Received: 21 July 2023 | Accepted: 6 November 2023

DOI: 10.1111/bjh.19208

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

ORIGINAL PAPER

# Haemochromatosis in children: A national retrospective cohort promoted by the A.I.E.O.P. (Associazione Italiana Emato-Oncologia Pediatrica) study group

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**Summary**

Haemochromatosis (HC) encompasses a range of genetic disorders. HFE-HC is by far the most common in adults, while non-HFE types are rare due to mutations of *HJV*, *HAMP*, *TFR2* and gain-of-function mutations of *SLC40A1*. HC is often unknown to paediatricians as it is usually asymptomatic in childhood. We report clinical and biochemical data from 24 paediatric cases of HC (10 cases of HFE-, 5 TFR2-, 9 HJV-HC), with a median follow-up of 9.6 years. Unlike in the adult population, non-HFE-HC constitutes 58% (14/24) of the population in our series. Transferrin saturation was significantly higher in TFR2- and HJV-HC compared to HFE-HC, and serum ferritin and LIC were higher in HJV-HC compared to TFR2- and HFE-HC. Most HFE-HC subjects had relatively low ferritin and LIC at the time of diagnosis, so therapy could be postponed for most of them after the age of 18. Our results confirm that HJV-HC is a severe form already in childhood, emphasizing the importance of early diagnosis and treatment to avoid the development of organ damage and reduce morbidity and mortality. Although phlebotomies were tolerated by most patients, oral iron chelators could be a valid option in early-onset HC.

**KEYWORDS**
haemochromatosis, hyperferritinaemia, iron chelation, juvenile haemochromatosis, phlebotomy

## INTRODUCTION

Haemochromatosis (HC) includes a group of genetic disorders caused by mutations in genes regulating hepcidin synthesis, which is the master controller of iron homeostasis, or in the ferroportin gene, which is both the receptor of hepcidin and the only known cellular iron exporter.[1] According to their role in iron homeostasis, protein defects lead to increased iron absorption and macrophage iron release, increased transferrin saturation (TSAT) and ferritin levels, progressive iron overload and secondary damage in different organs. There are five types of HC that have been classified on the basis of the involved protein, divided into HFE-related and non-HFE-related.[2]

HFE-HC is the most common inherited form of iron overload in Caucasian populations, and the p.C282Y homozygosity is the most frequent genotype. It is characterized by a wide range of phenotype variability, ranging from no abnormalities to severe iron overload with organ damage. A second genotype (compound heterozygosity for p.C282Y and p.H63D) is found in about 5% of the HFE-HC series but has a very low penetrance and expression with a very low risk of developing iron overload that is even less in patients homozygous for the p.H63D variant.[1] Rare *HFE* mutations have been reported in compound heterozygosity with p.C282Y in single families and delimited geographical clusters,[3] and rare *HFE* deletions have been described only in Sardinia (Italy).[4] Non-HFE-HC includes rare or ultra-rare genetic disorders: HJV-HC and HAMP-HC, also called juvenile HC, due to mutations in *HJV* or in *HAMP*,[5,6] TFR2-HC due to mutations in the transferrin receptor 2 gene (*TFR2*)[7] and SLC40A1 due to gain-of-function mutations of *SLC40A1* coding for ferroportin, which is the only form of HC with autosomal dominant transmission.[8] This form must be distinguished from ferroportin disease as it is due to loss-of-function mutations of *SLC40A1* and largely differs from HC by biochemical characteristics (hyperferritinaemia with normal TSAT) and tissue iron distribution (exclusive or predominant accumulation of iron in macrophages).[9] In the paediatric population, HFE-HC is asymptomatic, and the diagnosis is usually made incidentally after elevated levels of serum iron or ferritin are detected or by family screening. Even in the most severe HJV-HC form, symptoms usually develop after the age of 18, although iron overload can already be marked at that time.[10] A new form of severe iron overload simulating juvenile HC has been described in children with neurodegenerative X-linked epileptic encephalopathy and germinal *PIGA* mutations.[11] Diagnosis of HC is suspected in the presence of confirmed increased TSAT and ferritin once the causes of secondary overload have been excluded. Organ iron quantification by magnetic resonance imaging (MRI) and, in order, HFE and non-HFE-HC genetic testing are performed according to guidelines.[10,12] Liver biopsy is reserved for very selected cases. Phlebotomy is the main treatment for adult HC[13] and is tolerated on average in adolescents and even in school-aged children, although vascular access can be a problem after repeated procedures. Oral iron chelators, such as deferasirox (DFX), can be alternative options, although they are still not approved by the European Medicine Agency (EMA) and should be authorized by local committees as off-label therapies.

