Short Communication

Angelman syndrome and isovaleric acidemia: What is the link?

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A R T I C L E   I N F O

A B S T R A C T

We report a toddler affected with Angelman syndrome and isovaleric acidemia (IVA). Such association was due to paternal uniparental isodisomy (UPD) of chromosome 15 in which the proband inherited two paternal copies of an IVA gene point mutation. As both diseases may have severe impact on neurodevelopment, adequate treatment of IVA should be discussed. In our patient however, the variant identified likely causes asymptomatic organic aciduria. Such findings emphasize that paternal UPD 15 can rarely lead to co-occurrence of Angelman syndrome and potentially treatable inborn errors of metabolism.

1. Introduction

Angelman syndrome (AS) is a rare and complex neurodevelopmental disorder caused by a deficiency of Ubiquitin-protein ligase E3A (UBE3A) in the central nervous system (CNS). The *UBE3A* gene is located on chromosome 15 in the imprinted region 15q11–q12 and is expressed from both paternal and maternal chromosomes in most tissues but not in neurons where the gene is imprinted in a way that the paternally inherited copy of the gene is silenced and the maternally inherited copy of the gene is expressed [1]. Therefore, any genetic event leading to the presence of two paternal copies or the absence of the maternal copy of the gene may lead to a defect in *UBE3A* with subsequent AS. Four mechanisms can cause AS [1,2]. Approximately 70% of AS cases are caused by *de novo* maternal deletions within the 15q11.2–q13 region, 20% result from mutations in *UBE3A*, 5% from imprinting defects and 5% of cases are due to paternal uniparental isodisomy of the 15q11.2–q13 region. AS has 4 cardinal features: severe developmental disorder, minimal or even absent speech, a puppet-like and ataxic gait and a distinctive behavior that includes

hyperactivity, a happy appearance with frequent or inappropriate smiling and outbursts of laughter with hand flapping. The most typical EEG finding in patients with AS is the presence of a rhythmic triphasic two to three cycles per second delta activity of high voltage, especially over the frontal regions but EEG may also show nonspecific abnormalities [1]. AS due to uniparental disomy (UPD), expresses a milder phenotype than that due to deletion. AS is diagnosed on suggestive clinical presentation and EEG findings and is confirmed by molecular testing.

UPD arises when a diploid individual carries both homologs of a chromosomal pair from a single parent (uniparental heterodisomy) or two copies of a single parental chromosome (uniparental isodisomy). UPD results from the rescue, in the zygote, of parental meiosis and mitosis errors. In the case of AS due to paternal UPD, symptoms reflect homoallelism of a *UBE3A* gene silenced by genetic imprinting.

We report herein a toddler whose clinical story suggested AS and for whom a urinary organic acid profile performed as a systematic screen for developmental disorders was diagnostic for isovaleric acidemia (IVA). IVA is autosomal recessive inborn error of metabolism (IEM) resulting in a defect in leucine catabolism due to isovaleryl-CoA dehydrogenase (IVD) dysfunction. This deficiency leads to the accumulation of isovaleryl-CoA and its metabolites, which are toxic to the CNS and predispose to severe acidotic episodes, especially during catabolic stress potentially dangerous for an appropriate neurodevelopment.

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The *IVD* gene is located on chromosome 15q14–15, consisting of 12 exons [3]. Some mutations can lead to a paucisymptomatic phenotype.

Co-occurrence of IVA and AS has never been reported to date and is due to paternal uniparental isodisomy of chromosome 15.

2. Materials and methods

2.1. Case report

This 4-year-old boy was the first child from non-consanguineous parents without any particular family history. The child had been conceived through IVF as a result of male infertility. Pregnancy and delivery were uneventful as well as birth and neonatal period. Social smile appeared by 3 to 4 months contrasting with motor delay: head control appeared around 5–6 months and the child could sit unsupported by 10–11 months. The first concerns were reported around the age of 9 months with neuromotor delay. Audiometry and ophthalmoscopy were checked and found normal. He was able to stand upright with assistance at the age of 15 months and started walking assisted by 30 months of age. At the age of three years and three months, he was able to walk without assistance. At that age, the patient was admitted for further investigation: His language was minimal and was constricted to only babbling. A happy behavior was noticed. Growth parameters were average for age except head circumference (HC), which was at −2SD (normal HC at birth, starting to slow down at 6 months of age: −1SD and at −2SD from 9 months of age). Clinical examination showed an ataxic and puppet-like gait. Angelman syndrome was suspected on the association of psychomotor delay with speech delay, ataxia and acquired microcephaly along with a happy behavior. Because such features could also be representative of other etiologies in very young children, other genetic and metabolic tests, including urine organic acids, were performed at the same time to rule out frequent or treatable causes of psychomotor delay and ataxia.

