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Novel *SLC7A7* large rearrangements in lysinuric protein intolerance patients involving the same *AluY* repeat

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Lysinuric protein intolerance (LPI) is a rare autosomal inherited disease caused by defective cationic aminoacid transport 4F2hc/y⁺LAT-1 at the basolateral membrane of epithelial cells in the intestine and kidney. LPI is a multisystemic disease with a variety of clinical symptoms such as hepatosplenomegaly, osteoporosis, hypotonia, developmental delay, pulmonary insufficiency or end-stage renal disease. The *SLC7A7* gene, which encodes the y⁺LAT-1 protein, is mutated in LPI patients. Mutation analysis of the promoter localized in intron 1 and all exons of the *SLC7A7* gene was performed in 11 patients from 9 unrelated LPI families. Point mutation screening was performed by exon direct sequencing and a new multiplex ligation probe amplification (MLPA) assay was set up for large rearrangement analysis. Eleven *SLC7A7*-specific mutations were identified, seven of them were novel: p.L124P, p.C425R, p.R468X, p.Y274fsX21, c.625 + 1G > C, DelE4-E11 and DelE6-E11. The novel large deletions originated by the recombination of *Alu* repeats at introns 3 and 5, respectively, with the same *AluY* sequence localized at the *SLC7A7* 3' region. The novel MLPA assay is robust and valuable for LPI molecular diagnosis. Our results suggest that genomic rearrangements of *SLC7A7* play a more important role in LPI than has been reported, increasing the detection rate from 5.1 to 21.4%. Moreover, the 3' region *AluY* repeat could be a recombination hot spot as it is involved in 38% of all *SLC7A7* rearranged chromosomes described so far. *European Journal of Human Genetics* (2009) 17, 71–79; doi:10.1038/ejhg.2008.145; published online 20 August 2008

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Introduction

Lysinuric protein intolerance (LPI, MIM 222700) is a primary inherited aminoaciduria caused by defective dibasic amino acid transport activity, which affects renal reabsorption of cationic amino acids and its transport function in the small intestine. Lung, liver, immune system, muscle and brain are also affected to a varying

extent in LPI patients.¹ LPI has an autosomal recessive mode of inheritance and is relatively common in Finland, with a prevalence of 1/60 000. It also occurs in Southern Italy and Japan (prevalence of 1/50 000 in the northern part of Iwate).^{2–4} Sporadic cases have been described worldwide in other 22 countries.^{5,6}

Lysinuric protein intolerance is a multiorgan disease with a variety of clinical symptoms. Patients with LPI are usually asymptomatic while being breast fed, and symptoms appear only after weaning. After infancy, they reject a high-protein diet and show bone growth delay, osteoporosis, hepatosplenomegaly and muscle hypotonia. Most patients show normal mental development, but some have moderate retardation. About two-thirds of the patients have interstitial changes in chest radiographs, sometimes with acute or chronic respiratory insufficiency that can lead to fatal pulmonary alveolar proteinosis and multiple-organ dysfunction syndrome.^{1,7} Recently, some patients with end-stage renal disease have been reported.⁸ Further symptoms, such as glomerulonephritis and erythroblastophagia, suggest that the immune system is affected in some patients.⁹

Lysinuric protein intolerance is diagnosed by urine hyperexcretion of dibasic amino acids, especially lysine, and by their poor intestinal absorption. As a result, plasma levels of dibasic amino acids are low in patients.^{1,7,9,10} Arginine and ornithine are intermediates of the urea cycle that provide the carbon skeleton. Their reduced availability is responsible for hyperammonaemia, which is thought to cause acute symptoms such as nausea and vomiting. Protein malnutrition and lysine deficiency contribute to the patient's failure to thrive.⁹ Patients are treated with low-protein diet and supplementation of L-citrulline, a urea cycle intermediate, absorbed by neutral amino acid transporters, which is used to correct their urea cycle functional deficiency. Low-dose oral lysine supplementation is potentially beneficial to patients with LPI starting at an early age.¹¹

The human gene responsible for LPI, *SLC7A7* (MIM 603593; RefSeq: NM_003982), was localized to 14q11.2 by linkage.^{12–15} It contains 11 exons (9 coding exons), 10 introns and two promoter regions spanning ~46.5 kb of the genomic DNA.^{3,4,16} The cDNA is 2477 bp long with a 527 bp 5'-UTR and a 385 bp 3'-UTR, with a polyA site located at 2447.⁶ It encodes the y⁺LAT1 protein (RefSeq: NP_003973), which has 511 predicted amino acids and a molecular weight of ~56 kDa.¹⁷ y⁺LAT1 heterodimerizes with 4F2hc to express system y⁺L amino acid transport activity in the basolateral plasma membrane of the epithelial cells of the renal proximal tubule and the small intestine.¹⁷ System y⁺L mediates the electro-neutral exchange of dibasic amino acids (efflux) for neutral amino acids and sodium (influx).¹⁷

