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The Importance of Multiple Gene Analysis for Diagnosis and Differential Diagnosis in Charcot Marie Tooth Disease

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ABSTRACT

AIM: To investigate the genetic etiology of Charcot-Marie-Tooth (CMT) disease or hereditary motor and sensory neuropathy (HMSN).

MATERIAL and METHODS: We herein examined 55 non-related patients with a suspicion of CMT phenotype or HMSN using a customized multigene panel based on the next-generation sequencing technique. All cases were previously analyzed for PMP22 duplication with the Multiplex Ligand Probe Amplification (MLPA) method.

RESULTS: In 13 cases (7.15%), we identified a pathogenic/likely pathogenic variant. The affected genes were *MARS1, NDRG1, GJB1, GDAP1, MFN2, PRX, SH3TC2,* and *FGD4*. In six cases (10.9%), novel variants were identified: pathogenic variants in *GJB1* and *FGD4* genes, variants of unknown significance (VUS) in *HSPB3, CHRNA1, ARHGEF10,* and *KIF5A* genes. In 21 cases (11.55%), VUS with the genes *HSPB3, KIF1B, SCN11A, CHRNA1, HSPB1, FIG4, ARHGEF10, DHTKD1, SBF1, EGR2, SBF2, IGHMBP2, KIF5A,* and *DNAJB2* were identified.

CONCLUSION: In this study, we had a 7.15% diagnosis rate with the NGS (Next Generation Sequencing) method in the CMT disease. Targeted next-generation sequencing panels are beneficial, time-saving, and cost-effective in the diagnosis of CMT.

KEYWORDS: Charcot-marie-tooth, Next generation sequencing, Multigene testing, Hereditary neuropathy

INTRODUCTION

Peripheral neuropathy is a common neurological pathology that indicates any disorder of the peripheral nervous system. Polyneuropathy is a more specific term characterized by generalized, relatively homogeneous involvement of many peripheral nerves depending on the same cause and physiopathological processes (13). In polyneuropathies, motor, sensory, and autonomic nerve fibers can be held together or separately. Classification can be made based on the type of nerve fiber involved, whether the major change is in the axon, myelin, or the underlying cause.

Hereditary neuropathies are rare diseases that affect the peripheral nervous system and occur after genetic defects, and central nervous system involvement may be seen in some of its subtypes (16). The most common form is HMSN, also known as CMT, and its prevalence is 1/2500 (10). In the classification, clinical findings are evaluated together with hereditary transition form and electrophysiological findings (presence of axonal or demyelinating involvement findings) (15). CMT is inherited as autosomal dominant, autosomal recessive, and X linked with different types (18). The vast majority of cases are CMT-Type 1, CMT-Type 2, and CMT-Type 4 (19). CMT-Type 1 starts in the first or second decade and is inherited

