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Chromosomal Microdeletions and Genes' Functions: A Cluster of Chromosomal Microdeletions and the Deleted Genes' Functions

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ARTICLE

Global distribution of the most prevalent deletions causing hypotonia–cystinuria syndrome

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Hypotonia–cystinuria syndrome (HCS) is a recessive disorder caused by microdeletions of *SLC3A1* and *PREPL* on chromosome 2p21. Patients present with generalized hypotonia at birth, failure to thrive, growth retardation and cystinuria type I. While the initially described HCS families live in small regions in Belgium and France, we have now identified HCS alleles in patients and carriers from the Netherlands, Italy, Canada and United States of America. Surprisingly, among the nine deletions detected in those patients, only one novel deletion was found. Furthermore, one previously described deletion was found six times, another twice. Finally, we have investigated the frequency of both deletions using a random Belgian cohort. Given the global occurrence, HCS should be considered in the differential diagnosis of neonatal hypotonia.
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Introduction

The hypotonia–cystinuria syndrome (HCS; MIM 606407) is a recessive congenital disorder characterized by generalized hypotonia at birth, failure to thrive, growth retardation and cystinuria type I.[1,2] Recently, the genetic defect underlying this syndrome has been characterized in nine families.[1] The disease is due to microdeletions that disrupt two genes on chromosome 2p21, *SLC3A1* and *PREPL*. As mutations in *SLC3A1* cause isolated cystinuria type I and the flanking genes are normally expressed, the extended phenotype can be attributed to *PREPL*.[1]

A larger deletion of 179 kb at this locus has previously been reported,[3,4] which causes the recessive 2p21 deletion syndrome. At least four genes (*SLC3A1*, *PREPL*, *PPM1B* and *C2orf34*) are deleted, resulting in a much more severe phenotype. A detailed comparison between the two syndromes has been published.[5]

At least three additional deletions involving *PREPL* have been reported in patients with isolated cystinuria type I. However, they were not fully characterized.[6–8] Finally, one sporadic case with HCS symptoms, accompanied with a mitochondrial respiratory chain defect has been reported. Molecular analysis of the *SLC3A1/PREPL* locus was suggested but not performed.[9]

This report focuses on the molecular analysis of four new HCS families, including two previously described cases.

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Finally, the frequency of the most prevalent deletions causing HCS was estimated using a random Belgian population.

Materials and methods

Patient material

Material from family 10 (University Hospitals Leuven, Belgium), family 11 (University Medical Centre Leiden, The Netherlands) and family 12 (CentraCare Clinic, St Cloud, MN, USA) was sent for molecular diagnosis for the *SLC3A1/PREPL* locus. DNA samples for family 13 (University of Verona, Italy) and family 14 (McGill University, Montreal, Canada) were obtained upon request. As a random population, we used the CAREGENE[10] cohort.

Quantitative PCR

Quantitative PCR was performed using qPCR Master Mix for SYBR Green I detection and fluorescein as internal standard (Eurogentec, Seraing, Belgium) on the MyIQ system (Bio-Rad, Nazareth-Eke, Belgium), in accordance with the manufacturer's guidelines. Primers were developed with Primer Express software (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The relative number of alleles were calculated using the Livak ($2^{-\Delta\Delta C_t}$) method.[11] An amplicon within the p53 gene was used as reference.

Junction fragment PCR and sequencing

PCR products were amplified using 150 ng of genomic DNA, by the use of the Advantage 2 PCR kit (Clontech, Mountain View, CA, USA). Junction fragments were purified with the Montage PCR Centrifugal Filter Device (Millipore, Billerica, MA, USA) before sequencing (Bigdye Terminator v3.1, Applied Biosystems) on the ABI3130 system (Applied Biosystems). Primers used for amplifying deletions A and B have been described.[1] Primers for deletion E are 5′-TTGCTCCAAAAGTTCCTAACCAA-3′ and 5′-TTCTGTGTTGAGGTTGCACTCC-3′.

Haplotype analysis

Genotypes of microsatellites and single nucleotide polymorphisms (SNP) were determined as described.[1]

Results

The clinical and endocrinological data on the patients are similar to those reported earlier.[1] Details are listed in Table 1. In summary, the clinical picture is characterized by neonatal and infantile hypotonia, and anorexia, often necessitating nasogastric tube feeding or gastrostomy. Some dysmorphy is present, particularly dolichocephaly and ptosis of the eyelids, as well as a striking nasal speech. Anorexia and hypotonia ameliorate with age, but gradually growth velocity decreases due to IGF-1 insufficiency. Patients respond well to growth hormone treatment. Electromyography and brain magnetic resonance imaging are normal, when performed. In addition, patients have classical cystinuria type I causing nephrolithiasis at variable ages.

