Short Report

Clinical, biochemical and molecular characterization of Cystinuria in a cohort of 12 patients


Cystinuria is a rare autosomal inherited disorder characterized by impaired transport of cystine and dibasic aminoacids in the proximal renal tubule. Classically, Cystinuria is classified as type I (silent heterozygotes) and non-type I (heterozygotes with urinary hyperexcretion of cystine). Molecularly, Cystinuria is classified as type A (mutations on \( SLC3A1 \) gene) and type B (mutations on \( SLC7A9 \) gene). The goal of this study is to provide a comprehensive clinical, biochemical and molecular characterization of a cohort of 12 Portuguese patients affected with Cystinuria in order to provide insight into genotype–phenotype correlations. We describe seven type I and five non-type I patients. Regarding the molecular classification, seven patients were type A and five were type B. In \( SLC3A1 \) gene, two large genomic rearrangements and 13 sequence variants, including four new variants \( c.611-2A>C; c.1136+44G>A; c.1597T (p.Y533N); c.*70A>G \), were found. One large genomic rearrangement was found in \( SLC7A9 \) gene as well as 24 sequence variants including 3 novel variants: \( c.216C>T (p.C72C) \), \( c.1119G>A (p.S373S) \) and \( c.*82C>T \). In our cohort the most frequent pathogenic mutations were: large rearrangements (33.3% of mutant alleles) and a missense mutation \( c.1400T>C (p.M467T) \) (11.1%). This report expands the spectrum of \( SLC3A1 \) and \( SLC7A9 \) mutations and provides guidance in the clinical implementation of molecular assays in routine genetic counseling of Portuguese patients affected with Cystinuria.

Conflict of interest

The authors declare no conflicts of interest.

Key words: Cystinuria – MLPA analysis – silent mutation – \( SLC3A1 \) gene – \( SLC7A9 \) gene
Cystinuria (MIM #220100) is an autosomal inherited disorder characterized by impaired transport of cystine and dibasic amino acids (lysine, ornithine and arginine) in the proximal renal tubule and gastrointestinal tract. The overall prevalence of this disease is approximately 1 in 7000 neonates, ranging from 1 in 2500 neonates in Libyan Jews to 1 in 100,000 among Swedes (1). The impaired renal reabsorption of cystine and its low solubility causes the formation of calculi in the urinary tract, which leads to obstructive uropathy, pyelonephritis and, rarely, renal failure. Classically, Cystinuria was biochemically classified according to the excretion of cystine and dibasic amino aciduria of obligate heterozygotes into phenotype type I (in which heterozygotes have a normal pattern of amino acid excretion) which implies that the disease is transmitted by an autosomal recessive trait, and phenotype non-type I (in which heterozygotes can show urinary hyperexcretion of cystine), indicating that the disease is transmitted in a dominant recessive mode with incomplete penetrance (1–3).

More recently, two genes have been implicated in this disorder. The **SLC3A1** gene (solute carrier family 3, member 1; MIM *104614, locus 2p16.3) contains 10 exons and encodes the b\(^0\,+\) transporter-related protein (rBAT) (4). The **SLC7A9** gene (solute carrier family 7, member 9; MIM *604144, locus 19q13.1) contains 13 exons and encodes a protein that heterodimerises with rBAT (b\(^0\,+\)AT) (3). The rBAT/b\(^0\,+\)AT complex belongs to the heteromeric aminoacid transporter (HAT) family, which is formed by a heavy subunit (rBAT or 4F2hc) linked by a disulphide bridge to a range of light subunits (b\(^0\,+\)AT in the case of rBAT) (5).

A new classification of Cystinuria has emerged based on molecular genetics data: type A disease due to mutations in **SLC3A1** (genotype AA) and type B disease due to mutations in **SLC7A9** (genotype BB) (6); rare cases of possible digenic inheritance (type AB) have been described (2).

Molecular analysis in some families has revealed the co-occurrence of two mutations in one gene and one in the other, consistent with an AAB or BBA genotype (7, 8).

Previously, a straightforward correlation between the biochemical and molecular classifications has been proposed, in which **SLC3A1** mutations (type A) invariably lead to type I disease and **SLC7A9** mutations (type B) cause non-type I disease. However, there has been an increasing number of exceptions: E5_E9dup mutation of **SLC3A1** causes hyperexcretion of cystine and dibasic amino acids in carriers (6); several point mutations (e.g., p.I44T, p.G63R, p.W69X, p.T123M, p.A182T, p.G105R and p.P261L) in **SLC7A9** are present in silent heterozygotes (6). Hypothesis to explain these exceptions have been elegantly equated by the International Cystinuria Consortium (3, 7).