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as autosomal dominant. It constitutes 60% of CMT cases. In electrophysiological and histopathological examinations, demyelinating involvement is evident, accompanied by the loss of axons (21). CMT-Type 1A is caused by *PMP22* gene duplication (24), *MPZ* gene pathogenic variants are responsible for CMT-Type 1B (6), *LITAF* gene pathogenic variants cause CMT-Type 1C (14), and the *EGR2* gene handles CMT-Type 1D (12). The most common cause of CMT-Type 1 cases have *PMP22* duplication (5). Therefore, duplications containing the *PMP22* gene fragment should be investigated primarily in demyelinating cases. After this situation is excluded, other gene variants should be analyzed. CMT-Type 2 starts in older ages than CMT-Type 1 and is inherited as autosomal dominant (15). Axon involvement is evident in electrophysiological and histopathological examinations. CMT-Type 2A, 2B, 2C, 2D, 2I/J, 2F, 2H/K are reported with the pathogenic variants of *MFN2, RAB7, TRPV4, GARS, MPZ, HSPB1,* and *GDAP1* (2). The most common form of CMT-Type 4 is CMT-Type 4A (3). With autosomal recessive inheritance, it occurs due to a *GDAP1* gene mutation. Severe sensory-motor neuropathy and vocal cord paresis are seen in the early period. In nerve conduction studies, axonal and demyelinating involvement are observed together. The most common demyelinating form of CMT-Type 4 is CMT-Type 4C, which occurs due to *SH3TC2* gene pathogenic variants (3). Facial, bulbar, and diaphragmatic involvement in the early infantile period due to myotube-related protein-2 (MTMR2) pathogenic variant in CMT-Type 4B1 has been reported in some families (3). Early scoliosis due to the *SH3TC2* gene pathogenic variant in CMT-Type 4C chromosome 5q23-q33 is the first application finding in many cases. *EGR2* in CMT-Type 4E, periaxin (PRX) in CMT-Type 4F, and 12p11.21-q13.11 chromosome pathogenic variants in CMT Type 4H are known (3). The most common form of CMT-X occurs due to the *GJB1* gene point mutation in the dominant, X-inherited Xq13.1 chromosome (26). Connexin 32, encoded by *GJB1*, takes part in the transport of ions and small molecules in myelin gap junctions (4). Symptoms occur at a younger age in men and are more severe (26). In women, it starts between the ages of 20 and 30 and is not severe (26). The slowing of nerve conduction velocity is proportional to the clinical course. Unlike CMT-Type 1 pathologically, myelinated and regenerated fibers are more common, and onion skin appearance is rarely encountered (26). Electrophysiological studies are between demyelinating in CMT-Type 1 and axonal involvement in CMT-Type 2 (26).

In this study, our aim was to ascertain the frequency of different gene variants that cause different CMT types and to determine the genotype-phenotype correlation in cases where *PMP22* duplication is excluded with the NGS method. We also want to emphasize that analyzing many genes simultaneously with the NGS method supports a time-saving and cost-effective testing.

MATERIAL and METHODS

Samples

In this study, 55 cases (25 females, 30 males) with a suspicion of CMT/HMSN, were analyzed for CMT-neuropathy targeted gene panel in our Genetics Diagnosis Center between June 2017 and March 2020. Patient files were evaluated retrospectively. The study was approved by the Research Ethics Boards (number of 2020-167) of Trakya University Faculty of Medicine, and written informed consent forms were taken before being recruited to the study.

Genomic DNA was isolated from peripheral blood samples using the EZ1 DNA Investigator Kit (Qiagen, Hilden, Germany). Quality control of the DNA samples was performed on NanoDrop (Thermo Fisher Scientific, Waltham, MA), and samples having A260/280 values between 1.8 and 2.0 were used for downstream applications.

Next Generation Sequencing

DNA samples were sequenced using a QIAseq Targeted DNA Panel (Qiagen, Hilden, Germany). Libraries covering the target genes (Table I) were prepared according to the QIAseq Targeted DNA Panel protocol (Qiagen, Hilden, Germany). Following the target enrichment process, libraries were sequenced on the NextSeq550 System (Illumina, San Diego, CA, USA). QCI analysis (Qiagen, Hilden, Germany) was used for quality control and VCF generation from FASTQ files. Analysis of the VCF files was performed using QIAGEN Clinical Insight software (Qiagen, Hilden, Germany). ACMG-2015 guidelines were used for variant classification (22).

Multiplex Ligation Dependent Probe Amplification

According to the manufacturer's instructions, MLPA was applied to all samples using the MRC-Holland commercial kit to define possible deletions/duplications of the *PMP22* gene (SALSA MLPA P143-C1) (MRC-Holland, Amsterdam, the Netherlands). Fragments were separated using capillary gel electrophoresis in an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, USA). The fragments were analyzed using Coffalyser software (MRC-Holland, Amsterdam, the Netherlands).

RESULTS

With a pre-diagnosis of CMT or HMSN, 55 cases were analyzed with targeted gene sequencing after *PMP22* deletion duplication analysis with MLPA. Thirty male cases had a mean age of 26.6; 25 female cases had a mean age of 25.8; the mean age of 55 cases was 26.2 with a range of 3 to 62 years.

