The H63D Mutation of the Hemochromatosis Gene is Associated with Sustained Virological Response in Chronic Hepatitis C Patients Treated with Interferon-Based Therapy: A Meta-Analysis

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The hemochromatosis (HFE) gene encodes the HFE protein that regulates iron absorption. HFE mutations lead to the hemochromatosis disease of excessive iron absorption. HFE mutations may also influence the sustained virologic response (SVR, long-term virus suppression) in chronic hepatitis C patients treated with interferon-based antiviral therapy. We performed a meta-analysis of all English and Chinese language studies of HFE mutations and SVR in interferon-treated chronic hepatitis C patients indexed in the Medline, PubMed, Embase, and China National Knowledge Infrastructure databases to November 2011. Seven studies involving 605 patients with HFE mutations (homozygous or heterozygous mutation of C282Y, H63D or S65C) and 1279 with wild-type HFE (no mutation of C282Y, H63D or S65C for both alleles) were analyzed. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated with the fixed- or random-effect models. HFE mutations were associated with significantly higher SVR rate (vs. wild-type: OR = 1.56, 95% CI: 1.23 - 1.97, \( P < 0.001 \)), indicating that mutation carriers were likely to achieve SVR in response to interferon-based antiviral therapy. Stratification analysis by HFE mutation type revealed that the H63D mutation was associated with a significantly higher SVR rate (OR = 1.60, 95% CI: 1.09 - 2.34, \( P = 0.020 \)), while the C282Y mutation was not (OR = 1.19, 95% CI: 0.71 - 1.98, \( P = 0.510 \)). Our meta-analysis results indicate that the H63D mutation in HFE is associated with a higher SVR rate in chronic hepatitis C patients treated with interferon-based antiviral therapy.

Keywords: chronic hepatitis c; hemochromatosis; interferon-based therapy; mutation; sustained virological response

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Chronic hepatitis C virus (HCV) infection is a major cause of progressive liver fibrosis, cirrhosis, and hepatocellular carcinoma (World Health Organization 2000; Perz et al. 2006). It has been estimated that 3% of the world’s population, approximately 170 million people, suffer from HCV infection (Lauer and Walker 2001). The fact that many countries have reached epidemic levels has precipitated substantial research efforts toward developing efficacious therapies to combat HCV; however, the available therapeutic methods remain far from satisfactory (Manns et al. 2001; Fried et al. 2002; Ghany et al. 2009). Many studies have focused on identifying the factors underlying the antiviral drug-induced sustained virologic response (SVR, long-term virus suppression) in chronic HCV. As a result, several genetic factors have been identified, providing insights into the individualized nature of SVR in different patients (Gao et al. 2004; Dai et al. 2006; Li et al. 2011; Romero-Gomez et al. 2011).

The hemochromatosis (HFE) gene, located in the human leukocyte antigen (HLA) region of chromosome 6, encodes a major histocompatibility complex (MHC) class I–type protein that binds to β2-microglobulin, the primary function of which is to regulate absorption of iron from the gut by mediating the interaction of the transferrin receptor with the transferrin protein (Roetto et al. 1997; Jazwinska 1998). Three mutations have been identified in the HFE gene. One mutation produces an amino acid substitution of cysteine for tyrosine at position 282 (C282Y, nucleotide 845G>A), the second mutation replaces histidine with aspartic acid at position 63 (H63D, 187C>G), and the third mutation replaces serine with cysteine at position 65 (S65C, 187A>T) (Merryweather-Clarke et al. 1997; Arya et al. 1999; Merryweather-Clarke et al. 2000; Kucinskas et al. 2012). The prevalence of HFE mutations varies greatly among populations from different geographical regions. One study that evaluated 2,978 subjects to estimate the
global prevalence of \textit{HFE} mutations determined that C282Y and H63D occurred at allele frequencies of 1.9% and 8.1%, respectively. Subsequent stratification analysis revealed that the highest frequencies of C282Y occurred in the Irish (10.0%) and of H63D in the Basque (30.4%) (Merryweather-Clarke et al. 1997). In contrast, the frequency of S65C mutation was lower than that of C282Y or H63D in all regions examined (Candore et al. 2002; Kucinskas et al. 2012).

