

Role of Alpha-methylacyl-CoA racemase gene in pathogenecity of CMT patients

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Abstract

Objective: To find the causative mutation by linkage analysis of Charcot-Marie-Tooth disease while focussing on AMACR gene.

Methods: The case-control study was conducted from November 2016 to March 2017 in Kongju National University Korea. A family of 15 members with composite symptoms of peripheral neuropathy were enrolled. In addition, 50 healthy controls, which had no clinical features and family history of neuromuscular disorders, were also recruited. The family was selected for sequencing analysis by using capillary sequencing. It was sequenced for all the causative genes for CMT disease i.e. PMP22, MPZ, MFN2, GDAP1, NEFL, CX32, MYH14, LMNA, TRPV4, LITAF. Various regions of chromosome were suspected based on the logarithm of the odds score.

Results: Of the 15-member family, 7(47%) were affected and 8(53%) were unaffected. Those unaffected also acted as the controls. A missense mutation was found in exon 1 of the AMACR gene at p.Gly175Asp position. The mutation was also found in some of the unaffected members as well as in the control samples.

Conclusion: As the mutation was found in the healthy samples as well, it can be said that the current mutation AMACR can be involved in some other forms of peripheral neuropathy which can be with other phenotypes.

Keywords: CMT disease, Neuromuscular disorder, Peripheral neuropathy. (JPMA 68: 1039; 2018)

Introduction

AMACR is a biological catalyst which is essential for the oxidation of branched chain fatty acids and dihydroxycholestanic acid which is a bile acid intermediate. In enzymology, alpha-methylacyl-CoA racemase (AMACR) is an enzyme that catalyses chemical reactions. AMACR is essential for beta oxidation of branched-chain fatty acids and bile acid intermediates, such as dihydroxycholestanic acid and trihydroxycholestanic acid. AMACR-deficit is a turmoil that causes a diversity of neurological harms that start in maturity and gradually get severe.¹ People with AMACR insufficiency may have a steady defeat in cerebral functioning (cognitive decline), convulsion, and migraines. They might also have severe episodes of brain dysfunction (encephalopathy) like stroke, connecting distorted awareness and lesions in the brain.² The enzyme deficiency causes structural and functional brain changes, and disturbances in fatty acid and oxidative phosphorylation pathways observed in individuals with schizophrenia.³ Other features of AMACR deficiency may include weakness and loss of sensation in the limbs due to nerve damage (sensorimotor neuropathy), muscle stiffness (spasticity), and difficulty coordinating movements (ataxia). Vision problems caused by deterioration of the light-sensitive layer at the back of the

eye (the retina) can also occur in this disorder.⁴ Alterations in the gene programming peroxisomal AMACR grounds adult-onset neuromuscular disease.⁵ AMACR is highly overexpressed in prostate cancer (PCa) and its transcriptional regulators include various transcription factors and CTNNB1/ β -catenin. AMACR has been used as a diagnostic biomarker for CaP and is now a standard biomarker for needle biopsy specimens with ambiguous lesions.⁶ Mutations in AMACR gene has been responsible for various disorders identified by a homozygous S52P mutation in unrelated patients with adult-onset AMACR deficiency.³

As reported in literature, AMACR gene is associated with sensorimotor neuropathy, muscle stiffness, and difficulty in coordinating movements.⁵ Mutations found in AMACR gene show a variety of phenotypes, including seizures, visual failure, sensorimotor neuropathy, spasticity, and migraine.⁷ After sequencing all the genes related to Charcot-Marie-Tooth (CMT2) disease, the current study was planned to find the causative mutation by linkage analysis.

Subjects and Methods

The case-control study was conducted from November 2016 to March 2017 in Kongju National University Korea. A family of 15 members with composite symptoms of peripheral neuropathy were enrolled. In addition, 50 healthy controls, who had no clinical features and family history of neuromuscular disorders, were also recruited. All the participants provided written informed consent

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prior to the study.

Samples of peripheral neuropathy patients were collected from various hospitals. Subsequently, one CMT family was selected for mutational screening. After informed consent, blood samples from affected and unaffected family individuals were collected. deoxyribonucleic acid (DNA) was extracted using standard procedures. The family was initially sequenced to find out the mutation in CMT gene already discovered. By using a QIAamp DNA blood mini kit (Qiagen, Hilden, Germany), genomic DNA was isolated.

