EDITORIAL

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What is the future of research for hereditary hemochromatosis in Turkey?

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Although the condition was first described in 1865 by Armand Trousseau as "bronze diabetes"(1), the actual association with iron accumulation was established by von Recklinghausen in 1890 who also coined the term "hemochromatosis" (2). Hereditary hemochromatosis (HH) has since become one of the most extensively studied genetic disorders of metabolism. A major breakthrough regarding the pathogenesis of the autosomal recessive condition came with the identification of the HFE gene located on the short arm of chromosome 6 which codes for a membrane protein which plays an important role in the regulation of iron absorption in the small intestine (3). HFE-related HH was eventually classified as type 1, with mutations in several other genes (hemojuvelin, hepcidin, transferrin receptor 2, ferroprotin, etc.) later identified. More than 20 different mutations in the HFE gene have been found to result in clinical manifestation of the condition, the most commonly encountered mutations being the major allele C282Y (Cystein-282-Tryrosine or G845A) and the minor allele H63D (Histidine-63-Aspartate or C187G) (4).

Early epidemiological studies have reported on varying frequencies of HH with a higher incidence and prevalence at higher latitudes and in individuals of European descent. It is estimated that between 1 in 200-300 individuals in the US and Western Europe have iron overload due to HH with an even larger number of persons having genetic mutations associated with an increased risk for hemochromatosis (5-7).

Karaca et al. (8) reported on an overall prevalence of 43 per 100,000 individuals after evaluating 2304 outpatients from the Central Anatolia region of Turkey. In this study, patients with a fasting transferrin saturation (TS) of more than 45% for women and 50% for men were subjected to further evaluation of serum ferritin levels and genetic analysis for the two mutations C282Y and H63D, commonly associated with HH. Overall, only 14 patients (9 male and 5 female) had TS values suggestive of iron overload. While traditionally ferritin levels greater than 300 ng/mL in men and 200 ng/mL are considered suggestive of a diagnosis of hemochromatosis, in this study, only 1 patient with elevated TS had a serum ferritin concentration in excess of 200 ng/mL. Furthermore, mutations were detected in only 3 patients with ferritin levels of 28 ng/mL (25 y.o. female; H63D heterozygote), 111.4 ng/mL (53 y.o. male; C282Y homozygote) and 754ng/mL (43 y.o. male; C282Y heterozygote). None of the patients with ascertained mutations had clinically apparent hemochromatosis. The frequency of iron overload due to HH in Turkey has been evaluated in three previous studies with inconsistent results; 2 on blood-donors, and 1 on the general population. In one of the earlier studies from Turkey by Barut et al. (9), 26 out of 4633 healthy adults screened were found to have iron overload (fasting TS ≥ 50%). Homozygote H63D mutation was detected in only 1 male subject who had a normal serum ferritin level whereas H63D heterozygosity was observed in 10 males and 1 female. C282Y mutation was not detected in any of the patients. Only 1 patient who was heterozygote for the H63D mutation had a markedly elevated ferritin level, in whom a diagnosis of HH

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was confirmed by a liver biopsy. In another study by Bozkaya et al. (10) on 3060 blood donors, only 5 had low fasting unbound iron-binding capacity (< 28 μM) and a fasting TS>45%, consistent with iron overload. However, none of the patients had hyperferritinemia and genetic analysis reveled the presence of H63D heterozygosity in only two patients. A similar study by Simsek et al. (11) published in the same year evaluated the frequency of iron overload and common HFE-related HH mutations in 2677 blood donors. An interesting feature of this study was the relatively high rate of iron overload of 9.9% compared to other studies from Turkey. From the report, it is not clear whether blood samples were obtained in the fasting state or not, which could explain this discrepancy. Overall, genetic analysis was performed on 86 individuals with a TS≥ 45% and 57 individuals with a TS <45%. While H63D heterozygosity was detected in 42% and 29.1% of individuals with TS<45% and ≥45%, respectively (p>0.05), none of the participants with TS<45% were homozygote for the H63D mutation compared to a homozygosity rate of 12.8% in individuals with TS ≥45%. Information on the number of patients with elevated ferritin levels and elevated liver iron content was not included in the study. A most recent study awaiting publicationby Dulger et al. (12) produced an interesting result in that H63D mutations were detected in 5.6% of 159 seemingly healthy individuals, a rate much higher than any other study from Turkey. It was also observed that patients with mutations had significantly elevated levels both of serum iron and transferrin saturation. Epidemiological studies from Turkey on HH have been summarized in Table 1 (8-12).

Despite the obvious inconsistencies, a striking feature that is shared by available studies from Turkey is the absence of the C282Y mutation, and in this regard the study Karaca et al. (8) is the first to identify this mutation in a cohort of healthy individuals, although the same mutation has been reported once before in 6 members of the same Turkish family (13). In 2005, Simsek et al. (14) attempted to identify mutations in the HFE genes of a cohort of patients with confirmed hereditary hemochromatosis. All five patients tested were heterozygote for the H63D mutation, which is intriguing since H63D heterozygosity is not normally expected to result in iron overload. Investigators postulated that other non-HFE gene mutations may have contributed to the clinical picture in the patients involved. Considering that the only patient with HH in the Barut study was also heterozygote for the H63D mutation, and the 5 patients with iron overload in the Bozkaya study had H63D heterozygosity, it would seem that other mutations may indeed be present in the Turkish population.