There are few reports on children and adolescents affected by HFE and non-HFE-HC, and the disease is almost unknown to paediatricians.[14–18] We here report the clinical and biochemical data of 24 paediatric patients diagnosed with HC, the different therapeutic approaches and efficacy and their clinical evolution at follow-up. Our aim is to promote disease knowledge in the paediatric setting and facilitate prompt referral of the more severe cases to expert centres, where early diagnosis and treatment can modify the prognosis of the disease.

## MATERIALS AND METHODS

### Patients

This observational retrospective multicentre study was designed by the Red Cell Study Group of AIEOP and approved by the Ethics Committee of the *Fondazione Monza e Brianza per il Bambino e la sua Mamma*, Monza. Case report forms (CRFs) were sent to all the AIEOP centres to collect paediatric patients with HC. Reference centres for iron disorders in adulthood were also involved in the study to identify paediatric cases among their series. When the study was planned, both the loss-of-function and gain-of-function *SLC40A1* mutations were included in the HC group (HC type 4A and 4B),[19] but taking into account the current proposal of nomenclature and classification of HC and the more recent reviews,[1,2,8] we modified the inclusion and exclusion criteria of the present paper. Accordingly, a TSAT >45% confirmed in two consecutive samples was considered the indispensable inclusion criteria to suspect HC, independent of the presence of increased ferritin. As far as we know, most of the epidemiological studies on iron status in children and adolescents are aimed to define the prevalence of iron deficiency and not the presence of iron overload.[20–22] Thus, there is not a defined cut-off point for serum ferritin as an index of increased iron stores in children, and WHO adopted a cut-off of 150 and 200 μg/L in adolescent females and males respectively.[23]

Exclusion criteria included any other cause of secondary iron overload (transfusions, inherited or acquired iron loading anaemia, chronic liver diseases). Figure 1 summarizes the selection criteria.

