Reports of Biochemistry & Molecular Biology
Vol. 3, No. 2, Apr 2015

Original paper

Determination of haptoglobin genotype in an Iranian population with idiopathic generalized epilepsy

Sukaina Al-balaghee[1], Zeinab Al-balaghee[1], Ashraf Shabani[1], Parinaz Ghadam[1], Mojgan Bandehpour[2,3], Ali Askari Mehr[4], Bahram Kazemi*[2, 3]

Abstract

Background: Haptoglobin (Hp) is a plasma α_2-sialoglycoprotein that contains alpha and beta chains. It displays in three common phenotypes, Hp1-1, Hp2-1, and Hp2-2. Proteins expressed by polymorphic genes have grossly different molecular sizes resulting in different diffusion rates in the brain. Haptoglobin expressed by the Hp2-2 genotype has lower hemoglobin-binding capacity than Hp1-1 or Hp2-1 and is associated with idiopathic generalized epilepsy.

Methods: To determine polymorphism in haptoglobin genes in patients with idiopathic generalized tonic-clonic seizures, 42 men, 42 women, and 50 controls were selected for this study. Genomic DNA was extracted from blood and studied by polymerase chain reactions (PCR).

Results: The amplified fragments for the Hp1-1 and Hp2-2 genotypes were 1757 and 3481 base pairs (bp) respectively, and the Hp2-1 genotype had both fragments, in addition to a 349-bp fragment. The distribution of the three major Hp phenotypes in epilepsy patients was 28.6 (1-1), 38.1 (2-1), and 33.3% (2-2) in the men, and 31 (1-1), 40.5 (2-1), and 28.6% (2-2) in the women. The distribution of Hp genotypes in controls was 22 (1-1), 40 (2-1), and 38% (2-2).

Conclusion: We show that all Hp genotypes participate in idiopathic generalized epilepsy.

Keywords: Epilepsy, Haptoglobin, Iran

Introduction

Haptoglobin (Hp) is an -α_2-sialoglycoprotein acute-phase reactant that binds to free haemoglobin (Hb) and forms a stoichiometrically stable complex. The name haptoglobin comes from “hapto” to bind, and globin. Polonovski and Jayle discovered haptoglobin in 1938 (1) and later Smithies determined its genetic variations (2). The primary function of Hp is to bind Hb, thereby preventing renal excretion of iron and to protect blood vessels from Hb’s oxidative effects (3). Haptoglobin is a tetrameric protein that structurally resembles immunoglobulin. It has two light and two heavy chains covalently bound to each other by disulphide bridges (S-S) (4). Although present in all vertebrates, in humans Hp is characterized by molecular heterogeneity caused by genetic polymorphism. Smithies identified three main phenotypes: Hp1-1, Hp2-1, and Hp2-2 (2). Subsequently, Smithies and Walker showed that these phenotypes were controlled by two autosomal co-dominant alleles identified as HP1 and HP2 (5). The heavy (β-) chain of Hp has a molecular weight of 40 kDa and is not

1: Department of Biology, Al Zahra University, Tehran, Iran
2: Cellular and Molecular Biology Research Centre, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3: Biotechnology Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4: Iranian Epilepsy Association, Tehran, Iran
***Corresponding author: Bahram Kazemi; Tel: +98 21 22439957; Fax: ++98 21- 89784665; E-mail: kazemi@sbmu.ac.ir**

Received: Jul 15, 2014; Accepted: Oct 22, 2014

polymorphic. Haptoglobin polymorphism reflects inherited variations in the α-chain that result from differences between the α1 (8.9 kDa) and α2-chains (16 kDa) (6). The α1-chain can be further classified into α1S (slow) or α1F (fast), depending on the electrophoretic mobility. The difference between these chains lies in the amino acids at positions 52 and 53, which are asparagine and glutamic acid in α1S, and aspartic acid and lysine in α1F (7, 8). This polymorphism results in Hp's with different molecular masses of 86 kDa for Hp1-1, 86-300 kDa for Hp2-1, and 170-900 kDa for Hp2-2 (6, 9).

