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Original Article

Frequencies of HFE gene mutations associated with haemochromatosis in the population of Libya living in Benghazi

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Abstract: The studies of the HFE mutations: H63D and C282Y in North African populations have revealed the extreme rarity or even the absence of the C282Y mutation. We have examined 200 chromosomes (100 Libyan people live in Benghazi) for the presence of the two HFE mutations by PCR-RFLP analysis by using PCR conditions used to amplify both Autosomal and Y chromosomal STRs. We have found that the allele frequencies are, respectively, 17% for the H63D and 0% for the C282Y. These results are consistent with the worldwide spread of the H63D mutation and the north European restriction of the C282Y.

Keywords: HFE gene mutations, H63D, C282Y, haemochromatosis, allele frequency, Benghazi, Libyan population

Introduction

Hereditary hemochromatosis (HH) is an autosomal recessive blood disorder that is characterized by an increase in iron absorption, which, if untreated, can lead to excessive iron deposits in organs and eventual organ failure [1]. HH is caused by a mutation of the HFE gene that regulates iron absorption. Since the disease is recessive, a person must have two mutated genes in order for the disorder to be expressed (termed homozygous). The two main mutations that can occur are C282Y mutation (accounting for 90-95% of cases) and H63D mutation. H63D is associated with a less severe form of iron absorption than a C282Y mutation. Although fairly uncommon, people can also exhibit signs and symptoms of HH if they possess only one mutated gene (termed heterozygous), but generally they are only carriers in this case [2]. Most people absorb roughly one milligram of iron per day from their diets, which is then equally excreted through sweat, sloughing skin cells, urine, and the gastrointestinal tract. In contrast, people with HH can absorb anywhere from two to four milligrams per day, but the excretion rate remains the same [3]. Premenopausal women tend to have lower iron levels and fewer complications from HH than men since they lose blood (and iron) during menstruation [4]. Over time the body begins to deposit excess iron in organs such as the liver, heart, brain, pancreas, and lungs [2]. Common symptoms of HH include extreme fatigue, impotence and infertility, skin hyperpigmentation (also referred to as bronzing), depression, abdominal pain, arthritis/joint pain, diabetes, liver disease, and heart disease [5]. Diagnosis typically does not occur until between the ages 40 to 60, when iron levels have already reached toxic, damaging levels [2]. Since 1996, several studies have been performed in order to evaluate the H63D and C282Y frequencies in different populations and to provide information about the worldwide HFE mutations distribution. We notified that no or few information was found about the H63D and C282Y frequencies in Libyan and in North Africa generally. The aims of our study are to determine the two missense mutations frequencies in Libyan population living in Benghazi trying to use this phenotyping disease (correlation between phenotyping and genotyping) in positive samples in Human identification for forensic application and to com-

Figure 1. Map of Libya showing the city of Benghazi (http://en.wikipedia.org/wiki/Benghazi).

pare these results to other studies.

Population

Libya, a Northern African country, was first inhabited by Berbers, followed by Phoenicians, Greeks, Romans, Arabs and Ottomans. Libya became independent in 1951 after a brief period as an Italian colony; it had been invaded by Italy in 1911. In February 2011 an uprising against the government occurred in the city. Benghazi is the second largest city in Libya and the main city (or capital) of the Cyrenaica region (or ex-Province), located in the North of Africa. Benghazi is located half way between Tripoli in the West (a distance of approximately 1000 Km between these cities) and Cairo in the East (also approximately 1000 Km) (**Figure 1**). Cyrenaica is surrounded by desert on three sides; hence in ancient times the most accessible civilization was to the North, across the Mediterranean, in Crete and Greece, only 400 km away. The population of Benghazi was 500, 120 in 1995 (census) and increased to 670, 797 in the 2006 census. As with other cities in Libya, there is a reasonable amount of ethnic diversity in Benghazi. The people of eastern Libya, Benghazi included, have in the past always been of predominantly Arab descent. In recent times, however, there has been an influx of African immigrants into Benghazi. There are also many Egyptian immigrants in Benghazi. A small Greek community also exists in Benghazi; the Greek island of Crete is a short distance from Benghazi and many families in Benghazi today bear Cretian surnames.

In modern times, Benghazi has seen a lot of Libyans from different parts of the country move into the city, especially since the Kingdom era (1951-1969). Many Libyans came to Benghazi from Misrata (About 60% of the population have roots from Misrata, West of Benghazi).

Materials and methods

Informed consent was obtained from100 unrelated Libyan individuals (Benghazi region). Fifty of those samples are male and fifty are female.