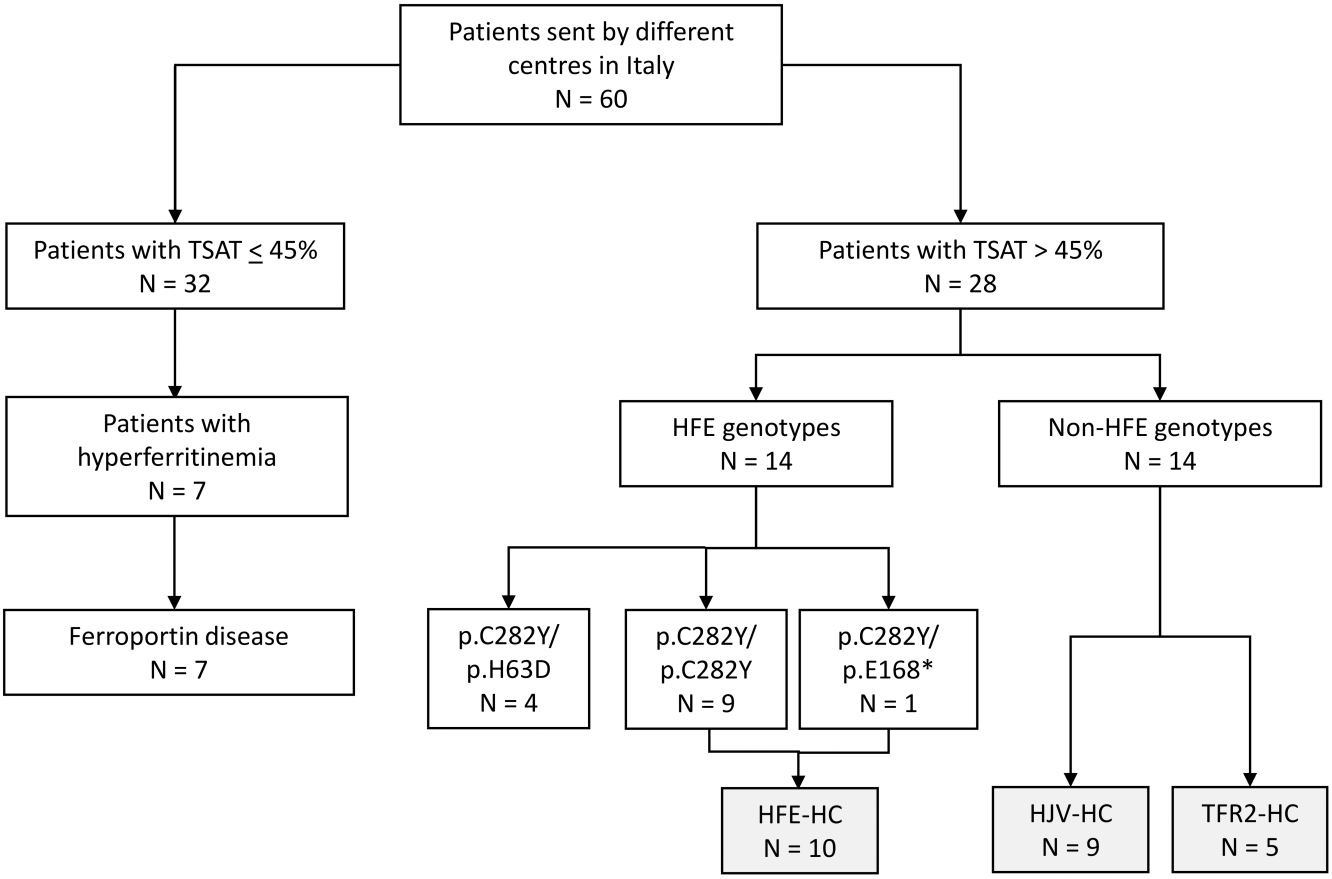


**FIGURE 1** Diagram showing the selection criteria of the patients. Patients with transferrin saturation ≤45% were excluded from subsequent analyses, as were those with the compound heterozygous p.C282Y/p.H63D genotype in *HFE* (see text for more details).

## Methods

Serum iron indices and liver function tests were measured by commercial kits in all subjects. Liver iron quantitation was done by MRI, superconducting quantum interference device (SQUID) or liver biopsy at diagnosis and during the follow-up according to the rules of good clinical practice (e.g. clinically relevant indices of iron overload and the requirement for therapy monitoring) and instrument availability. Heart MRI was limited to those patients with severe iron overload, as defined below. MRI, SQUID liver iron concentration (LIC) and heart T2* were measured as previously reported.[24,25] $LIC^{SQUID}$ levels expressed in mg/g liver wet weight were converted to mg/g dry weight by a conversion factor of 6.1, according to Pakbaz et al.[26] A liver biopsy was performed only for prognostic purposes in four HJV-HC patients according to guidelines.[10] Standard stains were used for histological assessment and Perls' stain and Scheuer's grading for iron evaluation. Genetic testing was performed on a step-by-step basis: searching for p.C282Y and p.H63D variants at first, and in the case of wildtype or heterozygous HFE genotypes, searching for causal mutations in *HFE*, *HJV*, *HAMP*, *TFR2* and *SLC40A1* by Sanger's sequencing. Abdominal ultrasound was performed at diagnosis and during follow-up according to clinical needs.

All the procedures were done in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the 1975 Declaration of Helsinki as amended in 2000. Informed consent for the participation in the study and for the genetic studies was obtained from parents or patients' legal guardians according to the institutional review boards of the hospitals involved in the study.

Continuous variables were described as medians and quartiles (Q1–Q3), and categorical variables were reported as counts and frequencies. The chi-squared test and the Kruskal–Wallis test were used to make comparisons between groups (HFE-, TFR2-, HJV-HC) in terms of categorical and continuous variables respectively. A post hoc Wilcoxon rank sum test was applied for pairwise comparisons in continuous variables. Finally, a one-sided Wilcoxon rank sum test was used to evaluate the median change score and IQR of the two-time points (before and after follow-up) within the three groups (HFE, TFR2 and HJV). The significance level was set as $p < 0.05$. All statistical analyses were performed using open-source R software v.4.4.2 (R Foundation for Statistical Computing, Vienna, Austria).

Taken into account the different methodology used, liver iron overload was classified into four different grades of severity as follows: very mild ($LIC^{MRI}$ >1.5 ≤3 mg/g; $LIC^{SQUID}$ >0.4 ≤ 0.8 mg/g wet weight), mild ($LIC^{MRI}$ >3 ≤ 7 mg/g; $LIC^{SQUID}$ >0.8 ≤ 1 mg/g wet weight), moderate ($LIC^{MRI}$ >7 ≤ 12 mg/g; $LIC^{SQUID}$ >1 ≤ 2 mg/g wet weight; Scheuer's grade 2) and severe iron overload ($LIC^{MRI}$ >12 mg/g; $LIC^{SQUID}$ ≥2 mg/g wet weight; Scheuer's grade 3–4).