It has also been suggested that the **SLC7A9** haplotype background may modify the expression of an **SLC7A9** pathogenic mutation, making the interpretation of the biochemical and molecular data even more complex (9). Only taking all these factors into account and adding the contribution from the remaining genetic background, the role of environment, and stochastic effects, is it possible to explain how Cystinuria, can have such a complex genotype–phenotype (biochemical and/or clinical) correlation.

To date, 128 different mutations in **SLC3A1** and 97 in **SLC7A9** are listed in the Human Genome Mutation Database (HGMD: http://www.hgmd.cf.ac.uk/ac/index.php; accessed on September 2010). There is striking allelic heterogeneity as missense, nonsense, splicing, small rearrangements (insertions and deletions), gross rearrangements (insertions and deletions) (10) and complex rearrangements have all been described.

The prevalence of some mutations varies significantly with the population or ethnic group studied. There is evidence that p.M467T in the **SLC3A1**

The aim of this study was to provide a comprehensive clinical, biochemical and molecular characterization of a cohort of 12 Portuguese patients affected with Cystinuria. It was also the authors’ intention to ascertain genotype–phenotype correlations and, taking into account the prevalence of the mutations found in this study, to delineate a strategy for the clinical implementation of molecular diagnosis of Portuguese Cystinuria patients.

**Materials and methods**

**Patients**

We investigated 12 patients affected with Cystinuria from 12 reportedly unrelated families from the Northwest of Portugal. The diagnosis had been established on the basis of kidney stone formation, urinary sediment and urinary aminoacids profile. Informed consent was obtained from all patients and their families.

**Aminoacids profile in urine**

Aminoacid excretion was determined, both in patients and their relatives, using morning urine samples and, whenever possible, a 24 h collection of urine. Urinary aminoacid profile was determined by liquid ion-exchange chromatography.

**DNA sequence analysis**

Genomic DNA (gDNA) from patients, relatives and controls was extracted from peripheral lymphocytes using standard protocols. The whole coding sequence and exon–intron junctions of the SLC3A1 and SLC7A9 genes (10 and 13 exons, respectively) were amplified using newly designed primers (primers and condition upon request).

DNA sequence analysis was performed by using the commercial kit BigDye Terminator Cycle Sequencing Kit 1.1 (Applied Biosystems, Foster City, CA).

Mutational analysis was performed using the SeqScape™ v.2.5 software (Applied Biosystems) and the reference sequences available at Ensemble Genome Browser (http://www.ensembl.org) (SLC3A1 gene: ENSG00000138079 and SLC7A9 gene: ENSG0000021488).

**Population screening**

For novel variants not previously described in the HGMD or in the literature, a population screen was performed using gDNA of 100 unrelated healthy individuals (200 alleles) from the same population, who served as controls. Controls were screened for the variants using high resolution melting curve analysis (hrMCA) (16).

**RNA analysis**

In cases C8 and C9, RNA was extracted from leukocytes and cDNA was obtained by reverse transcription - polymerase chain reaction (RT-PCR) amplification of total RNA using a reverse transcriptase included in the SuperScript™ III kit (Invitrogen™, Carlsbad, California, USA).

SLC3A1 and SLC7A9 transcripts were amplified in seven amplicons for each gene using primers specifically designed for this study.

**MLPA analysis**

In cases C1, C4, C6, C11 and C12 multiplex ligation-dependent probe amplification (MLPA) analysis was performed as previously described (10).

**Bioinformatic tools**

Apart from the bioinformatic tools already mentioned in this section, the following software was used: Human Splicing Finder v.2.3. (http://www.umd.be/HSF/) and GenScan (http://genes.mit.edu/GENSCAN.html) was used for analysis of splicing mutations; BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for sequence homology analysis; Polyphen (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://sift.jcvi.org/) were used to perform in silico prediction of the functional impact of missense mutations.

**Results**

**Clinical characterization**

A cohort of 35 individuals (18 females and 17 males), 12 index patients and 23 relatives (1st and 2nd degree) were included in this study. Table 1 summarizes personal data and family history of the probands.

