HFE gene mutation and transferrin saturation in very low birthweight infants

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Abstract

***Aim*—To determine if there is an association between high transferrin saturation and the C282Y HFE gene mutation in very low birthweight (VLBW) infants.**

***Methods*—One hundred and forty three VLBW infants receiving recombinant erythropoietin and 3 to 9 mg/kg/day of enteral iron were studied. Genomic DNA was extracted from filter paper cards. The C282Y mutation was determined by restriction fragment length polymorphism analysis.**

***Results*—Six infants were heterozygous for the mutation; none was homozygous. Ten infants had a transferrin saturation above 80% at least once. No infant was positive for both transferrin saturation above 80% and the mutation.**

***Conclusions*—The data strongly suggest that there is no association between high transferrin saturation and the HFE gene mutation in VLBW infants during the first weeks of life.**

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Keywords: transferrin saturation; HFE gene mutation

The G845A mutation of the HFE gene, resulting in a C282Y change in HFE protein, is strongly associated with hereditary haemochromatosis.[1] Patients homozygous for this mutation are characterised by increased intestinal iron absorption and excessive iron deposition in various organs, which eventually fail. A high transferrin saturation has been found in heterozygous individuals.[2] Around 1 in 400 caucasians are homozygous for this mutation and carrier frequency is estimated to be about 1 in 10 people of Northern Europe descent.[3]

In a European multicentre trial we observed a median transferrin saturation of 25% in extremely low birthweight infants during their first weeks of life, which is similar to that in healthy adults.[4 5] Ten per cent (19 out of 183) of these infants had a transferrin saturation above 80%. Thus we hypothesised that a transferrin saturation above 80% may be associated with the C282Y mutation in very low birthweight (VLBW) infants (birthweight under 1500 g).

Methods

From January 1995 to April 1997, 192 VLBW infants were admitted to the Department of Neonatology. Twenty nine (15%) died, blood samples were not available in 15, and records were incomplete for five, giving a study population of 143 infants.

Enteral iron (3 mg/kg/day) was scheduled to start on days 3 to 5 of life. The daily dose was increased to 6 mg/kg by days 12 to 14 and to 9 mg/kg by days 24 to 26 of life, if the transferrin saturation was less than 30%. If it was greater than 80%, iron supplementation was interrupted. Parenteral iron was not applied. Nine infants received erythropoietin (rhEPO) in a weekly dose of 1500 U/kg, according to the protocol of the third European multicentre trial.[4] All other infants were treated with rhEPO 750 U/kg per week.

For DNA extraction we used dried blood that has been routinely sampled on a filter paper card to screen for congenital errors of metabolism. For anonymity, all infants were coded using the random number generator of a personal computer. Small circles were cut out of the filter paper cards and coded independently. Before analyses and evaluation, the two codes were combined and the original lists identifying the infants were destroyed, thus excluding retrospective identification.

Genomic DNA was extracted using a commercially available kit (Qiagen, Hildesheim, Germany). Primers flanking the C282Y mutation in exon 4 (5-GTGACCACTCTACGGTGTCG-3 and 5-CAGCTCCTGGCTCTCATCAG-3) were synthesised based on the nucleotide sequence of the HFE gene (GenBank, Y09803). Polymerase chain reaction was performed and the amplified products digested with Rsa I at 37° C overnight, separated by polyacrylamide gel electrophoresis, and visualised using silver stain. The identity of the genetic amplifications was verified by dye terminator cycle sequencing on a ABI 373A fluorescence sequencer (Applied Biosystems Inc., Weiterstadt, Germany). The HFE genotype was determined in duplicate. Consistent results were obtained in all samples.

Since 1989 all VLBW infants admitted to intensive care have been recorded prospectively on an SPSS database (SPSS Inc., Chicago, Illinois, USA). For our analysis the following additional data were retrieved retrospectively by reviewing the infants' charts: serum iron and transferrin concentration; cumulative iron dose; number of transfusions; and cumulative transfusion volume.

Results

None of the infants was homozygous for the C282Y mutation, while six of 143 (4.2%) infants were heterozygous. A transferrin saturation above 80% at least once during the clinical

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course was observed in 10 infants (7.0%). None of the infants was positive for both a transferrin saturation above 80% and the C282Y mutation. Multiple regression analysis showed that the cumulative iron dose was significantly ($p < 0.05$) correlated with median transferrin saturation, but not the C282Y mutation, cumulative transfusion volume, birthweight, or sex.

Discussion

Very low birthweight infants have only small iron stores at birth, which are gradually depleted by diagnostic blood sampling during intensive care. Adequate iron supplementation is difficult to achieve. Enteral resorption is uncertain. The high osmolarity of enteral iron preparations may aggravate pre-existing problems caused by the immaturity of the gut and force enteral feeding to be abandoned. Parenteral iron administration may increase oxidative stress, leading to, or aggravating, free radical induced disease, such as bronchopulmonary dysplasia and necrotising enterocolitis.

For this study, no additional blood sampling was required. We were able to use blood from filter papers as a readily available source for DNA analyses. This is very important for studies in VLBW infants, in whom the cumulative amount of sampling losses often exceeds their own blood volume, thereby necessitating frequent blood transfusions.

The prevalence of heterozygous HFE mutation in our infants was lower than that observed in Northern European adults.[6] This, and the fact that we did not find any case of homozygous mutation, can be explained by our sample size. Nevertheless, our data strongly suggest that there is no association between a high transferrin saturation and the HFE gene mutation in VLBW infants during their first weeks of life. Other factors, such as feed tolerance, variable iron absorption rates due to immaturity, and infections, seem to have a higher impact on enteral iron absorption than the HFE gene mutation.

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