## RESULTS

We received CRFs from 60 subjects from eight different paediatric units and two adult centres in Italy, collecting demographic, laboratory and treatment data. Thirty-two patients showed normal TSAT and were excluded from the study. In the remaining 28 children with increased TSAT, 16 were males and 12 were females. Nine carried the p.C282Y homozygotes and one the compound p.C282Y/p.E168* genotype previously described as a deleterious *HFE* genotype[27] and were included in the HFE-HC group; five had TFR2-related HC, and nine had HJV-related HC. Four carried the compound heterozygote p.C282Y/p.H63D genotype in *HFE*. Notably, serum iron indices did not differ between HFE-HC patients carrying *HFE* deleterious genotypes (p.C282Y homozygous and p.C282Y/p.E168*) and children with the p.C282Y/p.H63D genotype (Table S1). However, in view of the recent guidelines and new nomenclature proposal, which consider this genotype at most as a susceptibility factor,[2,10] children with the p.C282Y/p.H63D genotype were not included in the subsequent analyses. The main data of the 24 children with HC finally selected are reported separately in Table 1 and grouped in Table 2. One TFR2-HC patient has been previously described as a single case report elsewhere.[28] The ethnicity was Caucasian in all cases: 20 were Italians, one HFE-HC was German and three HJV-HC were Albanians. There were four couples of affected siblings, one in the TFR2-HC group and three in the HJV-related HC respectively. The diagnosis was made before age 18 in all the patients. Median age at diagnosis was 12.9 years (first and third quartile: 9.9–15.0 years). At diagnosis, all cases were asymptomatic, except for one HJV-related patient who presented delayed puberty due to hypopituitary hypogonadism. Although the median age was lower in HJV-HC than in the other groups, there was no significant difference. TSAT was significantly higher in TFR2- and HJV-HC compared to HFE-HC. In particular, 100% TFR2-HC and HJV-HC had TSAT >70% compared to only four HFE-HC (40%). Serum ferritin and LIC were higher in HJV-HC compared to both TFR2- and HFE-HC. Accordingly, eight out of nine HJV-HC patients showed moderate or severe iron burden compared to only one HFE-HC and none TFR2-HC patients (Table 1; $p < 0.01$). Interestingly, the single case with HFE-HC with the highest liver iron overload ($LIC^{MRI}$ 11.2 mg/g), the highest ferritin (995 ng/mL) and TSAT (95%) was also heterozygous for the $\beta^0$-39 mutation in the β-globin gene.

Laboratory assays revealed a mild elevation of transaminases in five cases out of 23 evaluated, one HFE-HC and four HJV-HC; no endocrine dysfunction was reported except for the case of pituitary hypogonadism in a single HJV-HC patient. Abdominal ultrasound at diagnosis, performed in 21 cases, found mild liver steatosis in six cases (1 HFE-HC, 1 TFR2-HC and 4 HJV-HC), although none showed metabolic alterations and overweight, and a liver biopsy showed mild liver fibrosis in two HJV-HC patients. Table 3 reports information on iron removal therapies and follow-up. Overall, 19 patients (79%) started therapy at a median age of 15.3 years (range 11.5–18). All HJV-HC but one (88.9%) underwent iron removal treatment before 18 years, while the majority of HFE-HC (80%) began therapies after 18 years (Table 1) as they did not present a significant increase in ferritin levels that warranted therapeutic intervention in their childhood. Five children (three HFE-HC and two TFR2-HC) were not treated during the entire follow-up, showing no significant change in ferritin levels after a median follow-up of 3.4 years (1.8–3.7) (median ferritin [1–3 quartiles] at baseline: 174 ng/mL [151–210] and 164 ng/mL [96–255] at the end of follow-up). Seventeen cases were treated with phlebotomies whose regimen was tailored according to body weight, severity of iron overload and patient tolerability, the frequency ranging from one phlebotomy every 2 weeks to one every 6 months. In two cases, it was necessary to switch to DFX due to difficult venous access. No complications were observed during phlebotomy therapy. In two cases, oral iron chelation was started as the first therapy because of poor compliance and needle phobia. The dosage of DFX ranged between 2 and 10 mg/kg/day, according to a previous report in adult HFE-HC.[29] The drug, used as an off-label drug for the pathology, was efficient and well tolerated in all cases; no adverse events were reported.

One patient was lost at follow-up. After a median (1–3 quartiles) of 9.5 (3.6–13.1) years of follow-up, patients' median age was 22.1 (17–26) years. Of the 19 patients who underwent iron depletion (Table 3), 16 were still in maintenance treatment (phlebotomy in 14 and DFX in 2 cases) at the end of follow-up, while in three, the therapy was temporarily suspended because the ferritin level had fallen below 50 ng/mL. As expected, serum ferritin significantly decreased in treated patients: HFE-HC ($N = 7$) (median 123 [58–287] μg/L, $p < 0.0078$) and HJV-HC ($N = 8$, median 286 [169.8–519] μg/L, $p < 0.0080$), while there was no significant difference in TFR2-HC ($N = 4$), (median 22 [−69.25 to 41.50] μg/L, $p < 0.4375$).

