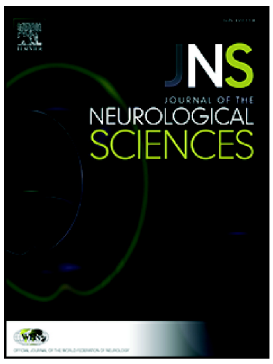

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Implication of the *SH3TC2* gene in Charcot-Marie-Tooth disease associated with deafness and/or scoliosis: illustration with four new pathogenic variants

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ABSTRACT

The autosomal recessive demyelinating form of Charcot-Marie-Tooth can be due to *SH3TC2* gene pathogenic variants (CMT4C, AR-CMTde-*SH3TC2)*. We report on a series of 13 patients with AR-CMTde-*SH3TC2* among a French cohort of 350 patients suffering from all type of inheritance peripheral neuropathy. The *SH3TC2* gene appeared to be the most frequently mutated gene for demyelinating neuropathy in this series by NGS. Four new pathogenic variants have been identified: two nonsense variants (p.(Tyr970*), p.(Trp1199*)) and two missense variants (p.(Leu1126Pro), p.(Ala1206Asp)). The recurrent variant p.Arg954* was present in 62%, and seems to be a founder mutation. The phenotype is fairly homogeneous, as all these patients, except the youngest ones, presented scoliosis and/or hearing loss.

KEY WORDS: *Charcot-Marie-Tooth; Neuropathy; Hearing loss; Scoliosis; SH3TC2; NGS*

Introduction

Charcot-Marie-Tooth disease (CMT) is the most frequent inherited peripheral neuropathies (1/2500). So far, variants in more than 90 genes have been identified causing either the demyelinating or the axonal form. Duplication of the *PMP22* gene is the most frequent cause of the autosomal dominant demyelinating form. The autosomal recessive demyelinating form is foremost due to *SH3TC2* gene pathogenic variants (CMT4C, or AR-CMTde-*SH3TC2)* [1]. Patients with AR-CMTde-*SH3TC2* suffer from early severe neuropathy starting in the first decade. Scoliosis and cranial nerve involvement, including hearing loss (HL), are frequently observed [2,3,4].

We report on a series of 13 patients with AR-CMTde-*SH3TC2* among a French cohort of 350 patients suffering from all type of inheritance peripheral neuropathy. Phenotype-genotype correlations of these specific features have been looked for.

Materials and Methods

After giving their informed consent, 350 French patients suffering from inherited peripheral neuropathy were screened by *PMP22* multiplex-ligation-dependent-probe-amplification, followed by targeted next-generation-sequencing using a 92-gene custom panel designed for the diagnosis of CMT and associated neuropathies (detailed in [5]; ***Supplementary data***).

Patients were selected from diagnostic registries of a French genetic reference center. Previously, a clinical questionnaire has been fulfilled. Patients' ascertainment could be precised thanks to medical records.

The NGS panel included the 44 known CMT genes, 27 genes involved in HSN (Hereditary Sensitive Neuropathy) and HMN (Hereditary Motor Neuropathy) and 21 other genes of interest involved in neuropathies of differential diagnosis. The amplified library was prepared with Ion P1 HiQ Template OT2 200 kit (Ampliseq Custom (Life technologies)), sequenced on Proton sequencer (Life technologies), and mapped to the human reference sequence hg19/GHCh37. Variants were evaluated with Alamut Mutation Interpretation Software (Interactive Biosoftware, Rouen, France) using the NM_024577.3 reference sequence for the *SH3TC2* gene. Databases such as ExAC Genome browser (http://exac.broadinstitute.org), dbSNP135 (National Center for Biotechnology Information [NCBI], Bethesda, Maryland, USA, http://www.ncbi.nlm.nih.gov/projects/SNP/), Clin Var (www.ncbi.nlm.nih.gov/clinvar), HGMD professional (www.hgmd.cf.ac.uk) and Mutalyzer

(https://mutalyzer.nl/) were also screened. Pathogenic variants of interest were verified by Sanger sequencing using forward and reverse primer pairs.