Since now a total of 44 different *SLC7A7*-specific mutations have been identified in 131 patients including

all different types of mutations distributed along the entire gene.^{5,6} Five polymorphisms within the *SLC7A7* coding region and two in the 5'-UTR have also been identified.⁶ A founder effect mutation has been demonstrated only in Finland, where LPI patients share the same homozygous mutation, c.895-2A>T.^{2,14} LPI patients show extreme variability in clinical presentation, and no genotype–phenotype correlations have been defined.¹⁸ This phenotypic variability and the lack of a specific clinical presentation have led to various misdiagnoses.⁶

Here we describe the spectrum of mutations in the *SLC7A7* gene in nine unrelated LPI families. An *SLC7A7* gene-specific multiplex ligation probe amplification (MLPA) assay was set up for the first time to search for large rearrangements in *SLC7A7* gene. Seven novel mutations have been found: two missense, a nonsense, a splice donor, a nucleotide duplication and two large deletions originated by *AluY*-mediated homologous recombination.

Materials and methods

Patients

We studied 11 LPI patients, and their relatives when available, from nine unrelated families with different geographic origins: Spanish (three), Chilean (two, half brothers), Moroccan (two), English-Argentinean (one), French (one) and Greek (two, brothers). According to the referring centres, the main clinical features of these patients were compatible with those of LPI (Table 1). Informed consent was obtained from all patients, their parents and 50 healthy controls. This research was prospectively reviewed and approved by the Ethics Committee of the IDIBELL.

Genomic DNA amplification, sequencing and mutation analysis

DNA was extracted from peripheral leukocytes from whole blood using standard protocols. Mutational screening of the whole coding region and all intron/exon boundaries was performed by PCR and direct sequencing, with PCR conditions and primers reported earlier.³ The promoter localized in intron 1, which is the active promoter in tissues with strong defects in LPI patients,¹⁶ was also amplified with primers (5'-CTGGCCTGATTCCTCATATT-3') and [5'-GAGGGTTAGCAAGGTAAGTGG-3'), following standard methods. The reaction started with an initial denaturation of 5 min at 94°C, followed by 36 cycles of 94°C 30 s, 58°C 30 s, 74°C 1 min and 5 min at 74°C.

All newly described mutations were checked on 100 control chromosomes to discard polymorphisms. All new mutations follow the nomenclature of the HGVS (<http://www.hgvs.org/mutnomen/>) and have been introduced in the HGMD database (<http://www.hgmd.org/>).

Table 1 Clinical features of LPI patients

Patient no.	1	2	3	4	5	6	8	9	10
Age	6 years	11 years	2 years	14 years	12 years	13 years	9 months	14 months	15 years
Sex	M	F	M	M	F	F	F	F	M
Basic hyperaminoaciduria	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Basic hypoaminoacidaemia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Developmental delay	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Protein intake aversion	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hepatosplenomegaly	Yes (mild)	Yes (mild)	Yes (mild)	Yes	Yes	Yes	No	No	Yes (mild)
Osteoporosis	Yes	Yes	—	Yes	Yes	—	—	No	Yes
Hypotony	No	No	Yes	No	No	Yes	Yes	Yes	—
Anaemia	Yes	No	Yes	Yes	Yes	—	No	No	—
Mental retardation	—	No	—	—	Yes	—	—	No	—
Pulmonary insufficiency	No	No	—	—	No	Yes	—	—	—

MLPA analysis

Probes within the MLPA set were designed following instructions by MRC Holland (http://www.mlpa.com/pages/support_desing_synthetic_probespag.html). The presence of repetitive sequences was excluded using the BLAT program from UCSC website (<http://genome.ucsc.edu>),¹⁹ and regions with SNPs or known LPI-specific mutations were avoided (<http://www.ensembl.org> and reported LPI data) (Supplementary Table 1). Oligonucleotides were ordered from Sigma Genosys (UK, <http://www.sigma-genosys.com>). MLPA reactions were performed essentially as described²⁰ with hybridization and ligation reagents purchased from MRC Holland. Genomic DNA (150–200 ng) in a final volume of 5 µl was heated at 98°C for 5 min. After cooling to room temperature, 1.5 µl probe mix and 1.5 µl SALSA hybridization buffer were added to each sample, heat-denatured at 95°C for 1 min, followed by hybridization for 16 h at 60°C. Ligation was performed at 54°C by adding 32 µl ligation mix. After 15 min, the reaction was stopped by heat inactivation at 95°C for 5 min. Amplifications were carried out in a 25 µl final volume containing 5 µl of ligation product, 2 µl of SALSA PCR buffer (MRC Holland, Amsterdam), 1 µl of Taq dilution buffer (Roche Diagnostics), 180 nM of each MLPA primer (Sigma), 1 mM of dNTPs (Ecogen, Spain) and 1.25 U of Taq Polymerase (Roche Diagnostics). The products were analysed on an ABI PRISM 3100 genetic analyser according to the manufacturers' instructions. MLPA data were analysed by visual examination of the peak profiles and by using the relative peak height (RPH) method as previously described.²¹ Heterozygous deletions and duplications should show an RPH of ~0.5 and 1.5, respectively, and no peak reveals a homozygous deletion.