## MUTATIONS AND GENOTYPE

HFE-HC included nine p.C282Y homozygotes (eight probands and one sibling) and one compound p.C282Y/p.E168* (Table 1). Among patients with HJV-HC, the p.Gly320Val mutation was the most represented[5,6] and found in two unrelated patients in the homozygous state,

**TABLE 1** Clinical and biochemical characteristics of patients at diagnosis.

| No. | Sex (F/M) | Age at diagnosis (years) | Gene | Variant (cDNA) | Variant (protein) | Hb (g/dL) | MCV (fL) | TSAT (%) | Ferritin (ng/mL) | Iron overload | LIC (mg/g) | Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| #1[a] | F | 12.4 | *HJV* | c.306delC<br>c.959G>T | p.Phe103Serfs*11<br>p. Gly320Val | 13.5 | 87 | 88 | 456 | Severe | 15.9 | Deferasirox |
| #2[a] | M | 10 | *HJV* | c.306delC<br>c.959G>T | p.Phe103Serfs*11<br>p. Gly320Val | 14.1 | 85 | 88 | 376 | Moderate | 7.6 | Deferasirox |
| #3 | F | 7.4 | *HJV* | c.959G>T<br>c.959G>T | p. Gly320Val<br>homozygote | 13.1 | 88 | 83 | 177 | Severe | 12.3 | Phlebotomy/Deferasirox |
| #4[a] | M | 11.9 | *HJV* | c.257A>G<br>c.959G>T | p.Tyr86Cys<br>p. Gly320Val | 13.5 | 85 | 81 | 2838 | Moderate | 7.4 | Phlebotomy |
| #5[a] | M | 8.1 | *HJV* | c.257A>G<br>c.959G>T | p.Tyr86Cys<br>p. Gly320Val | 13.1 | 82 | 72 | 735 | Mild | 3.1 | Phlebotomy |
| #6 | F | 9.8 | *HJV* | c.445delG<br>c.445delG | p.Asp149Thrfs*97 homozygote | 12.4 | 90 | 85 | 780 | Moderate | 7.0 | Phlebotomy |
| #7[a] | M[b] | 17.5 | *HJV* | c.445delG<br>c.445delG | p.Asp149Thrfs*97 homozygote | 16.6 | 90 | 74 | 1350 | Moderate (liver biopsy) | Scheuer grade: 2 | Phlebotomy |
| #8[a] | F | 14.1 | *HJV* | c.445delG<br>c.445delG | p.Asp149Thrfs*97 homozygote | 15.0 | 86 | 92 | 312 | Moderate (liver biopsy) | Scheuer grade: 2 | Phlebotomy |
| #9 | F | 19 | *HJV* | c.959G>T<br>c.959G>T | p. Gly320Val homozygote | 12.3 | 95 | 83 | 560 | Severe (liver biopsy) | Scheuer grade: 3 | Phlebotomy |
| #10 | M | 3.4 | *TFR2* | c.750C>G<br>c.750C>G | p.Tyr250* homozygote | 13.9 | 84 | 80 | 58 | Mild | 3.1 | — |
| #11 | M | 14.6 | *TFR2* | c.1540delG<br>c.671T>G | p.Leu224Arg p.Asp514Mefs*12 | 15.8 | 86 | 91 | 151 | Normal | 1.3 | — |
| #12[a] | M | 10.5 | *TFR2* | IVS17+5636G>A<br>IVS17+5636G>A | — | 14.8 | 91 | 93 | 104 | Very mild | 2.8 | Phlebotomy |
| #13[a] | F | 14.9 | *TFR2* | IVS17+5636G>A<br>IVS17+5636G>A | — | 12.5 | 80 | 86 | 47 | Mild | 3.7 | Phlebotomy |
| #14 | M | 15.4 | *TFR2* | c.2093_2096delGAGA<br>c.2093_2096delGAGA | p.Arg698Thrfs*4 homozygote | — | — | 85 | 792 | Mild | 5.8 | Phlebotomy |
| #15 | F | 8.1 | *HFE* | c.845G>A<br>c.845G>A | p.Cys282Tyr homozygote | 14.3 | 87 | 52 | 310 | Very mild | 2.9 | Phlebotomy/Deferasirox |
| #16 | M | 15 | *HFE* | c.845G>A<br>c.845G>A | p.Cys282Tyr homozygote | 16.4 | 96 | 62 | 174 | Very mild | 2.4 | — |
| #17 | M | 13 | *HFE* | c.845G>A<br>c.845G>A | p.Cys282Tyr homozygote | 16.0 | 93 | 66 | 122 | Very mild | 2.2 | Phlebotomy |
| #18 | F | 12.9 | *HFE* | c.845G>A<br>c.845G>A | p.Cys282Tyr homozygote | 13.2 | 91 | 81 | 210 | Mild | 3.5 | — |
| #19 | M | 15.3 | *HFE* | c.845G>A<br>c.845G>A | p.Cys282Tyr homozygote | 16.0 | 90 | 87 | 442 | Mild | 4.9 | Phlebotomy |