**Biochemical characterization**

Table 2 summarizes the biochemical data of the 12 probands. On the basis of biochemical criteria and reference values previously
<table>
<thead>
<tr>
<th>Patient and family history</th>
<th>Age</th>
<th>Clinic</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial symptoms</td>
<td>Dx</td>
<td>CD</td>
</tr>
<tr>
<td>Patient</td>
<td>G</td>
<td>C</td>
<td>Relatives w/lithiasis</td>
</tr>
<tr>
<td>Case</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>N</td>
<td>Father</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>N</td>
<td>Sister</td>
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<tr>
<td>3</td>
<td>M</td>
<td>N</td>
<td>Sister</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>N</td>
<td>Brother and Father</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>N</td>
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<td>M</td>
<td>N</td>
<td>N</td>
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<td>8</td>
<td>M</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;C,</td>
<td>N</td>
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<td>9</td>
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<td>M</td>
<td>N</td>
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<tr>
<td>11</td>
<td>M</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>N</td>
<td>Two maternal uncles</td>
</tr>
</tbody>
</table>

G, gender; C, consanguinity; w/, with; Dx, Diagnosis; CD, consultation date; UTI, urinary tract infection; F, female; M, male; N, No; M, Months; Y, years; BL, bilateral; UL, unilateral; U, unknown; 2<sup>nd</sup>C, parents are second cousins.

<sup>a</sup>To the best of our knowledge genes within this deletion are not related to Cystinuria.
### Table 2. Biochemical and molecular characterization of the 12 index patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Cystine (μmol/24 h)</th>
<th>Ornithine (μmol/24 h)</th>
<th>Lysine (μmol/mmol creat.)</th>
<th>Arginine (μmol/mmol creat.)</th>
<th>Phenotype of the patient</th>
<th>Phenotype of the parents</th>
<th>SLC3A1$^b$</th>
<th>SLC7A9$^b$</th>
<th>Classification$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>494$^{(1,2)}$</td>
<td>8–30</td>
<td>0–7</td>
<td>2382$^{(1,2)}$</td>
<td>10–46</td>
<td>412$^{(1,2)}$</td>
<td>0–9</td>
<td>Yes</td>
<td>Homozygote</td>
</tr>
<tr>
<td>2</td>
<td>1570$^{(1,2)}$</td>
<td>40–258</td>
<td>49–151</td>
<td>102$^{(1,2)}$</td>
<td>48–640</td>
<td>615$^{(1,2)}$</td>
<td>22–88</td>
<td>Undetermined</td>
<td>Homozygote</td>
</tr>
<tr>
<td>3</td>
<td>270$^{(1,2)}$</td>
<td>8–22</td>
<td>0–7</td>
<td>312$^{(1,2)}$</td>
<td>10–68</td>
<td>866$^{(1,2)}$</td>
<td>0–7</td>
<td>Undetermined</td>
<td>Homozygote</td>
</tr>
<tr>
<td>4</td>
<td>281$^{(1,2)}$</td>
<td>40–258</td>
<td>49–151</td>
<td>123$^{(1,2)}$</td>
<td>48–640</td>
<td>440$^{(1,2)}$</td>
<td>22–88</td>
<td>Undetermined</td>
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</tr>
<tr>
<td>5</td>
<td>384$^{(1,2)}$</td>
<td>40–258</td>
<td>49–151</td>
<td>178$^{(1,2)}$</td>
<td>48–640</td>
<td>479$^{(1,2)}$</td>
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<tr>
<td>6</td>
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<td>40–258</td>
<td>49–151</td>
<td>114$^{(1,2)}$</td>
<td>48–640</td>
<td>227$^{(1,2)}$</td>
<td>22–88</td>
<td>Yes</td>
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<tr>
<td>7</td>
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<td>0–6</td>
<td>106$^{(1,2)}$</td>
<td>10–44</td>
<td>85$^{(1,2)}$</td>
<td>0–6</td>
<td>Undetermined</td>
<td>Homozygote</td>
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<tr>
<td>8</td>
<td>88$^{(1,2)}$</td>
<td>6–34</td>
<td>75$^{(1,2)}$</td>
<td>106$^{(1,2)}$</td>
<td>10–46</td>
<td>21$^{(1,2)}$</td>
<td>0–9</td>
<td>Yes</td>
<td>Heterozygote non-type I</td>
</tr>
<tr>
<td>9</td>
<td>1471$^{(1,2)}$</td>
<td>40–258</td>
<td>49–151</td>
<td>55$^{(1,2)}$</td>
<td>48–640</td>
<td>29$^{(1,2)}$</td>
<td>22–88</td>
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</tr>
<tr>
<td>10</td>
<td>1750$^{(1,2)}$</td>
<td>7–23</td>
<td>77$^{(1,2)}$</td>
<td>190$^{(1,2)}$</td>
<td>10–56</td>
<td>57$^{(1,2)}$</td>
<td>0–6</td>
<td>Undetermined</td>
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<tr>
<td>11</td>
<td>921$^{(1,2)}$</td>
<td>40–258</td>
<td>49–151</td>
<td>27$^{(1,2)}$</td>
<td>48–640</td>
<td>241$^{(1,2)}$</td>
<td>22–88</td>
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<tr>
<td>12</td>
<td>1153$^{(1,2)}$</td>
<td>40–258</td>
<td>49–151</td>
<td>28$^{(1,2)}$</td>
<td>48–640</td>
<td>368$^{(1,2)}$</td>
<td>22–88</td>
<td>Undetermined</td>
<td>Heterozygote non-type I</td>
</tr>
</tbody>
</table>

