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Exploring the transcriptomic variation caused by the Finnish founder mutation of lysinuric protein intolerance (LPI)

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ABSTRACT

Lysinuric protein intolerance (LPI) is an autosomal recessive disorder caused by mutations in cationic amino acid transporter gene *SLC7A7*. Although all Finnish patients share the same homozygous mutation, their clinical manifestations vary greatly. The symptoms range from failure to thrive, protein aversion, anemia and hyperammonaemia, to immunological abnormalities, nephropathy and pulmonary alveolar proteinosis. To unravel the molecular mechanisms behind those symptoms not explained directly by the primary mutation, gene expression profiles of LPI patients were studied using genome-wide microarray technology. As a result, we discovered 926 differentially-expressed genes, including cationic and neutral amino acid transporters. The functional annotation analysis revealed a significant accumulation of such biological processes as inflammatory response, immune system processes and apoptosis. We conclude that changes in the expression of genes other than *SLC7A7* may be linked to the various symptoms of LPI, indicating a complex interplay between amino acid transporters and various cellular processes.

1. Introduction

Lysinuric protein intolerance (LPI [MIM 222700]) is a rare aminoaciduria with an autosomal recessive inheritance. It is a classic monogenic disease, caused by mutations in cationic amino acid (CAA) transporter gene *SLC7A7* [1,2]. Its gene product, y^+LAT1, heteromerizes with *SLC3A2*-encoded 4F2hc [3–5] to form an exchanger for cationic and neutral amino acids, thereby providing the main export route for CAAs from both epithelial and non-polar cells [6,7]. This transporter is involved in the absorption of CAAs in the small intestine and the proximal kidney tubules. Hence, LPI mutations impair both the intestinal and renal transport of CAAs [8]. Their effects in non-polar cells (for example lymphocytes) are currently less well-known [9,10].

To date, 51 different mutations have been discovered in *SLC7A7* world-wide, including insertions, deletions, point mutations and frame shift mutations [11–15]. The Finnish LPI patients, however, all share the same point mutation, LPI_{Fin}, a substitution of T for A at cDNA position 1181–2 [1,2] (IV5-2A-T [12], c.895-2A>T [14]), that obliterates a splicing site, which in turn results in a frame shift and consequent putative shortening of the polypeptide. This genetic uniformity of the Finnish patients offers an excellent basis for studying the impact of other genes and factors on the variable symptoms of LPI. An additional advantage is that the patients are clinically well characterized, and their follow-up is centralized at our clinic.

The symptoms of LPI are extremely variable even among patients sharing the same mutation. Therefore, no correlation between the genotype and the clinical manifestations has been detected. The connection between the CAA transport defect and variable multisystem symptoms remains poorly characterized, but it has been suggested that the accumulation of arginine in the cells with the defect and the subsequent increase of nitric oxide (NO) may be of essence [10]. The symptoms include failure to thrive, protein aversion, osteoporosis, hepatosplenomegaly and immunological abnormalities (e.g. deficient B cell functions: low concentrations of IgG subclasses and poor vaccination response), but some patients also develop severe life-threatening complications, such as pulmonary alveolar proteinosis and nephropathy [8,16–19]. Due to the transport defect, without treatment, the patients' plasma concentrations of lysine, arginine and ornithine are from one third to a half of normal values, and those of serine, alanine, glycine, proline, citrulline and glutamine are slightly raised [20]. Most of the Finnish patients suffer from slight thrombocytopenia and unexplained normochromic normocytic anemia, which is particularly severe and persistent in those who have undergone kidney transplantation (unpublished data).

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The aim of this study was to define the genetic factors and molecular mechanisms behind the clinical variability in the Finnish patients by examining their transcriptome by means of microarray technology. The exact cause of the variable symptoms still remains unclear but, based on our results, changes in the expression of genes other than *SLC7A7* may be linked to the LPI symptoms and their variability. More generally, our study demonstrates the complexity of the genetic network contributing to the clinical outcome of this monogenic disease.

2. Materials and methods

2.1. Study subjects

For the microarray study, thirteen Finnish LPI patients were chosen from a cohort of 38 patients in follow-up at the Department of Pediatrics, University of Turku and the Turku University Hospital. Patients with severe complications such as end-stage renal disease and active alveolar proteinosis were excluded from the study since our aim was to examine the patients with moderate symptoms representing the 'classic' clinical characteristics of LPI. Of the 13 patients included, seven were female. Their mean age was 29.9 years (range from 7 to 47 years).

The control samples were collected from 10 healthy volunteers (five female) who were age and sex-matched to the patients. The age of the controls varied from 9 to 48 years (mean 30 years). The diets of the control individuals were not controlled, but they can be expected to differ markedly from those of the patients, the patients being on a permanent protein-restricted diet. In addition to health reasons, the patients' diet is maintained by their strong natural protein aversion. Therefore, recruiting healthy volunteers on a similar diet was unfortunately impossible.