There is an increasing body of evidence supporting the presence of mutations that result in what is now known as non-HFE hemochromatosis (15). HH Type 2A and 2B have been linked with several mutations in the HJV gene which encodes a protein called hemojuvelin. Hemojuvelin has been shown to play a critical role in hepcidin regulation, and the resultant decrease in hepcidin levels disrupts iron hemostasis leading to excess iron absorption. Type 3 HH, which was first identified in 2000, has been attributed to a mutation in the TFR2 gene. The product of this gene, the transferrin receptor 2 protein, which is expressed exclusi-

Table 1. Summary of epidemiological studies from Turkey on hereditary hemochromatosis (8-10,12,13)

Study/year	Screening method for IO	Population	Overall frequency of IO	Genetic analysis*			
				C282Y/ C282Y	C282Y/ Wild	H63D/ H63D	H63D/ Wild
Barut et al. 2003	TS >50%	General population (volunteers)	0.56%	0	0	3.8%	42.3%
Bozkaya et al. 2004	Fasting unbound iron-binding capacity level of <28 microM	Blood donors	0.16%	0	0	0	20%
Simsek et al. 2004	T/S >45%	Blood donors	9.9%	0	0	12.79%	14.53%
Dulger et al. 2012	?	?	?	0	0	2%	7%
Karaca et al. 2012	TS >50% in men TS>45% in women	Outpatients (internal medicine)	0.043%	7.14%	7.14%	0	7.14%

IO, iron overload * genetic analysis only performed on consenting individuals who screened positive for iron overload and the results provided only represent those in whom mutations were investigated.

vely in the liver is believed to be a sensor of iron levels and is also involved in hepcidin synthesis. Inheritance of both TFR2 and HFE mutations are known to lead to an earlier onset of iron overload. Type 4 HH, also known as ferroportin disease, is different from the other types in that it is an autosomal dominant disorder. Mutations in the SLC40A1 gene affect the production of ferroportin which has been shown to be responsible for iron transportation across the enterocyte surface and for iron recycling in the reticuloendothelial system. Hepcidin also binds to ferroportin, thus promoting its internalization and degradation, leading to a decrease in iron absorption. Although patients with ferroportin disease typically present with low to normal TS and iron overload within macrophages, some cases may present with high TS and an iron overload predominantly in hepatocytes, similar to HFE-related HH.

Screening for HH has been a controversial topic, particularly in the USA and Western Europe where HFE mutations are quite prevalent. Although TS with the currently accepted cut-off (45% for women and 50% for men) seems to be a reliable screening test for iron overload, this may not be reflected in serum ferritin levels. The main issue regarding population-based screening is the low penetrance of HFE-related hemochromatosis which translates into the majority of individuals with a detected mutation neither clinically manifesting the condition nor likely to ever develop it. It has also been postulated that utilization of transferrin saturation as a screening test may not be cost effective, and that ferritin, albeit with varying cut-off points, offers a feasible alternative for screening (16). Following an assessment by the U.S. Preventive Services Task Force (USPSTF), the reported frequency of HFE-gene mutation homozygosity was 4.4 per 1000 among white persons, with much lower frequencies among Hispanic persons (0.27 per 1000), black persons (0.14 per 1000), and Asian-American persons (< 0.001 per 1000). Investigators concluded that "the potential harms of genetic screening for hereditary hemochromatosis outweigh the potential benefits", and that asymptomatic individuals should not be routinely screened. However, experts also stressed that this recommendation does not include individuals with a family history of clinically detected or screening-detected probands for HH (17). A similar message is reflected in national guidelines in the United Kingdom (18) and others European countries. Considering the low prevalence of HH in Turkey, screening may not be a cost effective option. However, the high rate of consanguineous marriages in Turkey suggests that couples would benefit greatly from screening and genetic counseling, especially in the presence of a positive family history of HH.

Focus of future research regarding HH in Turkey could be towards discovering other mutations (particularly non-HFE-related) that my lead to iron overload, as well as the potential role of gene therapy for correcting this inborn error of metabolism. An interesting article by Ezquer et al. explored several aspects of gene therapy aimed at regulating iron absorption (19). Inhibition of DMT-1 gene expression in intestinal cells, inhibition of ferroportin gene expression in enterocytes, increasing expression of the wild-type HFE gene in enterocytes, and prevention of over expression of the iron regulatory peptide hepcidin in the liver have all been investigated as potential targets. Gene delivery has mainly been established using AAV, a human parvovirus carrying a small, single-stranded DNA genome, and other adenoviral vectors and although initial results look promising, further research is needed before this option may be considered for the management of this condition.

Previous studies on non-European populations have demonstrated much lower rates of HFE gene mutations in Asia, Africa and Australia (20) compared to Europe and North America, and it would seem that the prevalence of HH follows a pattern similar to other parts of the world. Available studies have firmly established the frequency of HH and associated mutations, and although precise numbers from Turkey may not be available, current studies suggest that HFE-related HH mutations are rare. It would seem that undergoing a population-based study to establish the frequency of the condition Turkey is an expensive and unnecessary venture. Instead, research should perhaps be directed towards identifying non-HFE related mutations that may be responsible for iron overload in populations with a low rate of common HFE mutations.

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