Allelic Frequency of a 24-bp Duplication in Exon 10 of the CHIT1 Gene in the General Iranian Population by HRM Analysis

Behrooz Motlagh, PhD

Assistant Professor of Clinical Biochemistry
Zanjan University of Medical Sciences
Chitotriosidase (EC 3.2.1.14)

Chitotriosidase: A biomarker of macrophage-related diseases

Activated macrophage

Lysosomal diseases
Arterial sclerosis
Fungal infection
Respiratory diseases
Malaria

Chitotriosidase

20 Kb

E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12

5' Neutrophilic granulocyte

50 kDa

Macrophage

39 kDa
One of the most common lysosomal storage disorders is Gaucher’s disease, which results from autosomal recessive defects in acid β-glucosidase (glucocerebrosidase).

Since chitotriosidase activity is significantly increased in the plasma and tissues of Gaucher’s disease patients, it is important to determine whether chitotriosidase activity is a reliable disease marker because it has been shown that enzyme activity has a direct correlation with the number of Gaucher cells and disease severity.

Treatment of Gaucher patients with enzyme replacement therapy (ERT) can gradually reduce the level of chitotriosidase, suggesting that this may be a useful marker for disease diagnosis and indicator of response to therapy.
Homozygous duplication of a 24-bp region in exon 10 of the CHIT1 gene eliminates enzyme activity and may complicate disease monitoring.

The level of plasma chitotriosidase in carriers of the 24-bp duplication is approximately half of that observed in individuals carrying the wild-type CHIT1 gene.

Approximately 30-40% of the Caucasian population is heterozygous for this mutation, while approximately 6% are homozygous with a complete absence of chitotriosidase activity.
The purpose of this study was to determine the frequency of the 24-bp duplication in exon 10 of the CHIT1 gene in the general Iranian population by using different mutation analysis methods and identifying which methods provide the simplest, most accurate, and efficient means of detecting mutations in CHIT1.
The present study was performed on blood samples from 577 Iranian individuals who were selected based on their medical history and absence of the specific disease.

- Chitotriosidase enzyme activity was determined by the fluorometric assay and the activity range was used to detect heterozygosity of the 24-bp CHIT1 duplication.
To identify the 24-bp duplication in exon 10 of the CHIT1 gene (H allele), **genotyping** of DNA extracted from peripheral blood leukocytes was performed using **PCR amplification** and **High Resolution Melting (HRM) PCR techniques**.
### Genotypic and Allelic Frequencies of the CHIT1 Gene 24-bp Duplication Mutation

<table>
<thead>
<tr>
<th>Genotype frequencies N (%)</th>
<th>Allele frequencies N (%)</th>
<th>Hardy-Weinberg P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt/wt</td>
<td>wt/H</td>
</tr>
<tr>
<td>Population sample</td>
<td>577</td>
<td>359</td>
</tr>
</tbody>
</table>

The diagram shows a scatter plot of CHIT1 activity (nmol/hr/ml) against the CHIT1 genotype (wt/wt and wt/H).
**PCR products of CHIT1 polymorphisms**

- Lane 1, homozygote mutant (99-bp)
- Lanes 2 and 3, heterozygote wt/mutant (99- and 75-bp)
- Lane 4, homozygote wild type (75-bp)
HRM analysis of the 24-bp duplication in the CHIT1 gene exon 10
Difference graph of the HRM analyses

B

- Homozygote (H/H)
- Heterozygote (wt/H)
- Wild Type (wt/wt)
Melting graph of the HRM curves
For the first time, the present study determined the allelic frequency of the CHIT1 gene in a healthy Iranian population sample. A wide range of frequencies have been identified in other subject populations throughout the world.

The difference in CHIT1 allele frequency in the Iranian population was comparable to other populations and might be attributed to ethnic diversity.
Samples from wild-type as well as heterozygous and homozygous individuals could be differentiated by the significant differences in HRM curves.

Based on accuracy, reliability, cost, and time, the HRM method appears to be the most suitable and powerful tool for genotype screening to date.

Unlike other methods that scan for genetic changes, HRM does not require gel electrophoresis. Therefore, the advantage of the HRM method for assessing patient samples is that PCR tests and melting curve analysis processes are carried out in parallel in a closed system, which reduces the risk of product contamination and also saves time.
Our study indicates that genotype analysis by PCR-HRM is a fast, reliable, and highly accurate screening approach for identifying the 24-bp duplication in CHIT1 exon 10. Due to the wide range of duplication frequencies among different ethnic groups, new biomarkers are necessary for assessing genetic characteristics of lysosomal storage disorders in different populations.
Thank You...