The presence or absence of different exons of *SLC3A1* and *PREPL* was determined in patients and parents (when

Table 1 Clinical and endocrinological data on the patients

	Family 10	*Family 11*	*Family 12*	*Family 13*
Actual age (years)	16	$3^{4/12}$	9	15
Sex	M	M	M	F
Birth weight (g)	1220	3185	3205	3660
Birth length (cm)	38	NI	44	50
Gestational age (weeks)	31	40	40	40
Infantile hypotonia	+	+	+	+
Infantile anorexia	+	+	+	+
Nasogastric tube feeding	+	−	+	+
Gastrostomy	−	−	+	−
Dysmorphy	Dolichocephaly	Deep sunken eyes	Dolichocephaly, eyelid ptosis and tented upper lip	−
Nasal speech	+	−	+	+
Unsupported walking from (m)	18	23	14	14
Special school	−	NI	+	−
Growth retardation	+	−	+	+
From age (years)	1–2	NA	7	2.5
Growth hormone (basal/peak)	2.4/31 μU/ml	NI	NI	1.2/10.2 μU/ml
IGF-1	−3 SD	NI	NI	−3 SD
Growth hormone treatment	Yes	NA	Yes	No
Start of puberty (years)	14	NA	−	12
Age of first symptomatic nephrolithiasis	7 years (coral stone)	No lithiasis yet	No lithiasis yet	No lithiasis yet
Electromyography	Normal	−	Normal	NI
Brain CT/MRI	Normal MRI	−	Normal MRI	Normal MRI

NI, no information; NA, not applicable

available) using quantitative PCR. In families 11 and 13, the boundaries for both deleted alleles were located in intron 1 of *SLC3A1* and intron 1 of *PREPL*. As the same fragments were deleted in the previously described deletion B,[1] we performed a junction fragment PCR specific for this deletion followed by sequencing of the amplicons (Figure 1). Both patients are homozygous for deletion B, therefore confirming the diagnosis of HCS. The patient in family 10 is compound heterozygous for two deletions, one identical to deletion B, the other extending from exon 10 in *SLC3A1* to exon 2 in *PREPL*-like deletion A from Jaeken *et al*.[1] Junction fragment PCR and sequencing confirmed that the second allele is indeed deletion A (Figure 1). The patient in family 12 showed a homozygous loss from exon 10 in *SLC3A1* to exon 2 in *PREPL*. Junction fragment PCR and sequencing confirmed that the paternal allele is again deletion A. The proximal boundary of the deletion on the maternal chromosome is located in intron 1 of *SLC3A1*, whereas the distal break point is located between 45 and 50 kb upstream of *PREPL* (Figures 1 and 2). Sequencing of the junction fragment indicated that the deletion was 127 kb in size, with the distal break point located in intron 3 of *C2orf34*, the gene flanking *PREPL*. This is a novel allele (deletion E) involved in HCS.

The patient in family 14 (patient 936 in Saadi *et al*[6]) has isolated cystinuria type I, and has previously been

