Frequency Of The Common G985A Mutation In The Medium-chain Acyl-coa Dehydrogenase Gene In Turkish Population

Orta zincirli açil coa dehidrojenaz genında yaygın olan G985A mutasyonunun Türk popülasyonundaki taşıyıcı sıklığı

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Özet

Amaç: Orta zincirli açil-CoA dehidrojenaz (MCAD) orta zincirli yağ asitlerinin beta oksidasyonu için elzem olan bir tetramerik flavoproteindir. MCAD eksikliği mitokondrial beta oksidasyon defektinin en sık bilinen nedenidir. Fatal olabilmekle beraber geniş bir klinik spektrumu vardır.Bu çalışmanın amacı MCAD genindeki G985A mutasyon taşıyıcısı sıklığını Türk popülasyonunda tesbit etmektir. **Gereç ve Yöntemler:** 1400 sağlıklı bireyden DNA örneği analiz edilmiştir. Mutasyon tesbiti polimeraz zincir reaksiyonu sonrası G985A mutasyonu taşıyan PCR ürününün NcoI restriksiyon bölgesine sahip olması ve kesim sonrası %8'lik poliakrilamid jelle görüntülenmesi suretiyle yapılmıştır.
Bulgular:G985A heterozigot mutasyonu Türk popülasyonunda 1400 kişide 3 kişide tesbit edildi.
Sonuç: G985A taşıyıcılığının düşük olması MCAD eksikliğinin insidansının Türk toplumunda G985A taşıyıcılığı diğer popülasyonlara göre düşüktür. Bu çalışma Türk toplumunda akraba evliliğinin Avrupa ülkelerine göre sık olması ve hastalığın birikim göstermesi nedeniyle Türk toplumunda MCAD eksikliğinden farklı mutasyonların sorumlu olabileceğini önermektedir.
Anahtar Kelimeler: **Grup Popülasyonu; Nokta Mutasyonu; Orta zincirli açil-CoA dehidrojenaz; Sıklık; Türkiye.**

Abstract

Purpose: Medium chain acyl-CoA dehydrogenase (MCAD) is a tetrameric flavoprotein essential for the beta-oxidation of medium chain fatty acids. MCAD deficiency is the most commonly recognized defect of mitochondrial b-oxidation. It is potentially fatal, but shows a wide clinical spectrum.The aim of the present study was to investigate the frequency of the G985A mutation carrier in the medium-chain acyl-CoA dehydrogenase (MCAD) gene in Turkish population.
Material and Methods: We analyzed 1400 DNA samples. Mutation detection was performed with the polymerase chain reaction (PCR), in which a NcoI restriction site was created in the presence of a G985A mutation in the PCR product, followed by the digestion, and 8% polyacyrlamide gel electrophoresis.
Results: We detected a G985 carrier frequency of 3 in 1400 Turkish population.
Conclusion:The incidence of MCAD deficiency is lower in our population than other populations. The present study suggest that different MCAD mutations responsible for Turkish Population' MCAD phenotype, because the disorder shows a strong founder effect and consanquinity very common in our population than other European countries
Key Words:**Frequency; Grup Population; Medium chain acyl-CoA dehydrogenase; Point Mutation; Turkey.**

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Introduction

Fatty acid oxidation in mitochondria is an essential source of cellular energy. Medium chain acyl-CoA dehydrogenase (MCAD) deficiency (Mc Kusick 201450) is the most common defect of mitochondrial -oxidation in humans (1,2) and is particularly common in populations of northern European origin (3). It is a potentially fatal, autosomal recessively inherited defect, which most often presents in the first years of life.

The clinical manifestations of MCAD deficiency are diverse, but usually they include fasting induced non-ketotic hypoglycemia with lethargy which may develop into coma (2,4,5).

Between 20 and 25% of patients die suddenly at first presentation of the disease (5,6) and all deaths have been in previously undiagnosed patients. Although affected patients, who remain without symptoms for years have also been reported (6-9).

The human MCAD gene consists of 12 exons that span more than 44 kb encoding a precursor protein of 421 amino acids (10,11).

