

Association of SNP in JPH1 gene with severity of disease in Charcot Marie Tooth 2K patients

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Abstract

Phenotype varies among the various types of Charcot Marie Tooth Neuropathies (CMT), However the problem arises in cases of same gene but gives a huge variety of phenotype in terms of early and late onset and severity of the disease. To check the impact of rs139723190 SNP on severity of the CMT 2k patients; being a genetic modifier of GDAP1. In the current study CMT 2k patients with early and late onset were analyzed for association of rs139723190 SNP in JPH1 gene responsible for CMT type severe and mild phenotypes. Single nucleotide polymorphisms (SNPs) lead to genetic differences in CMT patients on the basis of severity of the disease. The results of the present study suggest that variants of JPH1 may contribute to the genetic susceptibility as it plays a vital role as genetic modifier in CMT 2K. Candidates risk variants should be further evaluated in studies with a larger sample size.

Keywords: Genetic Modifier, CMT2K, axonal CMT, MAF, HMSN.

Introduction

Charcot Marie Tooth (CMT) hereditary neuropathies are a cluster of turmoil distinguished by malfunction of peripheral motor and sensory nerves. Medical attributes of CMT comprise continuous distal muscle flax and degeneration, gentle to modest distal sensory failure, depressed tendon reflexes, and a distinctive foot distortion with pes cavus and hammer toes. Phenotype varies among the various types of CMT, However the problem arises in cases of same gene but gives a huge variety of phenotype in terms of early and late onset and severity of the disease. As this is a well established fact that JPH1 is a genetic modifier for the Charcot-Marie-Tooth disease 2k. To find the association of a JPH1 SNP (rs139723190) with GDAP1 phenotype, seven CMT patients having GDAP1 mutations were selected.

Junctional composites linking the plasma membrane

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and endoplasmic reticulum are a familiar trait of all impulsive cell types and mediate cross talk between cell exterior and intracellular ion channels.¹ Junctophilin (JP) can be further divided into subtypes, namely JP-1, 2, and 3, have been recognized in excitable cells and comprise a new family of junctional membrane complex proteins. Recent investigations have suggested that JPs take part in the formation of junctional membrane complexes by straddling the membrane of the intracellular Ca²⁺ store and intermingle with the cell-surface membrane.² Cell signaling mechanisms often transmit information via posttranslational protein modifications, most importantly reversible protein phosphorylation.³ The junctophilins have come into sight as a family of proteins that play a vital role in communication, maturation and maintenance of the ultrastructure's of the cell., junctophilin 1 and junctophilin 2 plays these roles Within skeletal and cardiac muscle, respectively, couple sarcolemmal and intracellular calcium channels.⁴ JPs dominantly affect Ca²⁺ signaling in striated muscle, and that's why they can be responsible for various disorders related to muscles.⁵

Charcot-Marie-Tooth disease (CMT) disease takes account of a inherited diverse group of inherited neuropathies, as well as recognized hereditary motor and sensory neuropathies (HMSN).^{6,7} Alterations in the GDAP1 gene sequences leads to various phenotypes of Charcot-Marie-Tooth (CMT) disease, and the main medical appearance of this disease is noticeably changeable in the dominant inheritance form (CMT type 2K; CMT2K), in which transporter of the GDAP1 p.R120W mutation can exhibit a wide range of clinical brutality. It was explored the JPH1 gene as a genetic modifier of clinical expression inconsistency because junctophilin-1 (JPH1) is a good positional and functional candidate.⁸ GDAP1 is mainly articulated in neurons,⁹ whereas JPH1 is predominantly expressed in skeletal muscle.¹⁰ Autosomal recessive axonal Charcot-Marie-Tooth disease type 2K is caused by homozygous or compound heterozygous mutation in the GDAP1 gene (606598) on chromosome 8q. In human neural crest cells, it was investigated that

Table: Clinical and molecular association of SNP with CMT 2K patients.