The patients' mean age at the time of the LPI diagnosis was 7.3 years (range from 0.1 to 31 years). Three patients suffered from chronic nephropathy with mild proteinuria, haematuria and a moderately elevated serum creatinine concentration. Two patients had experienced one or more episodes of alveolar proteinosis, but were currently in remission. One patient had been diagnosed with type 2 diabetes. All 13 patients suffered from moderate to severe osteopaenia, and six of them had a history of multiple fractures in childhood. Skeletal maturation was delayed in the children. Blood hemoglobin values were normal in all the patients, but two had microcytosis (Table 1, Supplementary Table 1A).

The amino acid supplementation of the patients had been adjusted according to the plasma amino acid concentrations. The treatment consisted of a protein-restricted diet, calcium and vitamin D supplements, and oral supplementation with citrulline, either alone or in combination with sodium benzoate or sodium phenylbutyrate if nitrogen scavengers were needed to control hyperammonaemia. In addition, 7 patients received low-dose oral lysine supplementation at meal times. Twelve of the 13 patients had combined hyperlipidemia and responded poorly to dietary therapy, and 5 were treated with statins. Six patients were on antihypertensive medication, and one patient received carnitine supplementation for hypocarnitinemia. The laboratory findings and amino acid supplementation of the 13 patients included in the microarray study are presented in more detail in Table 1.

The second part of the study consisted of quantitative real-time PCR verification of the genes of interest uncovered in the microarray study. For this part, we collected samples from an additional 22 LPI patients raising the number of study subjects to 35. The medication and symptoms of these patients are presented in Supplementary Table 1A and B, respectively [20].

Informed consent was obtained from all the patients or their parents. The investigation corresponds to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital District of Southwest Finland.

2.2. Methods

2.2.1. Blood collection and RNA extraction

Whole blood from the patients and control subjects was collected into PAXgene Blood collection tubes (PreAnalytiX, Hombrechtikon, Switzerland). The total RNA was subsequently extracted from the peripheral blood cells using the PAXgene Blood RNA Kit (PreAnalytiX) according to the manufacturer's instructions, apart from the elution step; instead of the elution buffer provided in the kit, we used sterile, pyrogen and RNAase-free water (Promega Corporation, Madison, WI, USA).

2.2.2. RNA amplification

5.0 μg of the total RNA of each patient (apart from one patient, from whom the yield of total RNA was only 4.5 μg) and of the pooled

Table 1
Laboratory findings and relevant medication of the patients in the microarray analysis (P1-P13).

		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	
Gender		F	F	F	F	M	M	F	F	M	M	M	F	M	
Age at dg		1.0	4.9	3.5	3.0	20.6	5.4	3.0	0.2	7.0	0.3	2.2	12.0	30.6	
Age at study		6.9	10.7	14.8	18.8	20.6	31.8	36.4	37.1	39.3	39.6	41.6	44.8	46.6	
Laboratory findings	Unit	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	Mean
P-arginine[a]	μmol/l	38	42	39	44	24	40	25	20	65	29	30	20	50	36
P-citrulline[a]	μmol/l	84	69	92	116	60	58	105	54	110	94	86	81	91	86
P-glutamine[a]	μmol/l	1717	1557	1056	1921	2761	1638	703	1112	1007	740	663	821	744	1211
P-lysine[a]	μmol/l	130	132	119	138	43	116	105	91	22	94	108	157	165	109
S-cholesterol[b]	mmol/l	5.0	5.7	7.3	5.6	5.4	7.2	6.4	6.8	6.3	5.3	8.3	4.7	5.3	6.1
Creatinine	μmol/l	51	60	104	75	67	105	75	73	146	108	60	80	156	95
Haemoglobin	g/l	118	131	154	123	111	151	146	132	137	133	155	139	132	136
Ferritine	μg/l	551	512	916	753	1057	3932	953	652	1698	3645	537	922	860	1501
Thrombocytes	E9/l	144	208	123	174	107	112	84	225	204	154	162	146	184	155
Leukocytes	E9/l	3.9	5.6	5.8	3.8	3.4	4.2	3.4	5.9	4.5	6.4	5.2	6.3	4.9	4.8
Medication	Dose	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	Mean
Statine		-	-	-	-	-	-	yes	-	yes	yes	-	yes	yes	
Citrulline suppl.	mg/kg/d	179	116	86	128	80	91	80	86	70	159	50	94	90	93
Lysine suppl.	mg/kg/d	-	-	13	28	15	22	-	-	21	10	-	34	-	22
Na benzoate	mg/kg/d	122	-	-	-	-	86	25	-	-	-	-	-	-	-

[a] P = Plasma concentration.
[b] S = Serum concentration.