Fourteen variants in 13 cases (7.15%) were assessed as pathogenic/likely pathogenic with the genes *MARS1, NDRG1, GJB1, GDAP1, MFN2, PRX, SH3TC2,* and *FGD4* (Table II). Twenty-two variants in 21 cases (11.55%) were assessed as variants of uncertain clinical significance with the genes *HSPB3, KIF1B, SCN11A, CHRNA1, HSPB1, FIG4, ARHGEF10, DHTKD1, SBF1, EGR2, SBF2, IGHMBP2, KIF5A,* and *DNAJB2* (Table III). Two novel pathogenic/likely pathogenic variants were detected in the *GJB1* and *FGD4* genes, and four novel VUS were detected in the *HSPB3, CHRNA1, ARHGEF10,* and *KIF5A* genes. Of our cases, 10.9 had a novel variant, and 3.63% of our cases had a pathogenic/likely pathogenic novel variant.

Table I: The Genes Sequenced in the Targeted NGS Panel and Their Corresponding Transcript/Protein Numbers

Gene Name	Transcript ID	Protein ID	Gene Name	Transcript ID	Protein ID
AARS	NM_001605.2	NP_001596.2	***INF2***	NM_022489.3	NP_071934.3
AIFM1	NM_145812.2	NP_665811.1	***KARS***	NM_005548.2	NP_005539.1
ARHGEF10	NM_014629.2	NP_055444.2	***KIF1B***	NM_015074.3	NP_055889.2
BSCL2	NM_032667.6	NP_116056.3	***KIF5A***	NM_004984.2	NP_004975.2
COX6A1	NM_004373.3	NP_004364.2	***LITAF***	NM_001136473.1	NP_001129945.1
DHTKD1	NM_018706.6	NP_061176.3	***LMNA***	NM_170707.3	NP_733821.1
DNAJB2	NM_006736.5	NP_006727.2	***LRSAM1***	NM_001005374.3	NP_001005374.1
DNM2	NM_001005360.2	NP_001005360.1	***MARS***	NM_004990.3	NP_004981.2
DNMT1	NM_001379.2	NP_001370.1	***MED25***	NM_030973.3	NP_112235.2
DYNC1H1	NM_001376.4	NP_001367.2	***MFN2***	NM_014874.3	NP_055689.1
EGR2	NM_000399.3	NP_000390.2	***MPZ***	NM_000530.6	NP_000521.2
FBLN5	NM_006329.3	NP_006320.2	***MTMR2***	NM_016156.5	NP_057240.3
FGD4	NM_139241.2	NP_640334.2	***NDRG1***	NM_006096.3	NP_006087.2
FIG4	NM_014845.5	NP_055660.1	***NEFL***	NM_006158.4	NP_006149.2
GAN	NM_022041.3	NP_071324.1	***PDK3***	NM_005391.4	NP_005382.1
GARS	NM_002047.2	NP_002038.2	***PLEKHG5***	NM_198681.3	NP_941374.2
GDAP1	NM_018972.2	NP_061845.2	***PMP22***	NM_153321.2	NP_696996.1
GJB1	NM_000166.5	NP_000157.1	***PRPS1***	NM_002764.3	NP_002755.1
GNB4	NM_021629.3	NP_067642.1	***PRX***	NM_181882.2	NP_870998.2
HINT1	NM_005340.6	NP_005331.1	***RAB7A***	NM_004637.5	NP_004628.4
HK1	NM_033497.2	NP_277032.1	***REEP1***	NM_022912.2	NP_075063.1
HOXD10	NM_002148.3	NP_002139.2	***SBF1***	NM_002972.2	NP_002963.2
HSPB1	NM_001540.3	NP_001531.1	***SBF2***	NM_030962.3	NP_112224.1
HSPB8	NM_014365.2	NP_055180.1	***SH3TC2***	NM_024577.3	NP_078853.2
IGHMBP2	NM_002180.2	NP_002171.2	***TRIM2***	NM_001130067.1	NP_001123539.1
IKBKAP	NM_003640.3	NP_003631.2	***TRPV4***	NM_021625.4	NP_067638.3
YARS	NM_003680.3	NP_003671.1	***VCP***	NM_007126.3	NP_009057.1

All 55 cases had MLPA analysis for *PMP22* deletion/duplication analysis before targeted gene sequencing, and none of these cases had a deletion or duplication in the *PMP22* gene.