Due to various clinical spectrum of the disease, much effort has been directed towards elucidating the molecular cell pathology in MCAD deficiency. More than 20 different disease-associated missense variations in the MCAD gene have been reported (9,12-15).

In particular the molecular defect of the mutant protein (K304E) resulting from the prevalent G985A mutation has been investigated (15-19). Several studies have shown that about 80% of clinically ascertained cases are homozygous for an A to G transition at position 985, resulting in a lysine to glutamate substitution(20-22) and a further 18% have this mutation in one of the two defective alleles (23). Tanaka et al. (3) found a heterozygotes frequency of 1 in 216 in Turkey.

Brackett and co-workers (24) have stated that compound-heterozygosity with the G985A mutation and one of the non-G985A mutations, A583, gives rise to a particularly severe presentation of the disease.

MCAD deficiency is a disorder with significant morbidity and mortality, so in the present study we have investigated carrier frequency of the G985A mutation in the medium-chain acyl-CoA dehydrogenase (MCAD) gene in Turkish population.

Materials And Methods

This study was approved by Erciyes University Ethics Committee. All patients gave informed consent. Blood samples obtained were used to investigate genetic polymorphism of this study only. We used proteinase-K method for DNA isolations (25). The DNA samples were collected from unselected healty newborns, who were admitted to paediatric departments clinical genetics or other deparments to our university hospital from different city in Middle Anatolia in Turkey. The primers used for the detection of the G985A mutation were synthesized according to Gregersen et al.(26): a base mismatch was introduced into one of the primers so that a NcoI restriction site was created in the presence of a G985A mutation in the PCR product. A part of the PCR product was used for overnight digestion with the NcoI restriction endonuclease. Mutation analysis was performed on a 8% polyacrylamide gel, and the bands were visualized by ethidium bromide staining (Figure 1) (27).

Results

MCAD deficiency is inherited in an autosomal recessive manner. G985A is reportedly found in 90% of all retrospectively identified MCAD deficient patients' alleles; 81% of all MCAD deficient patients are homozygous, and 18% of MCAD deficient patients are compound heterozygous for G985A (20-23). G985A has been shown that is rather common in some European countries (Great Britain 1 in 6,000, Switzerland 1 in 10,000). In Caucasoid populations, one mutation, the 985A>G transition, causing the amino acid substitution K329E, accounts for about 90% of all mutant MCAD alleles. Caucasians of Northern European decent exhibit the highest frequency of MCAD deficient genotypes. The carrier frequency of G985A mutation among this group is estimated to be 1:40-100 and the homozygote frequency is 1:6.500-20.000 (28).

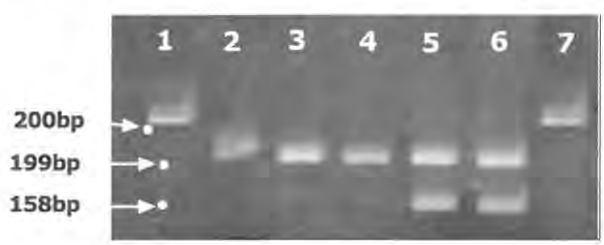

Figure1. PCR Products (199bp) were digested with the restriction enzyme NcoI and separated on 8% polyacrylamide gel. Lane1, 7 size marker (200bp ladder); Lane2,3,4 normal inviduals; Lane5,6, heterozygous control.

Discusison

Pollitt and Leonard (22) reported the findings of a prospective clinical study of MCAD deficiency in the UK. Between 1994 and 1996 there were 62 reported cases in 54 families, giving a minimum incidence of 4.5 in 100,000. In 46 cases, diagnosis followed an acute illness: 39 after a single episode, 6 after a second, and 1 after his third episode at the age of 12 years. The authors commented that the mortality and morbidity associated with MCAD deficiency remained high. Most patients have their first acute manifestation after the age of 3 months; this, the authors argued, supported the case for the introduction of a national neonatal screening program in the UK. Andersen et al.(23) determined the frequency of MCAD deficiency to be 1 in 15,001 in the U.S. population. De Vries et al. (29) detected a G985A carrier frequency of 1 in 55 in The Netherlands. Also other a lot of studies found the carrier frequency of the mutation are between the 40 to 276 in different populations (20,30,31). MCAD carrier frequency detected heterozygotes frequency of 1 in 216 in Turkey (3).

