Nota clínica

An update on the molecular analysis of classical galactosaemia patients diagnosed in Spain and Portugal: 7 new mutations in 17 new families

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INFORMACIÓN DEL ARTÍCULO

Historia del artículo:
Recibido el 15 de septiembre de 2008
Aceptado el 5 de noviembre de 2008
Available online 16 de abril de 2009

Keywords:
Classical galactosaemia
Mutations
Metabolic disorder

ABSTRACT

Background and objectives: Classical galactosaemia is an inherited metabolic disorder due to mutations in the galactose-1-phosphate uridyltransferase gene (GALT). We previously reported molecular analysis of 83 Spanish and Portuguese unrelated galactosaemic patients. Here we present the results of another seventeen unreported affected individuals.

Material and methods: DNA from patients was PCR-amplified and sequenced following standard protocols.

Results: Twelve patients diagnosed in Spain were studied. We detected five alleles carrying p.Q188R, accounting for 21%. Other six alleles (25%) were identified with the mutation p.K285N. We also identified six novel mutations: p.Q9X, c.328+2T>4C, p.I170N, p.C180F, p.V233L and p.P257L. Taking into account all the Spanish galactosaemic diagnosed patients, mutation p.Q188R is still the most frequent mutation identified (44.4%). In five new Portuguese patients, five alleles p.Q188R were detected, representing 50%. One novel mutation (p.F171C) was identified.

Conclusions: Our results confirm our previous observations that p.Q188R is the most frequent mutation in Iberian Peninsula galactosaemic patients (49%), and that Portuguese and Spanish genotypes differ.

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Actualización del análisis molecular de la galactosemia clásica en España y Portugal: 7 mutaciones nuevas en 17 nuevas familias

Resumen

Fundamento y objetivo: La galactosemia clásica es una enfermedad metabólica hereditaria debida a mutaciones en el gen de la galactosa-1-fosfato uridiltransferasa (GALT). Nuestro grupo ya publicó los resultados del análisis molecular de 83 pacientes galactosémicos de España y Portugal. Aquí se presentan los resultados de una nueva serie de pacientes.

Material y método: El ADN de los 17 pacientes estudiados se amplificó por PCR y se secuenció siguiendo métodos ya descritos.


Conclusiones: Estos resultados confirman nuestras observaciones previas, en las cuales se mostraba que la mutación más frecuente en la península ibérica es p.Q188R (49%), pero que los genotipos españoles y portugueses difieren entre sí.

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Introduction

Classical galactosaemia (MIM 230400) is an inherited autosomal recessive metabolic disorder caused by deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT; EC 2.7.7.12). The disease causes neonatal jaundice, cataracts, hepato-megaly, failure to thrive and seizures. If undiagnosed, it may lead to a fatal outcome, but the symptoms are reversed with the introduction of galactose-free diet. However, in spite of good dietary compliance, long-term outcome can be associated with growth and mental retardation, verbal dyspraxia, neurological defects and primary ovarian failure.1

Over 230 different base changes and disease-causing mutations have been reported in the GALT gene (ARUP galactosaemia database http://arup.utah.edu/database/galactosemia/GALT_welcome.php)2. The two most frequent mutations in European populations are p.Q188R and p.K285N, with frequencies depending on the population studied. They both have been associated with a severe form of the disease1,3.

Our previous report on the mutational spectrum of classical galactosaemia in Spain and Portugal4 analysed 51 Spanish and 32 Portuguese families. Our results indicated a high incidence of mutation p.Q188R (50% and 57.8%, respectively), and different frequencies for p.K285N (9.8% in Spanish alleles and absence in Portugal). In addition, we found a rather high frequency for p.L195P in Spanish patients (15.7%). Here we present the molecular results of another 17 unreported affected individuals. Twelve were from Spain and the other five came from Portugal. Our aim is to update the spectrum of GALT mutations in the Iberian Peninsula.

Patients and methods

The patients included were unrelated and came from all the different regions of Spain and Portugal. In all cases the diagnosis of galactosaemia was confirmed by reduced or absent GALT activity in erythrocytes.

Genomic DNA was extracted from patient leukocytes using standard protocols. Each exon and intron boundaries of the GALT gene were PCR-amplified. SSCP and RFLP analyses were performed as described4.

Gene nucleotide numbering was made according to RefSeq NM_000155.2, with +1 as A of the ATG start codon. The ATG codon represents +1 for the amino acid numbering according to GALT protein sequence NP_000146.2.