A duplication/deletion of 1.4 Mbp in length on 17p12 (including PMP22), which is the most frequent genetic cause of CMT, was pre-screened by genotyping six microsatellites within the 17p12 (22) and quantitative real-time PCR for PMP22 dosage. For this purpose EGR2, MPZ, PMP22, CX32, HSP22, HSP27, LITAF, LMNA, MFN2, NEFL and HSN2 were screened and we did not found any mutation. Sequencing analysis of all coding exons and flanking intronic sequences was performed. For sequencing analysis PCR products were sequenced on the automatic genetic analyser ABI3100 by using the BigDye terminator cycle sequencing kit.

The mutation in the AMACR gene was confirmed by DNA sequencing of all exons and contiguous flanking intronic sequences. The sequences were determined by sequencing the purified polymerase chain reaction (PCR) products

using an ABI3100 automatic sequencing analyser (Applied Biosystems, Foster City, CA). The mutation was identified in the proband. We used the SEQSCAPE (ver. 2.1) programme (Applied Biosystems) to detect the sequence variation and confirmed the sequence variations by analysing both strands of DNA. AMACR gene was screened for FC221 family.

Results

Of the 15-member family, 7(47%) were affected and 8(53%) were unaffected (Figure-1). Those unaffected also acted as the controls. In AMACR gene, there was a single nucleotide polymorphism (SNP)p. Gly175Asp found in

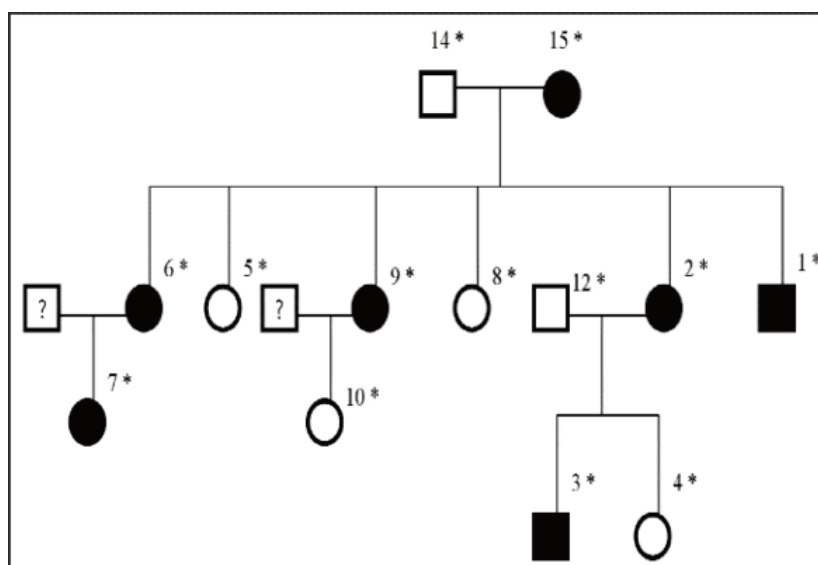


Figure-1: Pedigree of the Charcot-Marie-Tooth (CMT) Family. Affected individuals are denoted by blackened symbols, males are denoted by squares, females are denoted by circles, and unavailable sample are mentioned by I.

Table: Clinical and molecular description of the selected Patients.

Samples	Clinical phenotype	Amino Acid Change	Nucleotide Change	AA position
1	peripheral neuropathy	[Gly] ? D [Asp]	GGC ? GAC	175
2	peripheral neuropathy	[Gly] ? D [Asp]	GGC ? GAC	175
3		[Gly] ? D [Asp]	GGC ? GAC	175
4		[Gly] ? D [Asp]	GGC ? GAC	175
6	peripheral neuropathy	[Gly] ? D [Asp]	GGC ? GAC	175
7	peripheral neuropathy	[Gly] ? D [Asp]	GGC ? GAC	175
8		[Gly] ? D [Asp]	GGC ? GAC	175
9	peripheral neuropathy	[Gly] ? D [Asp]	GGC ? GAC	175
10		[Gly] ? D [Asp]	GGC ? GAC	175
11		[Gly] ? D [Asp]	GGC ? GAC	175
12		[Gly] ? D [Asp]	GGC ? GAC	175
13	NA			
14	NA			
15	peripheral neuropathy			