DISCUSSION

Hereditary neuropathies are an important cause of polyneuropathy and its prevalence is 1:2500 (23). Distal symmetric polyneuropathy is the dominant phenotype, and phenotype heterogeneity can be seen in the same family. For this reason, all neuropathy phenotypes should be considered while performing genetic tests (20). It is vital to have a detailed family history in the evaluation of polyneuropathy. It is necessary to suspect and investigate the hereditary neuropathy phenotype. Since molecular diagnostic tests are available, there is a need for genotype-phenotype correlation on the benefits of these tests in the diagnosis of polyneuropathy. Symptoms differ in polyneuropathy according to the affected nerve fiber. The most common symptoms are numbness, tingling, burning, or chills in the hands and feet, stabbing, sharp pains, loss of balance and coordination, and especially, weakness in the feet. When autonomic fibers are affected, constipation, diarrhea attacks, difficulty in controlling the bladder, impotence, changes in heart rhythm, orthostatic hypotension, decreased sweating, and difficulty in perceiving temperature changes can be seen. If the patients are diagnosed earlier, the factor causing nerve

Table II: Cases with Pathogenic/Likely Pathogenic Variants in Our Study

Sample ID	Gender/ Age	Test endication	Gene	OMIM phenotypes	Mutation Type	Nucleotide HGVS	Protein HGVS	dbSNP
1-SC	F/9	Hereditary Spastic paraplegia	MARS1	Charcot-Marie-Tooth disease, axonal, type 2U (AD) Interstitial lung and liver disease (AR)	Frameshift Heterozygous	NM_004990.4:c.2114dupT	(p.Leu705PhefsTer19)	rs777324495
2-EO	M/16	CMT?	NDRG1	Charcot-Marie-Tooth disease, type 4D (AR)	Intronic Heterozygous	NM_006096.4:c.205+1G>A	-	rs1060503092
			NDRG1 **(VUS)**	Charcot-Marie-Tooth disease, type 4D (AR)	Missense Heterozygous	NM_006096.4:c.964C>T	(p.Arg322Cys)	rs368404338
3-HD	F/14	CMT?	NDRG1	Charcot-Marie-Tooth disease, type 4D (AR)	Nonsense Homozygous	NM_006096.4:c.442C>T	(p.Arg148Ter)	rs119483085
			GJB1	Charcot-Marie-Tooth neuropathy, X-linked dominant, 1 (XLD)	Missense Heterozygous	NM_001097642.2:c.656G>A	(p.Arg219His)	rs199834862
4-CB	F/37	CMT?	GDAP1	Charcot-Marie-Tooth disease, axonal, type 2K (AD, AR) Charcot-Marie-Tooth disease, axonal, with vocal cord paresis(AR) Charcot-Marie-Tooth disease, recessive intermediate, A (AR) Charcot-Marie-Tooth disease, type 4A (AR)	Missense Heterozygous	NM_018972.4:c.653A>G	(p.Gln218Arg)	rs556827873
5-AAC	M/5	Polyneuropathy, gait disorder	MFN2	Charcot-Marie-Tooth disease, axonal, type 2A2A (AD) Charcot-Marie-Tooth disease, axonal, type 2A2B (AR) Hereditary motor and sensory neuropathy VIA (AD)	Missense Heterozygous	NM_014874.4:c.310C>T	(p.Arg104Trp)	rs119103268
6-ZB	F/20	CMT?	GDAP1	Charcot-Marie-Tooth disease, axonal, type 2K (AD, AR) Charcot-Marie-Tooth disease, axonal, with vocal cord paresis(AR) Charcot-Marie-Tooth disease, recessive intermediate, A (AR) Charcot-Marie-Tooth disease, type 4A (AR)	Frameshift Homozygous	NM_018972.4:c.786delG	(p.Phe263LeufsTer22)	rs1060500978
7-OE	F/5	Gait disorder	PRX	Charcot-Marie-Tooth disease, type 4F (AR) Dejerine-Sottas disease (AD, AR)	Nonsense Homozygous	NM_181882.3:c.3208C>T	(p.Arg1070Ter)	rs104894708
8-RUU	M/13	CMT?	NDRG1	Charcot-Marie-Tooth disease, type 4D (AR)	Nonsense Homozygous	NM_001135242.2:c.442C>T	(p.Arg148Ter)	rs119483085
9-SD	F/26	CMT?	GJB1	Charcot-Marie-Tooth neuropathy, X-linked dominant, 1 (XLD)	Missense Heterozygous	NM_000166.6:c.166C>G	(p.Leu56Val)	Novel
10-CP	F/58	CMT?	SH3TC2	Charcot-Marie-Tooth disease, type 4C (AR) Mononeuropathy of the median nerve, mild (AD)	Nonsense Homozygous	NM_024577.4:c.2860C>T	(p.Arg954Ter)	rs80338933
11-OG	M/18	CMT?	NDRG1	Charcot-Marie-Tooth disease, type 4D (AR)	Nonsense Homozygous	NM_001135242.2:c.442C>T	(p.Arg148Ter)	rs119483085
12-AE	F/33	CMT?	SH3TC2	Charcot-Marie-Tooth disease, type 4C (AR) Mononeuropathy of the median nerve, mild (AD)	Nonsense Homozygous	NM_024577.4:c.2860C>T	(p.Arg954Ter)	rs80338933
13-ZO	F/3	CMT?	FGD4	Charcot-Marie-Tooth disease, type 4H (AR)	Splicing Heterozygous	ENST00000534526.2:c.504-1G>A	-	Novel