Characterization of large rearrangements

Eleven PCR primers were designed along nonrepetitive sequences from intron 3, intron 5 and the 3' region, obtained in the RepeatMasker Web Server (<http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker>) (primers sequences are available upon request). PCR on patient nos. 3 and 5, with all possible combinations of forward primers

from the respective intron and reverse primers from the 3' region, was performed. Positive amplification was obtained with primers: LPI_I3F7 (5'-CTGAGCATCTGATCCAGAGT-3') and LPI_3'R1 (5'-CAGTCCTAGGAACTGTGGCA-3') for DelE4-E11, and E5F (5'-TGTCAGTCCCTTTACTTGACCATC-3') and LPI_3'R1 for DelE6-E11 characterization. PCR was performed as described above. An initial denaturation of 5 min at 94°C was followed by 10 cycles at 94°C 20 s, 58°C 20 s, 72°C 15 min and 30 cycles at 90°C 20 s, 58°C 20 s, 72°C 15 min and a final extension of 7 min at 72°C.

Results

Clinical data

All patients had plasma concentrations and amino acid urinary excretion patterns characteristic of LPI. Some patients had elevated serum ferritin and lactate dehydrogenase concentrations. They had shown hyperammonaemia after high-protein meals, and most of them had hepatosplenomegaly, developmental delay, bone growth delay, osteoporosis and muscle hypotonia (Table 1). Some patients presented anaemia, pulmonary insufficiency and/or mental retardation. All patients are under treatment with L-citrulline (treatment of patient no. 2 has been already reported²²).

Mutation analysis of *SLC7A7*

Eleven *SLC7A7* mutations were found, seven of which are novel.¹⁶ The mutations comprise two frameshift (c.820dupT, c.1185_1188delTTCT), three nonsense (p.R410X, p.Y457X, p.R468X), three missense (p.L124P, p.L334R, p.C425R), a splice donor (c.625 + 1G > C) and two large rearrangements identified by MLPA (DelE4-E11, DelE6-E11). All mutations segregated with the disease status in the respective families (Figure 1c). The novel mutations are p.L124P, p.C425R, p.R468X, c.625 + 1G > C, c.820dupT, and the two large deletions are DelE4-E11 and DelE6-E11 (Figure 1 and Table 2). None of them were detected in 100 control chromosomes tested (data not shown). In addition, none of the *SLC7A7* sequence changes

