Recurrence of neonatal haemochromatosis in half sibs born of unaffected mothers

Alain Verloes, I Karen Temple, Anne-Frédérique Hubert, Peter Hope, Stephen Gould, Christian Debauche, Gaston Verellen, Jean-Luc Deville, Lucien Koulischer, Etienne M Sokal

Abstract
We report two families in which neonatal haemochromatosis was observed in half sibs. In the first family, two successive girls were born of different fathers. In the second family, an affected brother and sister were followed by an affected half brother born after donor insemination. These observations, as well as a previous abstract describing two affected half sisters, revive the debate over the inheritance of neonatal haemochromatosis. Incomplete penetrance or gonadal mosaicism for a dominant disorder, a maternal "environmental factor", or mitochondrial defect may be more suitable explanations than autosomal recessive inheritance in this condition. Alternative modes of fertilisation, such as donor insemination or in vitro fertilisation with donor eggs, should be considered with caution.

(J Med Genet 1996;33:444-449)

Key words: neonatal haemochromatosis; half sibs; maternal inheritance.

Neonatal haemochromatosis (NH) is a polyvisceral iron storage disorder of prenatal onset. It is characterised by a rapidly progressive hepatic insufficiency with prenatal or perinatal onset and a specific distribution of iron overload similar to that seen in adult chromosome 6 linked haemochromatosis, in the absence of any known cause of prenatal liver disease. Serum iron, ferritin, and iron binding capacity saturation are usually moderately raised, whereas total transferrin is low. Liver histology discloses striking haemosiderin deposition in hepatocytes and significantly less in Kupffer cells and biliary epithelium. Liver damage may be widespread with diffuse fibrosis, often amounting to frank cirrhosis, together with regenerative changes, ductular proliferation, and cholestasis. Multinucleated hepatocytes are commonly observed, although inflammatory infiltrate is not striking. Other common sites of iron storage include adrenal cortex, endocrine and exocrine pancreas (together with Langerhans islet hyperplasia), and the epithelial cells of renal tubules, thyroid follicles, and most exocrine glands, but not the reticuloendothelial system (Kupffer cells, spleen, lymph nodes, bone marrow). Total hepatic iron is not always significantly greater than in control groups although most cases do show an absolute overload that can now be quantified in vivo by MRI or CT scanning. NH is thus established as a specific pathological diagnosis (that is, a phenotype) but its genetic or environmental (viral or toxic) bases are still unknown, no specific metabolic disorder is recognised, and aetiological heterogeneity is suspected. NH is usually considered as an autosomal recessive disorder (MIM 231100). We report here on two sibships where typical neonatal haemochromatosis was observed in half sibs, indicating that at least in some instances NH should not be considered as a recessive disorder.

Case reports
FAMILY 1
Patient 1
This female child was born to non-consanguineous, healthy, Belgian parents. An older sister was healthy. The pregnancy was uneventful and birth weight was 3500 g. From 4 hours of life, the child presented with diffuse mucosal bleeding and progressive jaundice. She rapidly deteriorated, with signs of liver insufficiency. On admission to a paediatric neonatal intensive care unit (NICU) on day 12, the child was deeply jaundiced, with ascites and a swollen abdomen. The liver and spleen could not be palpated. Bleeding diathesis was patent. Laboratory investigations showed: total bilirubin 243 μmol/l (normal <17), direct bilirubin 92 μmol/l (normal <17), aspartate transaminase (AST) 52 IU/l (normal <25), alanine aminotransferase (ALT) 30 IU/l (normal <32), blood ammonium 95 μmol/l (normal: 18 to 59), prothrombin time 10% (normal >65%), blood glucose 0.9 mmol/l (normal >3.3). Despite intensive management, the child died on day 13 from diffuse uncontrolled cutaneous and mucous bleeding. Liver histology at necropsy showed micronodular cirrhosis with positive iron staining (Perl's coloration). Iron overload was mainly observed in hepatocytes and giant hepatocytes.