CMT: *Charcot Marie Tooth,* ***AD:*** *Autosomal dominant,* ***AR:*** *Autosomal recessive,* ***XLD:*** *X Linked Dominant,* ***VUS:*** *Variants of Unknown Significance.*

damage can be detected and controlled, thus preventing the progression of the nerve damage and protecting it from other symptoms that may occur. Our study contributes to the correct and early diagnosis of cases with polyneuropathy and supports early management, treatment, and necessary genetic counseling.

The majority of genetically determined polyneuropathies are the CMT variants. Genetic tests are being developed in years for other types of neuropathy. The clinical phenotype of CMT is very variable, ranging from a severe polyneuropathy accompanied by respiratory distress to the classic type with pes cavus and stork leg or with minimal neurological findings (10). Also, different genetic mutations can cause similar phenotypes (genetic heterogeneity), and different phenotypes can result in the same genotype (phenotypic heterogeneity) (11).

Stojkovic reported that nearly 80 different genes are associated with CMT (23). Regarding CMT, it is strongly suggested to examine the *GDAP1, GJB1, HINT1, MFN2, MPZ, PMP22,* and *SH3CT2* genes (18). In the current retrospective study, we analyzed 55 genes in 55 cases with CMT/HMSN suspicion, and we detected pathogenic variants in 13 cases in *MARS1* (autosomal dominant-AD), *NDRG1* (autosomal recessive-AR), *GJB1* (X linked dominant-XLD), *GDAP1* (AD-AR), *MFN2* (AD-AR), *PRX* (AD-AR), *SH3TC2* (AD-AR), and *FGD4* (AR) genes. Some cases had a molecular diagnosis with pathogenic variants, but others still could not be certain, such as case 2 (EO) (Table II) that had a pathogenic variant and a VUS in the same gene *NDRG1*. The VUS variant would be pathogenic if de novo inheritance was proven, but for this patient, we could not do the segregation analysis. If the VUS variant is considered pathogenic with segregation, this case would be compound heterozygous for the *NDRG1* gene and could have the CMT diagnosis with the molecular results. Case 13 (ZO) had a novel heterozygous pathogenic variant in the *FGD4* gene, which has an autosomal recessive inheritance. Since the case had the heterozygous variant, we did not expect the CMT phenotype due to autosomal recessive inheritance. The segregation analysis showed that the mother had the same variant without any clinical findings related to CMT. Two siblings with mild CMT4H phenotype were reported with two novel *FGD4* variants (c.514delG and c.2211dupA) (1). The mild phenotype with compound heterozygosity may show that the *FGD4* gene has a mild effect on phenotype.