2.2. Molecular analysis

Genomic DNA was extracted using QIAamp® DNA blood midi kit columns (Qiagen, Venlo, The Netherlands) after receiving informed consent from the parents of the patient. MS-MLPA (Methylation Sensitive-Multiplex Ligation-dependent Probe Amplification) was performed to assess the methylation status and genomic dosage at numerous sites across the 15q11–q13 region with the SALSA® MS-MLPA® kit ME028-B2 Prader Willi/Angelman from MRC-Holland b.v. In order to determine the genetic defect involved in the abnormal methylation status observed, we performed a microsatellite analysis. 12 microsatellite markers (D15S128, D15S122, D15S210, PWS-196, AFMa309yg1,
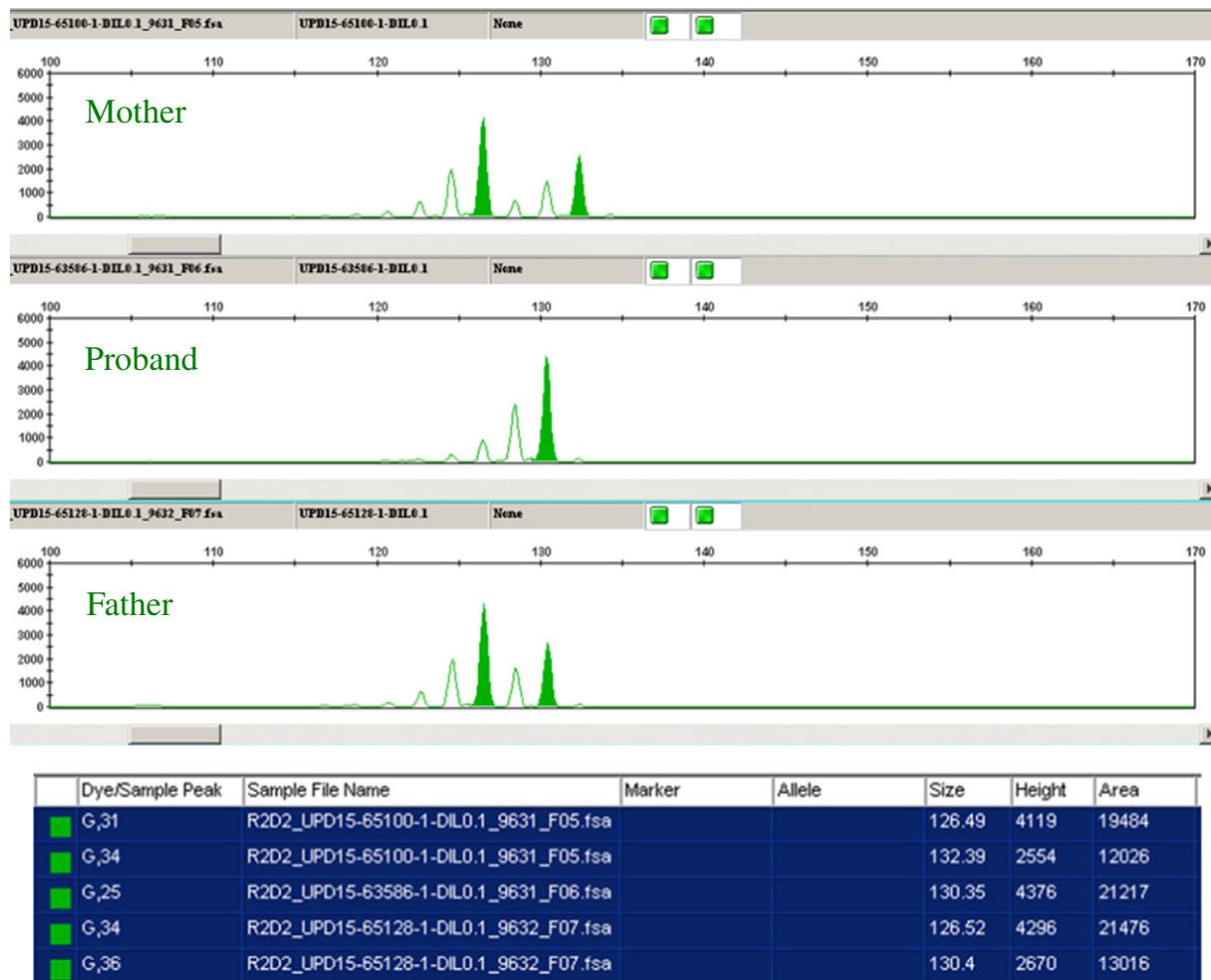