Numerous studies have shown that homozygosity for the C282Y mutation in Caucasians is associated with hereditary hemochromatosis, a disease characterized by excessive iron absorption. The reported rates of C282Y homozygosity have ranged from 64% in Italian hemochromatosis cases up to 100% of Australian hemochromatosis cases (Merryweather-Clarke et al. 1997; Piperno et al. 1998; Merryweather-Clarke et al. 2000). A study of almost 100,000 North American primary care patients investigated the distribution of \textit{HFE} mutations in a racially diverse group (Adams et al. 2005) and confirmed that C282Y homozygosity is most common in whites, consistent with the hypothesis that this mutation originated in a Caucasian “founder” individual. Individuals who are compound heterozygotes for C282Y and H63D may also present with iron overload in the diagnostic range for hemochromatosis, although the penetrance of this genotype is lower than that of C282Y homozygotes (Aguilar-Martinez et al. 1997; Robson et al. 1997; De Gobbi et al. 2004). The H63D mutation is variably distributed worldwide, but is more prevalent than the C282Y mutation. Approximately 20% of European populations are estimated to be H63D heterozygotes (Merryweather-Clarke et al. 1997; Rochette et al. 1999). The C282Y/H63D compound heterozygous and H63D homozygous mutations have mostly been associated with only biochemical evidence of mild iron overload. The clinical penetrance of these mutations is low, although there have been reports of varied phenotypic presentation (Aguilar-Martinez et al. 2001; De Gobbi et al. 2004). The S65C mutation, on the other hand, was originally considered to be a neutral mutation. To date, it has not been associated with any perturbations in transferrin receptor saturation (Arya et al. 1999).

Recently, mutations of \textit{HFE} have been suggested to influence the sustained virologic response in chronic hepatitis C patients treated with interferon-based antiviral therapy (Distante et al. 2002; Cardoso et al. 2004; Lebray et al. 2004; Bonkovsky et al. 2006; Yu et al. 2006; Carneiro et al. 2010; Sikorska et al. 2010). Thus, we performed a meta-analysis of the related studies published to date in the peer-reviewed literature to determine whether \textit{HFE} mutations play a significant role in the SVR of chronic HCV infection treated with interferon-based therapy.

\section*{Materials and Methods}

\subsection*{Search strategy}

The Medline, PubMed, Embase, and China National Knowledge Infrastructure (CNKI) databases were searched for relevant studies published up to November 2011. The following search terms were used in various combinations: “\textit{HFE} or C282Y or H63D or S65C” and “hepatitis C or hepacivirus or hepatitis C, chronic or HCV” and “sustained virological response or SVR” and “interferon”. The search was limited to only English-language and Chinese-language papers. Initially, the title and abstract of identified relevant studies were used to exclude any obviously irrelevant studies. The full-texts of the remaining articles were retrieved and perused to determine the relevancy of the study design and data, according to the inclusion and exclusion criteria detailed below. Additional studies were identified by screening the reference lists of each relevant study. Furthermore, reviews concerning the relevant topic were retrieved from the above-mentioned databases in order to potentially broaden the search by identifying additional relevant publications from the studies cited in the reviews.