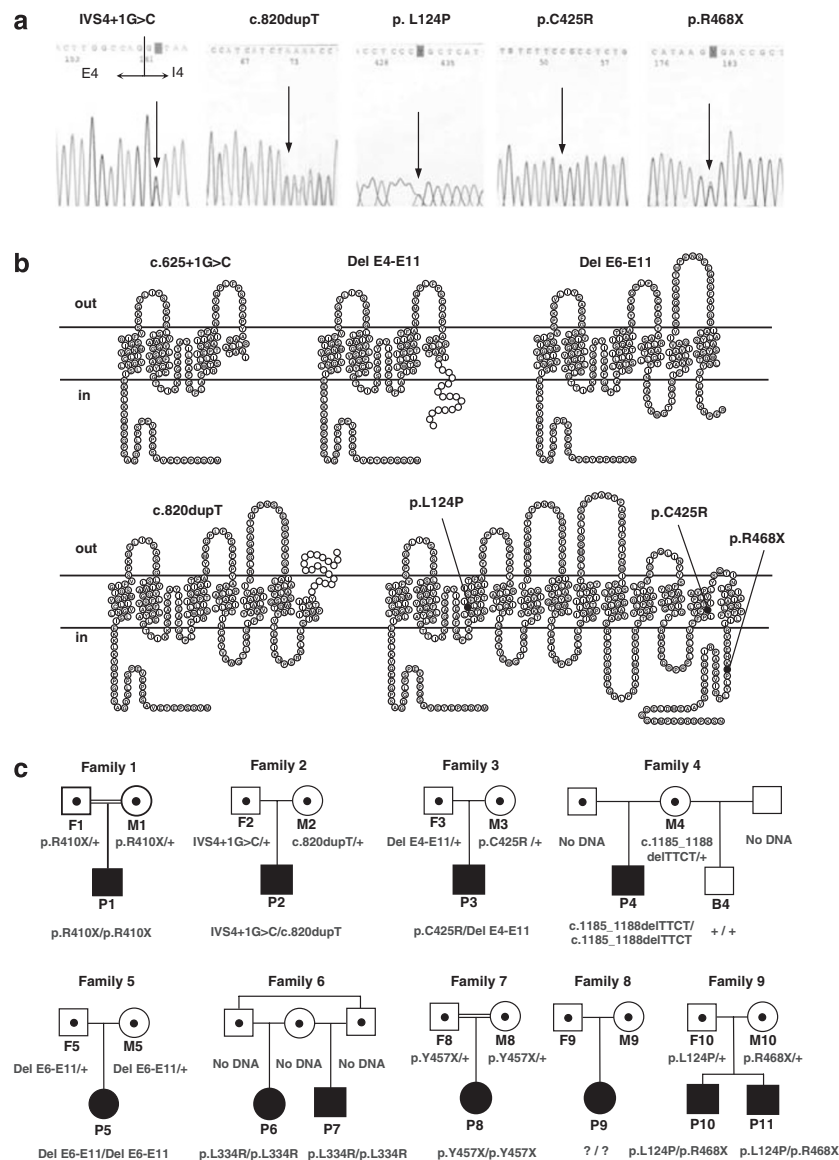


Figure 1 New *SLC7A7* point mutations in patients with LPI and segregation analysis of all the mutations detected in LPI families. (a) Electropherogram sections showing the new point mutations: p.L124P, p.C425R, p.R468X, c.625 + 1G > C and c.820dupT. (b) Scheme of the predicted mutant proteins in the topology model of human y^+ LAT-1 of 12 transmembrane domains. All the novel mutations but two encode truncated proteins, if translated. Interestingly, c.625 + 1G > C and DelE4-E11 mutations would encode nearly the same protein lacking the last nine transmembrane domains, but the differences at the mRNA level (presence or absence of polyA signal, 3'-UTR and so on) could give differences in its stability and translation. Notice that two of the frameshift mutations, Del E4-E11 and c.820dupT, have addition of missense residues (white circles). (c) Segregation of all the mutations found in 11 patients with LPI by direct sequencing. Parents of patient nos. 1 and 7 are closely consanguineous and patient nos. 6 and 7 have the same mother and their fathers are brothers. In families 4 and 6, the segregation could not be performed because there was no DNA available from the relatives, but normal MLPA pattern of these patients (Supplementary Table 1) confirmed the homozygosity of these patients. Square symbols are men and circles are women. The filled symbols are affected individuals and the half-filled symbols are obligated carriers. B, brother; F, father; M, mother; P, patient; +, wild-type allele.

underlying these novel mutations are listed in the NCBI Single Nucleotide Polymorphism database (dbSNPs; <http://www.ncbi.nlm.nih.gov/projects/SNP>) or in Ensembl (<http://www.ensembl.org>). Seven silent polymorphisms were also found, some of them reported earlier^{2,4} (Supplementary Table 2).

Figure 1 and Table 2 show the novel mutations found in this group of patients. c.625 + 1G > C mutation is a G-to-C transversion that modifies the splice GT donor site of intron 4. Interestingly, the mutation is located in the same nucleotide position as c.625 + 1G > A reported earlier, which was demonstrated to cause exon 4 skipping,⁴

Table 2 Mutations in the *SLC7A7* gene causing LPI in the patients analysed

Patient no.	Nucleotide change	Predicted protein change	Status	Exon/Intron	Mutation type	Geographic origin
1	c.1228C>T	p.R410X	Homozygote	9	Nonsense	Moroccan
2	c.820dupT	p.Y274fsX21	Compound	6	Frameshift	English/
3	c.625+1G>C	p.C167_G209delinsX	heterozygote	4	Splice site	Argentinean
	c.1273T>C	p.C425R	Compound	10	Missense	Spanish
4	c.500–4294_1908+1028 del12136	p.L168_N511delfsX19	heterozygote	13-3'region	Large rearrangement	
	c.1185_1188delTTCT	p.S396fsX121	Homozygote	9	Frameshift	Spanish
5	c.771–848_1908+718 del4647	p.N258_N511delinsX	Homozygote	15-3'region	Large rearrangement	Spanish
6 and 7	c.1001T>G	p.L334R	Homozygote	8	Missense	Chilean
8	c.1371C>A	p.Y457X	Homozygote	10	Nonsense	Moroccan
10 and	c.371T>C	p.L124P	Compound	3	Missense	Greek
11	c.1402C>T	p.R468X	heterozygote	10	Nonsense	

suggesting aberrant splicing for this mutation. This would result in a truncated γ^+ LAT1 protein with only three transmembrane domains and the re-entrant loop between TMD II and III (p.C167_G209del42insX). The c.820dupT mutation in exon 6 creates a frameshift after residue I273 with the addition of 20 missense residues before the first stop codon (p.Y274fsX21) eliminating half of the protein, resulting in a truncated protein with six TMD. The p.C425R mutation is caused by a C-to-T transition at nucleotide position 1228 of the cDNA in exon 10, resulting in a nonconservative amino acid exchange from a slightly polar amino acid (cysteine) to a positively charged amino acid (arginine) at position 425. The substituted cysteine residue is located in the transmembrane domain XI (residues 419–438) of γ^+ LAT-1, introducing a positively charged residue. The p.L124P mutation is caused by a T-to-C transition at nucleotide position 371 of the cDNA in exon 3, which is located in the transmembrane domain III (residues 117–140) of γ^+ LAT-1. The p.R468X is a C-to-T transition at nucleotide position 1402 of the cDNA in exon 10. The premature stop codon should originate a truncated protein lacking the last 44 cytoplasmic residues, if transcribed.