Patient 2
Two years after the death of patient 1, the mother sought genetic advice for a new pregnancy. She was divorced from the father of patient 1. It was clearly stated by the mother that her new partner was not the father of patient 1 and that she had no more contact with her former husband. In view of this situation, a very low recurrence risk was given, assuming that the disease of patient 1 was probably autosomal recessive. Patient 2, a girl, was born at 39 weeks of gestation with a birth weight of
Recurrence of neonatal haemochromatosis in half sibs born of unaffected mothers

3070 g. From the sixth hour of life, she developed multiple petechiae and mucosal bleeding. It soon appeared that she also had severe liver failure. On admission on day 1, she was jaundiced, with a distended abdomen, a hard enlarged liver 1.5 cm below the right costal margin, no splenomegaly, and multiple petechiae.

Biological investigations on day 1 showed:
- Total bilirubin 102 µmol/l, direct bilirubin 7 µmol/l, AST 84 IU/l, ALT 16 IU/l, prothrombin time 15%, blood glucose 2 mmol/l, ammonium 41 µmol/l, lactic acid 4.4 mmol/l. Total bilirubin increased to 530 µmol/l, direct bilirubin to 148 µmol/l, AST to 237 IU/l, ALT 69 IU/l, ammonium 171 µmol/l, and prothrombin time decreased to <10% during the following days. As for patient 1, screening for neonatal cholestasis and early liver failure was performed using appropriate investigations and was not contributory. Tyrosinosis was excluded. Serum iron concentration on day 2 was 62 µmol/l (normal 14 to 30), serum iron binding capacity 28 µmol/l (normal 47 to 68-5), serum ferritin 425 µg/l (normal 10 to 300), transferrin 4 g/l (normal 2-2 to 4), and α-fetoprotein 2 µg/l (normal 0-01 to 0-13). Liver ultrasound showed increased echogenicity and nodular transformation. Liver biopsy was not performed because of the e’ating defect.

A diagnosis of NH was considered and the child was treated with desferrioxamine (Desferal®) by a continuous subcutaneous infusion of 30 mg/day. Large amounts of iron were found in the urine: from 8 µmol/l before treatment to 326 µmol/l under treatment. However, serum iron increased to 228 µmol/l and liver insufficiency persisted. She had disseminated intravascular coagulation with fibrin degradation products at 160 µg/ml (normal <20). In addition to standard treatment for liver failure, she was given continuous infusion factors II, V, VII, IX, and fibrinogen at a dosage of 50 IU/kg/day without improvement. Death resulted from massive intracranial haemorrhage and multisvisceral failure on day 5.

Postmortem liver biopsy showed micronodular cirrhosis, mild ductular proliferation and portal inflammation, extramedullary haematopoiesis, and multinucleated hepatocytes. Iron overload was obvious and was concentrated in hepatocytes, without iron accumulation in Kupffer cells. Iron overload was found at necropsy in the pancreas, stomach, adrenal glands, kidneys, thyroid, heart, and salivary glands.

AETIOLOGICAL INVESTIGATIONS

As recommended elsewhere,9 appropriate blood and urine tests were performed before death in both children. Combined with postmortem pathological examination, tyrosinaemia, galactosaemia, hereditary fructose intolerance, organic acidemias, Niemann-Pick type C, glycogenosis I, III, and IV, α1 antitrypsin deficiency, peroxisomalous disorders, primary defects of bile acid synthesis, cystic fibrosis, Byler disease, Wolman disease, and neonatal infections were excluded.

The activities of the five mitochondrial respiratory chain complexes were assayed for patient 2. No anomalies were observed either in muscle or liver samples (Professor A Munnich, Paris). Fumarylacetoacetate lyase activity was normal in fibroblasts of patient 2.