\subsection*{Criteria for study inclusion and exclusion}

The following inclusion criteria were used to select relevant studies for the meta-analysis: (a) the study evaluated HCV patients receiving antiviral therapy based on interferon, regardless of HCV genotype or ethnic group; (b) the study reported data of the SVR rate; and (c) the study reported genotypic frequencies of \textit{HFE} mutations in patients. Studies were excluded based on the following criteria: (a) the reported data would not allow for correlation analysis between the \textit{HFE} mutation and SVR; (b) the study included human immunodeficiency virus or hepatitis B virus co-infected patients; (c) the study included liver transplant recipients; (d) the study was reported as a letter or case report; and (e) the study enrolled fewer than 10 subjects. If two or more studies used the same population resource or had overlapping subjects, only the study reporting the largest population was selected for inclusion in the meta-analysis.

\subsection*{Data extraction}

Two independent investigators (Shi-Hong Li and Hong Zhao) performed the data extraction from the seven included studies. Inconsistencies were resolved by discussion between the two, and consensus was achieved for all data prior to meta-analysis. Data for all \textit{HFE} mutations reported (C282Y, H63D, or S65C) and SVR were extracted. In total, 605 patients with \textit{HFE} mutations (with any mutation of C282Y, H63D or S65C, including both homozygotes and heterozygotes) and 1279 with wild-type \textit{HFE} (without any mutation of \textit{HFE}) were included. To facilitate subgroup analyses by \textit{HFE} mutation type stratification (including C282Y, H63D and S65C) and SVR, the genotypes of C282Y/WT and C282Y/C282Y, regardless of H63D and S65C, were grouped as the C282Y mutation; the genotypes of H63D/WT and H63D/H63D, regardless of C282Y and S65C, were grouped as the H63D mutation; and, the genotypes of S65C/WT and S65C/S65C, regardless of C282Y and H63D, were grouped as the S65C mutation. In addition,
the following information was collected from each publication: the first author’s name, publication year, country where the study was conducted, and number of participants.

**Endpoint**

The main endpoint was the SVR rate, which was defined as the percentage of patients who tested as HCV RNA-negative at six months after therapy completion (Ghany et al. 2009).

**Statistical analysis**

To evaluate the association between HFE mutations and SVR rate, the odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using pooled group and subgroup data from the studies. Data pooling was carried out by using the fixed effects model (based on the Mantel-Haenszel method) or the random effects model (based on the Dersimonian and Laird method) (Mantel and Haenszel 1959; DerSimonian and Laird 1986). The random effects model was used if heterogeneity existed between the studies from which the data was extracted; otherwise, the fixed effects model was used. Statistical heterogeneity between studies was assessed with the Chi-square-based Q test and I², and heterogeneity was considered significant when the two-tailed p-value was less than 0.05. I² was used to qualify variation in OR that was attributable to heterogeneity. Finally, the statistical significance of the OR was determined by using the Z test.

All analysis was performed by using the Review Manager v.4.2 software package (http://ims.cochrane.org/revman).

**Results**

**Meta-analysis of HFE mutations and SVR rate**

Seven related studies that fulfilled the inclusion and exclusion criteria were included in the meta-analysis (Distante et al. 2002; Cardoso et al. 2004; Lebray et al. 2004; Bonkovsky et al. 2006; Yu et al. 2006; Carneiro et al. 2010; Sikorska et al. 2010). The characteristics of each are summarized in Table 1. These studies involved a total of 1884 individuals, comprising 605 patients with HFE mutations and 1279 with WT HFE. Three of the seven studies had reported an increased rate of SVR in the HFE mutation group (Distante et al. 2002; Bonkovsky et al. 2006; Carneiro et al. 2010), while the other four studies found no evidence of different SVR rates between the two groups (Cardoso et al. 2004; Lebray et al. 2004; Yu et al. 2006; Sikorska et al. 2010). The overall rate of SVR in the pooled HFE mutations group was 28.60% (173/605) and 24.08% (308/1279) in the WT HFE group. Thus, in the meta-analysis, the HFE mutations were associated with a significantly higher SVR rate (OR = 1.56, 95% CI: 1.23 - 1.97, P < 0.001; Fig. 1).