For HL screening, MLPA and Sanger sequencing for *GJB2* and *GJB6* were performed.

Results and discussion

Diagnosis was positive for 201 patients (57%). As expected, the most frequent pathogenic variant was *PMP22* duplication detected in 52 patients (15%). Deletion of *PMP22* was observed in 29 patients (8%) and pathogenic point variant were detected in 120 patients (34%).

Among these 120 patients diagnosed with point variants, 40 patients presented with a demyelinating neuropathy and the *SH3TC2* gene appeared to be the most frequently mutated with 13 diagnosed patients (32.5%). Details of their phenotypes and genotypes are presented in ***Tables 1 and 2.***

Phenotype:

Although some clinical information is missing, we can see that all these patients, except for the youngest patients in our series, presented with scoliosis (n=10; 77%) and/or deafness (n=8; 62%). The phenotype is fairly homogeneous with sensori-motor demyelinating polyneuropathy with early onset before the age of 10, except for one patient with adulthood onset (patient XII).

Sensory ataxia with poor imbalance seems to be a prominent feature, as it was found in nine patients (70%), like Gooding *et al* [6].

Cranial nerve involvement is another key point of CMT4C, as it is shown in our study with eight patients reporting HL and one patient with increased latencies of visual brainstem responses. Recently, Kontogeorgiou *et al* reported cranial nerve involvement in 31% of the cases [7], and Yger *et al* in a French cohort in 71% [4]. HL is the foremost observed condition [8].

Pes cavus was found in eleven cases (85%). Foot deformities are very frequently described in this phenotype [9;10].

Only one patient had a proximal muscle involvement, whereas it has been reported in recent studies [11;12].

Patient		Polyneuropathy								Hearing loss			Scoliosis		Cranial nerve involvement	Other symptoms
Reference Family	Patient (gender/age in years)	Neuropathy	Median nerve motor velocity (m/s)	Age at onset (years)	Sensory nerve imapairment	Mild or severe weakness	Proximal muscle involvement	Pes Cavus	Loss of ambulation/ sensory ataxia	Present	Degree of severity	Age at onset (age in years)	Present	Degree of severity		
Patient I	F, 22	Sensori-motor demyelinating	28	3	**Y**	Moderate	N	**Y**	N / **Y**	**Y**	Moderate	16	**Y**	NC	NC	Sphincter disorders
Patient II	M, 24	Sensori-motor demyelinating	30	< 8	**Y**	Moderate	N	**Y**	N / N	N	/	/	**Y**	24°	N	/
Patient III	M, 43	Sensori-motor demyelinating	35	8	**Y**	Moderate	N	**Y**	N / **Y**	**Y**	NC	NC	**Y**	Severe	N	
Patient IV	F, 23	Sensori-motor demyelinating	25	1	**Y**	Moderate	N	**Y**	N / **Y**	N	/	/	**Y**	Mild	NC	/
Patient V	F, 47	Sensori-motor demyelinating	25	4	**Y**	Severe	N	**Y**	N / **Y**	**Y**	Severe	NC	N	/	Latency of Visual brainstem responses	/
Patient VI	F, 29	Sensori-motor demyelinating	25	6	NC	Severe	N	N	N / **Y**	NC	NC	NC	**Y**	NC	NC	/
Patient VII	M, 56	Sensori-motor demyelinating	32	Childhood	**Y**	Moderate	N	**Y**	N / **Y**	**Y**	Moderate progressive	25	**Y**	NC	N	/
Patient VIII	F, 68	Sensori-motor demyelinating	34	9	NC	Severe	NC	**Y**	NC / NC	**Y**	Moderate slope curve	NC	**Y**	NC	NC	/
Patient IX	F, 12	Sensori-motor demyelinating	34	3	**Y**	Severe	**Y**	**Y**	**Y** / **Y**	**Y**	Mild	11	N	/	N	/
Patient X	M, 9	Sensori-motor demyelinating	30	<5	N	Moderate	N	**Y**	**Y** / **Y**	N	/	/	**Y**	Mild <10°	N	Autism
Patient XI	F, 71	Sensori-motor demyelinating	30	Teens	**Y**	Severe	N	**Y**	N / **Y**	**Y**	Severe progressive	< 10	**Y**	NC	N	Bilateral cataract
Patient XII	M, 83	Sensori-motor demyelinating	31	73	**Y**	Severe	N	N	NC / NC	**Y**	Moderate U-shaped curve	NC	**Y**	NC	NC	/
Patient XIII	M, 27	Sensori-motor demyelinating	30	8	**Y**	Moderate	N	**Y**	N / N	N	/	/	N	/	N	Muscular pain

