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ORIGINAL ARTICLE

PREVALENCE OF THE HEREDITARY HEMOCHROMATOSIS MUTATIONS (C282Y, H63D AND S65C) IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

Hereditary hemochromatosis is considered to be one of the most frequent genetic diseases in Europeans. Mutations in the hemochromatosis (HFE) gene, which underlie this disease, have been characterized in the last decade. The most frequent HFE mutations are C282Y, H63D and S65C. The C282Y mutation is most frequent in the general population of northwest Europe; 1 in 8-10 persons being carriers and 1 in 200-400 being homozygotes. The prevalence of this mutation is much lower in southern Europe. The H63D mutation has an equally high prevalence in northwest and southern Europe. In this article we present data concerning the prevalence of the most frequent HFE mutations in Macedonians, Albanians and Gypsies in the Republic of Macedonia.

Key Words: hereditary hemochromatosis, HFE mutations, population screening.

INTRODUCTION

Hereditary hemochromatosis is a disease of abnormal iron metabolism (iron overload) characterized biochemically by increased transferrin saturation (>40%) and increased level of serum ferritin, and clinically, by early multi-systemic and non specific signs such as weakness, malaise, fatigue, impotence, abdominal pain, joint pain, and late sequelae which includes dark (bronze) skin pigmentation, diabetes mellitus, hepatic cirrhosis, primary liver cancer, hypogonadotrophic hypogonadism, cardiomyopathy and arthropathy. The mutations in the HFE gene responsible for hemochromatosis (C282Y and H63D) were first reported in 1996 [1-3], and they were shown to be the most prevalent mutations in the Caucasian population. Hereditary hemochromatosis is the most common genetic disease in the Caucasian population, far exceeding the combined incidence of cystic fibrosis, phenylketonuria and muscular dystrophies [4-6]. The relationship between the C282Y mutation and hemochromatosis is considered to be obvious (over 80-90% of the homozygotes have biochemical or clinical signs), whereas that between H63D and hemochromatosis is much more subtle (it has been shown that the prevalence of this mutation among patients with hemochromatosis is no higher than among healthy individuals) [7]. A much rarer mutation in the HFE gene, S65C, is associated with mild forms of hemochromatosis [8].

The prevalence of the C282Y mutation in the general population in Europe is estimated to be 9.2% (heterozygotes) and 0.4% (homozygotes). It is the highest in northwest Europe, where 1 in 8-10 (10.0-12.5%) are heterozygotes and 1 in 200-400 (0.25-0.5%) are homozygotes [4,6,8]. Heterozygotes are very common in Ireland (28.4%), Denmark (13.7%), Norway (12.8%) and Iceland (10.0%), and less common in southern Europe: Greece (2.6%), Italy (2.2%), Spain (4.5%), Turkey (0%) [9]. In the Slavic populations, prevalence differs in different populations (the former USSR 1.9%, Czech Republic 10.0%). This mutation is absent in populations outside Europe and America [9].

The prevalence of the H63D mutation is equally high in northwest and southern European populations, where

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22.0% are estimated to be heterozygotes and 2.0% are homozygotes, the highest in Spain (32.1%) and lower in Norway and the former USSR (18.0%). Apart from India, where the prevalence is reported to be 15.0%, the H63D mutation is very rare in the populations outside Europe and America [9].

The S65C mutation is rare in the studied populations: 2.5% in France [8], 1.5% in Denmark [10], and 1.1% in the USA [11].

The aim of this study was to determine the prevalence of HFE mutations (C282Y, H63D and S65C) in the general population of the Republic of Macedonia.

MATERIALS AND METHODS

A total of 200 random DNA samples from healthy individuals (100 Macedonians, 50 Albanians and 50 Gypsies), provided by the Macedonian human DNA bank (hDNAMKD), Institute of Immunobiology and Human Genetics at Skopje, Republic of Macedonia, were genotyped for HFE mutations (C282Y, H63D and S65C). Written consent was obtained from each individual enrolled in this study.