MLPA analysis

The presence of *SLC7A7* large rearrangements was assessed by MLPA with specific probes to find out copy number changes in these patients. Figure 2 shows the abnormal MLPA patterns found in two LPI families. In family no. 3, the patient and his father bore a deletion of exons 4–11, whereas the mother, who bore p.C425R mutation in heterozygosis, showed a normal MLPA pattern. In family no. 5, both parents showed half-dosage for exons 6–11, whereas the patient showed no peak for these exons. The rest of the patients and relatives and 10 control individuals showed a normal MLPA pattern (Supplementary Table 3).

Breakpoint characterization of the novel large rearrangements

Breakpoints of the two large *SLC7A7* deletions were mapped by standard PCR across the junction fragments with primers that specifically amplify the mutated allele in both cases. In patient no. 3, a 1 kb fragment was amplified, purified and sequenced using primers LPI-13F3 and LPI-3'R1, showing a deletion of 12 136 bp with a breakpoint at 4294 bp upstream of exon 4 and 1028 bp downstream of exon 11 (c.500–4294_1908+1028del12136) (Figure 3a). The protein product expected from the 12 kb deletion, if translated, would have a frameshift after residue C167 with the addition of 18 missense residues before the first stop codon (p.L168_N511delfsX19), lacking transmembrane domains IV–XII (Figure 1b). In patient no. 5, a 3 kb fragment was amplified with primers LPI-23FG and LPI-3'R1 showing a deletion of 4647 bp with a breakpoint at 848 bp upstream of exon 6 and 718 bp downstream of exon 11 (c.771–848_1908+718del4647) (Figure 3b). The protein product of the 4.6 kb deletion would have a stop codon after residue R257 (p.N258_N511delinsX), lacking transmembrane domains VII–XII (Figure 1b).

Both large deletions were originated by the crossing over between two direct *Alu* repeats: DelE4-E11 was originated by recombination of a 296 bp *AluSc* in intron 3 and a 316 bp *AluY* in the *SLC7A7* 3' region, whereas DelE6-E11 was originated by recombination of a 173 bp *AluJb* in intron 5 and the same *AluY* repeat in the *SLC7A7* 3' region (Figure 3). BLAST two sequences of *AluSc-AluY* and *AluJb-AluY* revealed 86 and 80% nucleotide identity, respectively. Analysis of the genomic regions implicated in these deletions showed a high content of repetitive elements. Intron 3 of *SLC7A7* gene contains 70 complex repetitive elements (47 *Alu*, 4 MIRs, 12 LINEs, 4 LTR elements, 2 MERs), which represent 67% of the 32.8 kb of the sequence. Intron 5 has six complex repetitive elements (four *Alu* and two MIRs) along 2.4 kb, which represent 45% of the intron, whereas in the *SLC7A7* 3' region, there are eight repetitive

