β-Globin gene cluster haplotypes of Hb D-Los Angeles in Mazandaran Province, Iran

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Several types of hemoglobin D (Hb D) are distinguishable by DNA analysis, and the aim of this study was to identify the types of Hb D variant and β-globin gene haplotypes linked to Hb D in Mazandaran Province, northern Iran. Fifty five individuals were identified as Hb D carriers, and PCR-RFLP analysis revealed that all 55 had the Hb D-Los Angeles type. To identify haplotypes associated with the βD allele, family linkage analysis, using the PCR-RFLP method for seven polymorphisms in the β-globin gene cluster, was carried out on families of 23 of these 55 individuals. We observed three different haplotypes in association with Hb D-Los Angeles. In most cases (91.4%) βD alleles were linked to haplotype I [+ – – – + +]. Haplotype II [– + + – + +] and an atypical haplotype [– + + – – + –] were each in association with the βD allele in only one case (4.3%). This is the first report worldwide of the [– + + – – + –] haplotype in association with Hb D-Los Angeles. We conclude that more than 90% of the evaluated Hb D-Los Angeles alleles in Mazandaran have the same origin, and the two rare haplotypes may represent different genetic origins and/or other molecular events, such as gene conversion or recombination, in the region.

Key words: haplotype, hemoglobin D, hemoglobin D-Los Angeles, PCR-RFLP

Hemoglobin D (Hb D) has an electrophoretic mobility similar to Hb S, but normal solubility (Itano, 1951). Several hemoglobins with the same electrophoretic pattern and solubility have been detected with mutations in the β-globin gene, including Hb D-Bushman (β16 Gly→Arg), Hb D-Ouled Rabah (β19 Asn→Lys), Hb D-Granada (β22 Glu→Val), Hb D-Iran (β22 Glu→Gln), Hb D-Ibadan (β87 Thr→Lys), Hb D-Los Angeles (β121 Glu→Gln) and Hb D-Neath (β121 Glu→Ala) (Huisman et al., 1996; Hardison et al., 1998a, b). Hb D variants are asymptomatic in the heterozygous or homozygous state, but in compound heterozygous form, in association with Hb S, sickle cell disease occurs (Perea et al., 1999).

Two variants of Hb D, D-Los Angeles and D-Iran, are common in Iran. Hb D-Los Angeles, which is also known as Hb D-Punjab, -North Carolina, -Portugal, -Chicago and -Oak Ridge (Kinney and Ware, 1994; Platt et al., 1995), has a particularly high incidence rate in Punjab, India where 3% of the Sikh population are carriers of Hb D (Kinney and Ware, 1994). Hb D-Iran is mainly found in Iranian and Pakistani families in the heterozygous state and associated with no clinical abnormality (Platt et al., 1995). The aim of this study was to determine the Hb D type in patients previously identified as Hb D carriers based on the Hb electrophoresis method, and subsequently to identify the β-globin gene cluster haplotype linked to each Hb D allele.

This study was done in Mazandaran, a northern province of Iran with 4 million inhabitants that is located on the southern coastline of the Caspian Sea. From 2011 to 2013, individuals suspected to have Hb S or Hb D were selected and Hb electrophoresis was carried out using a capillary device (Minicap, Sebia, France) following complete blood count (Table 1). To differentiate Hb D from Hb S, both of which yield the same peak in capillary electrophoresis, alkaline (pH 8.4–8.6) or citrate agar (pH 6.1) gel electrophoresis, a solubility test, and a sickle cell prep screening test were done. Individuals identified as Hb D carriers and who agreed to take part in our study underwent DNA analysis and identification of associated mutations. This study was approved by the Ethics Committee of Mazandaran University of Medical Sciences, and written informed consent for the present study was obtained from all the participants. To identify the mutations causing Hb D variants, the presence of Hb D-Los
Table 1. Hematological indices of 55 cases with Hb D-Los Angeles

| Hematological index | Mean ± SD | Minimum | Maximum | Normal range |
|---------------------|-----------|---------|---------|--------------|
| RBC (10⁶/μl)        | 4.9 ± 0.5 | 4.2     | 6.0     | 4.10–5.10    |
| Hb (g/dl)           | 13.2 ± 1.2| 11.3    | 15.8    | 12.0–15.0    |
| Hct (%)             | 40 ± 3.8  | 33.7    | 48      | 36.0–46.0    |
| MCV (fl)            | 80.2 ± 4.5| 68      | 86      | 80.0–97.0    |
| MCH (pg)            | 26.9 ± 1.3| 23      | 28.6    | 27.0–32.0    |
| MCHC (g/dl)         | 32.8 ± 2.1| 23      | 35      | 32.0–36.0    |
| Hb A1 (%)           | 59.3 ± 4.1| 52      | 66      | 94.5–98.0    |
| Hb A2 (%)           | 2.2 ± 0.7 | 0.5     | 3.2     | < 3.5        |
| Hb F (%)            | 0.84 ± 0.3| 0.2     | 5.9     | < 2.0        |
| Hb D (%)            | 37.7 ± 3.8| 32      | 45      |              |

Fig. 1. Sequencing of PCR fragments showing the codon 121 (G→C) mutation that causes the Hb D-Los Angeles variant.

