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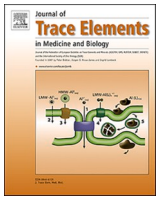

Letter to the Editor

Hereditary hemochromatosis: The complex role of the modifier genes

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Dear Editor,

we read the interesting article "Hereditary Hemochromatosis: an update of the laboratory diagnosis" by Molina et al. [1] about the role of the laboratory in the diagnosis of hemochromatosis (HC), which is an inherited disease characterized by iron overload and caused mainly by decreased production of hepcidin, the hormone peptide regulating iron metabolism.

The Authors reviewed the state of art of the genetic causes of hereditary HC that are mainly linked to the mutations of HFE gene responsible for the "HC related to HFE". This new denomination has substituted the previous definition "HC type 1″ in the update proposed by the BIOIRON society in 2022 [2]. The most common cause of this form of HC related to HFE (NM_000410.4) is the mutation c.845G>A p.Cys282Tyr (rs1800562) in homozygosity which is responsible of the 85–90 % of the cases of genetic HC. However, this pathogenetic genotype is characterized by incomplete penetrance. Other HFE mutations associated to HC are c.189C>G p.His63Asp (rs1799945) and the rare c.193A>T p.Ser65Cys (rs1800730, GMAF = 0.00399) and they have been widely studied in various pathological conditions related to iron metabolism including HC. These variations may cause mild forms of hereditary HC only when associated with the mutation of p.Cys282Tyr in double heterozygosity.

HC unrelated to HFE is rare and often caused by private mutations principally in the HJV, HAMP, TFR2 and SLC40A1 genes. HJV and HAMP mutations affectthe function respectively of Hemojuvelin and Hepcidin and may cause a juvenile form of HC with an early onset in the first or in the second decade of life. TFR2 gene mutations usually cause a form of HC that could develop from the third decade of life and its clinical expression can overlap with the classic form depending on the type of mutation. The SLC40A1 (FPN) gene should be discussed separately because it codifies ferroportin the only known iron transporter and its mutation could be responsible for Ferroportin Disease. In addition to these, other novel genes have emerged from numerous studies published to investigate the role of the possible modifier genes in cases of anomalous or unexplained HC [3].

In defining the biochemical picture of HC, we agree with the Authors when discussing the pivotal role played by the iron metabolism parameters (serum ferritin, serum iron and saturation of transferrin or TS) with the addition to CRP (C Reactive Protein) in order to exclude a contribution of inflammation to hyperferritinemia. As a diagnostic tool, also MRI has gained importance as a non-invasive imaging technique to determine liver iron overload in substitution to liver biopsy when possible.

In line with what just said, the Authors described a very interesting clinical case of a 56 year-old male patient in follow up for HC, carrier of the HFE p.Cys282Tyr homozygous mutation and showing particularly high iron overload parameters such as serum iron 243 μg/dL (60–180 μg/dL), serum ferritin 4242.4 ng/mL (15–300 ng/mL) and TS 89.67 % (20–40 %). MRI imaging confirmed a higher than usual liver iron overload of 350 (+/–) μmol/g (<36 μmol/g). The liver biopsy evidenced some histological characteristics typical of HC in transition phase to cirrhosis. This clinical and biochemical presentation seemed to be indicative of a too heavy iron overload to be justified only by the homozygosity of HFE p.Cys282Tyr mutation. As a result, the Authors suspected a case of digenic HC caused by the contribution of possible mutations in other genes in addition to the HFE p.Cys282Tyr and they enlarged the genetic study analyzing HJV, HAMP, TFR2, SLC40A1 and BMP6 genes.

As we have been interested in the study of the role played by modifier genes in HC for many years, we wish to share some observations about this interesting case.

In doing so, though, we assume that the common non-genetic contributing factors to iron overload (e.g alcohol consumption etc.) were excluded by the Authors even if not stated.

In addition, we believe that the onset age of the iron overload and the ethnicity of the patient may be informative in order to better understand the clinical presentation. On the same level, the family history assessment in the inheritance of HC may be explored in order to better investigate the causative background.

It is also noteworthy that p.Cys282Tyr homozygous patients when in the cirrhotic phase of the disease could have higher values of serum ferritin (>3000–4000 ng/mL) and higher liver iron overload (around 350 μmol/g) without evident correlation to digenic HC [4].

On the other hand, when taking into account only the genetic causes, the analysis of the little panel of genes mentioned above may be adequate, but the description of the additional variations reported

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should have been better specified in order to establish their possible contribution to the phenotype of the patient. For example, it is essential to refer to these variations by using an mRNA code to correctly associate the right mRNA isoform and to report if the mutations were in homozygous or in heterozygous state. We assume that the Authors implied that the patient carried these mutations in the heterozygous state.

Nevertheless, we find that it is scarcely plausible that the patient phenotype could be influenced by the non-HFE variations reported in the article.

The first SLC40A1 (NM_014585.6) variant c.–8C>G (rs11568351) located in the 5′UTR region is characterized by a Minor Allele Frequency (MAF) of 0.095 and seems to be too frequently represented to be significant. In fact, it is classified as benign in ClinVar.

The second variant reported in the SLC40A1 gene c.176–264 does not exist if it is referred to as the standard SLC40A1 transcript (NM_014585.6). In fact, the base in position c.176 is a T in the triplet codifying for the amino acid p.Leu59. It should be clarified whether the numeration of the variation reported in the article was due to a typing error and whether the correct identification of the variation was c.761–264G>C (rs28365783).

In any case, this base change is deeply positioned into intron 6 and it is characterized by a MAF around the warning cut-off value of 0.01 depending on the database used (MAF = 0.008 gnomAD; MAF = 0.02 1000G). As a consequence, this variation would not change the standard splice site region and, although not reported in ClinVar, it may be classifiable as a Variant of Uncertain Significance (VUS) and thus unlikely to be a causative mutation. Further functional studies on mRNA splicing or stability would be needed to confirm the possible effect of this variation.

The last mutation c.1281+24T>C (rs267172) can be found in intron 5 of the BMP6 gene (NM_001718.6) and it is characterized by an average MAF of 0.4 (MAF = 0.439 gnomAD; MAF = 0.353 1000G) showing to be too frequently represented to have some pathological effect.

For these reasons, we find that the genetic profile of the patient described in the article may be worthy of some further investigation, maybe by expanding the panel of genes analyzed.

As a possible suggestion, we have implemented in our laboratory a panel of 29 genes comprehensive of some causative or modifying genes related to iron metabolism (ACO1/IRP1, ACO2, ALAS2, BMP2, BMP6, CP, CYBRD1/DCYTB, EPO, FAM132B/ERFE, FTH1, FTL, IRE-FTL (FTL-5′UTR), HAMP, HAMP-5′UTR, HEPH, HFE, HFE2/HJV, HMOX1, HP, IL6, IL6–5′UTR, IL6R, IREB2/IRP2, NCOA4, SLC11A1/NRAMP1, SLC11A2/NRAMP2, SLC40A1/FPN1, SOD2, TF, TFRC, TFR2, TMPRSS6) with the aim to try to explain similar cases.

In conclusion, HC related to HFE is characterized by a heterogeneous clinical presentation due to the incomplete penetrance of the HFE mutations and the contribution of both environmental and other genetic factors. Various studies have focused their attention on this clinical variability in recent years. They have shown in particular that the severity of the presentation may be due to forms of undiagnosed digenic HC caused by mutations difficult to recognize [5]. Maybe, in the next few years, the Next Generation Sequencing (NGS) technology could contribute to a better understanding of these novel genetic variants [6].

Declaration of Competing Interest

The authors declare no conflicts of interest.

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