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Intragenic Deletion as a Novel Type of Mutation in Wolman Disease

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Abstract

Two clinically distinct disorders, Wolman disease (WD) and cholesteryl ester storage disease (CESD), are allelic autosomal recessive disorders caused by different mutations in lysosomal acid lipase (*LIPA*) which encodes for an essential enzyme involved in the hydrolysis of intracellular cholesteryl esters and triglycerides. We describe a case of lysosomal acid lipase deficiency in an infant with WD and report on a novel mutation type, intragenic deletion.

Keywords

Wolman disease; *LIPA*

1. Introduction

Lysosomal acid lipase is an essential enzyme involved in the hydrolysis of intracellular cholesteryl esters and triglycerides. The gene, *LIPA*, maps to 10q23.2–23.3 [1–3], and mutations result in two clinically distinct autosomal recessive disorders, Wolman disease (WD) and cholesteryl ester storage disease (CESD). WD is a severe disorder with infantile-onset that presents with failure to thrive, feeding intolerance, diarrhea, and hepatosplenomegaly. Adrenal calcifications are a hallmark of the disease [4]. It is almost always universally fatal by the first year of life although there are now reported cases of successful bone marrow transplantation [5–7].

To date, over 40 mutations have been identified in WD and CESD. In approximately 70% of CESD cases a guanine to adenine substitution at the exon 8 splice junction results in exon skipping and the loss of 24 amino acids [8]. Other mutations, including point mutations, insertions, and deletions have also been reported in CESD [8–12]. WD appears to be more

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genetically heterogeneous [13]. In most reported cases, the proband was from a consanguineous family and was found to be homozygous for a familial mutation [14–16].

2. Methods and Results

2.1 Clinical summary

The patient was a full term infant born to non-consanguineous parents. He presented at six weeks with fever, emesis, abdominal distention, and failure to thrive. Imaging studies showed hepatosplenomegaly and calcified adrenals. Enzymatic assay demonstrated undetectable LIPA activity.

Over the course of four weeks he developed acute liver failure with disseminated intravascular coagulation and cortical adrenal insufficiency. Due to the poor prognosis and limited treatment options, the parents elected palliative care. His liver function continued to worsen and in the days preceding death, the patient became less responsive and developed anuria and respiratory insufficiency secondary to multi-organ failure.

2.2 Molecular summary

DNA sequence analysis of all coding exons demonstrated a paternally inherited c.482delA mutation in exon 5 that results in a frameshift mutation at amino acid 161 (Figure 1a). This mutation leads to premature truncation of the protein at amino acid 179. We screened 200 chromosomes from ethnically matched controls by sequencing and did not detect any carriers of the c.482delA deletion.

Initially, sequencing did not identify a second mutation so known SNPs were genotyped in *LIPA* to serve as markers to screen for lack of biparental inheritance to support a possible deletion mutation. Using this strategy, at rs2071509 the father was C/C, the mother appeared to be G/G, and the proband appeared to be C/C. Because the proband appeared to be homozygous for a paternal allele not carried by the mother, this data suggested that the mother had a deletion leading to hemizyosity in the proband and mother.

By genotyping additional SNPs, the deletion was found to include intron 3 but not to extend into exon 3. This finding was confirmed by real-time quantitative PCR using probes in exons 3, 4, and 10 which showed that the proband and mother have one copy of exon 4 compared to male and female controls (Figure 1b). Probes located in exons 3 and 10 demonstrated two copies in the proband and mother. This deletion, in conjunction with the c.482delA mutation, is the likely genetic cause of WD in this infant (Figure 1c).

3. Discussion

Aside from another report of a deletion that includes exon 4 [17], this is the only other reported case of a moderately sized intragenic deletion associated with WD. However, several other cases of WD have been previously described with only one identifiable mutation after sequence analysis [11, 12, 17, 18]. Our results suggest that deletions may be responsible for failure to identify the second mutation in those cases. New methodologies to identify copy number should be considered as a reflex test when sequencing only identifies a single mutation.

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Abbreviations

CESD	cholesteryl ester storage disease
LIPA	lysosomal acid lipase
WD	Wolman disease

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Highlights

- > Wolman disease is caused mutations in *LIPA*.
- > In most reported cases probands are homozygous for a familial mutation.
- > Several cases have been described with only one identifiable mutation after sequence analysis.
- > Deletions may responsible for failure to identify the second mutation.

A.

B.

C.

Figure 1.

A. Electropherogram of proband (top), father (middle) and mother (bottom) demonstrates a paternally inherited c.482delA mutation (arrow) in exon 5 that results in a frameshift mutation at amino acid 161. B. Quantitative PCR results for a probe within exon 4 show only one copy number for the proband and mother compared to two in the controls. C. On the top, the arrow indicates the paternally inherited c.482delA and the dashed line approximates the location of the maternally inherited deletion. The bottom shows sequencing results with a SNP (rs2071509) 95 bps 5' of exon 4 that was hemizygous in the proband suggesting the presence of a maternally inherited deletion. Another unreported SNP found 2,969 bp 5' of exon 4 (IVS4-2,969) is outside the area of the deletion. The deletion involved at least part of exon 4 but not exon 3.