The human Hp gene consists of three structural alleles: Hp1F, Hp1S, and Hp2. The products of the Hp1F and Hp1S alleles differ by only one amino acid; Lys54 of the Hp1S-chain is replaced by Glu in the Hp1F-chain (10). The Hp2 allele, probably originated by a non-homologous crossing-over event, is the result of a fusion of the Hp1F and Hp1S alleles, and is present only in humans (11). Hp1-1 is biologically the most effective gene product in binding free hemoglobin and suppressing inflammatory responses associated with extracellular (free) hemoglobin (6). In contrast, Hp2-2 is the least effective. The plasma concentrations of haptoglobin are highest in individuals with Hp1-1 and lowest in those with Hp2-2, with intermediate concentrations in Hp2-1 individuals (6). Haptoglobin also has antioxidant (12, 13), angiogenic (14), and anti-inflammatory effects (15, 16). If hemoglobin (or its iron) is involved in the etiology of seizures, then inadequate removal of hemoglobin by haptoglobin may be important (9). Haptoglobin expressed by the Hp2-2 genotype has lower hemoglobin-binding capacity than Hp1-1 or Hp2-1, and studies have associated this genotype with idiopathic generalized epilepsy (15, 17).

In this study the relationship between haptoglobin phenotypes with normal and idiopathic epileptic populations were determined by polymerase chain reaction (PCR). Amplification of genotypes Hp1-1 and Hp2-2 produced single bands of 1757 and 3481 bp's, respectively, and genotype Hp2-1 produced both the 1757 and 3481 bp bands. Additionally, a 349 bp Hp2-1-specific product was amplified.

Materials and Methods

Samples

In this study DNA samples from 42 male and 42 female patients with idiopathic generalized tonic-clonic seizures, and 50 healthy controls were analyzed. The epileptic patients were between 20 and 50 years of age with body mass indexes (BMI's) of 2.27 to 2.97. Forty-eight percent of the men were smokers. The patients' systolic and diastolic blood pressures were 140-145 and 81-87 mmHg, respectively. Patients' blood cholesterol values were 232-231 mg/dl, LDLs were 140-145 mg/dl, and HDLs were 35-38 mg/dl. All 84 patients were from the Iranian Epilepsy Association and Tehran laboratory. Blood samples were collected in the presence of EDTA, and the plasma was stored at -70 ºC.

DNA Extraction and PCR amplification

DNA was extracted from whole blood using DNG ^{TM} plus solution (Cinnagen, Tehran, Iran). Two PCR's were performed on each DNA sample:

1) Primers

A: 5'- GAGGGGAGCTTGCCTTTCCATTG- 3' and B: 5-' GAGATTTTTGAGCCCTGGCTGGT-3' amplified 1757 and 3481-bp fragments from Hp1-1 and Hp2-2, respectively, and both bands from Hp2-1.2) Primers

C: 5'-CCTGCCTCGTATTAACTGCACCAT-3' and D: 5' CCGAGTGCTCCACATAGCCATGT-3' amplified a 349-bp fragment from Hp2-1 only.

Reaction mixtures included 1 µl of DNA, 150 µM dNTP's, 4 pmol each of the forward and reverse primers, 1.5 mM MgCl2, 1X PCR buffer, and 1.25 units of Taq DNA polymerase in a final volume of 30µl. Amplifications were performed using the following parameters: denaturing at 94 °C for 30 sec, annealing at 65 °C for 30 sec, and extension at 72 °C for 45 sec, repeated for 30 cycles. Reactions were incubated at 94 and 72 °C for 5 min each before and after PCR cycling.

Detection of PCR products

PCR products were analyzed by electrophoresis on 1% agarose gels and DNA bands were observed on UV Trans-illuminator after SYBR green staining.

Results

After electrophoresis of the PCR products in 1% agarose gels, Hp genotype-specific fragments were observed; the 1757 and 3481-bp fragments for genotypes Hp1-1 (Fig. 1 lane 3) and Hp2-2 (Fig. 1 lane 2) respectively, and both bands for genotype Hp2-1 (Fig. 1 lane 1). The 349-bp Hp2-1-specific product was amplified with primers C and D (Fig. 2).

Table 1 shows the distribution of Hp genotypes and alleles in idiopathic generalized epilepsy and controls.

The distribution of the three major Hp phenotypes in epilepsy patients was 28.6% [1-1], 38.1% [2-1], and 33.3% [2-2] in the men, and 31% [1-1], 40.5% [2-1], and 28.6% [2-2] in the women. The distribution of the Hp genotypes in control subjects was 22% [1-1], 40.0% [2-1], and 38% [2-2]. The chi- square test determined that the influence of Hp phenotypes in epilepsy was gender-independent (χ^2=0.8 p>0.05), and also a correlation was observed between Hp2 allele and the risk of epilepsy (χ^2=45.01 p<0.001).

