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Variable *β*-globin haplotypes in Saudi *β* thalassemia population*

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ABSTRACT

Twenty two haplotypes were generated from a pool of 60 unrelated Saudi *β* thalassemia major patients using previously described restriction sites in the *β* globin gene. Linkage disequilibrium analysis of the polymorphic sites was also conducted, a few identified haplotypes were novel while the remainder was previously reported, haplotype1222212 was the most frequent haplotype in the study group and a strong linkage disequilibrium between two polymorphic restriction sites in these *β* thalassemia patients was uncovered.

Keywords: SNPs; Haplotype; Linkage Disequilibrium; Restriction Sites

1. INTRODUCTION

Single Nucleotide Polymorphisms (SNPs) are the most common and easily detected type of variation that occurs throughout the genome [1]. Models of DNA polymorphisms along a single chromosome are known as haplotypes and are generated from combining the genotypes of closely related polymorphisms that are not separated by recombination. Naturally defined haplotypes arise as a result of genetic variations in different populations [2], such event appears clearly in the human *β*-globin gene cluster that is extremely polymorphic and variable in different populations, however, the polymorphic sites can act as genetic markers used to generate chromosomal haplotypes [3]. Generally, a mutation in the *β* globin gene that occurs on a chromosome with a specific haplotype is strongly linked to that haplotype [4], moreover, as many polymorphisms are found in the *β*-globin gene cluster [5], more than one *β* globin mutation can be associated with one haplotype and a single mutation may be found on several chromosome haplotypes [3]. Although seventeen sequence polymorphisms can be detected using restriction endonucleases for haplotyping of the *β*-globin gene, yet in most haplotyping studies only seven sites are examined because the remaining polymorphisms are specific to a particular racial group or linked uninformatively to sites already described. Haplotype analysis tracks the history of a mutation in order to know if there is a founder effect or a mutational hotspot in a specific population. The polymorphic site can be described as 1 or (+) if a restriction enzyme cuts at the position, and if it does not cut then the site is described as 2 or (−). Thus, the sequence of polymorphic sites can be arranged to define the haplotype following the 5' to 3' order of the genes in the complex [6], the 5' subhaplotype is made up of 5 restriction sites and the 3' subhaplotype is made of two restriction sites covering the *β*-globin gene cluster.

2. METHOD

2.1. RFLP Genotyping of *β*-Globin Gene

60 Saudi *β* thalassemia major patients who were attending clinics at King Faisal Specialist Hospital were taken into consideration, 10 samples were homozygous and 3 were heterozygous for the *β*-thalassemia mutations. Blood samples were collected by venipuncture in EDTA tubes and DNA was extracted from whole blood using the DNA purification system: Puregene™, (Gentra sys-

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#Both authors contributed equality to the manuscript.
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tem) according to the manufacturers' instructions. Five fragments of the DNA were amplified by PCR using specific primers [7], and the PCR-products were separately restricted using *Hinc* II, *Hind* III, Ava II and *Hinf* I endonucleases to determine the presence or absence of the seven restriction sites [8]. Approximately 10 - 17 µl of PCR product were digested with 1U of the appropriate restriction endonuclease under conditions recommended by the manufacturer and the samples were subjected to agarose gel electrophoresis to determine the presence or absence of the polymorphic site. The electrophoretic pattern was obtained for each fragment and restriction sites were symbolized by numbers (1 - 7).

2.2. Linkage Disequilibrium and Haplotype Construction

Haplotypes of the polymorphic sites along the *β*-globin gene cluster were constructed and their frequencies were calculated using Haploview program [9]. Linkage disequilibrium analysis of the seven restriction sites was conducted using the default settings (confidence interval [CI] minima for strong LD: upper, 0.98; lower, 0.7; upper CI maximum for strong recombination, 0.9; fraction of strong LD in informative comparisons must be at least 0.95) [9].

3. RESULTS

Twenty-two haplotypes were obtained from the study population, **Figure 1** presents these haplotypes and the frequency of their occurrence in the Saudi patients, the most frequent haplotype in the study group was 1222212 with a probability of 0.135, linkage disequilibrium analysis defined a single block with linkage between restriction sites 2 & 3 (Hind III G*γ* and Hind III A*α*) with an LOD value of 5.82 and D' value of 0.89 (89%) (**Figure 2**).