**TABLE 1** (Continued)

| No. | Sex (F/M) | Age at diagnosis (years) | Gene | Variant (cDNA) | Variant (protein) | Hb (g/dL) | MCV (fL) | TSAT (%) | Ferritin (ng/mL) | Iron overload | LIC (mg/g) | Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| #20 | M | 15.1 | *HFE* | c.845G>A c.845G>A | p.Cys282Tyr homozygote | 15.8 | 96 | 70 | 41 | Normal | <1.5 | Phlebotomy |
| #21 | M | 10.7 | *HFE* | c.845G>A c.845G>A | p.Cys282Tyr homozygote | 13.8 | 81 | 78 | 69 | Normal | <1.5 | Phlebotomy |
| #22 | F | 13.5 | *HFE* | c.845G>A c.845G>A | p.Cys282Tyr homozygote | 14.6 | 79 | 66 | 211 | Very mild | 2.8 | — |
| #23 | M[c] | 14 | *HFE* | c.845G>A c.845G>A | p.Cys282Tyr homozygote | 12.4 | 61 | 95 | 995 | Moderate | 11.2 | Phlebotomy |
| #24 | M | 17.8 | *HFE* | c.845G>A c.502G>T | p.Cys282Tyr p.Glu168* | 15.7 | 86 | 69 | 494 | Mild | 3.9 | Phlebotomy |

*Note*: For the classification criteria for iron overload, see the text.

Abbreviations: Hb, haemoglobin; LIC, liver iron concentration; MCV, mean cell volume; TSAT, transferrin saturation.

[a]Siblings.

[b]Pituitary hypogonadism.

[c]Beta-thalassaemia trait.

in two couples of siblings in the compound heterozygous state with the novel p.Phe103Serfs*11 and in the p.Tyr86Cys mutation (ClinVar variation ID 1507976) respectively. The recurrent Italian pathogenic variant p.Asp149Thrfs*97[30] was found in the homozygous state in two siblings and in a single proband. Two brothers with TFR2-HC carried the IVS17+5636G>A variant affecting RNA splicing[31] in homozygosity; one patient was homozygous for the already described p.Tyr250*,[28] one compound was heterozygous for the p.Asp514Metfs*12 and p.Leu224Arg[32] and one was homozygous for the p.Arg698Tyrfs*4 mutation, already described elsewhere.[33]

## DISCUSSION

This is the first report on a series of paediatric patients with HC, collecting 24 subjects from different Italian paediatric units and the Centers for Disorders of Iron Metabolism. It provides novel information regarding the presentation of different forms of HC during childhood and adolescence. First, differently from the adult Caucasian population, where HFE-HC predominates and non-HFE-HC are rare or ultra-rare disorders, in our series, non-HFE-HC represented more than 50% of the population. Second, TSAT was confirmed to be significantly higher in non-HFE patients than in HFE-HC patients, even at paediatric age. Third, most of the HFE-HC subjects presented with relatively low ferritin and liver iron overload at diagnosis, allowing them to postpone therapies after the age of 18 in most cases. Interestingly, the single HFE-HC patient with clinically relevant iron overload carried the beta-thalassaemia trait. Fourth, although TFR2-HC patients were characterized by very high TSAT, serum ferritin and LICs were normal or slightly increased, like in HFE-HC. Fifth, HJV-HC was confirmed as a severe form already in paediatric age, stressing the importance of an early diagnosis and treatment to avoid the development of organ damage, thus reducing morbidity and mortality. Last, although phlebotomies were tolerated in most of the patients, the oral iron chelator resulted very efficient at low dosage in reducing iron overload.

Unlike the more severe juvenile form of HC, HFE-HC is a low-penetrant disorder and is likely to rarely manifest, even at a biochemical level, in the paediatric age. Accordingly, 90% of HFE-HC showed normal to mild iron overload in the liver and ferritin values below 500 ng/mL at diagnosis. Also, three of them were not addressed to iron depletion therapy as they did not show changes in ferritin level during the follow-up, and the other five showed only minor changes, allowing them to postpone phlebotomies after the age of 18 years, indicating that the progression of iron overload in children with HFE-HC is very mild. These allow the child to take the due time for genetic testing and start therapy without any risk to the child, postponing investigations until the subject has reached the age of legal consent and increasing awareness