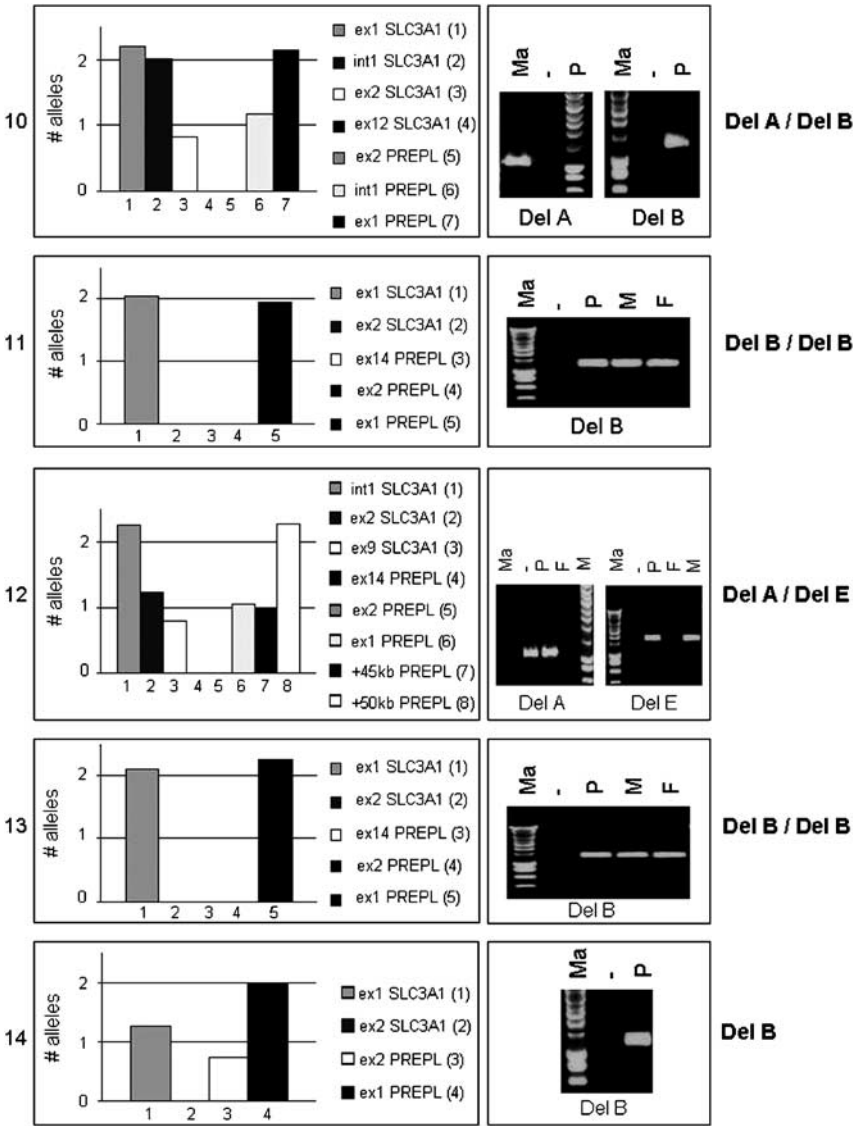

Figure 1 Genetic analysis of the HCS patients. (A) Quantitative PCR analysis on genomic DNA of HCS patients. Only relevant amplicons are shown. (B) Junction fragments spanning the break point in patients and parents (when available). –, negative control; P, patient; M, mother; F, father. (C) Genotype of the patients.

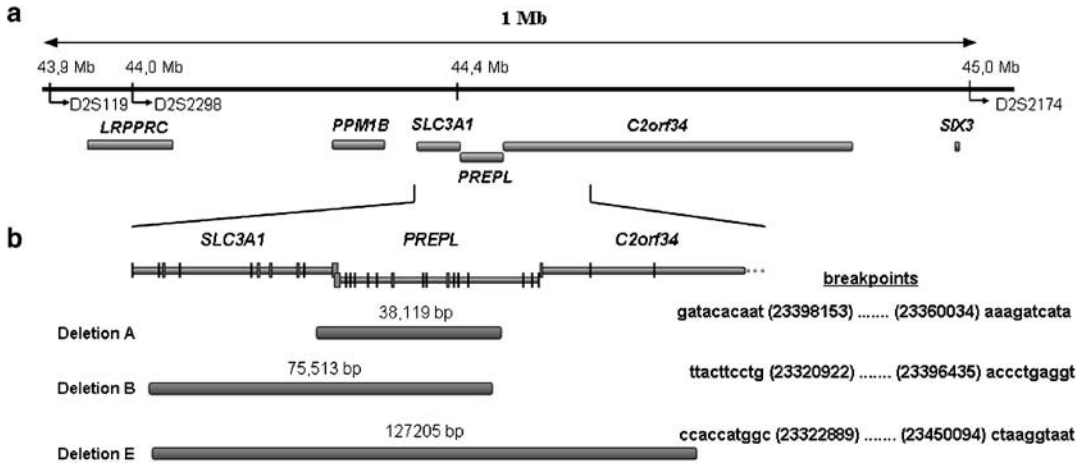

Figure 2 The 2p21 locus. (**a**) Genomic organization of the 2p21 locus. The flanking microsatellite markers used in the haplotype analysis are indicated with their genomic coordinates. (**b**) Schematic representation of the deletions detected in the novel HCS families, presented here. Deletions (**a** and **b**) have previously been described,[1] whereas deletion E represents a new allele. Sequences flanking the break points are shown and are numbered in accordance with the numbering in the database (Homo sapiens, build 36; http://www.ncbi.nlm.nih.gov/).

deletion A	ancestral haplotype 1	ancestral haplotype 2	family 10 p	family 13 p
D2S119	215	215	215	221
D2S2298	209	209	209	221
D2S174	200	216	214	214
D2S2280	173	173	169/173[a]	169
D2S2291	227	237	227	233/239[a]

deletion B	ancestral haplotype 1	ancestral haplotype 2	family 10 m	family 11 m	family 11 p	family 12 m	family 12 p
D2S119	217	217	223	-	-	221	221
D2S2298	217	197	217	217	217	213	209
D2S174	218	218	214	212	214	202	212
D2S2280	169	169	169/173[a]	169	167	173	173
D2S2291	237/233	237/233	233	235	227	241	241