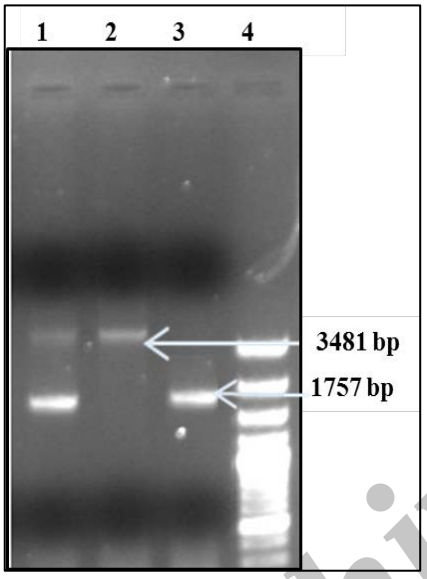

Fig. 1: Haptoglobin genotyping using primers A and B. Lane 1: genotype Hp2-1 produced both the 1757 and 3481 bp bands, lane 2: genotype Hp2-2 produced single band of 3481 bp, lane 3: genotype Hp1-1 produced single band of 1757 bp, lane 4: 100 bp DNA ladder marker.

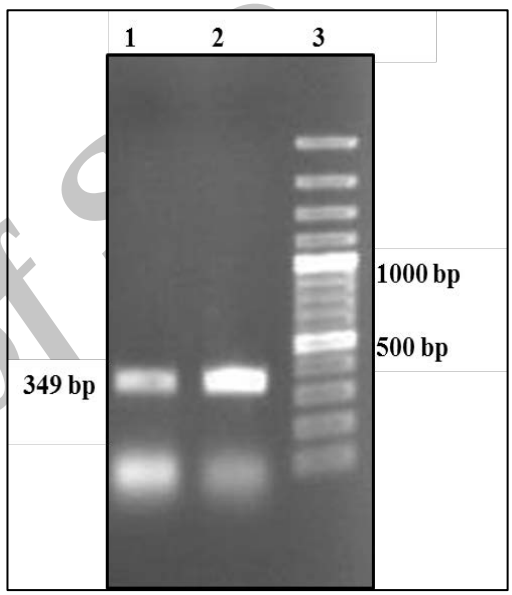

Fig. 2: Haptoglobin genotyping using primers C and D Lanes 1 and 2: Hp2 –specific amplification product, lane 3: 100 bp DNA ladder marker.

Table 1. The relationship between epilepsy and haptoglobin genotypes

Hp genotype / Sex	Hp1-1 (%)	Hp2-1 (%)	Hp2-2 (%)	Hp alleles 1 (%)	Hp alleles 2 (%)
Women	31	40.5	28.6	51.19	48.6
Men	28.6	38.1	33.3	47.60	52.38
Healthy control	22	40	38	42	58

Discussion

Hemoglobin is involved in the etiology of seizures; therefore, removal of hemoglobin by haptoglobin may be important (9). In addition, haptoglobin expressed by the Hp2-2 genotype has lower hemoglobin-binding capacity than Hp1-1 or Hp2-1, and this genotype may be related to idiopathic generalized epilepsy (15, 17).

In this study an association between epilepsy and Hp gene polymorphism has been found in both males and females. An increased proportion of Hp2-2 frequency has been previously observed in familial epilepsy (18). Major depression has been also found to be associated with increased Hp2-2 allele frequency (16, 19). Zara and co-workers suggest that all haptoglobin genotypes participate in idiopathic generalized epilepsy (20, 21). Delanghe and co-worker have reviewed the Hb polymorphism; vitamin C deficiency and scurvy are codetermined by the Hb polymorphism (22).

Haptoglobin acts as a potent balancing factor for helper T-cell type 1 and type 2 (Th1/Th2) within the body (23). Haptoglobin polymorphisms affect body iron turnover. Although not seen in females, in healthy males, the Hp 2-2 phenotype is associated with higher serum iron, higher transferrin saturation, and higher ferritin than Hp1-1 and 2-1 (24, 25).

Indeed free Hb mediates OH free radical formation which produces brain lipid peroxidation, increased neuronal excitability, and cellular damage. Owing to its higher production and diffusibility, the Hp1-1 phenotype may be more protective against oxidative damage than the other Hp phenotypes. These properties make Hp a functional gene for convulsive disorders (15, 16).

Acknowledgements

This article extracted from MSc thesis of Sukaina Al-balaghee and Zeinab Al-Balaghee and was supported by AL Zahra University and was done in the Cellular and Molecular Biology Research Centre, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The authors thank the Iranian Epilepsy Association and Tehran Laboratory for their support of our work.

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