	Dye/Sample Peak	Sample File Name	Marker	Allele	Size	Height	Area
	G,31	R2D2_UPD15-65100-1-DIL0.1_9631_F05.fsa			126.49	4119	19484
	G,34	R2D2_UPD15-65100-1-DIL0.1_9631_F05.fsa			132.39	2554	12026
	G,25	R2D2_UPD15-63586-1-DIL0.1_9631_F06.fsa			130.35	4376	21217
	G,34	R2D2_UPD15-65128-1-DIL0.1_9632_F07.fsa			126.52	4296	21476
	G,36	R2D2_UPD15-65128-1-DIL0.1_9632_F07.fsa			130.4	2670	13016

Fig. 1. Microsatellite analysis showing paternal isodisomy. The mother's electropherogram shows two peaks at 126 and 132 base pairs (bp) corresponding to her two alleles for marker D15S128. The father's profile shows 2 peaks, at 126 bp and 130 bp. The patient's profile presents only one peak at 130 bp corresponding to one of the paternal allele. None of the maternal alleles are present (126 or 132 bp) demonstrating the absence of maternal contribution. As MLPA ruled out a maternal deletion, the mechanism is paternal isodisomy.

GABRB3, D15S102, D15S1012, D15S118, D15S994, D15S101, D15S117) located inside and outside the critical 15q11–q13 region on chromosome 15 were amplified by PCR and analyzed on a ABI 3730XL DNA Sequencing Analyzer (Applied Biosystems, Foster City, CA, USA).

All 12 exons and intron-exon boundaries of the *IVD* gene were PCR-amplified and Sanger-sequenced under standard conditions (primers available on request).

3. Results

MS-MLPA analysis showed an absence of deletion and an abnormal methylation pattern with a loss of methylated copies (not shown). Microsatellite analysis showed an exclusive paternal contribution (Fig. 1) within and outside the critical AS region. These results confirm the diagnosis of AS due to paternal uniparental isodisomy (UPD) of chromosome 15.

Urine organic acid profile showed isovalerylglycine at 135 μmol/mmol creatinine (nv < 5) with isovalerylcarnitine in plasma (1.7 μM) and normal carnitine levels: free carnitine 23 μM (nv 23–40) and total carnitine 32 μM (nv 25–50). Isovaleric acidemia was confirmed by molecular analysis of the *IVD* gene which revealed a homozygous missense mutation in exon 9: c.941C>T, responsible for an alanine to valine substitution on position 314: p.Ala314Val. This mutation (formerly known as p.Ala311Val or also as p.Ala282Val) is associated with a mild phenotypic expression [4]. Data from newborn screening suggest that this particular variant likely causes organic aciduria without clinical symptoms. The father's sequence analysis revealed the same heterozygous mutation (p.Ala314Val) while no mutation could be found in the mother.

4. Discussion

Co-occurrence of two rare genetic diseases affecting the same patient is extremely rare and results, in this case, from paternal uniparental isodisomy of the chromosome 15 where a paternal *IVD* point mutation is homozygous in the proband.

This is the first report of such an association while AS has been reported in association with tyrosinemia type 1 (*FAH*) due to the same genetic mechanism [5]. Chromosome 15 contains other IEM genes; therefore, in the case of paternal uniparental isodisomy 15, similar association of AS with other IEMs could theoretically also occur for *CLN6* (neuronal ceroid lipofuscinosis), *HEXA* (Tay-Sachs disease), *ETFA* (glutaric aciduria type 2), *GAMT* (guanidino acetate methyltransferase deficiency, inborn error of creatine biosynthesis), *IDH2* (D-2-hydroxyglutaric aciduria) and *POLG* (polymerase γ).

Uniparental isodisomy of other chromosomic regions has been reported as the molecular mechanism at the origin of other recessive disorders [6].

In our patient, IVA had remained asymptomatic and discovered by chance after a systematic metabolic workup for ataxia, whereas AS was typical. Data from newborn screening suggest that such p.Ala282Val *IVD* mutation is likely responsible for an asymptomatic IVA phenotype thus questioning the need for any IVA therapy. However, due to the potential additional burden of untreated IVA on neurodevelopment, we chose to treat IVA minimally with only glycine and carnitine without any protein restriction.

In countries with expanded newborn screening, IVA would have been identified at birth. AS could have been overlooked and the neurologic symptoms erroneously ascribed to IVA, even if the absence of parental segregation for *IVD* would have raised suspicion of a non-classic Mendelian transmission.

In conclusion, this report emphasizes that uniparental isodisomy may be the cause for the co-occurrence of two genetic diseases in the same patient. In the case of AS, the treatment of IVA may prevent further additional neurocognitive deterioration, even if in the setting of this particular *IVD* variant, organic aciduria is likely to remain asymptomatic.

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