We present the carrier frequency of G985A mutation in the Turkish population. We analyzed 1400 DNA samples. We found 3 G985A heterozygotes but no homozygotes. It means that the heterozygotes frequency of the G985A mutation is 1/466 in Turkish population.

Lower frequency of G985A carriers (1/466) suggests that the incidence of MCAD deficiency is lower in our population than we expected or may be our population have a different mutation type related to MCAD deficiency. Although the present study suggest that Turkish Population' MCAD phenotype, may contribute with different MCAD mutations, because the disorder shows a strong founder effect and consanquinity very common in our population than other European countries and estimates of incidence based on mutation testing suggest the defect remains underdiagnosed.

References

1. Gregersen N, Andresen BS, Bross P, Bolund L and Kolvraa S. Disorders of mitochondrial fatty acid oxidation especially medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. In: Farriaux, J.P. and Dhondt, J.L. (eds) New Horizons in Neonatal Screening. Elsevier Science B.V. 1994; 247-255.

2. Roe CR and Coates PM. Mitochondrial fatty acid oxidation disorders. In: Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D. (eds) The Metabolic and Molecular Basis of Inherited disease. McGraw-Hill, New York. 1995; 1501-1533.

3. Tanaka K, Gregersen N, Ribes A, et al. A survey of the newborn populations in Belgium, Germany, Poland, Czech Republic, Hungary, Bulgaria, Spain, Turkey, and Japan for the G985 variant allele with haplotype analysis at the medium chain acyl-CoA dehydrogenase gene locus: clinical and evolutionary consideration. Pediatr Res. 1997;41:201-9.

4. Vianey-Liaud C, Divry P, Gregersen N and Mathieu M. The inborn errors of mitochondrial fatty acid oxidation. J. Inher. Metab. Dis., 1987; 10 (suppl 1): 159-198.

5. Iafolla AK, Thompson RJ and Roe CR. Medium-chain acyl-coenzyme A dehydrogenase deficiency: clinical course in 120 affected children. J. Pediatr.1994;124: 409-415.

6. Wilcken B, Hammond J and Silink M. Morbidity and mortality in medium chain acyl coenzyme A dehydrogenase deficiency. Arch. Dis. Child. 1994;70: 410-412.

7. Gregersen N, Winter V, Lyonnet S, et al. Molecular genetic characterization and urinary excretion pattern of metabolites in two families with MCAD deficiency due to compound heterozygosity with a 13 basepair insertion in one allele. J. Inher. Metab. Dis. 1994;17: 169-184.

8. Duran M, Hofkamp M, Rhead WJ, Saudubray JM and Wadman SK. Sudden child death and 'healthy' affected family members with medium-chain acyl- CoA dehydrogenase deficiency. Pediatrics 1986; 78:1052-57.499-506.

9. Andresen BS, Jensen TG, Bross P, L. et al. Disease-causing mutations in exon 11 of the medium-chain acyl-

CoA dehydrogenase (MCAD) gene. Am. J. Hum. Genet. 1994;54: 975-988.

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

11. Zhang Z, Kelly DP, Kim J, et al. Structural organization and regulatory regions of the human medium-chain acyl-CoA dehydrogenase gene. Biochemistry 1992;31:81-89.

12. Andresen BS, Bross P, Jensen TG, et al. A rare disease-associated mutation in the medium-chain acyl-CoA dehydrogenase (MCAD) gene changes a conserved arginine, previously shown to be functionally essential in short-chain acyl-CoA dehydrogenase (SCAD), Am. J. Hum. Genet.1993;53:730-739.

13. Andresen BS, Bross P, Udvari S, et al. The molecular basis of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in compound heterozygous patients: is there correlation between genotype and phenotype? Hum. Mol. Genet. 1997;6: 695-707.