DNA extraction

DNA was extracted from blood stains collected on FTA® cards (Whatman, Kent, UK) using FTA® Purification Reagent (Whatman) following the manufacturer's protocol and from buccal swabs using QIAamp®DNA Blood Mini Kit (QIAGEN, Hilden, Germany). DNA was quantified using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, USA).

PCR amplification

About 1.2 mm FTA® disc and 1 ng of DNA purified from buccal swabs was used .Separate PCR are conducted for the two mutations using the pairs of primers previously described by Feder, et al; 1996 [6]. The PCR mixtures contained 16 pmol of primers, 200 µmol of each desoxy nucleotide triphosphate, 100 ng of genomic DNA, 2.5 µl 10×buffers and 0.2 U of Taq polymerase in a final volume of 25 µl. These amplifications were done for 5-min denaturizing at 95 °C followed by 30 cycles of 30-s denaturation at 94° C, 30-s annealing at 58°C and 1-min extension at 72°C. Finally an extension cycle of 5 min at 72°C. Then RFLP analysis [7, 8]. Were made in order to determine the HFE genotype of these samples.

We have tested some amplification of these mutations with PCR conditions used to amplify Autosomal and Y Chromosomal kits and good results were obtained.

PCR was carried out in a Thermocycler 9700

from Applied Biosystems. PCR conditions were as follows: starting temperature was 95°C for 11 min, (denaturing temperature 95°C for 1min, annealing 59°C for 1min, extension 60° C for 1min, 27 cycles), then 60°C for 80 minutes and hold at 4°C, according to the manufacturer's protocol (AmpFlSTR® Yfiler™ and Identifiler Amplification KitsUser's Manual).

C282Y and H63D mutations were screened using enzymatic digestion of which PCR products encompassing the mutation sites.' The C282Y mutation creates a found in new RsaI restriction site. The 390 bp PCR reaction product (forward primer 5'-TGGCAAGGGTAAACAG ATCC-3' and reverse primer 5' CTCAGGCACTCCT-CTCAACC-3') digested with RsaI (E coli strain, gene from Rhodopseudomonas shaeroides) shows cites of two fragments of 249 and 141 bp in normal DNA, while mutant DNA generates two new fragments (112 and 29 bp). The H63D mutation destroys an MboI (E coli strain, gene from Moraxella bovis) site 294 bp PCR product (forward primer 5'-ACATGGTTAAGGCCTGTTGC-3' and reverse primer 5'-CTTGCTGTGGTTGTGATT TTCC-3'), while normal DNA generates three fragments of 138, 99, and 57 bp.

13 µl of the PCR product of 208 bp (H63D) and 390 bp (C282Y) were digested using, respectively, 4 U of MboI (Biolabs, R0147S) and 2U RsaI (Biolabs, R0167S) enzyme. Added to 4U NEBuffer 4 In a final step, the digested products were incubated at 37°C. Over night and run on a 2 % agarose gel for 1 h and photographed under UV light.

Results

Two hundred Libyan chromosomes from Benghazi were analysed. H63D and C282Y mutation were observed at frequencies of 17% and 0 %, respectively.

Of 100 samples from Benghazi (50 females and 50 males) analysed for both H63D and C282Y mutation.

For H63D mutation 17% were positive for site 294 bp and other samples Amplify normal H63d sites (138, 99, and 57 bp) (**Figure 2**).

And 0% for C282Y mutation (in all 100 samples only normal two sites observed 141bp and 249bp) positive mutation sites not detected (112 and 29bp) (**Figure 3**).

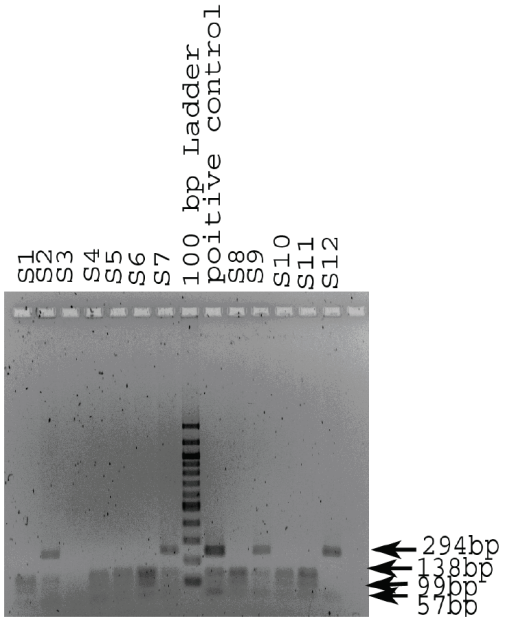

Figure 2. Determination of genotype in the HFE gene codon 282 region. The normal genotype gives rise to 2 RsaI fragments of the codon 282 region PCR product (203bp and 152bp) these were seen for all samples tested on this and other gels. The C282Y mutation, if present, introduces a second RsaI site resulting in three bands (203bp, 123bp and 29bp).