Table 1: Phenotypes of our 13 patients presenting with a demyelinating hereditary neuropathy among a cohort of 350 French patients

(F: Female; M: Male; NC: Not Communicated; /: not applicable; Y: Yes; N: No)

Genotype:

In addition to already known pathogenic variants, four new pathogenic variants have been identified: two nonsense variants (c.2910C>A, p.(Tyr970*) and c.3596G>A, p.(Trp1199*)) and two missense variants (c.3377T>C, p.(Leu1126Pro) and c.3617C>A, p.(Ala1206Asp)).

Analysis at the DFNB1 locus did not reveal any pathogenic variant for all diagnosed and known deaf patients, which is in favour of the implication of the *SH3TC2* gene in hearing loss.

The recurrent variant p.Arg954* was present in 62% of our patients. This is similar to another French study by Yger *et al* (62.5%) and a Czech study by Lassuthova *et al* with 63.2% [4; 10].

Thus, this pathogenic variant is prevalent in 5% in Northern Europe, such as Norway [13], and is also frequent in Italy [8;14]. As it is also identified in North America, p.Arg954* seems to be a founder mutation [15;16]. Haplotype analysis should be performed to confirm this hypothesis.

Patient			Genotype				
Reference Family	**Patient (gender/age in years)**	**Country**	**Mutation type**	**Zygosity**	**Nucleotide change**	**Amino acid change**	**Localization**
Patient I	F, 22	France	Nonsense	Homozygous	c.2860C>T	p.Arg954*	Exon11
Patient II	F, 29	France	Nonsense	Homozygous	c.2860C>T	p.Arg954*	Exon11
Patient III	M, 43	France	Nonsense	Homozygous	c.2860C>T	p.Arg954*	Exon11
Patient IV	F, 23	France	Nonsense + Nonsense	Compound heterozygous	c.2860C>T + c.3325C>T	p.Arg954*+ p. Arg1109*	Exon11+ Exon14
Patient V	F, 47	France	Nonsense	Homozygous	c.3325C>T	p.Arg1109*	Exon14
Patient VI	F, 29	France	Nonsense + Nonsense	Compound heterozygous	c.2860C>T + c.2910C>A	p.Arg954* + **p.Tyr970***	Exon11+ Exon12
Patient VII	M, 56	France	Nonsense	Homozygous	c.3321C>A	p.Tyr1107*	Exon14
Patient VIII	F, 68	France	Nonsense + Missense	Compound heterozygous	c.2860C>T + c.3377T>C	p.Arg954* + **p.Leu1126Pro**	Exon11+ Exon 15
Patient IX	F, 12	France	Nonsense + Missense	Compound heterozygous	c.2860C>T +c.3511C>T	p.Arg954* + p.Arg1171Cys	Exon11+ Exon 16
Patient X	M, 9	France	Nonsense +	Compound	c.2860C>T +	p.Arg954* +	Exon11+

			Missense	heterozygous	c.3511C>T	p.Arg1171Cys	Exon 16
Patient XI	F, 71	France	Missense + Nonsense	Compound heterozygous	c.2642A>G + c.3596G>A	p.Asn881Ser + **p.Trp1199***	Exon11+ Exon16
Patient XII	M, 83	France	Missense	Homozygous	c.3617C>A	**p.Ala1206Asp**	Exon16
Patient XIII	M, 27	France	Missense + Missense	Compound heterozygous	c.1969G>A + c.2642A>G	p.Glu657Lys + p.Asn881Ser	Exon11+ Exon11

