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Scottish Frequency of the Common G985 Mutation in the Medium-chain Acyl-CoA Dehydrogenase (MCAD) Gene and the Role of MCAD Deficiency in Sudden Infant Death Syndrome (SIDS)

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Summary: Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, is an autosomal recessive inborn error of metabolism associated with various clinical presentations, including sudden unexplained death in young children. We have determined the Scottish frequency of the common G985 mutation found in Caucasians and in samples from Scottish patients with sudden infant death syndrome (SIDS). The heterozygote frequency of the mutation was found to be 1 in 276 (95% confidence interval: 1/76–1/2279) in 552 healthy controls and 1 in 74 (95% confidence interval: 1/27–1/377) in 233 SIDS patients: a difference that was not statistically significant (Fisher's exact test; two-sided; $p = 0.316$). None of the SIDS samples was found to be homozygous for the G985 mutation.

The structural gene for MCAD (McKusick 201450) has been cloned and mapped to human chromosome 1p31 (Matsubaru et al 1986; Kelly et al 1987) and analysis of this gene in patients with MCAD deficiency has revealed one common mutation with an allele frequency of more than 90% (Yokota et al 1991) and several rare point mutations (Tanaka et al 1992). The frequency of G985 heterozygotes in Scotland is unknown and large-scale DNA analyses of SIDS cases for this mutation have not been reported.

METHODS

We have used small-scale DNA extractions from clotted bloods obtained from our α-fetoprotein screening laboratory followed by a combination of PCR and restriction enzyme digests of the amplified product to look for the mutation. We also extracted DNA from the post-mortem livers of 233 SIDS patients from the same geographical area. The DNAs from clotted bloods were extracted by a proteinase K method (Williams et al 1988) and the DNAs from SIDS liver samples were extracted by a method for isolating high-molecular-weight DNA (Hogan et al 1986).

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The DNAs from the clotted bloods and SIDS patients were subjected to a modified polymerase chain reaction (PCR) (Gregersen et al 1991), where 199 bp DNA fragments, including position 985, were synthesized. The sense and antisense primers used were (5′-TTT ATG CTG GCT GAA ATG GCC ATG-3′) (bp 961 to 984 of the cDNA) and (5′-CAG GAT ATT CTG TAT TAA ATC CAT GGC CTC-3′) (bp 1130 to 1159 of the cDNA), respectively (Gregersen et al 1991). The products were directly digested with *Nco*I and analysed in 8% polyacrylamide gels (Figure 1). G985 can be distinguished from A985 by the size of the DNA restriction fragments produced (A985 = 178 bp: G985 = 158 bp) (Kolvraa et al 1991).

Standard statistical methods were used (Daly et al 1991).

RESULTS AND DISCUSSION

We analysed 552 DNA samples of women from the west of Scotland, and found 2 heterozygotes and no homozygotes. In addition, we found 3 G985 heterozygotes in a sample of 233 Scottish patients with SIDS and no homozygotes. This means that, the heterozygous frequency of the G985 mutation is 1/276 (95% CI: 1/76–1/2279) in the Scottish population and 1/74 (95% CI: 1/27–1/377) in the Scottish patients with SIDS. When the results were compared statistically, no significant difference in the heterozygous frequency was found between the two groups (Fisher's exact test; two-sided; $p = 0.316$). The carrier frequency of G985 as shown by DNA analysis has been reported in two earlier studies. Blakemore et al (1991) found that the heterozygous frequency of the mutation is 1 in 68 (6/410) in Trent, UK, and Matsubaru et al (1991) found a heterozygous frequency of 1 in 40 (12/479) in Birmingham, UK. They also found a frequency of 1 in 118 in Australia, 1 in 107 in the USA, and no carriers amongst 500 Japanese neonates. From the two UK studies, homozygote frequencies of between 1 in 13 400 and 1 in 9760 were expected. The difference in heterozygote frequencies between our results (2/552) and the published data of Blakemore and