**TABLE 2** Clinical and biochemical characteristics of HC patients at diagnosis. Data are reported as number (%) or median (1–3 quartiles).

| | HFE-HC | TFR2-HC | HJV-HC | All patients | *p* |
|---|---|---|---|---|---|
| *N* (%) | 10 (41.7) | 5 (20.8) | 9 (37.5) | 24 | |
| Males, *N* (%) | 7 (70) | 4 (80.0) | 5 (55.6) | 16 (66.7) | NS |
| Age (years) | 13.7 (12.9–15.1) | 14.6 (10.5–14.9) | 10.0 (9.0–12.3) | 12.9 (9.97–15.0) | NS |
| Hb (g/dL) | 15.15 (13.93–15.95) | 14.35 (13.55–15.05) | 13.50 (13.10–14.10) | 14.10 (13.15–15.75) | NS |
| MCV (fL) | 88.50 (82.25–92.50) | 85.00 (83.00–87.25) | 87.00 (85.00–90.00) | 87.00 (84.50–90.50) | NS |
| Clinical symptoms at diagnosis, *N* (%) | — | — | 1 (11.1) | 1 (3.5) | |
| Transferrin saturation (%) | 69.4 (66.1–80.6) | 86* (84.6–91) | 83** (80.6–87.7) | 82 (71.4–87.1) | 0.044 |
| Serum ferritin (ng/mL) | 210.5 (105–409) | 104 (58–151) | 560# (376–780) | 311 (143.7–603.7) | 0.026 |
| Liver iron concentration (mg/g dry weight) | 2.85 (2.28–3.80) | 3.16 (2.80–3.76) | 7.50° (7.11–11.13) | 3.50 (2.80–7.01) | 0.0242 |
| AST > 45 U/L or ALT > 40 U/L, *N* (%) | 1/10 (10) | 0/5 | 4/8 (50.0) | 5/23 (21.7) | |
| Abdominal ultrasound: liver steatosis, *N* (%) | 1/10 (10.0) | 1/4 (25.0) | 4/7 (57.1) | 6/21 (28.5) | |

*Note*: Liver iron concentration was measured in 21 patients (10 HFE-HC, 5 TFR2-HC, 6 HJV-HC) by MRI or SQUID; three HJV-HC children underwent liver biopsy.
*$p = 0.055$ versus HFE-HC. **$p = 0.043$ versus HFE-HC.
°$p = 0.022$ versus HFE-HC, $p = 0.042$ versus TFR2-HC.
#$p = 0.015$ versus HFE-HC, $p = 0.030$ versus TFR2-HC.

**TABLE 3** Iron depletion therapies and follow-up. Data are reported as number (%) and median (first and third quartiles).

| | HFE-HC (*N* = 10) | TFR2-HC (*N* = 5) | HJV-HC (*N* = 9) | All patients (*N* = 24) |
|---|---|---|---|---|
| Iron depletion therapy, *N* (%) | 7 (70) | 3 (60.0) | 9 (100.0) | 19 (79.2) |
| Median age at the start of therapy (years) | 18.4 (16.1–19) | 16.0 (15.8–17.0) | 11.9 (9.8–15.0) | 15.3 (11.5–18) |
| Age <18 years at the start of therapy, *N* (%) | 2 (20) | 2 (66.7) | 8 (88.9) | 12 (50) |
| Phlebotomy, *N* | 7[a] | 3 | 7[a] | 17[b] |
| Deferasirox, *N* (%) | — | — | 2 | 2 |
| Median duration of follow-up (years) | 9.6 (5.6–11.9) | 3.7 (3.45–10.6) | 9.7 (7.8–14.1) | 9.56 (3.65–13.1) |
| Lost at follow-up, *N* (%) | — | 1 (20.0) | — | 1 (4.1) |
| Median age at last follow-up (years) | 23.6 (17.6–26.3) | 18.2 (17.7–23.0) | 20.3 (17.4–29.0) | 22.1 (17.0–26.0) |
| On maintenance therapy at last follow-up, *N* | 6 | 2 | 8 | 16 |
| Phlebotomy | 6 | 2 | 6 | 14 |
| DFX | — | — | 2 | 2 |

Abbreviations: DFX, deferasirox; HC, haemochromatosis.
[a]One patient shifted to deferasirox during follow-up.
[b]Two patients shifted to deferasirox during follow-up.