**Subgroup analysis of HFE mutations and SVR rate**

**Meta-analysis of the C282Y mutation and SVR rate:**

Six of the seven studies investigated the potential association between the common C282Y mutation and SVR. The remaining study did not provide sufficient information to determine the exact number of C282Y mutation cases. Therefore, the meta-analysis for this comparison was based upon 74 individuals in the C282Y mutation group, of whom 32 had SVR, and 591 individuals in the WT group, of whom 212 had SVR. The different effect of C282Y mutation on SVR rates between the two groups was not statistically significant (OR = 1.19, 95% CI: 0.71 - 1.98, P = 0.510; Fig. 2).

**Meta-analysis of the H63D mutation and SVR rate:**

Five of the seven studies investigated the potential association between the common H63D mutation and SVR. The remaining two studies did not; one study did not provide sufficient information to extract the exact number of H63D mutation cases, and the other did not report on the association between H63D mutation and SVR. Therefore, the meta-analysis for this comparison was based on 171 individuals in the H63D mutation group, of whom 68 had SVR, and 375 individuals in the WT group, of whom 110 had SVR. In contrast to the meta-analysis results for C282Y, H63D was associated with a significantly higher SVR rate (OR = 1.60, 95% CI: 1.09 - 2.34, P = 0.020; Fig. 3).

**Meta-analysis of the S65C mutation and SVR rate:**

Only two of the seven studies analyzed herein reported information on this mutation. One of those studies did not provide sufficient information for correlation analysis of SVR. As a result, we were unable to investigate the potential association between S65C mutation and SVR in chronic

<table>
<thead>
<tr>
<th>Authors</th>
<th>Country</th>
<th>Year</th>
<th>Patients with HFE mutations (C282Y/H63D)</th>
<th>Patients with wild-type HFE</th>
</tr>
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<tbody>
<tr>
<td>Distante et al.</td>
<td>Norway</td>
<td>2002</td>
<td>38 (38/0)</td>
<td>216</td>
</tr>
<tr>
<td>Cardoso et al.</td>
<td>Portugal</td>
<td>2004</td>
<td>4 (1/4)*</td>
<td>8</td>
</tr>
<tr>
<td>Lebray et al.</td>
<td>France</td>
<td>2004</td>
<td>45 (6/39)</td>
<td>101</td>
</tr>
<tr>
<td>Bonkovsky et al.</td>
<td>USA</td>
<td>2006</td>
<td>363 (0/0)**</td>
<td>688</td>
</tr>
<tr>
<td>Yu et al.</td>
<td>China</td>
<td>2006</td>
<td>7 (2/6)*</td>
<td>11</td>
</tr>
<tr>
<td>Carneiro et al.</td>
<td>Brazil</td>
<td>2010</td>
<td>83 (9/74)</td>
<td>21</td>
</tr>
<tr>
<td>Sikorska et al.</td>
<td>Poland</td>
<td>2010</td>
<td>65 (18/48)*</td>
<td>181</td>
</tr>
</tbody>
</table>

*Among the patients with HFE mutations, one individual was a compound heterozygote for C282Y/H63D. This patient was assigned to both the C282Y mutation and H63D mutation groups in genotype stratification analysis.

**The study did not provide sufficient information to determine the exact number of individuals with C282Y or H63D mutations.**
The results of the meta-analysis suggest that HFE mutations, especially the H63D mutation, are associated with a significantly increased rate of SVR in chronic hepatitis C patients treated with interferon-based therapy. At present, there is no obvious explanation for this.