Table 2: Genotypes of our 13 patients presenting with a demyelinating hereditary neuropathy due to SH3TC2 among a cohort of 350 French patients. Strong grey color corresponds to nonsense variants, middle grey color to nonsense variant associated to missense variant, mild grey color to missense variants.(F: Female; M: Male; in Red: novel variants found)

Conclusion

SH3TC2 appears to be the most frequently implicated gene in autosomal recessive demyelinating form of CMT in the French population, often associated with scoliosis and/or HL. Sensory ataxia and pes cavus are prominent features of CMT4C. Moreover, p.Arg954* appears to be a founder mutation.

Conflict of interest: None.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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References

[1] Mathis S, Goizet C, Tazir M, Magdelaine C, Lia A-S, Magy L, Vallat JM (2015) Charcot-MarieTooth diseases: an update and some new proposals for the classification. J Med Genet 52(10):681‐90.

[2] Senderek J, Bergmann C, Stendel C, Kirfel J, Verpoorten N, De Jonghe P, Timmerman V, Chrast R, Verheijen MH, Lemke G, Battaloglu E, Parman Y, Erdem S, Tan E, Topaloglu H, Hahn A, Müller-Felber W, Rizzuto N, Fabrizi GM, Stuhrmann M, Rudnik-Schöneborn S, Züchner S, Michael Schröder J, Buchheim E, Straub V, Klepper J, Huehne K, Rautenstrauss B, Büttner R, Nelis E, Zerres K (2003) Mutations in a gene encoding a novel SH3/TPR domain protein cause autosomal recessive Charcot-Marie-Tooth type 4C neuropathy. Am J Hum Genet 73(5):1106‐19

[3] Azzedine H, LeGuern E, Salih MA (2008). Charcot-Marie-Tooth Neuropathy Type 4C. In: GeneReviews® (ed) Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJ, Stephens K, et al. 1993-2018. http://www.ncbi.nlm.nih.gov/books/NBK1340/

[4] Yger M, Stojkovic T, Tardieu S, Maisonobe T, Brice A, Echaniz-Laguna A, Alembik Y, Girard S, Cazeneuve C, Leguern E, Dubourg O (2012) Characteristics of clinical and electrophysiological pattern of Charcot-Marie-Tooth 4C. J Peripher Nerv Syst 17(1):112‐22.

[5] Lerat J, Cintas P, Beauvais-Dzugan H, Magdelaine C, Sturtz F, Lia AS (2017) A complex homozygous mutation in ABHD12 responsible for PHARC syndrome discovered with NGS and review of the literature. J Peripher Nerv Syst 22(2):77‐84.

[6] Gooding R, Colomer J, King R, Angelicheva D, Marns L, Parman Y, Chandler D, Bertranpetit J, Kalaydjieva L (2005) A novel Gypsy founder mutation, p.Arg1109X in the CMT4C gene, causes variable peripheral neuropathy phenotypes. J Med Genet 42(12):e69.

[7] Kontogeorgiou Z, Nikolaou K, Kartanou C, Breza M, Panas M, Karadima G, Koutsis G (2019) Mutational screening of the SH3TC2 gene in Greek patients with suspected demyelinating recessive Charcot-Marie-Tooth disease reveals a varied and unusual phenotypic spectrum. J Peripher Nerv Syst 24(1):125-130. doi: 10.1111/jns.12305. Epub 2019 Feb 6.

[8] Piscosquito G, Saveri P, Magri S, Ciano C, Gandioli C, Morbin M, Bella DD, Moroni I, Taroni F, Pareyson D (2016) Screening for SH3TC2 gene mutations in a series of demyelinating recessive Charcot-Marie-Tooth disease (CMT4). J Peripher Nerv Syst 2016 Sep;21(3):142-9. doi: 10.1111/jns.12175.