FAMILY HISTORY

The mother was aged 22 at the birth of patient 1. Her liver function and basal iron metabolism, assessed six months after the birth of patient 2, showed slightly raised iron (2-04 mg/l, normal range 0-6–1-5) and transferrin (4-12 g/l, normal range from 2-4–3-81). On CT scan, attenuation of the liver was normal and the difference from splenic attenuation was not significant. Hepatic, renal, splenic, and adrenal iron storage, estimated through abdominal MRI scan, by T1, T2, and gradient echo sequences, was in the normal range. Striated muscle-liver signal ratio was higher than 1-6 in T2 and between 1-3 and 1-5 in T1. Iron load was estimated as <50 µmol/kg. Chronic viral infection and autoimmune disorders were excluded.

Because of lack of proper material from patient 1, paternity testing could not be performed by DNA analysis. The parents were very concerned by the implications of maternal inheritance and accepted all investigations. We had the opportunity to discuss genetic aspects several times with the mother, alone or with her husband. The possibility of a single father was categorically excluded and, considering their involvement and distress, the author has no doubt about that statement.

FAMILY 2

Patient 1

This boy was born at 39 weeks of gestation after a normal pregnancy. Birth weight was 2877 g, OFC 33-5 cm. He was noted to have spontaneously resolving hypoglycaemia on day 1. Jaundice appeared within 24 hours and severe conjugated hyperbilirubinaemia with signs of liver dysfunction was obvious by day 7. The liver remained non-palpable. Progressive hepatic failure and convulsive encephalopathy led to death at 5 weeks. Necropsy showed widespread liver fibrosis and biliary ductular proliferation, the latter containing bile in many places. Large amounts of stainable iron pigment were present predominantly in the hepatocytes with the macrophages being less affected. A mild chronic lymphomonocytic inflammatory infiltrate was seen in the fibrous tissue. Considerable iron was also shown in the myocardiun and a few renal tubular cells. Neither thyroid, adrenal, or other tissues examined showed iron. The pancreas was not available for examination. There was no other significant pathology except for severe brain swelling.

Patient 2

This girl, the sib of patient 1, was born by normal delivery with a birth weight of 1984 g. Intrauterine growth retardation had been diagnosed late in the pregnancy. She died on day
2 of overwhelming liver failure. Blood taken within the first day of life showed a serum iron within the normal range and a serum ferritin of 1270 mg/l (normal range up to 950 mg/l). A liver biopsy showed an identical picture to that of her brother with severe destruction of hepatic architecture and widespread fibrosis and loss of hepatocytes. There was extensive biliary transformation and hepatocytes contained much stored iron. Necropsy was refused.

Patient 3
Following genetic counselling, the couple was given a 1 in 4 risk of recurrence based on probable autosomal recessive inheritance. The parents decided to opt for artificial insemination by an anonymous donor (AID). A male infant with a birth weight of 2536 g was born after a normal pregnancy but died of fulminating liver disease on day 2. A liver biopsy showed an identical picture to that of his two half sibs. Necropsy was not performed. Paternity by donor was confirmed by a five multi-allelic loci DNA probe analysis.

AETIOLOGICAL INVESTIGATIONS
Multiple investigations were undertaken on patients 1 and 2. Liver function became grossly disordered in patient 1 over the first seven days. There was raised aspartate transaminase (654 IU/l on day 1 and 1255 IU/l by day 8). Alpha fetoprotein levels were grossly raised in patient 1 (>44 800 IU/l). There was no evidence of haemolytic anaemia and no erythropagocytosis on bone marrow biopsy. A sweat test and immunoglobulin estimation were normal. Metabolic screening excluded tyrosinaemia, galactosaemia, glutamic aciduria type 2, and Niemann-Pick disease type C. No fatty oxidation defects or inborn errors of bile acids were discovered, excluding most peroxisomal disorders. There was no evidence of a storage disorder and bone marrow and spleen specimens from the first child were normal. Mitochondrial DNA investigations showed no major deletions or any of the common mutations (MELAS, MERRF, and NARP). Viral studies including hepatitis B, CMV, rubella, and Coxsackie serology were normal. There was no evidence of toxoplasmosis or mycoplasma infection.