**Discussion**

The results of the meta-analysis suggest that HFE mutations, especially the H63D mutation, are associated with a significantly increased rate of SVR in chronic hepatitis C patients treated with interferon-based therapy.
association. It is possible that the H63D mutation might contribute to an overall advantageous immunogenetic profile, which may influence the response to interferon-based therapy. Recently, linkage disequilibrium was identified between the HFE-mutated gene and other proximal loci on chromosome 6p, and was implicated in improved virological response (Lebray et al. 2004). The HLA A29 haplotype, and to a lesser extent the HLA B44 haplotype, exhibited linkage disequilibrium with the H63D mutation. The precise role of these haplotypes, however, has yet to be elucidated (Porto et al. 1998). It is interesting to note that several MHC class I and class II loci appear to contribute to SVR under interferon treatment conditions (Puppo et al. 1995; Miyaguchi et al. 1997; Almarri et al. 1998; Kikuchi et al. 1998; Sim et al. 1998; Thursz et al. 1999). In addition, some genes for the interferon regulatory factors, such as IRF4, located in the same chromosomal region and not very far from the HFE gene, may also contribute to this phenotype (Lebray et al. 2004). However, further study is required to determine the linkage disequilibrium between HFE mutations and these genes. Moreover, recent studies of C282Y/C282Y patients have identified immunologic changes, such as lower CD8 T-lymphocyte numbers (Cruz et al. 2006) and reduced expression of MHC class I molecules on the surface of blood mononuclear cells (de Almeida et al. 2005). Finally, it was recently reported that patients infected with HCV genotype 3a had higher expression of hepatic MHC class I and HFE (Cardoso et al. 2004).

Considering that the C282Y mutation is reported more rarely than the H63D mutation (Candore et al. 2002; Kucinskas et al. 2012), it is not surprising that the number of C282Y-carrying patients was small in the studies in our meta-analysis. Meanwhile, in three of the enrolled studies, patients with the C282Y mutation displayed no SVR; however, the group of C282Y patients was too small to allow for detection of a statistically significant difference from the WT group (*i.e.* risk of type II error). The results of our meta-analysis showed a trend for patients with the C282Y mutation to have better responses than patients with the WT genotype (OR = 1.19, 95% CI: 0.71 - 1.98, *P* = 0.510), suggesting an effect similar to that observed for the H63D mutation. However, the potential association between C282Y mutation and SVR rate could not be verified by our analysis and further investigation with a much larger group of patients is required.

Similarly, the S65C mutation has a very low frequency (Candore et al. 2002; Kucinskas et al. 2012). For this reason, we could not investigate the potential association between S65C mutation and SVR rate in chronic hepatitis C patients treated with interferon-based therapy.

Based on these results, HFE mutations, especially the H63D mutation, appear to be associated with a significantly increased rate of SVR in chronic hepatitis C patients treated with interferon-based therapy. This association may prove useful as a predictive marker of a given HCV patient’s response to interferon-based therapy. Further investigation of this mechanism may also provide new strategies for HCV treatment.

It is important to note that this meta-analysis has some inherent limitations that may affect the generalizability of our results. Many other non-genetic and genetic factors are known to influence a chronic HCV patient’s response to antiviral therapy (Ikura et al. 1996; Kaserer et al. 1998; McHutchison et al. 1998; Poynard et al. 1998; Griffiths and Olynyk 2002; Fujita et al. 2007; Eslam et al. 2011; Li et al. 2011). Heterogeneity of these factors in the enrolled studies could have impacted the meta-analysis results. For example, HCV genotype, iron overload, insulin resistance, and other gene mutations (such as those in the IL28b gene) have been demonstrated as negative or positive risk factors of responsiveness to antiviral therapy (Ikura et al. 1996; Kaserer et al. 1998; McHutchison et al. 1998; Poynard et al. 1998; Griffiths and Olynyk 2002; Fujita et al. 2007; Eslam et al. 2011; Li et al. 2011). However, the heterogeneity of these influencing factors in the included studies could not be evaluated or adjusted, due to the limitation in the data extraction method.

In conclusion, the meta-analysis described herein provides evidence that the H63D mutation of the HFE gene is associated with a higher rate of SVR in chronic hepatitis C patients who were treated with interferon-based therapy.

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**Conflict of Interest**

All authors declare no conflict of interest.

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