[9] Baets J, Deconinck T, De Vriendt E, Zimoń M, Yperzeele L, Van Hoorenbeeck K, Peeters K, Spiegel R, Parman Y, Ceulemans B, Van Bogaert P, Pou-Serradell A, Bernert G, Dinopoulos A, Auer-Grumbach M, Sallinen SL, Fabrizi GM, Pauly F, Van den Bergh P, Bilir B, Battaloglu E, Madrid RE, Kabzińska D, Kochanski A, Topaloglu H, Miller G, Jordanova A, Timmerman V, De Jonghe P (2011) Genetic spectrum of hereditary neuropathies with onset in the first year of life. Brain 134(Pt 9):2664-76. doi: 10.1093/brain/awr184. Epub 2011 Aug 11.

[10] Laššuthová P, Mazanec R, Vondráček P, Sišková D, Haberlová J, Sabová J, Seeman P (2011) High frequency of SH3TC2 mutations in Czech HMSN I patients. Clin Genet 80(4):334-45. doi: 10.1111/j.1399-0004.2011.01640.x. Epub 2011 Mar 1.

[11] Arntzen KA, Høyer H, Ørstavik K, Tallaksen C, Vedeler C, Østern R, Nebuchennykh M, Braathen GJ, Fagerheim T (2018) Charcot-Marie-Tooth disease type 4C in Norway: Clinical characteristics, mutation spectrum and minimum prevalence. Neuromuscul Disord 28(8):639-645. doi: 10.1016/j.nmd.2018.06.004. Epub 2018 Jun 15.

[12] de Oliveira CM, Fussiger H, Winckler PB, Saute JAM (2019) Dropped head syndrome as a manifestation of Charcot-Marie-Tooth disease type 4C. Neuromuscul Disord 29(2):138-141. doi: 10.1016/j.nmd.2018.11.010. Epub 2018 Nov 29.

[13] Høyer H, Braathen GJ, Busk ØL, Holla ØL, Svendsen M, Hilmarsen HT, Strand L, Skjelbred CF, Russell MB (2014) Genetic diagnosis of Charcot-Marie-Tooth disease in a population by next-generation sequencing.Biomed Res Int 2014:210401. doi: 10.1155/2014/210401. Epub 2014 Jun 16.

[14] Manganelli F, Tozza S, Pisciotta C, Bellone E, Iodice R, Nolano M, Geroldi A, Capponi S, Mandich P, Santoro L (2014) Charcot-Marie-Tooth disease: frequency of genetic subtypes in a Southern Italy population. J Peripher Nerv Syst. 19(4):292-8. doi: 10.1111/jns.12092.

[15] Lupski JR, Gonzaga-Jauregui C, Yang Y, Bainbridge MN, Jhangiani S, Buhay CJ, Kovar CL, Wang M, Hawes AC, Reid JG, Eng C, Muzny DM, Gibbs RA (2013) Exome sequencing resolves apparent incidental findings and reveals further complexity of SH3TC2variant alleles causing Charcot-Marie-Tooth neuropathy. Genome Med. 5(6):57. doi: 10.1186/gm461. eCollection 2013.

[16] Varley TL, Bourque PR, Baker SK (2015) Phenotypic variability of CMT4C in a French-Canadian kindred. Muscle Nerve. 52(3):444-9. doi: 10.1002/mus.24640. Epub 2015 May 14.

Supplementary data

Supp. Table S1: 92-gene panel used for NGS. It includes the 44 known CMT genes, 27 genes involved in HSN (Hereditary Sensitive Neuropathy) and HMN (Hereditary Motor Neuropathy) and 21 other genes of interest involved in neuropathies of differential diagnoses.

HIGHLIGHTS

- *SH3TC2* was the most frequent gene for autosomal recessive demyelinating neuropathy
- Four new pathogenic variants have been identified by NGS
- The phenotype is homogeneous as all patients presented deafness and/or scoliosis