FAMILY HISTORY
The parents were healthy, non-consanguineous, and British, aged 29 at the birth of patient 1. Clinical examination was normal. Basal iron metabolism in the parents (serum iron, iron binding capacity, and ferritin) was normal other than low serum iron levels in the mother (9-5 \(\mu\)mol/l, normal range 14–31 \(\mu\)mol/l). Liver function tests in both parents were normal other than minimally raised bilirubin levels in the father (25 \(\mu\)mol/l, normal range 3–17 \(\mu\)mol/l). Basic metabolic screening (urea and creatinine, electrolytes, amino acids, caeruloplasmin) was all normal as were the karyotypes of both parents. Chronic immune/infectious disorder was excluded in the mother, who in addition had normal glycosylation of transferrin.

Although its levels were normal, in the mother (1-2 g/l, normal range 1-2-2-6) and the father (1-3 g/l), \(\alpha\) antitrypsin phenotyping indicated that the mother was heterozygous for a new mutation which she had inherited from her father. The mutant protein was transmitted to patients 2 and 3, but patient 1 had a normal Pi M phenotype. The significance of this rare allele is not yet known (D Whitehouse, personal communication).

Discussion
A diagnosis of neonatal haemochromatosis was established in the five children reported here on histopathological and clinical grounds. At least 65 patients with NH have been observed and exhaustive reviews are available. The most important features are a high prevalence of prematurity (median gestational age 36 weeks, 55% <37 weeks), IUGR (median birth weight 2300 g, 60% <2500 g), early onset (88% during the first day), and an usually fulminating course of the liver dysfunction (median survival seven days, 80% dead <40 days using data from reference 5). Some typical cases of NH were diagnosed beyond the perinatal period. Longer survival was recently reported in early onset cases owing to more effective intensive care or deferoxamine chelation, although the only curative treatment remains orthotopic liver transplantation, successfully performed in some children since 1992. Some sibs of typical cases have been shown to have a milder course and to survive without graft. These cases were nevertheless perinatally symptomatic. In a 16 month old surviving girl, control liver biopsy showed spontaneous disappearance of iron overload, and hepatic fibrosis.

Up to now, no consensus has been reached as to whether the iron storage is the primary defect or a phenomenon secondary to a primitive liver disease, and whether it is causally heterogeneous. Several physiopathological mechanisms have been proposed for NH, including infratherine infection with an unknown pathogen, abnormal transplacental iron transfer from mother to fetus (possibly related to an abnormal iron metabolism in the mother), or an inherited or acquired disorder affecting the hepatic binding, the intracellular handling, or the control of iron distribution. Iron does not reaccumulate in transplanted liver, suggesting that the anomaly of iron metabolism is not because of a multisystemic defect. The fetal liver plays a crucial role in the pathogenesis, either as the primitive pathological site, or as the major target of a transplacental transfer anomaly. This differs from adult onset, chromosome 6 linked haemochromatosis, in which iron reaccumulates in the allograft.

Some cases of NH appear to differ from the usual phenotype or show unusual biochemical features which illustrate the heterogeneity of NH. For example, in a familial observation, the liver was the only site of iron storage.
Recurrence of neonatal haemochromatosis in half sibs born of unaffected mothers