$^{u1}$, μmol/24 h; $^{u2}$, μmol/mmol creat.

$^a$Mutations described for the first time in this work.

$^b$Mutations that might contribute to the expression of the disease.

$^c$Classification based on cystine excretion by index case and progenitors (1).

$^d$Classification based on mutations found in SLC3A1 and SLC7A genes (5).

$^e$Polymorphisms in SLC3A1 described for the first time in this work were not included in the table for ease of reading: c.*70A>G, c.1136+44G>A, c.797T>C (p.F266S).

$^f$Polymorphisms in SLC7A9 described for the first time in this article were not included in the table for ease of reading: c.425T>C (p.V142A), c.667C>A (p.L223M), c.216C>T (p.C72C), c.1119G>A (p.S373S), c.*82C>T.
described (17–20), patients were assigned to two groups – homozygous or heterozygous non-type I – according to the concentration of cystine in urine. Moreover, when urine from both parents was available, the information about their excretion of cystine and dibasic amino acids allowed us to classify patients into type I or non-type I Cystinuria. Even though the fathers of C1 and C6 allegedly presented calculi, biochemical analysis of urine has shown that they have a normal urinary aminoacid excretion and hence were classified as having type 1 phenotype. The etiology of the calculi in these individuals was not established and their workup study should be broadened to include other causes of lithiasis.

Molecular characterization

DNA sequencing analysis of all exons and intron–exons boundaries of SLC3A1 and SLC7A9 genes was performed for the index patients (data showed in Table 2). Fifteen variations to reference sequence were identified in SLC3A1 gene including: six previously described polymorphisms (p.G38G, p.S77S, p.E345E, c.1136+3delT, c.1332+7C>T and p.M618I); four previously described pathogenic mutations (p.T216M, p.M467T, p.Y397C and p.C673W); one variant (p.F266S) with conflicting evidence in the literature concerning its pathogenicity and four new variants (c.611-2A>C; c.1136+44G>A; p.Y533N and c.*70A>G).


SLC3A1 mutations

To evaluate the biological impact of the sequence variants, the following studies were performed.

The c.*70A>G variation was identified in heterozygosity in three patients (C6, C7 and C11). This substitution occurs in the 3’ untranslated region (UTR) of SLC3A1. Population screening in 100 alleles showed a frequency of 5% for this variant. Hence, c.*70A>G is most probably a polymorphism.

Likewise, c.1136+44G>A was identified in heterozygosity in three patients (C8, C9 and C12). This is an intronic nucleotide substitution that does not involve the canonical splice sites and no cryptic splice site was predicted by in silico analysis. cDNA sequencing of the three patients did not reveal any change. This variant is also most probably a polymorphism.

The c.1597T>A (p.Y533N) variation was found in homozygosity in one patient (C5). This mutation occurs in exon 9 and leads to the substitution of a Tyrosine (aromatic polar) by an Asparagine (neutral). The p.Y533N mutation affects the extracellular domain of rBAT and most probably has a deleterious effect. Mutations affecting the extracellular domain of rBAT are predicted to lead to changes in the protein folding with subsequent degradation in the endoplasmic reticulum (21). This substitution was not identified in a population screening (200 alleles) and occurs in a highly conserved region. Polyphen and SIFT predictions also favored the deleterious nature of this missense mutation.