Vaeth et al. detected 193 samples with targeted NGS for 63 CMT-associated genes, and they reported 6.7% pathogenic/likely pathogenic variants and 17.6% variants of unknown significance (25). In this report, which is from Denmark, pathogenic/likely pathogenic variants were in the *AARS, INF2, PMP22, NEFL, SH3TC2, DNM2, GARS, MPZ,* and *PRPS1* genes (25). DiVincenzo et al. reported the frequencies of pathogenic/likely pathogenic variants of 14 genes in the data of 2,338 individuals, and the most common reported variants were in the *PMP22, GJB1, MPZ,* and *MFN2* genes (8). In our study, the genes with pathogenic/likely pathogenic variants were *MARS1, NDRG1, GJB1, GDAP1, MFN2, PRX, SH3TC2,* and *FGD4.* The genes detected in our study are not similar to these studies, perhaps due to ethnic differences or the variant in the study sample size. The most frequent pathogenic variants were in the *NDRG1* gene (30.7%), which has an autosomal recessive inheritance in our study. Three variants were homozygous NM_001135242.2:c.442C>T and one variant was heterozygous NM_006096.4:c.205+1G>A. The cases with the NM_001135242.2(*NDRG1*):c.442C>T variant were not related to each other. The frequency of this variant may be high in Turkey because it features an increased rate of consanguineous marriage. Parents of two cases had first degree cousin marriages, parents of one case were not consanguineous, but they were from the same village in our study.

In recent years, the VUS detection rate has increased with multiple gene panel tests among all sequenced genes. Consequently, different comments were reported for the test results in various laboratories for the same variant. Therefore, the proper review of VUS results is inevitable, so laboratories need to require a good command over these variants. As a result, the effects of the VUS data should be known exactly, and the patient should have clear genetic counseling. Once sufficient data has been obtained, the vast majority of VUS can be interpreted as benign polymorphisms. Improvements in the areas of more patient screenings, the creation of a widespread and public database, and successful implementation of new generation sequencing will be a step toward appreciating the high VUS rate. Since the majority of VUS are ultimately reclassified as benign, treatment management in individuals with VUS should be evaluated not only regarding the presence or absence of the variant, but also based on their personal and family backgrounds. In the current study, we detected VUS in 11.55% of cases in the *HSPB3, KIF1B, SCN11A, CHRNA1, HSPB1, FIG4, ARHGEF10, DHTKD1, SBF1, EGR2, SBF2, IGHMBP2, KIF5A,* and *DNAJB2* genes. The most frequent gene with VUS was *KIF1B* (33.3%), who had an autosomal dominant inheritance. All cases with *KIF1B* VUS variants were heterozygous for this gene. In a targeted NGS study, 220 individuals with the CMT phenotype were examined for 50 genes, and 98 VUS were reported in 73 cases (33.18%) in *SH3TC2, NTRK1, PRX, NGF, PLEKHG5, AARS, DYNC1H1,* and *SPTLC1* genes (7). In another study, 98 individuals were analyzed for 88 genes with targeted NGS, and 10 variants were reported as VUS in nine cases in the *DGAT2, DYNC1H1, INF2, KIF1B, MFN2, NEFL, PRX, SBF1,* and *SH3TC2* genes (5). As expected, diversity is seen in different studies conducted in various regions. An important priority should be to reduce the prevalence of reported VUS by screening larger and more diverse populations.