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sibship</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td></td>
<td>Abstract (incomplete pedigrees)</td>
</tr>
<tr>
<td>13</td>
<td>〇〇</td>
<td>One case surviving at 16 months</td>
</tr>
<tr>
<td>19</td>
<td>〇〇</td>
<td>3 with histologically proven NH, one with &quot;severe liver disease&quot; (iron not investigated), two babies dead at day 2</td>
</tr>
<tr>
<td>20</td>
<td>〇〇〇〇</td>
<td>First affected case died in utero at 34 weeks: no iron deposition in extrahepatic tissue</td>
</tr>
<tr>
<td>21</td>
<td>〇〇〇〇</td>
<td>First child possibly affected (died day 3 with generalised haemorrhage)</td>
</tr>
<tr>
<td>22</td>
<td>〇〇〇〇</td>
<td>Abstract (incomplete pedigrees)</td>
</tr>
<tr>
<td>23</td>
<td>〇〇〇〇</td>
<td>Two maternal half sibs in good health</td>
</tr>
<tr>
<td>24</td>
<td>〇〇〇〇</td>
<td>Older affected sib: successful liver transplantation</td>
</tr>
<tr>
<td>25</td>
<td>〇〇〇〇</td>
<td>Younger affected sib alive and stable aged 6 months</td>
</tr>
<tr>
<td>12</td>
<td>〇〇</td>
<td>Mother with post-transfusional non-A, non-B chronic hepatitis. Histologically proven giant cell hepatitis without iron overload in a 25 week fetus</td>
</tr>
<tr>
<td>14</td>
<td>〇〇〇〇</td>
<td>Two half sibships. Second boy survived. Abnormal ferritin</td>
</tr>
<tr>
<td>11</td>
<td>〇〇〇〇</td>
<td>Two unaffected sibs died perinatally: one &quot;dysmorphic&quot;, the other with polycystic renal disease</td>
</tr>
<tr>
<td>26</td>
<td>〇〇〇〇</td>
<td>Abnormal iron metabolism in parents</td>
</tr>
<tr>
<td>27</td>
<td>〇〇〇〇</td>
<td>One unaffected anencephalic boy</td>
</tr>
<tr>
<td>7</td>
<td>〇〇</td>
<td>Girl: successful liver transplantation. Complete clearing of iron overload in exocrine glands 5 months later</td>
</tr>
<tr>
<td>28</td>
<td>〇〇〇〇</td>
<td>Incomplete pedigrees. No family history available</td>
</tr>
<tr>
<td>1</td>
<td>〇〇〇〇</td>
<td>Last affected child in each family is maternal half sib of the previous case(s)</td>
</tr>
</tbody>
</table>

□□□□ normal, 〇〇〇〇 affected, 〇〇〇〇 unaffacted malformed boy/girl/child of unknown sex, □ miscarriage.

Familial cases of typical NH.

Deficiency of Δ1-3-oxosteroid 5-β-reductase was postulated in two unrelated patients who presented with late onset liver insufficiency (1 and 6 weeks). Paucity of primary bile acids and a predominance of some derivatives of chenoanoic acid were noted in the serum. Whether these represent a primary defect, or an anomaly secondary to the liver disease is still unknown. In the family described by Jacknow et al., the mother and an affected surviving child were shown to have low serum transferrin and absent ferritin, so that a dominant mutation of ferritin with abnormal iron affinity and structure facilitating hepatic uptake was suggested to be causative. A single newborn was reported with NH, facial dysmorphism, complex cyanotic heart disease, syndactyly, and postaxial polydactyly. We have observed two sibs with delayed NH, intractable diarrhoea, facial dysmorphism, and hair anomalies.

NH is usually considered to be a recessive disorder. Recurrence of NH in sibships has been reported in at least 24 sibships, including ours. However, in familial reports, parental consanguinity was never mentioned. The total number of affected children is 57. In addition, although complete pedigrees were probably not always published, the frequency of fetal losses is striking (figure): among 18 sibships where familial data were available, there were 18 probands, 27 affected sibs, 19 unaffected sibs, and 10 miscarriages. In an abstract, Jacknow et al. was the first to describe NH in half sibs born to the same mother. A girl died at birth, a male sib, although affected early, was alive at the age of 5 years, and a male half sib died on day 4. Another case occurring in half sibs is briefly mentioned by Knisely, without clinical data. Although no formal paternity testing was performed in family 1, it was verified in family 2. Considering the rarity of NH, recurrence in maternal half sibs in these two further families requires a less unlikely explanation than fortuitous recurrent matings between heterozygotes.

