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Short clinical report

2p21 Deletions in hypotonia–cystinuria syndrome

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ABSTRACT

The significant role of the *SLC3A1* gene in the aetiology of cystinuria is meanwhile well established and more than 130 point mutations have been reported. With the reports on genomic deletions including at least both *SLC3A1* and the neighboured *PREPL* gene the spectrum of cystinuria mutations and of clinical symptoms could recently be enlarged: patients homozygous for these deletions suffer from a general neonatal hypotonia and growth retardation in addition to cystinuria. The hypotonia in these hypotonia-cystinuria (HCS) patients has been attributed to the total loss of the PREPL protein. Here we report on the clinical course and molecular findings in a HCS patient compound heterozygote for a new deletion in 2p21 and a previously reported deletion, both identified by molecular karyotyping. The diagnostic workup in this patient illustrates the need for a careful clinical examination in context with powerful molecular genetic tools in patients with unusual phenotypes. The identification of unique genomic alterations and their interpretation serves as a prerequisite for the individual counselling of patients and their families. In diagnostic strategies to identify the molecular basis of both cystinuria and hypotonia 2p21 deletions should be considered as the molecular basis of the phenotype.

1. Introduction

The hypotonia–cystinuria syndrome (HCS; OMIM 606407) is an autosomal recessive disease associated with deletions affecting the *SLC3A1* and *PREPL* genes on chromosome 2p21. The main clinical features consist of a generalised hypotonia at birth, failure to thrive, growth retardation and cystinuria. Meanwhile, 13 HCS patients homozygous or compound heterozygous for deletions in 2p21 have been reported [1,2]. Among the five different HCS identified so far, two (deletions "A" and "B") are globally distributed. Despite their different sizes from ~38 to ~127 kb, all these deletions affect the *SLC3A1* and *PREPL* genes. A further deletion carrier with cystinuria as the only clinical feature was detected by screening classical cystinuria patients for *SLC3A1* mutations; the patient was compound heterozygous for a large *SLC3A1*/*PREPL* deletion and a small deletion in *SLC3A1* restricted to exons 1–7 [3]. *SLC3A1* encodes the heavy subunit of the renal amino acid transporter $b^{0,+}$ and mutations in this gene are generally associated with the autosomal recessive type I cystinuria, mutations restricted to the *PREPL* gene have not yet been reported. Nevertheless, it has been postulated that the phenotype in HCS patients can be attributed to the lack of *PREPL* which encodes a putative serine oligopeptidase with a currently unknown physiological function [1].

A second microdeletion syndrome also affecting 2p21 but larger in size (~179 kb) is referred to as 2p21 deletion syndrome [4]. This deletion has been detected in a large Bedouin pedigree. Homozygous deletion carriers in this family showed HCS and additional features including neonatal seizures and a severe global retardation, indicating that the loss of a third gene in 2p21, *PPM1B*, also contributes to the clinical outcome in this family. Recently, Chabrol et al. [5] identified patients with an intermediate phenotype between HCS and the 2p21 microdeletion syndrome who carried deletions intermediate in size.

Here we report on a HCS patient who is compound heterozygous for a recently reported variant and a new microdeletion in 2p21. Our case illustrates the necessity of a detailed phenotypic characterisation to delineate the molecular defect. By microarray typing we could define the deletion breakpoint as the basis for targeted genetic counselling.

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2. Clinical report

The boy M1260 is the first child of healthy, non-consanguineous German parents. There were no further cases of cystinuria in the family. The child was born at term, with normal growth parameters at the 40th gestational week (APGAR 9/10/10). After birth, the patient showed muscular hypotonia and feeding difficulties. Sonography of the cranium and abdomen as well as EEG did not reveal any irregularities. Eyes, ears and heart were normal. Biochemical investigations were normal except for urine amino acid analysis which led to the diagnosis of classical cystinuria. In the first months of life, he fed poorly requiring gavage feeding, and exhibited frequent vomiting (3–4 times per day). At the age of 7 months, brain MRI revealed mild frontotemporal atrophy without any additional abnormalities.

Free sitting was achieved at age 9 months, unattended walking at age 15 months. In the course of the first years of life, failure to thrive and muscular hypotonia persisted, requiring an increased caloric intake and physiotherapy, respectively.

At the age of 2 $^{3}/_{4}$ years, weight was 11.12 kg (<3rd centile), length 90 cm (10th centile), OFC 49 cm (10–25th centile). He had a pale skin and was noted to have a long face with mild bitemporal narrowing and frontal bossing (Fig. 1), a slight ptosis could be observed. He had a high and narrow palate. His abdomen was mildly protruding, without any pathological findings. He was reported to run rarely and fall frequently. However, early speech development was reported to be adequate. He was described as shy.

