



## Hereditary Hemochromatosis Mutation Analysis by PCR

*Marcy L. Hoffmann, Ph.D. and William A. Dittman, Jr., M.D.*

PAML AND SHMC ARE NOW OFFERING a polymerase chain reaction (PCR)-based test to detect the two most common mutations associated with hereditary hemochromatosis (HHC), a genetic disorder of iron metabolism resulting in iron overload.

HHC is an autosomal recessive disorder characterized by abnormally high intestinal iron absorption. This condition can lead to severe and potentially lethal iron overload with damage to a number of organs, including the liver, pancreas, heart, joints, and endocrine glands.<sup>1</sup> Even though the disease may be fatal, in its early stages it is easily treated by iron removal by phlebotomy, making early appropriate diagnosis desirable. The disease is caused by mutations in the human leukocyte antigen (HLA)-linked iron-loading gene (HFE). Although HHC is a common disease, it is significantly underdiagnosed. Within the population of individuals of northern European descent, it is estimated that HHC affects 1 in every 200 to 400 individuals.<sup>2</sup> The corresponding rate for non-symptomatic heterozygotes within this population is 10% to 16%.

Two mutations within the HFE protein have been described. The most common is a cysteine (C) to tyrosine (Y) substitution at position 282 (C282Y). This mutation has been extensively studied and is definitively associated with HHC. Studies conducted in the United States, Australia, France and Italy have shown that 64% to 100% of HHC patients are homozygous for the C282Y mutation. In contrast, the mutation at amino acid 63 resulting in a histidine (H) to aspartate (D) (H63D) remains controversial as a cause of iron overload and hemochromatosis. Approximately 4% of HHC patients are compound heterozygotes for C282Y and H63D. However, a small percentage of patients with clinically diagnosed HHC do not carry either mutation, suggesting that iron overload can result from other unidentified genetic and/or environmental causes.<sup>3</sup> This means that a negative study for these two mutations does not entirely rule out disease.

Expression of HHC is influenced by environmental factors including amount of iron in the diet, physiologic and pathologic blood losses and excessive alcohol intake. In countries where iron deficiency is prevalent largely due to low meat consumption, iron overload is rare, even in those individuals homozygous for the HFE mutation. In contrast, phenotypic expression of iron overload is more common in industrialized countries where individual consumption of meat is relatively

higher. Although HHC gene mutation frequency occurs equally in males and females, iron overload is significantly less frequent in females as a result of blood lost through menstruation and pregnancy. Alcohol abuse does not, in itself, lead to iron overload, although it is clearly associated with the development and severity of clinical manifestations in HFE homozygotes.<sup>3</sup>

HHC is a disease with somatic manifestations including but not limited to liver disease, arthropathies, endocrine and cardiac abnormalities. Laboratory diagnosis of patients suspected of having HHC should include assessing transferrin saturation concentration and serum ferritin concentration. Many experts recommend liver biopsy for histologic evaluation of siderosis and quantitative measurement of iron concentration. Confirmation of the diagnosis of HHC may be demonstrated by showing that an individual is homozygous for the C282Y mutation or heterozygous for C282Y and H63D.<sup>3</sup> As with all genetic disorders, genetic counseling and evaluation of other family members is important to identify other at-risk family members and to discuss appropriate therapeutic options.

Specific identification of the C282Y and H63D mutations in the evaluation of HHC involves isolating DNA from patient cells (usually white blood cells) then performing PCR using specific DNA sequences for the normal and abnormal HFE alleles. The current methods allow identification of heterozygotes as well as homozygotes. As described above, however, there are rare patients with HHC who will not have mutations at these two sites. This means a normal result does not absolutely rule out HHC.

### References

1. **Andrews NC, Levy JE.** Iron is hot: an update on the pathophysiology of hemochromatosis. *Blood* 1998. **92**(6):1845-1851.
2. **Fleming MD.** Hepatic iron overload in the age of hereditary hemochromatosis mutation analysis. *Am J Clin Pathol* 1998. **109**(5):505-507.
3. **Bothwell TH, MacPhail AP.** Hereditary hemochromatosis: etiologic, pathologic, and clinical aspects. *Semin Hematol* 1998. **35**(1):55-71.

## Test Information

DESCRIPTION HEREDITARY HEMOCHROMATOSIS  
METHOD PCR  
WORKPAR HHCP  
CPT CODE 83890, 83894, 83901×4, 83912  
SPECIMEN 5 mL EDTA whole blood (lavender top tube). Store and transport at room temperature. If delayed more than 72 hours, store and transport refrigerated. Do not freeze specimen.  
COMMENTS *Minimum amount:* 1 mL or 1 full EDTA microtainer.  
*Other acceptable specimens:* sodium citrate or ACD whole blood (blue or yellow top tube).  
*Unacceptable conditions:* heparinized whole blood, serum, grossly hemolyzed, or frozen specimens, specimens over 5 days old, specimens in leaking containers.  
*Stability:* 72 hours at room temperature, 5 days refrigerated, unstable frozen.  
SCHEDULE Varies  
TURNAROUND Varies  
RANGES Hereditary Hemochromatosis Result  
Genotype  
Interpretation  
Comment

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