Analysis of the hemochromatosis mutations C282Y and H63D in infertile men

The aim of this study was to find out whether C282Y and H63D mutations in the hemochromatosis (HFE) gene are associated with male infertility and whether the prevalence of the HFE mutations is higher in a group of 262 infertile men in comparison to 200 fertile men. Because the C282Y and H63D HFE gene distributions in infertile men were not significantly different from fertile controls, our data suggest that the C282Y and H63D HFE gene mutations are not risk factors for male infertility and are not associated with clinical manifestations of male infertility. (Fertil Steril® 2006;86:1796–8. ©2006 by American Society for Reproductive Medicine.)

Hereditary hemochromatosis is an autosomal recessive inherited disorder, with disease frequency estimated at 1/300 and a carrier rate 1/7 (1). It is a metabolic disorder of iron metabolism that is characterized by inappropriately high absorption of iron by the gastrointestinal mucosa. The excess of iron increases to toxic levels in tissues of liver, heart, brain, pancreas, and lungs. Hemochromatosis can develop into diseases such as diabetes, heart trouble, arthritis, liver disease, neurological problems, depression, impotence, infertility, and cancer. The gene for hereditary hemochromatosis (HFE) was cloned (2), and 2 of the 37 allelic variants of the HFE gene described to date (C282Y and H63D) are significantly correlated to HFE (3).

Recent studies of patients with hemochromatosis have shown significantly higher prevalence of severe sexual dysfunction (4) and changes in hormonal parameters due to impairment of hypothalamic–pituitary function by iron deposition (5, 6). Clinical hypogonadism was found in 46% of patients with hereditary hemochromatosis (7).

The animal models have shown that iron overload induce oxidative stress and the impairment of spermatogenesis, declined sperm production, as well as several morphological changes in seminiferous epithelium (8, 9).

Pathophysiology of male subfertility involves the complex network of genetic and environmental factors. Because the prevalence of both male infertility and hemochromatosis is high and increased iron level is in relationship with spermatogenesis impairment, we designed this study to test the hypothesis that mutations of the hemochromatosis gene are associated with male infertility.

We analyzed a group of 262 infertile men with normal spermiograms who have attended the Andrology Centre of the outpatient infertility clinic, Department of Obstetrics and Gynecology in Ljubljana, and have underwent an andrological examination. The testicular volume was measured using a Prader’s orchidometer. From the study we excluded infertile men with anamnesis of testis carcinoma, congenital bilateral absence of deferens, acquired vassal obstruction, cytogenetic abnormalities, and microdeletions of the Y chromosome. The study group consisted of 105 men with nonobstructive azoospermia and 157 with oligozoospermia or oligoasthenoteratozoospermia (OAT). As controls, the blood of 200 men with proven fertility was analyzed. Informed consent was obtained from each participant. All patients were Slovene or of Slavic origin.

Semen was assessed according to the World Health Organization guidelines (10) with regard to sperm concentration (normal ≥20 × 10⁹ spermatozoa/mL), rapid progressive sperm motility (normal ≥25%), and normal morphology (normal ≥30%). Plasma FSH was measured using the microparticle enzyme immunoassay (AxSYM System, Abbott Laboratories, Abbott Park, IL). Normal ranges were FSH <8 IU/L.

The analysis of HFE gene mutations were performed using standard polymerase chain reaction (PCR) with the primers described by Feder et al. (11). After an initial denaturation step at 94°C for 5 minutes, cycle parameters were 30 cycles at 94°C for 1 minute, 55,5°C for 80 seconds, and 72°C for 1 minute. The programs were followed by the final extension step at 72°C for 7 minutes. After amplifications, the PCR products were digested with RsaI (343 bp; C282Y) and Mbol (294 bp; H63D) restriction enzymes and visualized on a 3% Nusieve 3:1 agarose gel. The products carrying the mutation gave restriction fragments of 203, 111, and 29 bp for C282Y and 237 and 57 bp for H63D, whereas fragments without mutation yielded products of 203, 140 bp and 138, 99, 57 bp, respectively.

Due to only four homozygotes for H63D and no homozygotes for C282Y identified in our patients, we pooled...
the heterozygotes and homozygotes in the analysis. Based on the number of 262 patients and 200 control subjects, the statistical power was 80% to find a 2.25-fold increase in the frequency of C282Y heterozygosity (carriers in control subjects, 6.5%). For H63D (carriers in control subjects, 25.0%), the statistical power was 80% to find a 1.5-fold increase in frequency. Statistical analysis was performed with the SPSS program (version 12.0; SPSS Inc., Chicago, IL). The χ² test was used to confirm that the observed genotype frequencies were in Hardy-Weinberg equilibrium, and to compare genotype distributions and allele frequencies for the polymorphisms in the group of infertile men with controls. The clinical characteristics of infertile men were expressed as mean ± SD. Spearman’s method was undertaken to assess the correlation of gene polymorphisms in infertile patients to clinical characteristics using Spearman’s correlation coefficient.

The mean clinical characteristics of 262 infertile men are shown in Table 1. The C282Y and H63D genotype distributions in both study groups were compatible with Hardy-Weinberg expectations. C282Y and H63D HFE gene distributions in infertile men (C282Y: YY 0.0%, CY 8.4%, CC 91.6%; H63D: DD 1.9%, HD 24.4%, HH 73.3%) were not significantly different (P = .45, P = .64, respectively) from fertile controls (C282Y: YY 0.0%, CY 6.5%, CC 93.5%; H63D: DD 3.0%, HD 22.0%, HH 75.0%). C282Y mutation were revealed at allele frequencies of 4.2% (22/524) in the group of infertile men and of 3.3% (13/400) in the group of fertile men (P = .45). In men with azoospermia and those with oligozoospermia or OAT, the C282Y mutation allele frequency in comparison to controls was 4.3% (9/210) (P = .52) and 4.1% (13/314) (P = .53), respectively. In the groups of infertile and fertile men, H63D mutation was present at allele frequencies of 14.5% (74/524) (P = .68) and of 14.0% (56/400) (P = .81), respectively. The compound C282Y/H63D heterozygotes in infertile men did not differ significantly from fertile controls (P = .45).

We have not found any correlation between the tested polymorphisms genotypes and clinical characteristics of infertile man (sperm concentration, rapid progressive sperm motility, normal morphology, testicular volume, FSH levels).

The lack of association between C282 and H63D mutations and male subfertility in our study could be explained either by the very low relative risk contributed by the two mutations (our study had a 80% power to detect 2.25-fold increase in C282Y and 1.5-fold increase in H63D heterozygosity frequencies) or simply because the increase of iron serum levels associated with the two mutations is not sufficient to significantly impair spermatogenesis in human.

In conclusion, we provide evidence that the C282Y and H63D HFE gene mutations are not important risk factors for male infertility.
for male infertility and are not associated with clinical manifestations of male infertility.

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REFERENCES