Growth retardation persisted in the following years. At the age of 4.5 years weight was 15.9 kg (10th centile), length 99.5 cm (<3rd centile) and OFC 50.2 cm (10–25th centile). Neurological examination revealed truncal hypotonia. Global developmental delay of all motor and social skills and speech was evident. Despite intact hearing speech was incomprehensible.

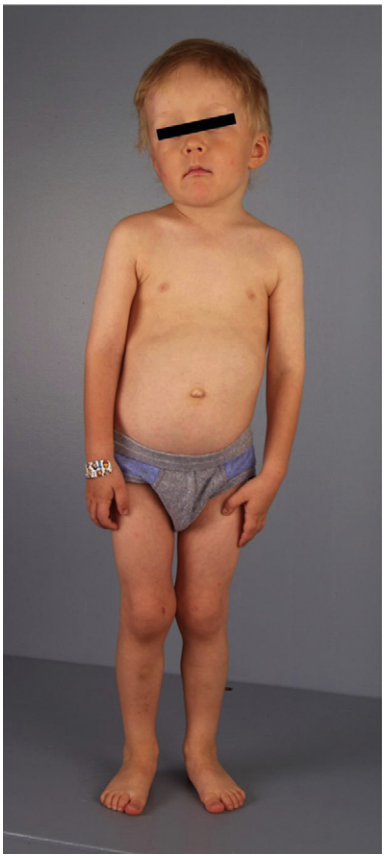

Fig. 1. Phenotype of the patient at the age of 3.5 years. Note the pale skin and the long face with mild bitemporal narrowing and frontal bossing.

Multiple diagnostic analyses were unremarkable and did not enlighten the aetiology of the psychomotor retardation and failure to thrive. Renal and/or bladder calculi were not detectable by sonography until now, probably due to the daily fluid supply of 2 l/day. Seizures were not observed.

Based on the biochemical diagnosis of cystinuria we initially screened the patient's DNA for *SLC3A1* and *SLC7A9* point mutations by Sanger sequencing. Whereas we did not detect any sequence variations in *SLC7A9* and in exon 1 of the *SLC3A1* gene, PCR amplification of exons 2 to 10 of the *SLC3A1* gene generally failed despite several PCR modifications. Based on the clinical data we assumed that our patient should be homozygous for a deletion affecting 8 exons of the *SLC3A1* gene as well as the telomeric region of the *PREPL* gene. We therefore tested the patients' DNA for genomic imbalances by the GenomeWideSNP_6 array system (Affymetrix, High Wycombe, UK). Indeed we could confirm that our patient carries deletions in 2p21 (Fig. 2): a region of up to 53.5 kb (NCBI36/hg18:44,360,636–44,413,046 bp) including the exons 2 to 10 of the *SLC3A1* gene and the last 4 exons of the *PREPL* gene was homozygously deleted. On both sides of this homozygously deleted region hybridisation patterns corresponding to the presence of only one chromosomal copy were visible. We therefore concluded that our patient is compound heterozygous for two different deletions in 2p21 which overlap over a region of <53.5 kb.

Segregation analysis confirmed this assumption: By testing the DNA of the patient and his parents for the known deletions by junction-fragment specific PCR [1] we could show that one allele corresponded to a previously described 75 kb deletion (deletion B) including exons 2 to 10 of the *SLC3A1* and the last four exons of *PREPL*. This deletion could also be demonstrated in the maternal DNA sample by junction fragment specific PCR but not in the paternal DNA sample. By further microarray typing of the paternal DNA sample we confirmed that the father is carrier for the second, so far unreported 63 kb deletion in 2p21 in our patient, spanning from chr2:44342498–44405883 (NCBI36/hg18).

3. Discussion

The identification of two different deletions affecting both the *SLC3A1* and the *PREPL* genes in 2p21 confirms the clinical diagnosis of HCS in our patient. Indeed, the maternal deletion B is the most frequent mutant allele detectable in HCS patients with a calculated frequency of 1/333 in the Belgium population [2]. The other paternally inherited deletion has not yet been reported and confirms the assumption that further HCS alleles exist and are often familial [2]. Our case confirms that deletions in 2p21 might either be restricted to the *SLC3A1* gene in case of classical cystinuria [for review: [6]] but might also affect several kilobases resulting in HCS or large genomic imbalances as in the 2p21 microdeletion syndrome.