| Patient ID | Sex | CMTNS Total | Age | CMT Type | Causative gene | Onset Age | Genotype rs139723190 |
|------------|-----|-------------|-----|----------|----------------|-----------|----------------------|
| 1 | F | 17 | 4 | CMT2K | GDAP1 | 16 | ACG ⇒ ATG |
| 2 | F | 25 | 17 | CMT2K | GDAP1 | 25 | ACG ⇒ ATG |
| 3 | F | 14 | 42 | CMT2K | GDAP1 | 2 | ACG ⇒ ATG |
| 4 | F | 18 | 28 | CMT2K | GDAP1 | 15 | ACG ⇒ ATG |
| 5 | M | 11 | 2 | CMT2K | GDAP1 | 30 | ACG ⇒ ATG |
| 6 | M | 17 | 38 | CMT2K | GDAP1 | 3 | ACG ⇒ ATG |
| 7 | M | 9 | 22 | CMT2K | GDAP1 | 31 | ACG ⇒ ATG |

SNA=Single Nucleotide Polymorphism

CMT= Charcot Marie Tooth

JPH1 primarily co localizes with markers in the endoplasmic reticulum (ER) and partially co localizes with mitochondria in the same region as GDAP1.⁸

Materials and Methods

Samples: CMT 2K is caused by alteration in GDAP1 gene primarily however it shows a variety of phenotypes among CMT 2K patients. The study was undertaken to check the impact of rs139723190 SNP on severity of the CMT 2k patients; a genetic modifier of GDAP. The MAF for the current SNP is very low so it can be involved as a pathogenic as well as genetic modifier. In the current study seen CMT 2k patients with early and late onset were analyzed for association of rs139723190 SNP in JPH1 gene responsible for CMT type severe and mild phenotypes. Table:

DNA extraction and PCR: Blood samples were collected from the patients. Written informed consent was sought from all caregivers of enrolled children. Genomic DNA was purified from peripheral blood using a QIAamp blood DNA purification kit (Qiagen, Germany). All patient samples were prescreened for the 17p12 (PMP22) duplication, which is the most frequent genetic cause of demyelinating CMT, using a hexaplex microsatellite PCR. The present study was started in September 2016 and was finished in April 2017.¹¹

Capillary sequencing was the technique in this study. DNA extraction was carried out from blood lymphocytes using salting out protocol with proteinase k (Garner, 2000). The primer set used for rs139723190 amplification was: forward 5'- GGCCAGAGTACTTGGACTGG -3' and reverse, 5'- ACACAGCCACTCTCTGCTT -3'. Polymerase chain reaction was performed for this polymorphism with the following programme: 94°C for 5min, 94°C for 30 seconds, 58°C for 30 sec, 72°C for 30 sec, 72°C for 10 sec.

Conclusion

The mutations identified in JPH1 were evaluated using online available programmers to predict whether variants are deleterious. PolyPhen classifies an amino acid substitution as probably damaging, possibly damaging, benign, or unknown. Proven predicts whether an amino acid substitution affects protein function. Single nucleotide polymorphisms (SNPs) lead to genetic differences in CMT patients on the basis of severity of the disease. A study was performed to evaluate genetic variants of JPH1 gene. The results of the present study suggest that variants of JPH1 may contribute to the genetic susceptibility as it plays a vital role as genetic modifier in CMT 2K. In current study the SNP rs139723190 did not showed any association with the early and late onset CMT type 2K patients as the alleles were present in all the patients of type 2K. However due to the limited number of samples we can suggest that large number of samples are required to establish the fact. As the JPH1 gene mutations are responsible for the CMT 4A and 2B2 so the given SNP is also a pathogenic and can be responsible for the disease as it was also evaluated by the Phyre2 and SIFT analysis. A large number of sample are therefore needed from various ethnic origin for further studies.

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Conflict of Interest: None

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