A Large Intragenic Deletion in the *ACADM* Gene Can Cause MCAD Deficiency but is not Detected on Routine Sequencing

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Abstract We report of a family who has three members affected by medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, one of whom sadly died in the neonatal period prior to diagnosis. Routine sequencing, available on a service basis in the UK, identified only a heterozygous mutation in *ACADM* gene (c.985A>G, p.Lys329Glu) in this family. Linkage analysis suggested a possible intragenic deletion which was confirmed by the use of array-based comparative genomic hybridization (aCGH). This second mutation was a large intragenic deletion encompassing at least exons 1–6 of the *ACADM*

gene. Now that this deletion has been identified, several family members have come forward for carrier testing which was not possible previously. Larger deletions (20bp or more) have only previously been reported twice, but these may be a more frequent cause of MCAD deficiency than hitherto believed, due to fact that these are not anticipated and, therefore, the routine diagnostic techniques used will not identify them. This finding represents a useful learning point in the management of families with MCAD deficiency, and highlights that we should be routinely looking for larger deletions, when only one of the mutations can be identified on standard sequencing.

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Abbreviations

MCAD Medium Chain Acyl-CoA Dehydrogenase

MLPA Multiplex Ligation-dependent Probe Amplification

Introduction

Medium-chain acyl-CoA dehydrogenase (MCAD; OMIM 201450; EC 1.3.99.3) deficiency (MIM 201450) is an autosomal recessive disorder resulting from a defect of fatty acid oxidation. Clinical presentation of the condition is varied. Some with the condition will never present with symptoms, others, after metabolic stress, will present with vomiting, lethargy, and coma. Unfortunately, a few are diagnosed after death in childhood. A newborn screening programme is in place in the UK. If treatment is established early, then long-term morbidity and mortality is low. Mutation screening by means of direct sequencing of exons 1 to 12 of the *ACADM* gene will not detect large deletions when present in the heterozygous state. We present a family

where a large deletion in the *ACADM* gene had wider implications.

Case Report

This family had three members affected by MCAD deficiency as depicted in Fig. 1. The twin brothers (C2 and C3) were diagnosed in 1994, at the age of 18 months, when one of them presented to hospital acutely unwell with a low glucose level. The second twin has never had any acute episodes requiring hospital admission. Sadly the other affected member of this family died, in 2009, at the age of 2 days from undiagnosed MCAD deficiency (C4).

Routine sequencing, available on a service basis in the UK, identified only the common heterozygous mutation in the *ACADM* gene (c.985A>G p.Lys329Glu) in this family. This testing was reported to exclude the vast majority (>99%) of all reported mutations (Andresen et al. 2001; Maier et al. 2005; Morris et al. 1995; Andresen et al. 1993; Maegawa et al. 2008).

To try and find the other mutation linkage analysis was performed (Fig. 2). Patient C4 was found to be heterozygous for the polymorphic silent variation c.1161 A>G in exon 11[4] of the *ACADM* gene. This polymorphic silent variation, in our experience, is normally observed in linkage with other polymorphic variations, including intron 1 (IVS1-32G), intron 3 (IVS3+10C), intron 5 (IVS5+32G), and intron 6 (IVS6-22A). Patient C4 was apparently homozygous for polymorphisms normally only seen with the c.985A>G haplotype, which included intron 1 (IVS1-32C), intron 3 (IVS3+10T), intron 5 (IVS5+32C), and intron 6 (IVS6-22C). This suggested the presence of a deletion spanning at least intron 1 to intron 6 in C4 c.1161G allele. The analysis found the mother (B6) to be heterozygous for the c.985A>G mutation. The analysis in the father (B5) found him to be heterozygous for the polymorphic silent variation c.1161 A>G in exon 11, but homozygous for the polymorphisms, which are usually not linked to c.1161A>G. This indicates that he also may have a deletion affecting exons 1–6 of the *ACADM* gene (Fig. 2). Targeted array-based comparative genomic hybridization (aCGH) with exon-level resolution (ExonArrayDx) was then employed to establish if such a deletion was present in the father. It confirmed the presence of an intragenic deletion encompassing at least exons 1–6 of the *ACADM* gene would have knocked out standard primer sites used in routine PCR-based sequencing in at least exons 2–5. The array contained multiple oligonucleotide probes in all exons and/or their flanking regions in the *ACADM* gene. Hybridization data was analyzed with Genomic Workbench v5 software (Agilent Technologies) to evaluate the copy number at the exon level. The Exon Array was designed