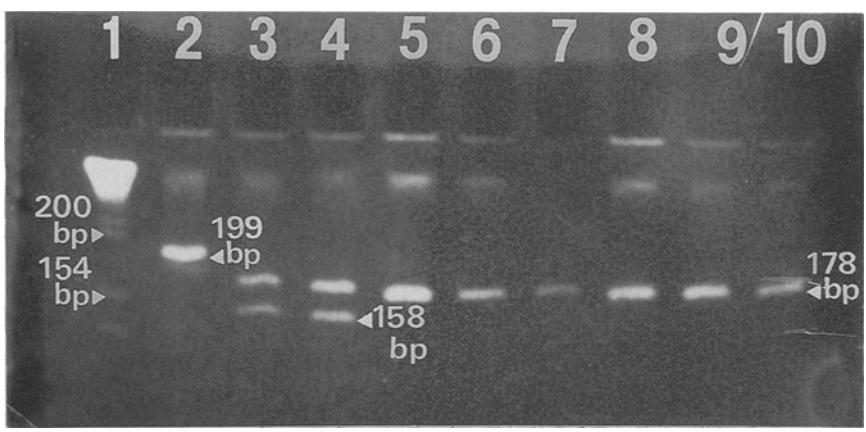

Figure 1 Genomic DNA fragments (199 bp) were amplified and digested with *Nco*I and subjected to electrophoresis in an 8% polyacrylamide gel. Lane 1, size marker (1 kb ladder); lane 2, control (undigested); lane 3, heterozygous control; lane 4, heterozygous individual; lanes 5–10, normal individuals

colleagues (1991) and Matsubaru and colleagues (1986) (18/889) is significant (Z test; $p = 2.62$), indicating a lower frequency of this mutation in the west of Scotland.

Our data show that, statistically, there is no increase in the heterozygous frequency of the common G985 MCAD mutation in Scottish SIDS when compared with the frequency found in a sample of women from the west of Scotland or with published data from England. This leads to the conclusion that MCAD deficiency, caused by the common G985 mutation, is not a common cause of SIDS in Scotland.

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REFERENCES

Blakemore AIF, Singleton H, Pollitt RJ, Engel PC, Kolvraa S, Gregersen N (1991) Frequency of the G985 MCAD mutation in the general population. *Lancet* **337**: 298–299.

Daly LE, Bourke GJ, McGilvray J (1991) Confidence intervals and hypothesis tests: two-group comparisons. In: *Interpretation and Uses of Medical Statistics*, 4th edn. London: Blackwell Scientific Publications, 102–138.

Gregersen N, Andersen BS, Bross P et al (1991) Molecular characterisation of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: Identification of a lys^{329} to glu mutation in the MCAD gene, and expression of inactive mutant enzyme protein in *E. coli*. *Hum Genet* **86**: 545–551.

Hogan B, Constantini F, Lacy E (1986) Introduction of new genetic information into the developing mouse embryo. In: *Manipulating the Mouse Embryo: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory, 174–176.

Kelly DP, Kim JJ, Billadello JJ, Hainline BE, Chu TW, Strauss AW (1987) Nucleotide sequence of medium-chain acyl-CoA dehydrogenase mRNA and its expression in enzyme-deficient human tissue. *Proc Natl Acad Sci USA* **84**: 4068–4072.

Kolvraa S, Gregersen N, Blakemore AIF et al (1991) The most common mutation causing medium-chain acyl-CoA dehydrogenase deficiency is strongly associated with a particular haplotype in the region of the gene. *Hum Genet* **87**: 425–428.

Matsubaru Y, Kraus JP, Yang-Feng TL, Francke U, Rosenberg LE, Tanaka K (1986) Molecular cloning of cDNAs encoding rat and human medium-chain acyl-CoA dehydrogenase and assignment of the gene to human chromosome 1. *Proc Natl Acad Sci USA* **83**: 6543–6547.

Matsubaru Y, Narisawa K, Tada K et al (1991) Prevalence of K329E mutation in medium-chain acyl-CoA dehydrogenase gene determined from Guthrie cards. *Lancet* **338**: 552–553.

Tanaka K, Yokota I, Coates PM et al (1992) Mutations in the medium chain acyl-CoA dehydrogenase (MCAD) gene. *Hum Mutat* **1**: 271–279.

Williams C, Weber L, Williamson R, Hjelm M (1988) Guthrie spots for DNA-based carrier testing in cystic fibrosis. *Lancet* **2**: 693.

Yokota I, Caetes PM, Hale DE, Rinaldo P, Tanaka K (1991) Molecular survey of a prevalent mutation 985 A-to-G transition, and identification of five infrequent mutations in the medium-chain acyl CoA dehydrogenase (MCAD) gene in 55 patients with MCAD deficiency. *Am J Hum Genet* **49**: 1280–1290.