Saint Luke's Regional Laboratories
Clinical Laboratory Letter

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Hemochromatosis Genotypes and Risk of Ischemic Stroke

Hemochromatosis, an autosomal recessive disorder of iron metabolism, is characterized by progressive accumulation of iron stores throughout the body that eventually compromise hepatic or cardiac function. The disease occurs in 0.2-0.5% of individuals of Northern European descent and is caused by point mutations in the hemochromatosis (HFE) gene. The unmutated and predominant form of the HFE gene is designated Wild Type (WT). Most patients with hemochromatosis are homozygous for a C282Y mutation. A second HFE mutation, H63D, is also associated with biochemical indicators of iron overload in homozygous or compound heterozygous (C282Y/H63D) form.

C282Y and H63D mutations are thought to influence iron metabolism via different molecular mechanisms. The HFE protein coded by an H63D mutation, unlike the C282Y mutation, forms stable complexes with the transferrin receptor and lowers its affinity for transferrin binding. Transferrin carries iron from blood into brain tissue via transferrin receptors located in the brain's microvasculature. Although HFE mutations have been linked in some studies with diseases of the brain, including Alzheimer and Parkinson diseases, ALS, MS and stroke, the relative association of C282Y and H63D mutations with brain disease has not been extensively studied.

A recent report in the journal Neurology (2007;68:1025-31) describes a well-controlled prospective study that investigated the association of HFE genotypes with ischemic cerebrovascular disease (ICVD) and ischemic stroke (IS) in 9,081 HFE genotyped individuals randomly selected to reflect the Danish adult population. Over a follow-up period of 24 years, 504 individuals developed ICVD, including IS (n=393), amaurosis fugax (n=7) and TIA (n=104). The remaining 8,577 individuals were not diagnosed with cerebrovascular disease. Outcome data is summarized in the table. Incident rates are listed as events per 1000 person-years; hazard ratios are multifactorially adjusted.

Cumulative incidence of ICVD as a function of age was increased in H63D homozygous individuals compared to WT/WT (log-rank, $p = 0.003$). A highly significant association was observed between H63D homozygotes and IS. Cumulative incidence of IS as a function of age was increased in H63D homozygous individuals compared to WT/WT (log-rank, $p < 0.001$). The hazard ratio for IS in H63D homozygotes was 2.8 ($p < 0.001$). Hazard ratios for all other HFE genotypes compared to WT/WT were not significant. Based on an H63D homozygote population frequency of 1.7% and the hazard ratio of 2.1 for ICVD and 2.8 for IS, the population attributable risk for ICVD was 2% and the risk for IS was 3%.

HFE Genotype	Population Incidence (%)	IS Incident Rate	IS Hazard Ratio (95% CI)
H63D/H63D	1.7	5.4	2.8 (1.7-4.6)
C282Y/C282Y	0.25	2.5	1.5 (0.2-11)
C282Y/H63D	1.4	2.0	1.0 (0.4-2.5)
H63D/WT	20.5	2.3	1.0 (0.7-1.2)
C282Y/WT	9.2	2.2	1.0 (0.7-1.4)
WT/WT	66.9	2.3	1.0

The authors concluded that H63D/H63D genotype is strongly associated with ICVD and IS. The increased risk does not appear to correlate with development of atherosclerosis, since no association was observed in a previous case-controlled study. They also discount the intrinsic influence of iron overload because C282Y homozygosity or heterozygosity was not significantly associated with ICVD or IS. These well-controlled studies appear to provide further evidence that the C282Y and H63D hemochromatosis mutations affect the brain through different mechanisms.

Umbilical Cord Blood Stem Cells

Stem cells from umbilical cord blood have increasingly become a viable alternative source of progenitor cells for hematopoietic cell transplantation (HCT). To date, cord blood stem cells have been used in over 6000 unrelated-donor transplantations throughout the world.

When autologous stem cells are not an option, the ideal source of progenitor cells is from the bone marrow or peripheral blood of an HLA-matched sibling. An HLA match decreases the risk of severe graft-versus-host disease (GVHD) and increases the chances of a successful engraftment. However, a full HLA-match occurs in only 25% of sibling donors. While HLA-matched allogeneic donors may be used, the process of finding a suitable donor can be lengthy and a fully HLA-matched donor may never be found for some patients. A full match from an unrelated donor is even less likely for potential recipients with a non-Northern European heritage due to fewer non-white donors and genetic heterogeneity. For these reasons, umbilical cord blood from unrelated, partially HLA-mismatched donors has become a reasonable alternative source of stem cells for bone marrow reconstitution.

Transplantation across HLA barriers, rapid availability and decreased risk of transmission of infectious diseases are advantages of the use of cord blood over peripheral blood or bone marrow HCT. In addition, studies have shown that the incidence and severity of GVHD decreases with partially HLA-mismatched cord blood HCT when compared to partially mismatched unrelated bone marrow or peripheral blood HCT.

The New York Blood Center (NYBC) recently collaborated with the Center for International Blood and Marrow Transplant Research (CIBMTR) in the analysis of HCT treatment of children (under 16 years old) with leukemia or myelodysplasia. They found similar three-year survival rates for those receiving unrelated cord blood transplants and those receiving equally well-matched unrelated bone marrow transplants.

Adults have also been found to be good candidates for cord blood HCT. According to a study published in the November 25, 2004 issue of the *New England Journal of Medicine* (NEJM), *"HLA-mismatched cord blood should be considered an acceptable source of hematopoietic stem-cell grafts for adults in the absence of an HLA-matched adult donor."* This study involved 600 adults and resulted in participants who received cord blood with a 5/6 or 4/6 HLA match doing as well as those who received bone marrow with a 5/6 HLA match.

Cord blood transplantation can be limited by the number of cells present in a given unit, particularly for adults or larger pediatric patients. If a single unit is inadequate for engraftment, multiple cord blood units are sometimes attempted. A double umbilical cord blood transplantation has been shown to have success (in terms of GVHD, rates of engraftment, survival and transplant-related mortality) similar to single umbilical cord blood transplantation and unrelated bone marrow transplantation. Other studies involving ex vivo expansion of cord blood stem cells to increase their numbers per unit are also under investigation.

During the past year, the Cell Processing Laboratory at Saint Luke's Cancer Institute processed three umbilical cord blood units for HCT in pediatric patients. The laboratory hopes to expand the number of cord blood stem cells processed in the future.

Cystic Fibrosis Newborn Screening Pilot Program

The Missouri State Public Health Laboratory is currently conducting a pilot program for newborn cystic fibrosis screening utilizing a fluroimmunoassay to measure immunoreactive trypsinogen (IRT). Newborns with cystic fibrosis have persistently elevated IRT levels, whereas unaffected infants may have transiently elevated levels at birth that return to normal after the first week of life.

The Missouri State laboratory will request a repeat screen for all infants that have elevated IRT levels on the initial newborn screen. These will be collected at no charge to the parents. If the IRT levels are elevated on the repeat screen, the physician of record will be contacted by a cystic fibrosis center for additional follow up testing.