Hb D-Los Angeles was first checked.

Genomic DNA was extracted from peripheral blood samples using the standard phenol/chloroform method. To identify the Hb D-Los Angeles mutation, a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-based method using EcoRI restriction enzyme was applied (Rahimi et al., 2006). The presence of a mutation causing the Hb D-Los Angeles variant was confirmed using DNA sequencing (Fig. 1). DNA analysis of all 55 cases having the βD allele showed the Los Angeles type of Hb D variant.

Linkage analysis of 5’ to 3' of β-globin gene cluster haplotypes associated with the βD allele was applied to the families of 23 of the 55 subjects. Seven polymorphic sites, HincII 5’ to ε, HindIII in Gγ, HindIII in ζγ, HincII 5’ and 3’ to ψβ, AvaII in β, and BamHI 3’ to β, were investigated using the PCR-RFLP method (Cabeda et al., 1999; Lee et al., 2002). The haplotype classification of Orkin et al. (1982) was adopted.

After haplotype analysis of 23 families carrying the βD allele, Hb D-Los Angeles was observed in association with three different haplotypes, and in 91.4% of subjects this variant was linked to haplotype I [+ – – – – + +]. In the βA allele, linkage to seven haplotypes was detected, and 56.6% of the subjects were associated with haplotype I [+ – – – – + +] (Table 2).

Hb D-Los Angeles has been observed in many countries in the Middle East (Turkey, Saudi Arabia, United Arab Emirates and Kuwait) (Yavarian et al., 2009). In Fars Province of Iran, around 51% of individuals with Hb D had the Hb D-Los Angeles mutation and 49% had the Hb D-Iran mutation (Zakernia et al., 2011), while in our study in the northern region, as in the Kurdish provinces of Iran (Rahimi et al., 2006), no individuals with Hb D-Iran were found.

Haplotype analysis of βD alleles in western provinces of Iran showed that the βD allele is in complete linkage disequilibrium (100%) with haplotype I [+ – – – – + +] (Rahimi et al., 2006), and the result of our study showed similarities between the origin of Hb D-Los Angeles in two populations (91.4% vs. 100%). Haplotype I [+ – – – – + +] is the most common haplotype associated with Hb D-Los Angeles worldwide, and subjects from Italy, Mexico, Turkey and India were reported in linkage with this haplotype (Yavarian et al., 2009).

In southern provinces of Iran (Fars and Hormozgan), βD alleles were associated with four haplotypes, haplotype I [+ – – – – + +], haplotype VII [+ – – – – –], haplotype V [+ – – – + +] and haplotype IX [+ – – ++ +] (Yavarian et al., 2006). Table 2 shows the β-globin gene cluster haplotypes associated with Hb D-Los Angeles and βA alleles in 23 families.

Table 2. β-globin gene cluster haplotypes associated with Hb D-Los Angeles and βA alleles in 23 families

| Haplotype name | Haplotype (5'→3') | Number of βD alleles (percent) | Number of βA alleles (percent) |
|----------------|-------------------|--------------------------------|--------------------------------|
| I              | [+]               | 91.4%                          | 91.4%                          |
| II             | [+]               | 67.5%                          | 67.5%                          |
| Atypical       | [+]               | 100%                           | 100%                           |
| VII            | [+]               | 0%                             | 0%                             |
| V              | [+]               | 0%                             | 0%                             |
| IX             | [+]               | 0%                             | 0%                             |

Table 3. β-globin gene cluster haplotypes in association with Hb D-Los Angeles in different parts of Iran

| Haplotype name | Haplotype (5'→3') | Northern Iran (Mazandaran Province) | Southern Iran (Fars and Hormozgan Provinces) | Western Iran (Kurd population) |
|----------------|-------------------|-----------------------------------|--------------------------------------------|--------------------------------|
| I              | [+]               | 91.4%                             | 67.5%                                      | 100%                           |
| II             | [+]               | 4.3%                             | 0%                                         | 0%                             |
| Atypical       | [+]               | 4.3%                             | 0%                                         | 0%                             |
| VII            | [+]               | 0%                               | 17.5%                                     | 0%                             |
| V              | [+]               | 10%                              | 0%                                         | 0%                             |
| IX             | [+]               | 5%                               | 0%                                         | 0%                             |
Based on that study and ours, the \( \beta^0 \) allele is now linked to six different haplotypes in Iran (Table 3). These finding represent the varieties of ethnicities and the multi-centric origin of Hb D-Los Angeles in Iran.

In the present study the atypical haplotype \([- + - - + \cdots -\] is reported for the first time in association with Hb D-Los Angeles, and family analysis showed that this mutation is inherited through the generations and is not a de novo mutation. This haplotype has not been reported anywhere else in the world linked to Hb D-Los Angeles. This rare haplotype could reflect different genetic origins and/or other molecular events such as gene conversion or recombination in the region.

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