DNA was isolated from peripheral blood leukocytes by the phenol-chloroform extraction method [12]. The mutations were detected by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method [1,2,12]. Exon 4 was amplified in a 50 µL PCR reaction ($MgCl_2$ 1.5 mM, 2.5 U Taq Gold; Perkin Elmer, Roche Molecular Systems, Inc., Branchburg, NJ, USA; Tm 62°C, 40 cycles) and exon 2 in a 100 µL PCR reaction ($MgCl_2$ 2.0 mM, 2.5U Taq Gold; Perkin Elmer; Tm 62°C, 40 cycles), on a 96-well thermal cycler (PTC-100; MJ Research, Inc., Waltham, MA, USA) [14,15]. Twenty µL of the PCR products and 5 U of the corresponding restriction enzyme were used for the digestion reaction (buffer, temperature and time according to the manufacturer's recommendations; New England Biolabs (UK), Ltd., Hitchin, Hertfordshire, UK). The restriction fragments were visualized after electrophoresis in 3% agarose gel stained with ethidium bromide. The primers and restriction enzymes used are shown in Table 1. Quality control was ascertained by participation in the UK NEQAS HFE quality control scheme 5, 2001 (an international system of external quality control provided by UK NEQAS; 20 external quality control samples analyzed; accuracy result 100%).

RESULTS

The C282Y mutation was detected in the heterozygous state in one of the 100 genotyped Macedonian samples and was absent in the 50 Albanian and 50 Gypsy samples. No C282Y homozygotes were found in any group. The H63D mutation was found in the heterozygous state in 21.0% of the Macedonians, 24.0% of the Albanian and 14.0% of the Gypsy samples, and in the homozygous state in 2.0% of each of the three groups. The S65C mutation was found in the heterozygous state in 5.0% of the Macedonian and 2.0% of the Albanian samples, but in none of the Gypsy samples. No C65S homozygotes were found in any group (Fig. 1.)

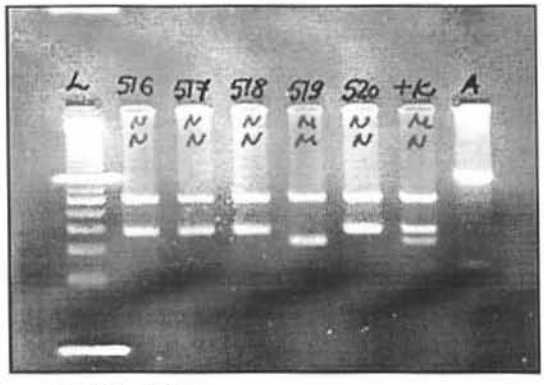
DISCUSSION

Hereditary hemochromatosis is the most prevalent monogenic disease in the Caucasian European population, the highest prevalence of HFE mutations being reported in European studies, and the C282Y mutation being the most prevalent in northwest Europe. This indi-

Table 1. Primers and restriction enzymes used for the diagnosis of HFE mutations [8,14-16]

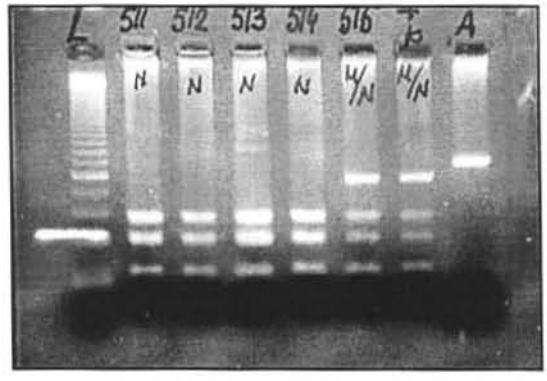
Mutation	Forward Primer (5'÷3')	Reverse Primer (5'÷3')	Restriction enzyme
C282Y *exon 4*	TGGCAAGGGTAAACAGATCC	CTCAGGCACTCCTCTCAACC	*Rsa*I; *Sna*BI
H63D *exon 2*	ACATGGTTAAGGCCTGTTGC	CTTGCTGTGGTTGTGATTTTCC	*Mbo*I, *Bcl*I
S65C *exon 2*	ACATGGTTAAGGCCTGTTGC	CTTGCTGTGGTTGTGATTTTCC	*Hin*fI