4. DISCUSSION

Discovering 22 different haplotypes in a single population is quite significant in terms of the rate of mutational event, however, the Saudi population is genetically heterogenous and therefore the genetic diversity may have originated from gene flow resulting from population migration. In addition, since *β*-thalassemia is known to offer a protection against malaria [9], it is believed to have a high mutation frequency in Saudis, as malaria was introduced a few thousand years ago. After exclusion of the *Ava* II and *Hinf* I restriction sites the most common haplotype in Saudi *β*-thalassemia patients was (12222) with a frequency of 13.5% followed by 12212 with a frequency of 11 %. Haplotypes 12222, 21121, and 21211 are known to be the most common haplotypes worldwide [9]. In addition, it appears from comparison of the present study haplotype frequencies with populations frequencies reported previously, that the frequency of haplotype 12222 in the Saudi *β*-thalassemia patients (13.5%) is close to the Africans (6.3%) but very low compared to all the other populations (frequency of at least 50%); haplotype 21211 is similar to the Southeast Asians (about 1.7%) but lower than Europeans, Indians, East Asians, Australian Aborigines (about 20%); haplotypes 21122 (frequency 1.7%), 21111 (frequency 1.7%), 22211 (frequency 5.7%) and 21222 (frequency 1.7%) are similar to all the other population frequencies [12]. It is well known that different ethnic groups carry their own set of mutations and therefore specific *β*-thalassemia mutations are strongly associated with specific haplotypes within an ethnic group [13]. Linkage disequilibrium D' value of 0.89 signifies a high LD between markers 2 and 3 indicating that 89% of the chromosomes had no evidence of historical recombination and 11% of chromosomes had evidence of historical recombination between markers 2 and 3. Therefore either these haplotypes are too recent leaving limited time for recombination to separate the markers or a recent admixture between different ethnic groups has taken place in the Saudi population and inter-

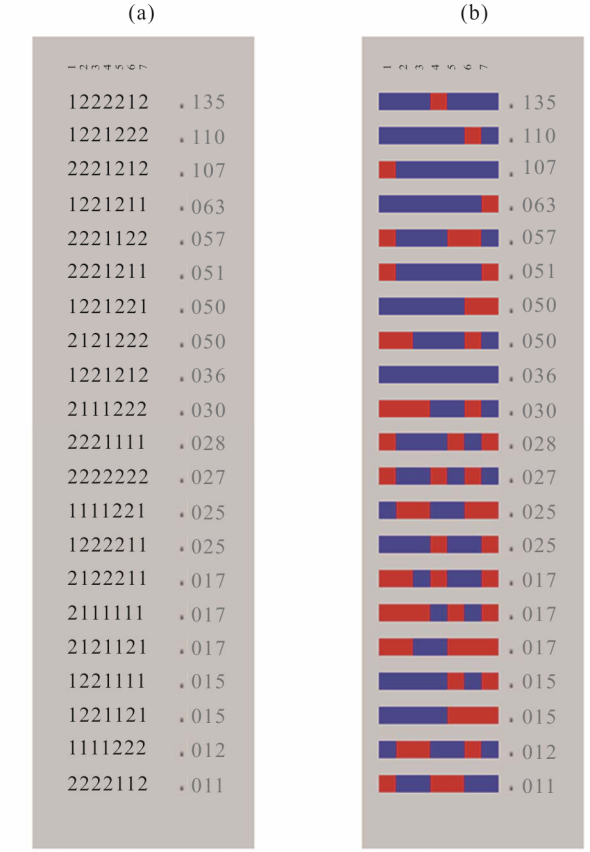

Figure 1. List of haplotypes identified in patients and their corresponding frequencies: (a) Haplotypes in number format; (b) Haplotypes in colored format.

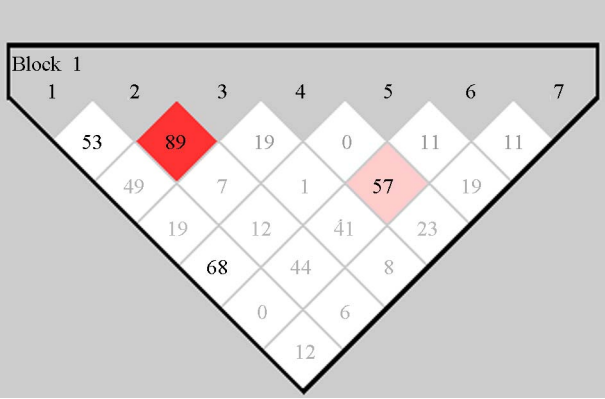

Figure 2. Linkage disequilibrium plot (LD plot) showing a strong linkage between marker 2 and 3 in the patient's samples: The numbers in the blocks represent multi-allelic D' with values ranging from 0 to 1(shown in percentage), Red squares indicate statistically significant (LOD >2) allelic association (linkage disequilibrium, LD) between pairs of markers, White squares indicate D' values of <1 with no statistically significant evidence of LD, markers are numbered from 1 to 7 to the top.

breeding between groups with different alleles has distorted the haplotype frequencies, causing linkage disequilibrium [14].

Performing similar studies on a larger β-thalassemia cohort would be essential for allowing the conduction of a significant linkage between haplotypes and disease causing mutations in the Saudi population.

5. ETHICAL APPROVAL

This work was approved by KFSHRC IRB, Research Advisory Council (RAC) and Research Ethics Committee (REC) (RAC# 2080012).

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