The lack of consanguinity in familial reports of this exceptional disease may also indicate that autosomal recessive inheritance is not the
correct pattern for at least a subgroup of NH. A maternal factor has been suggested by some. In the family of Jacknow et al.6 the mother and the surviving child had low serum iron, increased serum transferrin, and undetectable ferritin. The authors concluded that a mutant ferritin, transmitted as a dominant trait, was responsible for the hepatic iron sequestration (although an abnormality of the ferritin metabolism rather than a primary defect of ferritin was also possible). Driscoll et al.20 hypothesised that NH could result from increased transplacental transport of iron facilitated by maternal heterozygosity for a still heal homozygosity for a recessive gene. Others have suspected a purely maternal disorder, either genetic or acquired. Rand et al.17 suggested the presence of maternal isomime antibodies directed against a placental protein regulating the iron flux. A sporadic case was suspected to be related to the presence of anti-Ro/SS-A and anti-La/SS-B ribonucleoprotein antibodies in the mother who had Sjögren syndrome.29 Because of the hepatic aspect of the liver, an unknown viral infection has been suspected by some authors. No direct proof has been obtained for this, although some cases of NH have been observed in children whose mother had non-A, non-B post-transfusional hepatitis.24 As normal children were born after the affected child in some pedigrees, a persistent immune or infectious factor is unlikely. Absence of recurrence in the allograft and correction of serum markers of iron storage disease argues against the viral hypothesis.

Mitochondriopathy has recently been observed in sibs with neonatal cholestasis and early liver insufficiency, without NH.30 As no paternal half sibs have been observed, a mitochondrialia could also explain the unusual pattern of familial occurrence of NH. Investigations of the respiratory chain and mitochondrial DNA screening in our patients failed to confirm this hypothesis. Moreover, there is no report of iron storage in other clinical variants of mitochondrialopathies (Kearn-Sayres, Pearson, Wolfram syndromes, etc), and no reports mention compatible manifestations in patients.

Dominant inheritance with extreme clinical variation seems the more convincing model but no constant biological anomalies of iron metabolism have been shown in first degree relatives of NH patients. Abnormal liver function was noted in some parents.9 This has been considered by some as a marker of heterozygosity,23 but could reflect minimal expression of a dominant trait as well. In two instances, liver biopsies were taken from mothers. No iron overload was found in the specimens.10,24 In most cases, the only abnormality is a maternal iron depletion (low serum iron and low saturation) that corrects itself after delivery and is thought to reflect the fetal steal of iron.21 Gonadal mosaicism, an imprinted gene with maternal expression, or cryptic familial translocation are other possible variations on the dominant model. Confirmation of this hypothesis may be difficult to obtain, as most known surviving cases (either because of a milder phenotype or after hepatic allograft) are children. Finally, a maternally dependent disorder of fetoplacental iron metabolism could be considered.

In summary, a review of familial cases of NH, and observations of three sets of affected half sibs indicate that at least a subgroup of NH patients cannot be considered as suffering from a recessive disorder. As causal heterogeneity is still likely, systematic screening of the parents should be recommended for abnormal iron metabolism and evaluation of parental iron storage with MRI. Mitochondrial anomalies could reflect minimal mosaicism and dominant inheritance, although exact risk figures cannot be given at this moment. Alternative modes of fertilisation, such as sperm donor or oocyte gift, should be considered with caution. Prenatal diagnosis is possibly feasible through fetal MRI scan of liver, pancreas, and heart,22 and eventually percutaneous blood cord sampling.23

The authors wish to thank Dr S Gosseye for the pathological investigation of family 1, Dr S Huson for her help with family 2, and Dr D Whitehouse for his useful comments on the manuscript.

1 Wittke CL, Uri A. Perinatal hemochromatosis: entity or end result? Hum Pathol 1989;20:335-40.
Recurrence of neonatal haemochromatosis in half sibs born of unaffected mothers