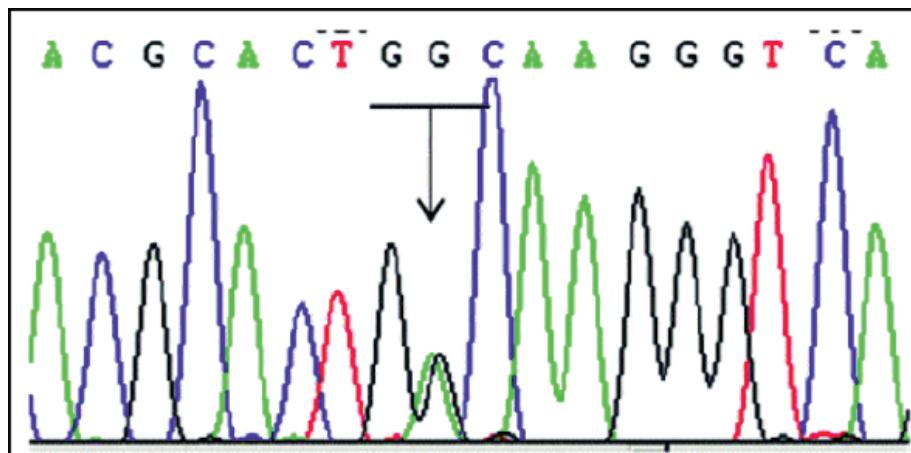


Figure-2: Chromatogram of the mutation found in the AMACR).

Exon 1 (Figure-2). Later, the whole family was sequenced to check the association with the other affected members. In unaffected sample (4,5,8,10,12) transition was also found as well as one WT sample had the same transition (Table).

Discussion

A family with CMT1 complex phenotype was selected to find the causative mutation. All known CMT1 genes were sequenced to find the responsible mutation. CMT is heritably and clinically diverse hereditary motor and sensory neuropathy with a predictable prevalence of 1/2500.⁸ Frequent clinical symptoms of CMT patients are distal muscle weakness, sensory loss, and foot deformities. Based on the nerve transmission velocities (NCVs), CMT is typically alienated into two types; the demyelinating form (CMT1) and axonal form (CMT2).⁹ By and large 70 genes or loci have been accounted as the fundamental reason of CMT.^{10,11}

On the basis of the previous studies, we selected the AMACR gene as a causative gene.⁵ Our data shows that the p.Gly175Asp mutation in AMACR was not a causative mutation and was not responsible for the complex phenotype of peripheral neuropathy, myopathy because of lack of co-segregation of the mutation with affected members in the pedigree, and the detection of the same mutation in 50 ethnicity-matched control chromosomes. In most cases the gene was involved in colorectal neoplasia.¹² However, in some reports the AMACR gene alterations are involved in the adult-onset sensory prostrate cancer. Axonal sensorimotor polyneuropathies hold a broad list of differential diagnoses.¹³ Analysis is based on comprehensive history, objective examination, recognition of associated neurologic and non-neurologic features, and suitable testing. Hereditary sensorimotor

neuropathies or CMT encompass a group of diseases with diverse clinical, electrophysiological and genetic expression.¹⁴ Gathered evidences show that decreased expression of AMACR results in neurological disorders. The symptoms are similar to those of adult Refsum disease and usually appear in the late teens or early 20s.^{15,16}

We studied only one family for the AMACR gene, and the subject should be further studied as the gene is responsible for peripheral neuropathy as already reported in literature.⁵ Since mutation in

AMACR have been shown to cause peripheral neuropathy, we therefore suggest that the identified mutation in AMACR can be associated with other forms of the neuromuscular disorder as the gene has already evidence of being involved in sensorimotor disorder. A large number of samples also needed to confirm the pathogenicity of the reported disease. AMACR gene can be involved in peripheral neuropathy but the current variant was not found to be involved in the CMT disease in the family studied.

Conclusion

AMACR gene mutation was also found in healthy and control samples as well. The current mutation AMACR can be involved in some other forms of peripheral neuropathy which can be with other phenotypes.

Disclaimer: None to declare.

Conflict of Interest: None to declare.

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