This case furthermore illustrates that the interpretation of molecular data needs a careful clinical documentation as a prerequisite for the interpretation of genetic data. In case of additional clinical features larger imbalances should be considered affecting not only the gene of interest but also neighboured factors. In this respect the use of DNA microarrays allows the detection and characterisation of genomic imbalances: these arrays should have a high resolution and cover the region of interest to identify even small imbalances (<100 kb). Bioinformatic parameters of array interpretation also have to be adapted to these conditions. In addition to the focused analysis of specific genomic regions the use of microarrays generally allows the detection of small genomic imbalances ("molecular karyotyping") which would have escaped

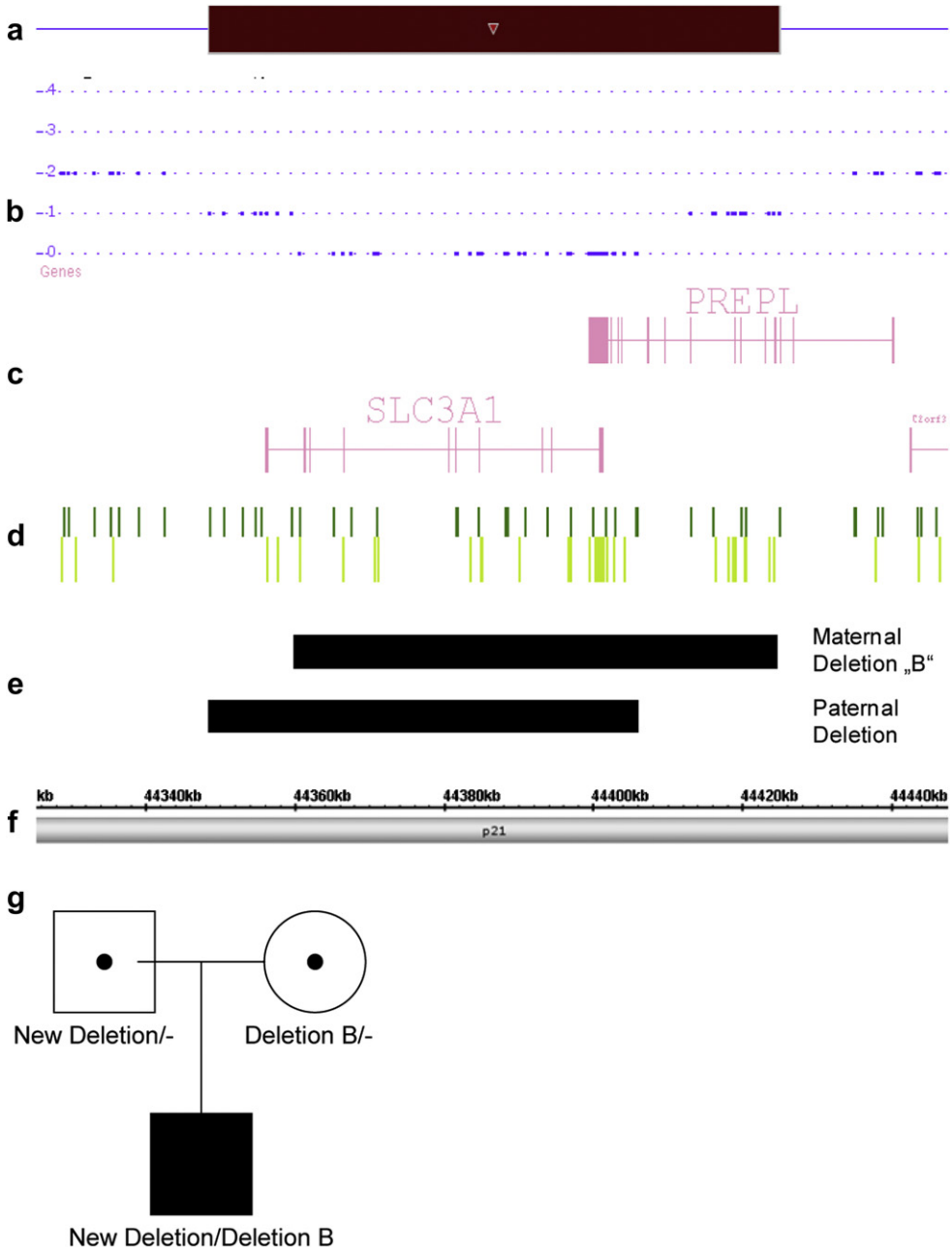

Fig. 2. Local GenomeWideSNP_6 array signal distribution pattern and segmentation result in our patient with two different 2p21 deletions. a) Schematic presentation of the copy number segments. b) Illustration of the copy number state. c) Affected genes and their genomic structures. d) Distribution of markers of the SNP_6 Array. e) Illustration of the two deletion alleles in our patient. f) Physical position and chromosomal band at 2p21. g) Pedigree of the family.

the formerly conventional cytogenetic analyses, an analysis indicated in syndromic disorders.

In conclusion, the critical evaluation of clinical features in the context with powerful molecular genetic tools allow the identification of unique genomic alterations and their interpretation as a prerequisite for individual counselling of patients and their families.

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