A. C282Y detection with Rsa I



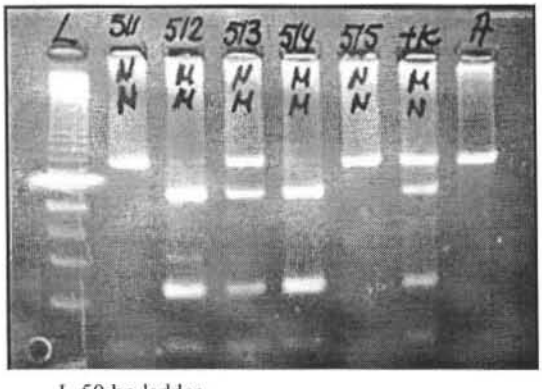
L-50 bp ladder
A-non-digested PCR fragment (4. exon)
lines 516-8, 520 are normal results
line +k is a C282Y heterozygote
line 519 is a C282Y homozygote

C. H63D detection with Mbo I



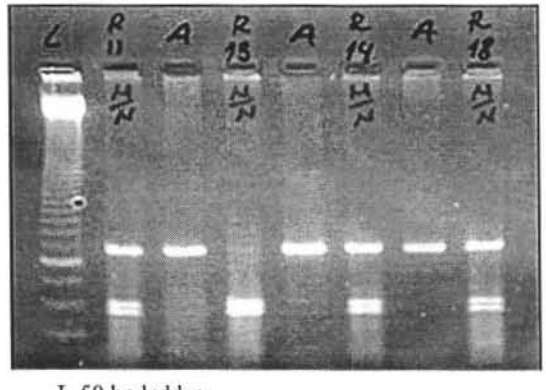
L-50 bp ladder
A-non-digested PCR fragment (2. exon)
lines 511-4, 520 are normal results
lines 516 and +k is a H63D heterozygote

B. C282Y detection with Sna BI



L-50 bp ladder
A-non-digested PCR fragment (4. exon)
lines 511 and 515 normal results
lines 513 and +k are C282Y heterozygotes
lines 512 and 514 C282Y homozygotes

D. H63D detection with Bcl I



L-50 bp ladder
A-non-digested PCR fragment (2. exon)
lines R 11, 13, 14 and 18 are H63D
heterozygotes

Fig. 1. Photographic reproduction of the agarose gels obtained after electrophoresis of the digested PCR products. A) C282Y detection with *Rsa*I; B) C282Y detection with *Sna*BI; C) H63D detection with *Mbo*I; D) H63D detection with *Bcl*I.

cates a European origin for this mutation. Prevalence of the H63D mutation is equally high in northwest and southern Europe [9]. Some studies have demonstrated that combined heterozygosity for C282Y/S65C is associated with a mild form of hemochromatosis [8], the S65C mutation prevalence being low in healthy individuals [8,10,11].

The results presented here reveal a low prevalence of the C282Y mutation in Macedonians and its absence among Albanians and Gypsies in the Republic of Macedonia. This is consistent with the reported low prevalence of this mutation in southern Europe (Greece, Italy, Spain) [9]. The prevalence of the H63D mutation is higher in Macedonians, Albanians and Gypsies in the Republic of Macedonia. This agrees with published results from other studies [9,13]. The prevalence of the S65C mutation among the Macedonians is higher than that reported from France, Denmark and the USA [10,11].

Given the fact that the consequences of iron overload in hereditary hemochromatosis are preventable by phlebotomy, the knowledge of the presence of these HFE mutations, and of the low C282Y prevalence in the Republic of Macedonia, raises the question of systematic HFE mutation screening in clinically relevant cases [4-6].

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