In our retrospective study, we reported two novel pathogenic/likely pathogenic variants in the *GJB1* and *FGD4* genes and four novel VUS in the *HSPB3, CHRNA1, ARHGEF10*, and *KIF5A* genes. Of our cases, 10.9% had a novel variant, and 3.63% of our cases had a pathogenic/likely pathogenic novel variant. Also, 250 Austrian CMT patients were directly sequenced for the *PMP22, MPZ,* and *GJB1* genes, and 14 novel pathogenic variants were detected (17). Dohrn et al. reported 34 (5.55%) novel likely pathogenic variants in 612 individuals (9). It is suggested that NGS panels are leading

Table III: Cases with variants of unknown significance (VUS) in our study

Sample ID	Gender/ Age	Test endication	Gene	OMIM phenotypes	Mutation Type	Nucleotide HGVS	Protein HGVS	dbSNP
1-KCU	M/9	CMT?	HSPB3	?Neuronopathy, distal hereditary motor, type IIC (AD)	Missense Heterozygous	NM_006308.3:c.18G>C	(p.Leu6Phe)	Novel
2-IB	F/51	CMT?	KIF1B	?Charcot-Marie-Tooth disease, type 2A1 (AD) {Neuroblastoma, susceptibility to, 1} (AD) Pheochromocytoma (AD)	Missense Heterozygous	NM_015074.3:c.5C>T	(p.Ser2Leu)	rs1272900057
3-BG	F/5	CMT?	SCN11A	Episodic pain syndrome, familial, 3 (AD) Neuropathy, hereditary sensory and autonomic, type VII (AD)	Missense Heterozygous	NM_014139.3:c.2759C>T	(p.Ala920Val)	rs759694252
4-MK	F/3	CMT?	KIF1B	?Charcot-Marie-Tooth disease, type 2A1 (AD) {Neuroblastoma, susceptibility to, 1} (AD) Pheochromocytoma (AD)	Missense Heterozygous	NM_015074.3:c.2731A>G	(p.Ser911Gly)	rs556117169
5-EO	M/8	CMT?	CHRNA1	Multiple pterygium syndrome, lethal type (AR) Myasthenic syndrome, congenital, 1A, slow-channel (AD) Myasthenic syndrome, congenital, 1B, fast-channel (AD,AR)	Missense Heterozygous	NM_000079.4:c.672C>A	(p.His224Gln)	Novel
6-BK	F/10	CMT?	HSPB1	Charcot-Marie-Tooth disease, axonal, type 2F (AD) Neuronopathy, distal hereditary motor, type IIB (AD)	Missense Heterozygous	NM_001540.5:c.562C>T	(p.Arg188Trp)	rs772767500
			KIF1B	?Charcot-Marie-Tooth disease, type 2A1 (AD) {Neuroblastoma, susceptibility to, 1} (AD) Pheochromocytoma (AD)	Intronic Heterozygous	NM_015074.3:c.-5T>C	-	rs200491456
7-ST	M/8	CMT?	FIG4	?Polymicrogyria, bilateral temporooccipital (AR) Amyotrophic lateral sclerosis 11 (AD) Charcot-Marie-Tooth disease, type 4J (AR) Yunis-Varon syndrome (AR)	Missense Heterozygous	NM_014845.5:c.535C>T	(p.Leu179Phe)	rs141116831
8-HK	M/44	CMT?	ARHGEF10	?Slowed nerve conduction velocity, AD (AD)	Missense Heterozygous	NM_014629.4:c.1528A>G	(p.Thr510Ala)	rs201912073
9-MSO	M/5	CMT?	DHTKD1	?Charcot-Marie-Tooth disease, axonal, type 2Q (AD) 2-aminoadipic 2-oxoadipic aciduria (AR)	Missense Heterozygous	NM_018706.7:c.1721C>T	(p.Ala574Val)	rs757993723
10-YK	M/29	CMT?	KIF1B	?Charcot-Marie-Tooth disease, type 2A1 (AD) {Neuroblastoma, susceptibility to, 1} (AD) Pheochromocytoma (AD)	Missense Heterozygous	NM_015074.3:c.2731A>G	(p.Ser911Gly)	rs556117169
11-MNV	F/20	CMT?	KIF1B	?Charcot-Marie-Tooth disease, type 2A1 (AD) {Neuroblastoma, susceptibility to, 1} (AD) Pheochromocytoma (AD)	Missense Heterozygous	NM_015074.3:c.2480C>T	(p.Thr827Ile)	rs121908162
12-EK	M/17	Gait disorder	HSPB1	Charcot-Marie-Tooth disease, axonal, type 2F (AD) Neuronopathy, distal hereditary motor, type IIB (AD)	Missense Heterozygous	NM_001540.5:c.562C>T	(p.Arg188Trp)	rs772767500
13-BC	M/18	CMT?	SBF1	Charcot-Marie-Tooth disease, type 4B3 (AR)	Missense Heterozygous	NM_002972.4:c.4768A>G	(p.Thr1590Ala)	rs200488568
14-ZE	M/58	CMT?	KIF1B	?Charcot-Marie-Tooth disease, type 2A1 (AD) {Neuroblastoma, susceptibility to, 1} (AD) Pheochromocytoma (AD)	Missense Heterozygous	NM_015074.3:c.4712G>A	(p.Arg1571Gln)	rs775481158