Results and discussion

Molecular results are shown in Table 1. Twelve Spanish patients were analysed. We detected five alleles carrying the frequent mutation p.Q188R, accounting for 21%. Other six alleles (25%) were identified with the described second most frequent mutation p.K285N. Remarkably, two patients who were homozygous for this change were of North African origin. We also identified six novel mutations: p.Q9X (c.25C>T), c.328+2T>C, p.I170N (c.509T>A), p.C180F (c.539G>T), p.V233L (c.697G>C), p.P257L (c.770C>T). The four missense mutations change conserved amino acids and they are predicted to affect the protein function after different prediction programs. The change c.328+2T>C causes the complete abolition of the donor splice site in intron 3 after splice site prediction programs and splice site score calculators; studies on cDNA are under way to elucidate the effect on the protein.

<table>
<thead>
<tr>
<th>Patient Origin</th>
<th>Allele 1</th>
<th>Allele 2</th>
</tr>
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<tbody>
<tr>
<td>Spain</td>
<td>p.Q188R</td>
<td>p.Q188R</td>
</tr>
<tr>
<td>Spain</td>
<td>p.Q188R</td>
<td>p.Q188R</td>
</tr>
<tr>
<td>Spain</td>
<td>c.253-2A&gt;G</td>
<td>p.V233L</td>
</tr>
<tr>
<td>Spain</td>
<td>c.253-2A&gt;G</td>
<td>p.S135L</td>
</tr>
<tr>
<td>Spain</td>
<td>p.Q9X</td>
<td>p.I170N</td>
</tr>
<tr>
<td>Portugal</td>
<td>p.Q188R</td>
<td>p.Q188R</td>
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<tr>
<td>Portugal</td>
<td>p.Q188R</td>
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<td>p.R204X</td>
</tr>
<tr>
<td>Portugal</td>
<td>p.S135L</td>
<td>p.F171C</td>
</tr>
</tbody>
</table>

In bold, novel changes. * North-African ancestry

Other frequent mutations such as p.L195P and p.R148Q, were also identified in this patient series. In patient 12, the mutation c.328+2T>C was present in the Duarte1 variant (p.[N314D; L218L] ) background1,3.

Considering all the 63 Spanish galactosaemic patients diagnosed, p.Q188R is still the most frequent mutation identified (44.4%). The second most frequent mutation is p.L195P (13.5%) followed by p.K285N (12.7%). It is worth mentioning that the two homozygous patients for mutation p.K285N are of Northern Africa origin. The increase of the immigration experimented in Spain during the last years is clearly responsible for the change in mutation frequencies of some inherited diseases such as galactosaemia.

In the five new Portuguese patients, five alleles p.Q188R were detected, representing 50%. All other changes were only in one allele each. One novel mutation was identified, p.F171C (c.512T>G), while mutations p.L195P and p.K285N still remain undetected in Portuguese patients.

In patient 14 we identified the frequent mutation p.Q188R in one allele and a combination of two novel changes ([c.328+33g>a; c.*105A>G]) in the other. No other changes were detected in this patient’s DNA and splicing or transcription sites prediction programs failed to predict any creation of a new splicing or transcription factor site for the intrinsic change. The variant c.328+33g>a was not identified in 100 control chromosomes, while the change c.*105A>G was detected in one out of 200 control alleles tested (0.5%). The change c.*105A>G affects the third 5’ nucleotide of the highly conserved polyadenylation signal AATAAA. Changes in the 3’ regulatory region and, specifically, in the USS region (upstream region; between the translational termination codon and the upstream core polyadenylation signal sequence)5, have been described in different genes. Some of them have been demonstrated to be the cause of a disease. The effect of these two variants remains unclear. Further studies must be done to elucidate which of both is the second disease-causing change in this patient. For the moment, patient cDNA is not available though its analysis might be very useful.

In the whole group of 37 Portuguese patients analysed to date, mutation p.Q188R remains the most frequent identified (56.7%). This is the only frequent mutation as the rest of changes were found in one or two alleles each.
If we take into account all the 100 galactosaemic patients diagnosed in the Iberian Peninsula, p.Q188R is the most frequent mutation (49%). Nevertheless, as mentioned before, the differences observed between the genotypes identified in Portuguese and Spanish galactosaemic populations continue to be notable.

Acknowledgments

We would like to thank Drs. Castro, Forga, Gacio, García, García-Silva, García-Valdecasas, Pérez-José, Pujals, Rodríguez, Sousa, Tavares, Ventura, Vilarinho for sending us samples from their patients.

Financing

The CIBER Enfermedades Raras is an initiative of the ISCIII. Ester Quintana is a recipient of a fellowship of FIS-ISCIII of the Ministerio de Sanidad y Consumo of Spain and she is a PhD student of the Department of Biochemistry and Molecular Biology of the Universitat Autònoma de Barcelona.

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