The c.611-2A>C variation was identified in heterozygosity in one patient (C3) and in his affected sister. It occurs in the 3’ splicing consensus sequence (AG) of intron 3 – exon 4. In silico analysis using Human Splicing Analyser and GenScan tools suggests that this substitution leads to exon skipping of exon 3. This nucleotide substitution was not identified in the population screening (200 alleles). c.611-2A>C is most probably a pathogenic mutation by affecting the splicing of exon 3.

The c.797T>C (p.F266S) was also identified in C3 and his sister. Even though F266S figures in HGMD as being pathogenic, functional studies (22) have proved that this mutation doesn’t affect significantly the transport of Cystine. Hence, p.F266S was considered benign.

SLC7A9 mutations

Two sequence variations – c.425T>C (p.V142A) and c.667C>A (p.L223M) – were identified in five families (C1, C4, C5, C6 and C11). These variants are listed in HGMD and classified as pathogenic. However, there is increasing body of evidence against this: population screening has revealed a high prevalence of these variants in the general population and functional studies suggest that these mutations in homozygosity and in compound heterozygosity only slightly decrease the transport of cystine (but never below 50% of wild type) (15, 23). In our families we found both these variants. They were present in patients
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(in addition to other two pathogenic mutations) and in their relatives. In fact, there were healthy individuals with simultaneous homozygosity for both p.V142A and p.L223M that had a normal excretion of cystine. Taking all these facts into account there is a robust evidence to consider these variants as polymorphisms.

The c.216C>T (p.C72C) nucleotide substitution, causing a putative silent substitution in exon 3, was found in heterozygosity in one patient (C3). Polyphen and SIFT predict a benign nature of this silent mutation. It was not identified in a population screening of 200 alleles. Therefore, p.C72C was interpreted as a very low prevalence polymorphism.

The c.1119G>A (p.S373S) change was found in heterozygosity in one patient (C12). This nucleotide substitution occurs in exon 11 and is also predicted to be silent. Polyphen and SIFT agreed on the benign nature of this silent mutation. Sequencing of cDNA of the patient did not reveal any abnormality. This substitution was not identified in a population screening (200 alleles). As a consequence p.C72C was also classified as a rare polymorphism.

c.*82C>T was identified in heterozygosity in 1 patient (C6). This change occurs in the 3′ UTR region of SLC3A1 gene and was not identified in a population screening of 200 alleles. However, taking into account that this variant is segregating in cis with a known pathogenic mutation (M467T), c.*82C>T was considered a rare single nucleotide polymorphism (SNP).

The c.972G>A (p.A324A) change was identified (Fig. 1) in three patients (C7, C8 and C9). A population screening in 100 alleles showed a frequency of 2%. This mutation occurs in exon 9 and was, apparently, silent. However, the analyses of cDNA of these patients revealed, besides the normal transcript, a deletion of four nucleotides (GCAG) most probably to cause a truncated protein due to frameshift. In fact, Human Splicing Finder showed that this mutation activates a cryptic sequence recognized by the splicing machinery leading to the above described four nucleotides deletion (Fig. 1). This deletion is predicted to cause a truncated protein (p.Gly325AspfsX36) shortened in 289 residues.

Even though, it was performed a careful sequencing of both SLC3A1 and SLC7A9 coding regions and boundaries of the 12 patients, in 8 of them (C1, C4, C6, C7, C8, C9, C11 and C12) the molecular characterization was still incomplete. In these patients, SLC3A1 and SLC7A9 genes were subsequently analyzed by MLPA which provided important data to understand the molecular basis of Cystinuria in such cases.

Discussion

Cystinuria is a hereditary heterogeneous disorder affecting cystine and dibasic amino acids transporter. In our cohort of 12 patients, 7 had a urinary excretion of cystine suggestive of homozygosity for Cystinuria (Group 1) and the remaining 5 were classified as heterozygotes non-type I (Group 2).

