSS6* cause iron-refractory iron deficiency anemia (IRIDA). *Nature Genetics*, **40**, 569-71.

Girelli, D., Nemeth, E. & Swinkels, D.W. 2016. Hepcidin in the diagnosis of iron disorders. *Blood*, **127**, 2809-13.

Goswami, T. & Andrews, N.C. 2006. Hereditary hemochromatosis protein, HFE, interaction with transferrin receptor 2 suggests a molecular mechanism for mammalian iron sensing. *Journal of Biological Chemistry*, **281**, 28494-8.

Hentze, M.W., Muckenthaler, M.U., Galy, B. and Camaschella, C. 2010. Two to tango: regulation of mammalian iron metabolism. *Cell*, **142**, 24-38

Hershko, C. & Camaschella C. 2014. How I treat unexplained refractory iron deficiency anemia. *Blood*, **123**, 326-33

Johnson, M.B. & Enns, C.A. 2004. Diferric transferrin regulates transferrin receptor 2 protein stability. *Blood*, **104**, 4287-93.

Le, H.T., Brouwer, I.D., Verhoef, H., Nguyen, K.C. & Kok, F.J. 2007. Anemia and intestinal parasite infection in school children in rural Vietnam. *Asia Pacific Journal of Clinical Nutrition*, **16**, 716-23.

Meynard, D., Kautz, L., Darnaud, V., Cannone-Hergaux, F., Coppin, H. & Roth, M-P. 2009. Lack of bone morphogenetic protein BMP6 induces massive iron overload. *Nature Genetics*, **41**, 478-81.

Nai, A., Pagani, A., Silvestri, L., Campostrini, N., Corbella, M., Girelli, D., Traglia, M., Toniolo, D. & Camaschella, C. 2011. TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum hepcidin levels in normal individuals. *Blood*, **118**, 4459-62.

Rausa, M., Pagani, A., Nai, A., Campanella, A., Gilberti, M.E., Apostoli, P., Camaschella, C. & Silvestri, L. 2015. Bmp6 expression in murine liver non parenchymal cells: a mechanism to control their high iron exporter activity and protect hepatocytes from iron overload? *PLoS ONE*, **10**, e0122696.

Scientific Advisory Committee on Nutrition. 2010. Iron and Health, 360 pp. The Stationary Office, London, p 328.