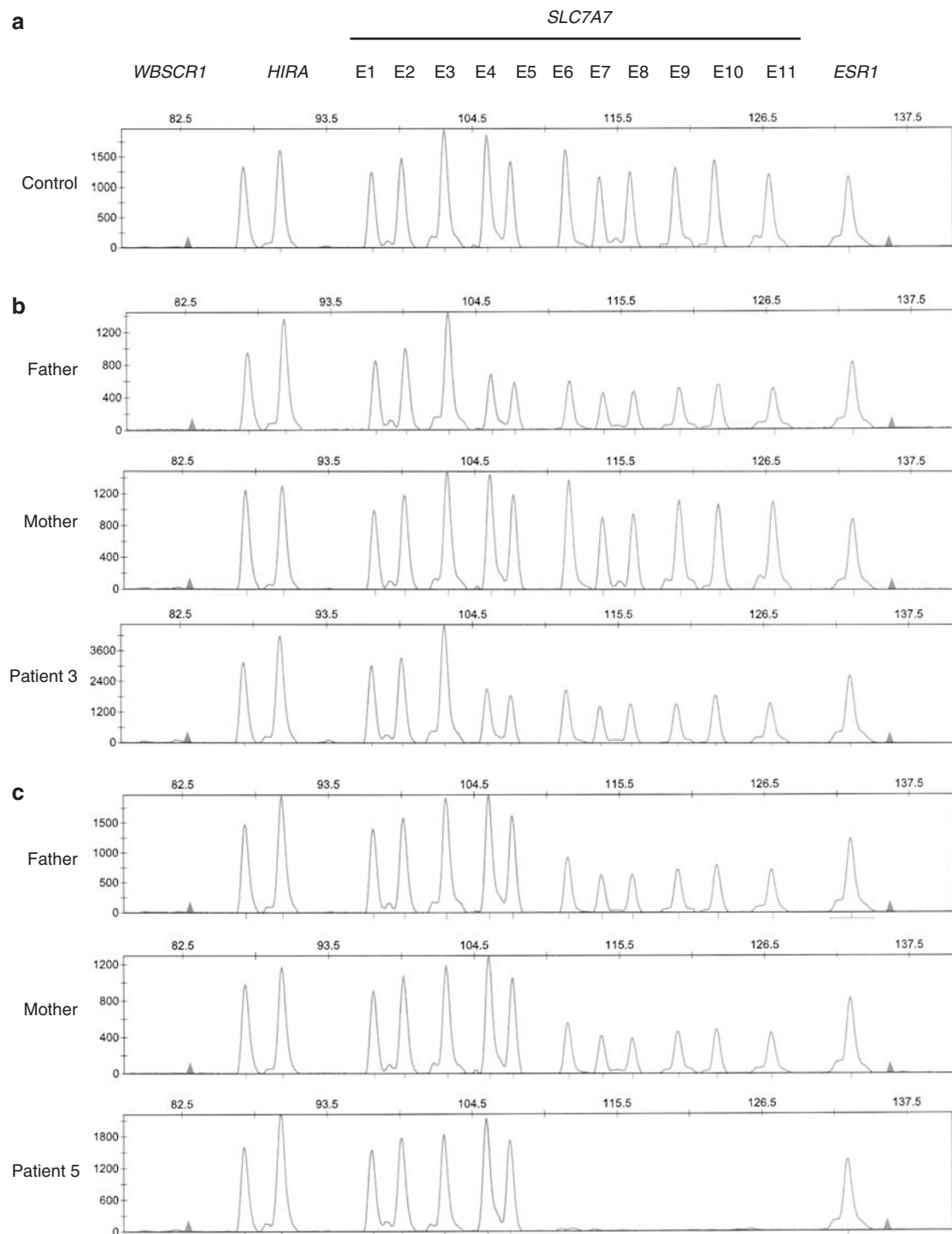


Figure 2 MLPA patterns of families 3 and 5. Samples were subjected to MLPA with 11 specific oligonucleotide probes for *SLC7A7* exon sequences. Three control probes for genes located on different chromosomes were also added in the assay as external controls. Each individual region's peak height is normalized by dividing it by the sum of control gene peak heights (*WBSR1*, *HIRA*, *ESR1*). Each normalized peak height is then divided by the corresponding average normalized peak height from control samples to obtain the RPH. (a) Normal *SLC7A7* MLPA pattern obtained from a control individual. (b) Deletion of the two-thirds of the *SLC7A7* gene detected by MLPA in family no. 3. The father and the patient show an abnormal pattern. MLPA peak pattern from the mother is the same as that of the control. (c) Deletion of the last six exons of *SLC7A7* gene detected by MLPA in family no. 5. Both parents carry the deletion in heterozygosis and the patient is a homozygote.