Concerning clinical characterization, three patients showed first symptoms early in infancy, whereas in five patients lithiasis started later in life. Noteworthy, in three of the patients there was a significant time lag between the beginning of Cystinuria-related symptoms and the establishment of clinical diagnosis. Four of the patients

![Fig. 1. Sequencing of SLC7A9 genomic DNA (gDNA) and cDNA of wild type (a) and patient with c.972G>A (p.A324A) mutation in heterozygosity (b). Human Splicing Finder revealed a score of 0.78 for the normal allele (c) and a score of 2.53 for the c.972G>A (p.A324A) mutated allele (d).](image-url)
have not presented cystine calculi to date and were ascertained through metabolic screening in the context of developmental delay diagnostic workup. Concerning the medical treatment, only one patient (C6) is taking a chelating agent. Patient 5, which had an excretion of 3,842 mmol/day (thus above the threshold of 3 mmol/day) had taken a chelating agent for a few months but this was discontinued due to adverse side effects (which are common and can indeed limit the treatment in 20–50% of the patients) (20). Indication for taking a chelating agent in C3 and C4 will be reassessed as the benefits of taking the drug would now outweigh the risks related to side effects. Two patients (C2 and C12) were taking captopril according to the guidelines on urolithiasis released by the European Association of Urology, which states that positive effects on urinary cystine and stone formation have been reported with a daily dose of 75–100 mg of captopril (24). Half of the eight symptomatic patients have already required urologic intervention (three had a nephrolithotomy and one needed both nephrolithotomy and unilateral nefrectomy), which clearly shows that the management of this disorder is still challenging. Nefrectomy is an extreme and rare measure. However, in this case because Cystinuria was not a well known clinical identity at the time the patient presented with the first symptoms, there was a significant time lag between clinical onset and diagnosis, which may have compromised patient management.

In Group 1 fully molecular characterization was possible: six patients presented biallelic in SLC3A1 and in one patient a mutation was identified on one allele of SLC7A9 (Table 2). Interestingly, this last patient also had the c.972G>A (p.A324A) mutation. Unfortunately, parental DNA was not available for testing, therefore it was not possible to establish whether these mutations were on cis or trans. Defects in regulatory or promoter sequences of SLC7A9 or eventually mutations in a third (still unidentified) gene related to Cystinuria could also account for this result.

Group 2 is composed of five heterozygous non-type I patients and in all of them the molecular analysis was successful (1 mutation detected). Interestingly, patient C8 has a pathogenic mutation and also the p.A324A mutation. As both parents have a normal profile of aminoacids in urine and each parent has one of the above mutations, we can confidently say that in this case the mutations are in trans. C11 is the only patient with a non-type I phenotype that presents a mutation in SLC3A1. The mutation identified – E5_E9dup – has long been recognized as being capable of causing hyperexcretion of cystine in heterozygous state (4, 6).

With regards to the genetic classification of cystinuric patients, proposed by Dello Strologo, seven patients were type A and five patients type B. Thirteen of 21 mutated alleles identified in this study were in SLC3A1 gene, whereas the remaining 8 were in SLC7A9. There was no obvious relationship between the clinical and biochemical phenotype and the genotype. A significant difference in disease severity between genders was also not identified.

In keeping with previous reports, great allelic heterogeneity was found in this study. The most frequent mutations in our cohort were large rearrangements: deletions and duplications (accounting 33.3%) specially E5_E9 dup (the most common) – and c.1400T>C (p.M467T) accounting for 11.1% of mutated alleles. These results clearly show that any laboratory performing molecular diagnosis of Cystinuria must include assays for screening large deletions and duplications. Future studies may clarify whether any of the mutations here identified constitute Portuguese founder mutations.

Concerning the c.972G>A (p.A324A), it is possible that both (normal and aberrant) splicing sites can be used alternatively in cells, as this mutation was found in heterozygosity in all patients. However, this type of situation has been receiving progressive attention (9): a mutation in SLC7A9 that is not per se pathogenic but that certainly contributes to the phenotype and that should be taken into account when predicting the severity of the phenotype. In parallel, there has been increasing acknowledgment that many apparently silent mutations have significant deleterious consequences, causing aberrant splicing or disrupting regulatory sequences (25–27). Functional studies in cells homozygous for these mutations and the study of mRNA from kidney cells would shed more light in this discussion.

In summary, as the knowledge of biological systems becomes more solid, it is understood that some mutations are deleterious, others are innocuous and others can predispose or protect to a phenotype. This fluid vision of the interaction of pathogenic mutations and its genetic background can provide a more accurate genetic counseling.

This study also emphasizes the importance of being cautious in the interpretation of apparently silent mutations that are frequently identified as being functionally neutral, but can in fact disrupt regulatory or splicing sequences with significant consequences in the function of the encoded protein.

Finally, this report expands the spectrum of SLC3A1 and SLC7A9 mutations and provides...
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guidance in the clinical implementation of molecular assays in routine genetic counseling of Portuguese patients affected with Cystinuria.

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