Figure 3 (A and B) Haplotype analysis for deletion. Completely conserved haplotypes are shaded dark gray, partially conserved haplotypes are shaded light gray. Dashed lines indicate the break point. Note '[a]' phase unknown.

described as compound heterozygous for two deletions involving *SLC3A1*. The deletion on the paternal allele had been shown to extend from exon 1 to exon 7 of *SLC3A1*, whereas the deletion on the maternal allele starts in intron 1 of *SLC3A1* and extends beyond exon 10. We have now determined the exact break points on the latter deletion. Quantitative PCR analysis revealed that the proximal boundary was located in intron 1 of *SLC3A1*, whereas the distal break point was localized in intron 2 of *PREPL*. Again, this pointed toward the presence of deletion B (Figure 1).

The different deletion alleles were haplotyped using microsatellites and SNPs. Only family 10 showed a partially conserved ancestral haplotype for deletion A (Figure 3). SNPs genotyped in families 11–13 identified the same alleles as present in the ancestral haplotype of deletion B; however, the genotypes for families 11 and 13 were not informative. The SNP data for family 10 could not be obtained due to a lack of parental DNA (data not shown).

Because of the high incidence of deletions A and B, we have screened a random Belgian cohort for the presence of these deletions. We found 1 allele with deletion A and 1 allele with deletion B in a total of 936 random samples (1872 chromosomes). This leads to an allele frequency estimate for deletions A or B between 1/333 and 1/7700 (binomial distribution; 95% confidence interval).

Discussion

HCS was initially described in nine families living in Belgium and France. On 18 independent chromosomes, only four different deletions (deletions A–D) were found.

The frequency of deletions A and B was 4/18 and 11/18, respectively, due to founder effects.[1] In the four novel HCS families and the one carrier of a HCS allele described here, the frequency of deletions A and B were 2/9 and 6/9, respectively. The allele frequencies of deletions A or B in a random Belgian cohort were estimated between 1/333 and 1/7700.

Deletions A and B are globally distributed, as they were found in patients from The Netherlands, Italy, Canada and the United States America. However, the ancestral haplotypes of the microsatellite markers flanking the break points were completely decayed in these families, indicating that the deletions are old. However, it cannot be excluded that a genetic mechanism is promoting deletions in this region. If this is the case, however, it is remarkable that the break points are exactly the same.

The patient in family 12 appeared to be compound heterozygous for deletions A and E, the latter being a novel deletion involved in HCS. This deletion does not only disrupt the coding region of *SLC3A1* and *PREPL*, but also of *C2orf34*. C2orf34 has a putative methyltransferase domain, but its function has not yet been described. It is, however, not expected that a loss of one allele is modulating the phenotype in this HCS patient, because the mother showed no phenotype that might be due to the loss of one allele of *C2orf34*.

Unexpected results were obtained for family 13. The proband in this family showed all HCS symptoms, accompanied with a mitochondrial respiratory chain defect. As a mitochondrial respiratory chain defect was also observed in the 2p21 deletion syndrome,[3] one might have expected that besides *SLC3A1* and *PREPL*, at least one other gene (*PPM1B* or *C2orf133*) was involved. However, this patient is homozygous for deletion B, and therefore her genotype is identical to some of the previously published patients. She has been the only HCS patient with a reported respiratory chain defect. This brings up an intriguing question about the cause of the respiratory chain defect in this patient. A possible explanation could be the presence of variations in modifier genes, either in *cis* or in *trans*, yet to be discovered. Currently, in-depth analysis of mitochondrial respiratory chain function in fibroblast cells from other HCS patients is being performed to determine potential minor defects that might previously have been overlooked.

Three deletions involving *PREPL* have been described in patients with isolated cystinuria type I. We characterized the deletion initially reported in Saadi *et al*.[6] It turns out to be the most frequent HCS allele, deletion B, only disrupting the coding region of *SLC3A1* and *PREPL*. This leaves the total of unique alleles where *PREPL* is deleted at six: five deletions present in HCS and one causing the 2p21 deletion syndrome. Possibly, two other alleles exist, but the break points have not been determined.[7,8]

In conclusion, HCS is a relatively homogenous syndrome, with common ancestral alleles found in different populations. On the basis of the allele frequencies, this disease will be rare (1/1 000 000 for an allele frequency of 1/1000); hence, our observations are compatible with a founder effect in the regions from where the HCS patients originate.

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