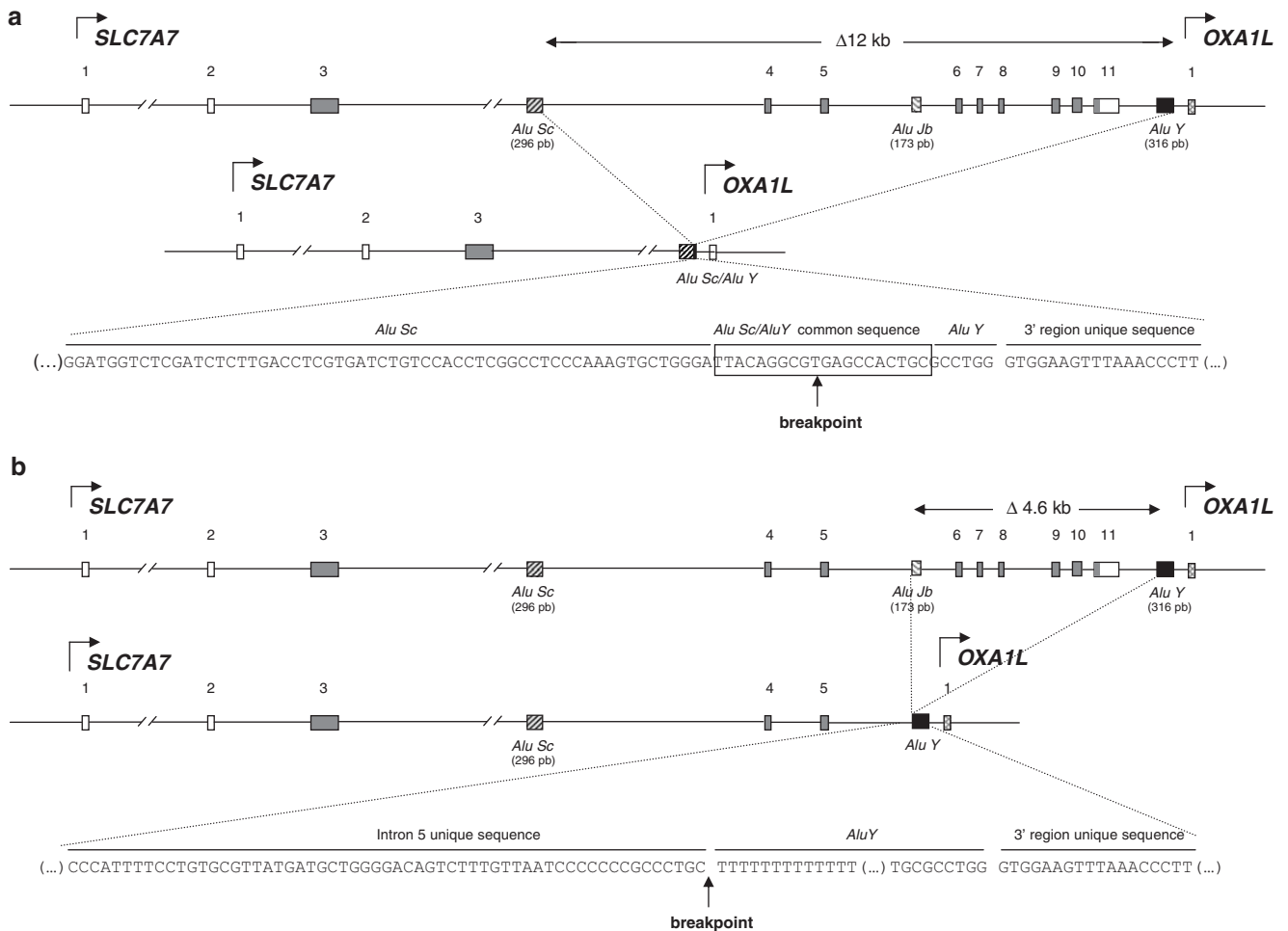


Figure 3 Scheme of the two large deletions in *SLC7A7* originated by the crossing over between two direct *Alu* repeats, found in LPI patient nos. 3 and 5. **(a)** Genomic scheme of the *SLC7A7* gene and the 12 kb deletion (DelE4-E11) found in patient no. 3, with a breakpoint at 4294 bp upstream exon 4 and 1028 bp downstream exon 11 (c.500–4294_1908 + 1028del12136). Notice that the breakpoint could be generated in any of the nucleotides in the box, as this 20 nt sequence is the same in both the *Alu* repeats involved in this genomic rearrangement. The mutation nomenclature localizes the breakpoint at the 3' end of the 20 nt sequence. **(b)** The 4.6 kb deletion (DelE6-E11) found in patient no. 5, had the breakpoint at 848 bp upstream of exon 6 and 718 bp downstream of exon 11 (c.771–848_1908 + 718del4647). In DelE4-E11, the breakpoint was at the 3' end of *AluY*, whereas in DelE6-E11, the breakpoint was located at the first nucleotide of the *AluY* sequence.

sequences (five *Alu* and three LINES) along the 5 kb downstream exon 11.

Discussion

To date, 51 LPI-specific *SLC7A7* mutations have been described (this study and Cimbalistiene *et al*⁵ and Sperandeo *et al*⁶), including 21 missense, 6 nonsense, 5 splice site, 4 small duplications/insertions, 10 small deletions and 5 large genomic rearrangements. The mutation screening of *SLC7A7* gene performed in this study revealed seven novel mutations and four mutations described earlier in eight LPI-unrelated families, which explain 89% of the studied alleles. Patient no. 9 bore only three polymorphisms in heterozygosis: rs8019656:G>A, c.1-86C>T

and c.159A>G. She showed the mildest phenotype with good clinical evolution, indicating that she could have been misdiagnosed. Alternatively, missing mutations in this patient might be located in unexplored intronic regions. We considered the possibility of finding mutations in the heavy-chain 4F2hc very unlikely, as no LPI-associated mutation had been found in *SLC3A2*,¹⁸ and *Slc3a2* knockout mouse is lethal.²³ Indeed, 4F2hc serves, in addition to system y^+L , as the heavy subunit of five other amino acid transporters and has a role in $\beta 1$ -integrin function.²⁴