and tolerance of phlebotomy.[34] These observations support the indications of the survey conducted by the European Molecular Quality Network (EMQN),[12] promoted by the European Federation of the Associations of Patients with Haemochromatosis (EFAPH), showing a prevalent policy of not testing minors, and are in agreement with the indication of the European Association of Liver Disease guidelines that only adults should be tested for HFE-HC.[10] Exceptions to the rule are rare cases referred for diagnosis when there is a clear clinical indication, for example a very high TSAT and a high ferritin level suspect for a form of non-HFE-HC. It is not easy to define such a warning threshold for both TSAT and ferritin, as the number of patients reported so far is limited. In our series, 100% of non-HFE-HC had TSAT >70% compared to only 40% of HFE-HC, suggesting it might constitute a hallmark of non-HFE-HC.[35] Taking as reference the threshold levels defined by the WHO for ferritin (150 and 200 μg/L in adolescent females and males, respectively), we observed that all HJV-HC patients had increased ferritin compared to 60% of HFE-HC and 20% of TFR2-HC. The coexistence of the beta-thalassaemia trait with the p.C282Y homozygous genotype that has been previously reported to aggravate the clinical manifestations of iron overload in adults[36] seems to have some relevance in the paediatric age, leading to severe iron overload and requiring early diagnosis and treatment even in childhood. Based on these findings, it is advisable not to underestimate the finding of high serum iron and ferritin levels in the paediatric age and, when necessary, to complete the investigations with a full assessment of the iron indices to define the percentage of TSAT and serum ferritin concentration. In the case of high

values, it is advisable to refer the child to specialized centres for appropriate genetic and clinical investigations. A TSAT exceeding 70% is often associated with the presence of non-transferrin-bound iron (NTBI) and labile plasma iron (LPI), the redox-active component of NTBI, increasing the risk of rapid and uncontrolled accumulation of iron in parenchymal cells and favouring iron-related tissue damage.[37]

Phlebotomy therapy remains the best therapeutic option in HC[13,38] and has been applied in children.[39,40] However, not all patients are candidates for phlebotomy due to underlying poor venous access, anaemia or heart disease. Compliance with regular phlebotomy may be an issue, as a number of patients report negative experiences related to treatment in the induction and maintenance phases.[40] We have applied extreme care in adjusting the frequency and quantity of bloodletting for each patient, generally achieving a good tolerance for the treatment. However, we needed to shift to deferasirox in two cases and to start with deferasirox in the other two because of low compliance. Deferoxamine has been used successfully in a limited number of HC patients,[13] but due to its short plasma half-life, it must be given by a slow subcutaneous infusion, which limits patients' compliance with treatment. Oral chelation therapy with deferasirox at a dosage of 5–10 mg/kg is an efficient alternative in HFE-HC, as shown by a phase 1/2 dose-escalation trial,[29] and is efficient in removing iron excess in children and adults affected by juvenile HC.[41] Overall, these findings indicate that the oral iron chelator deferasirox could be a valid option in early-onset HC, avoiding the development of clinical complications and changing the natural history of the disease.

## CONCLUSIONS

In this paediatric cohort, non-HFE-HC patients represent more than 50% of the population, with significantly higher levels of TSAT in comparison to HFE patients. HJV is a severe form of HC that develops at a young age, and early detection and referral are critical to avoiding organ damage and thereby lowering morbidity and mortality. Phlebotomy was generally tolerated in most of the patients, but oral iron chelators were efficient in reducing iron overload, representing a valid alternative option in early-onset HC.

### AUTHOR CONTRIBUTIONS

Paola Corti, Giulia Maria Ferrari, Martha Caterina Faraguna, Alberto Piperno: *study conception and design*; Paola Corti, Martha Caterina Faraguna, Filomena Longo, Elena Corradini, Tommaso Casini, Gianluca Boscarol, Valeria Maria Pinto, Giovanna Russo, Raffaella Colombatti, Raffaella Mariani: *data collection*; Paola Corti, Martha Caterina Faraguna, Alberto Piperno: *analysis and interpretation of results*; Paola Corti, Martha Caterina Faraguna, Alberto Piperno: *draft manuscript preparation*; Giulia Capitoli: *statistic analysis*. All authors reviewed the results and approved the final version of the manuscript.

### ACKNOWLEDGEMENTS

The project is supported (not financially) by the European Reference Network on Rare Hematological Diseases (ERN-EuroBloodNet)—Project ID No. 10108571. ERN-EuroBloodNet is partly co-funded by the European Union within the framework of the Fourth EU Health Programme.

### CONFLICT OF INTEREST STATEMENT

No authors have any conflicts of interest to disclose.

### DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

### ETHICS STATEMENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Corti P, Ferrari GM, Faraguna MC, Capitoli G, Longo F, Corradini E, et al. Haemochromatosis in children: A national retrospective cohort promoted by the A.I.E.O.P. (Associazione Italiana Emato-Oncologia Pediatrica) study group. Br J Haematol. 2024;204(1):306–314. https://doi.org/10.1111/bjh.19208