14. Bross P, Jespersen Jensen C, Andresen BS, et al. Ects of two mutations detected in medium chain acyl-CoA dehydrogenase (MCAD)-deficient patients on folding, oligomer assembly, and stability of MCAD enzyme, J. Biol. Chem. 1995;270:10284-10290.

15. Udvari S, Bross P, Andresen BS, Gregersen N, Engel PC. Mutations of human medium-chian acyl-CoA dehydrogenase, Biochem. Soc. Trans. 1998; 26: S65.

16. Saijo T, Welch WJ and Tanaka K. Intramitochondrial folding and assembly of medium-chain acyl-CoA dehydrogenase (MCAD). J. Biol.Chem. 1994; 269: 4401-4408.

17. Bross P, Andresen BS, Winter V, et al. Co-overexpression of bacterial GroESL chaperonins partly overcomes non-productive folding and tetramer assembly of E.coli expressed human medium-chain acyl-Coa dehydrogenase (MCAD) carrying the prevalent disease-causing K304E mutation. Biochim. Biophys. Acta 1993;1182: 264-274.

18. Jensen TG, Bross P, Andresen BS, et al. Comparison between medium-chain acyl-CoA dehydrogenase (MCAD) mutant proteins over-expressed in bacterial and mammalian cells. Hum. Mutat. 1995; 6: 226-231.

19. Yokota I, Saijo T, Vockley J and Tanaka K. Impaired tetramer assembly of variant medium-chain acyl-coenzyme A dehydrogenase with a glutamate or aspartate substitution for lysine 304 causing instability of the protein. J. Biol. Chem. 1992;267: 26004-26010.

20. Blakemore AI, Singleton H, Pollitt RJ, et al. Frequency of the G985 MCAD mutation in the general population letter.Lancet 1991;337:298-9.

21. Curtis D, Blakemore AI, Engel PC, et al. Heterogeneity for mutations in medium chain acyl-CoA dehydrogenase deficiency in the UK population. Clin Genet 1991;40: 283-6.

22. Pollitt RJ and Leonard JV. Prospective surveillance study of medium chain acyl-CoA dehydrogenase deficiency in the UK. Arch Dis Child 1998;79:116-19.

23. Andresen BS, Dobrowolski SF, O'Reilly L, et al. Medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by MS. MS-based prospective screening of newborns differ from those observed in patients with clinical symptoms: identi.cation and characterization of a new, prevalent mutation that results in mild MCAD deficiency. Am J Hum Genet. 2001;68: 1408-1418.

24. Brackett JC, Sims HF, Steiner RD, et al. A novel mutation in medium-chain acyl-CoA dehydrogenase causes sudden neonatal death. J. Clin. Invest. 1994; 94:1477-1483.

25. Williams C, Weber L, Williamson R Guthrie spots for DNA-based carrier testing in cystic fibrosis. Lancet 1988;2:693

26. Gregersen N, Andresen BS, Bross P, et al. Molecular characterization of medium chain acyl CoA dehydrogenase(MCAD) deficiency: identification of a lys329-to-glu mutation in the MCAD gene, and expression of inactive mutant enzyme protein in E. Coli. Hum Genet. 1991; 86:545-551

27. Kolvraa S, Gregersen N, Blakemore AIF, et al. 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. 1991; 87: 425-428.

28. Wang S, Khoury M. Epidemiologic assesment between the G985A MCAD allelic variant and sudden infant death sydrome(SIDS). Pediadrics 2000;105:1175-76

29. De Vries HG, Niezen-Koning K, Kliphuis J W, et al. Prevalence of carriers of the most common medium chain acyl CoA dehydrogenase(MCAD) deficiency mutation (G985A) in the Netherlands. Hum. Genet.1996;98:1-2

30. Matsubara Y, Narisawa K, Tada K, et al. Prevalence of K329E mutation in medium-chain acyl-CoA dehydrogenase gene determined from Guthrie cards. ancet 1991; 338: 552-553

31. Dundar M, Lanyon WG, Connor JM. Scottish frequency of the common G985 mutation in the Medium chain acyl-CoA dehydrogenase (MCAD) deficiency in sudden infant Death Sydrome (SIDS). J Inher Metab Dis. 993;16: 991-993