All the new mutations identified but two encode for severely truncated proteins, if translated, suggesting a dramatic loss of transport function in these cases. The novel large deletions (DelE6-E11 and DelE4-E11) were associated with the most severe phenotypes. A plausible

explanation would be that truncated transcripts lacking 3'-UTR sequence and polyA signal would end up in rapid degradation.²⁵ In contrast, frameshift (c.820dupT), splice-site (c.625 + 1G>C) and nonsense (p.R468X) mutations associate with less severe phenotypes in this group of patients. These mutations also introduce premature termination codons into the mRNA, which in most cases result in degradation through a mechanism known as nonsense-mediated decay and loss of function of the mutated allele.²⁶ However, c.1185_1188delTTCT mutation found in homozygosis and c.820dupT mutation found in heterozygosis are associated with a milder phenotype, suggesting that some of the truncated transporter may reach the plasma membrane and may retain partial activity in these patients. The missense mutation p.L124P is located in the transmembrane domain III in a highly conserved amino acid region among the known eukaryotic members of LAT family. The missense mutation, p.C425R, introduces a charged residue in transmembrane domain XI. None of the >80 known eukaryotic members of the LAT family, to which γ^+ LAT1 belongs, has a charged residue in this position or in the entire transmembrane domain XI.²⁷ This suggests a loss of transport function for these mutated transporters.

A detection system for SLC7A7 rearrangements by MLPA was established, and two novel large deletions of 12 and 4.6 kb were detected in two families. Interestingly, both deletions were originated by Alu-mediated recombination with identical AluY repeat at the 3' region. These deletions involve the 5' region of the adjacent gene OXAIL, as AluY element localizes at 388 bp of this gene, overlapping the SLC7A7 3' region. OXAIL gene encodes a mitochondrial integral membrane protein required for the correct biogenesis of F(1)F(o)-ATP synthase and NADH:ubiquinone oxidoreductase.²⁸ Considering that the homozygote patient with DelE6-E11 (patient no. 5) has a severe but characteristic LPI phenotype, the deletion of this sequence from the OXAIL 5' region may not affect the transcription of this contiguous gene, but further enzymatic deficiency studies of the OXPHOS system should be performed in this patient to confirm this.

Alu repeats are short interspersed nuclear elements, which are the most common repetitive DNA units dispersed throughout the genome. The 280-bp repeat unit occurs approximately once every 4 kb in the human genome.²⁹ Regions with high Alu repeat content are prone to recurrent genomic rearrangements through Alu–Alu recombination events.³⁰ In fact, there are several inherited disorders for which Alu-mediated recombination is a common cause.³¹ Given the high Alu density in introns 3, 5 and the 3' region, and the high degree of homology between Alu repeats, a recombination event could potentially occur anywhere within this region. However, the same AluY at the 3' region is involved in 38% (three out of eight) of all unrelated chromosomes with SLC7A7 large

rearrangements described so far,⁶ suggesting that it could be a recombination hot spot, as it has been observed at the duplication and deletion breakpoints in a number of human genetic diseases.^{30–33} The Alu core sequence itself has been suggested to promote DNA-strand exchange and genomic rearrangement, and it has striking sequence similarity to chi (which has been shown to stimulate recBCD-mediated recombination in *Escherichia coli*).³⁰ Although repeat DNA sequences may predispose to abnormal chromosome pairing and unequal crossing over, the fact that this region could be a hot spot implies that there must be other cellular mechanisms (sequence signals close to this AluY, chromatin structure³³ and so on) that may play a more prominent role in the initiation of chromosomal rearrangements.

Our results demonstrate that the percentage of chromosomes with large rearrangements in SLC7A7 gene in this study is more than four times higher (21.4%) than that the previously reported by all other non-Finnish groups of patients (5.1%).^{5,6} This demonstrates that deletions within this gene are more common than those have been reported so far, suggesting that the latter percentage is probably underestimated. In fact, there are some LPI patients with no mutated alleles reported in the literature: a patient with suspected LPI in which no mutations were found² and five patients with only one mutant allele identified.⁶ In these cases, the screening strategy was based on sequencing of coding regions and intronic boundaries. These patients could have large rearrangements considering our results, although mutations in the intronic regions cannot be discarded. In addition, some of the previously reported homozygotes with no segregation performed could in fact be compound heterozygotes with a large deletion in the other allele. The MLPA technique has allowed us not only to find novel large rearrangements but also to discard hemizygosities in families in which there was no DNA from the relatives.

In conclusion, our study shows that deletions of the SLC7A7 gene are a common cause of LPI in non-Finnish populations, and therefore we highly recommend this MLPA assay for molecular diagnosis of LPI patients, as it covers the whole gene in one simple and reproducible assay. We estimate that it would detect around 15–20% partial or whole gene deletions in non-Finnish patients. Our data also suggest that recombination between Alu repeat elements is likely to be the major mechanism underlying SLC7A7 gene rearrangements. Moreover, the AluY repeat localized at 3' of the SLC7A7 gene is involved in the majority of large rearrangements described in LPI patients.

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