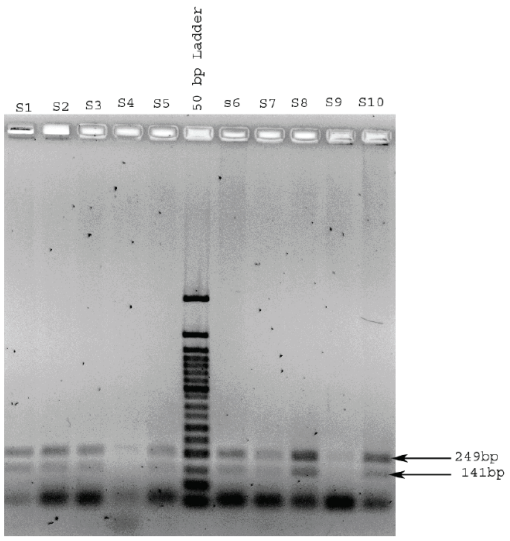

Figure 3. Determination of genotype in the HFE gene codon 63 region. The normal genotype gives rise to 3 MboI fragments of the codon 63 region PCR product (138bp, 99bp and 57bp) (H1, H3, H4, H5, H7, H9 and H10). The H63D mutation abolishes one MboI site resulting in the 237bp band. Heterozygotes give all 4 bands (H2, H6, H8 and the positive control). H11 is homozygous for H63D (237bp and 57bp only).

Table 1. Estimated prevalence of Haemochromatosis mutation in the general population

	N	H63D allele frequency	C282Y frequency	References
Europe				
North				
North Ireland	404	14.10%	9.9%	Murphy, 1998
Denmark	200	12.75%	6.75%	Steffen, 1998
Norway	94	11.20%	6.4%	Clarke, 1997
Sweden	117	12.39%	3.84%	Cardoso, 1998
South				
France	503	12.23%	1.07%	Piperno, 1998
Germany	153	14.07%	2.96%	Gottschalk, 1998
Greece	196	13.50%	1.3%	Clarke, 1997
Spain	420	20.40%	3.09%	Sanchez, 1998
Turkey	70	13.57%	0%	Clarke, 1997
Africa/Middle East				
Middle East				
Saudi Arabia	118	8.5%	0%	Clarke, 1997
North				
North Africa	171	13.2%	9.0%	Aguilar, 1997
Benghazi-Libya	100	17%	0%	Current study
South				
Gambia	39	1.3%	0%	Clarke, 1997
Senegal	130	0%	0%	Clarke, 1997
Kenya	78	1.3%	0%	Clarke, 1997
Nigeria	80	1.9%	0%	Clarke, 1997
Zambia	76	0.7%	0%	Clarke, 1997
Asia				
China	72	2.8%	0%	Clarke, 1997
Indonesia	90	2.8%	0%	Clarke, 1997
Taiwan	80	1.9%	0%	Clarke, 1997
Australasia				
Australia (aboriginals)	93	0%	0%	Clarke, 1997
Australia (vanuatuans)	90	0.6%	0%	Clarke, 1997
America				
Mexico	54	6.5%	0%	Clarke ,1997
Jamaica	90	2.2%	1.1%	Clarke, 1997
United States	5171	13.5%	5.4%	Karen, 2001

Discussion and conclusion

The discovery of the HFE gene and the C282Y, H63D mutations implicated in the onset of HH have been pursued researchers to study the prevalence of these two missense mutation around the world (**Table 1**). However, few information was found about the H63D and C282Y mutation distribution in North Africa. [9-12] and in the Arabian population [13]. This indicated the low prevalence or the absence of the C282Y mutation in these populations. Our study included 100 samples collected from Benghazi (Eastern Libya). The H63D allele frequency in Libyan population seems to be higher compared with Algerian Mzab population (9%) [11] and that found in Saudi Arabians population (8.5%) [13]. that frequency is getting closer to that reported from European population like British Irish (18.9%) [13] but remains lesser than the highest European frequency found in Spain (**Table 1**). Our results agree with the fact that the mutation responsible for the His 63 Asp substitution is not restricted to the European population.

Roth et al. thought that the presence of the H63D around the world and the restriction of the C282Y to European populations may suggest that appears of the first preceded the second.

We have found that the H63D allele frequency is 17% and the C282Y allele frequency is 0%. More detailed studies are needed to identify the correlation between genotype and the phenotype of HH in our population and to determine whether the H63D mutation itself can results in clinical haemochromatosis, or whether other associated risk factors are required in Human

identification.

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