Table III: Cont.

Sample ID	Gender/ Age	Test endication	Gene	OMIM phenotypes	Mutation Type	Nucleotide HGVS	Protein HGVS	dbSNP
15-SB	M/62	CMT?	ARHGEF10	?Slowed nerve conduction velocity, AD (AD)	Missense Heterozygous	NM_014629.4:c.826A>G	(p.Ser276Gly)	Novel
16-FF	F/58	Gait disorder	EGR2	Charcot-Marie-Tooth disease, type 1D (AD) Dejerine-Sottas disease (AD,AR) Hypomyelinating neuropathy, congenital, 1 (AD,AR)	Missense Heterozygous	NM_000399.5:c.1352G>A	(p.Gly451Asp)	rs138967272
17-DT	F/16	CMT?	SBF2	Charcot-Marie-Tooth disease, type 4B2 (AR)	Missense Heterozygous	NM_030962.3:c.1424A>G	(p.Gln475Arg)	rs199894823
18-IN	F/36	CMT?	IGHMBP2	Charcot-Marie-Tooth disease, axonal, type 2S (AR) Neuronopathy, distal hereditary motor, type VI (AR)	Missense Heterozygous	NM_002180.2:c.790C>T	(p.Arg264Cys)	rs139497493
19-EC	M/20	CMT?	KIF5A	{Amyotrophic lateral sclerosis, susceptibility to, 25} (AD) Myoclonus, intractable, neonatal (AD) Spastic paraplegia 10, autosomal dominant (AD)	Missense Heterozygous	NM_004984.4:c.668A>C	(p.Lys223Thr)	Novel
20-SG	M/70	CMT?	KIF1B	?Charcot-Marie-Tooth disease, type 2A1 (AD) {Neuroblastoma, susceptibility to, 1} (AD) Pheochromocytoma (AD)	Missense Heterozygous	NM_015074.3:c.2120A>G	(p.Lys707Arg)	rs1440406190
21-EB	F/11	Gait disorder	DNAJB2	Spinal muscular atrophy, distal, autosomal recessive, 5 (AR)	Missense Homozygous	NM_006736.6:c.700A>T	(p.Thr234Ser)	rs1212994119

CMT: *Charcot Marie Tooth,* ***AD:*** *Autosomal dominant,* ***AR:*** *Autosomal recessive,* ***XLD:*** *X Linked Dominant*

to increased identification of novel variants in known CMT-associated genes. With more studies and larger sample sizes, the genotype-phenotype relationship will become more comprehensible.

CONCLUSION

NGS has become the target of applications, especially as an indispensable tool in the diagnosis of multigenic diseases and multigenic studies. The main point is that bioinformatics analysis of variants obtained with NGS should be correlated simultaneously with the clinical findings of the patients. With this consideration, for diagnosis of neuropathies, a molecular analysis should be evaluated together with clinical phenotype, hereditary pattern, and electrodiagnostic testing features. Thus, accurate diagnosis and genetic counseling will be available for patients. Our study contributes a 7.15% diagnosis rate for CMT and six novel variants in the *GJB1, FGD4, HSPB3, CHRNA1, ARHGEF10,* and *KIF5A* genes, which were first reported in the literature.

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