to detect most single exon deletions and duplications. This result was confirmed by repeat analysis. Multiplex ligation-dependent probe amplification (MLPA) analysis with probes that test for deletions that span at least exons 2–4 was then employed to confirm the presence of the deletion in the father (B5), B4, C2, and C3. Testing by means of MLPA is now available on request through the Leeds Cytogenetics Laboratory in the UK.

Now that this deletion has been identified, several family members have come forward for carrier testing which was not possible previously.

Discussion

Larger deletions (20bp or more) have only previously been reported twice in *ACADM* (Morris et al. 1995; Maegawa et al. 2008), but these may be a more frequent cause of MCAD deficiency than hitherto believed, due to fact that these are not anticipated, and therefore, the routine diagnostic techniques used will not identify them. Arnold et al. reported on the mutational spectrum in newborns identified by routine screening for MCAD deficiency in New York (Arnold et al. 2010). They reported one case with a similar deletion to the one found in our family being a large deletion of exons 1–6 and heterozygosity for the c.985A>G mutation in the other allele. This patient demonstrated profound fasting intolerance in the first years of life, requiring gastrostomy feeding for several years. They also reported 7 cases out of 23 cases where the second mutation in the *ACADM* gene was unknown. We, therefore, speculate that some of these unknown second mutations could be due to deletions not usually detected by use of a service genetic laboratory. Another study of the mutational spectrum in newborns with MCAD deficiency (Kennedy et al. 2010) also reported a high proportion (5/25) of individuals with MCAD deficiency, who have an unknown second mutation, whereas a recent study from Denmark (Andresen et al. 2012) did not find such high proportions of individuals with MCAD deficiency who have an unknown second mutation. This may be due to differences in the mutational spectrum in the investigated populations.

Conclusion

This finding represents a useful learning point in the management of families with MCAD deficiency. It highlights that we should be routinely looking for larger deletions, when only one of the mutations can be identified on standard sequencing, in patients where we have high clinical suspicion or biochemical evidence of MCAD deficiency.

Fig. 1 Pedigree. Family members affected by MCAD deficiency are in *black*

Fig. 2 Linkage analysis of the *ACADM* gene

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Take-Home Message

Large deletions in *ACADM* can cause of MCAD deficiency, and routine diagnostic techniques in common use will not identify them.

Details of the Contributions of Individual Authors

1. Claire Searle – Co-wrote the article, collated the information about the family and test results, and met with several family members.
2. Prof Brage Storstein Andresen – Gave very valuable advice pertaining to the family, performed the linkage analysis, and co-wrote the article.
3. Prof Ed Wraith – Headed up clinical care for a number of affected family members and proofread the article.
4. Jamie Higgs GeneDx, performed the array CGH analysis of the *ACADM* gene and proofread the article.
5. Deborah Gray – Performed the MLPA analysis and proofread the article.
6. Alison Mills – Designed the MLPA primers and proofread the article.

7. K. Elizabeth Allen – Reported the initial sequencing work of the *ACADM* gene and proofread the article.
8. Emma Hobson – Identified and counselled all family members involved, collated the clinical information, instigated testing to confirm the presence of the deletion and co-wrote the article.

Guarantor for the Article

Claire Searle

Conflict of Interests Statement

The authors confirm independence from the sponsors; the content of this article has not been influenced by the sponsors.

Ethics Approval

No ethics approval was required for this case report.

Patient Consent Statement

No patient-identifiable data has been included in this article.

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