Original article

Folia Neuropathologica

Charcot-Marie-Tooth type 1C disease coexisting with progressive multiple sclerosis: a study of an overlapping syndrome

Anna Potulska-Chromik[1]*, Elena Sinkiewicz-Darol[2]*, Anna Kostera-Pruszczyk[1], Hanna Drac[1], Dagmara Kabzińska[2], Beata Zakrzewska-Pniewska[1], Marek Gołębiowski[3], Andrzej Kochański[2]

*These authors contributed equally to the study

[1]Department of Neurology, Medical University of Warsaw, Poland, [2]Neuromuscular Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, [3]Department of Radiology, Medical University of Warsaw, Poland

Folia Neuropathol 2012; 50 (4): 369-374 DOI: 10.5114/fn.2012.32366

Abstract

Charcot-Marie-Tooth type 1C disease (CMT1C) is a rare form of hereditary demyelinating neuropathy caused by mutations in the LITAF (lipopolysaccharide-induced tumor necrosis factor-α) gene. CMT1C disease was mapped to chromosome 16p12-p 13.3. To date only a few mutations in the LITAF gene have been reported. Due to a small group of CMT1C reported patients, the phenotype of CMT1C is poorly characterized. CMT1C disease is a pure demyelinating neuropathy limited to the peripheral nervous system with a mild clinical course, manifesting without any additional symptoms.
To the best of our knowledge, in this study, for the first time we present a three generational CMT1C family in which in the proband, CMT1C disease coexists with central demyelination fulfilling criteria of primary progressive multiple sclerosis (PPMS). The coexistence of PPMS and CMT1C in one family may not result from a common pathogenetic trait, however only in the proband with central demyelination and CMT1C we have detected a –308G>A sequence variant in the promoter of the TNF-α gene.

Key words: *CMT1C, primary progressive multiple sclerosis, overlapping syndrome LITAF, TNF-α.*

Introduction

CMT1C disease is caused by mutations in the *lipopolysaccharide-induced tumor necrosis factor-α* (*LITAF*) gene on chromosome 16p12-16p13.3 [1,18,19]. To date only nine pathogenic mutations in the *LITAF* gene have been reported [6].

All to-date reported CMT1C patients harbouring mutations in the *LITAF* gene manifest with a phenotype of mild, slowly progressive demyelinating neuropathy, which is not accompanied by additional features/symptoms [16,18].

Some sequence variants identified in the *LITAF* gene perfectly segregate with the CMT1C phenotype, whereas other variants are found in the patients suffering from chronic acquired neuropathies. Thus, the Ile92Val sequence variant was reported in the familial case of multifocal, acquired, demyelinating sensory and motor polyneuropathy (MADSAM) and was also considered to contribute to the phenotype of CMT1A disease caused by duplication of the *PMP22* gene [10,17].

Central demyelination caused by multiple sclerosis is rare in patients with genetic neuropathies.

Similarly to the *GJB1* and *Mfn2* genes responsible for CMTX1 and CMT2A forms for which central demyelination has been proved, mutations in the *LITAF* gene expressed in the brain, cerebellum and spinal cord

Communicating author:
Andrzej Kochański, Neuromuscular Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5, 02-106 Warsaw, Poland, phone +48 22 608 65 36, e-mail: andko@cmdik.pan.pl

might be expected to mediate central demyelination in a molecular mechanism independent from peripheral neuropathy [2,12].

In all to-date reported CMT1C-affected patients harbouring the Gly112Ser mutation, a homogenous phenotype of mild slowly progressive demyelinating neuropathy was present without symptomatic central nervous system involvement [8,16,18].

The role of genetic factors in development of multiple sclerosis is still debated. The published results are conflicting. Grey *et al.* suggested a role of TNF-α in the outcome of multiple sclerosis [5]. The results of the association of MS with DRB1*15(2) and TNF-α in the Russian population, reported by Favorova *et al.* indicate the interplay of three loci in susceptibility to multiple sclerosis [3].

To the best of our knowledge, we present for the first time a patient with CMT1C disease and clinical picture dominated by symptoms of central demyelination, fulfilling diagnostic criteria of primary progressive multiple sclerosis (PPMS).

Family report

A 25-year-old right-handed woman was admitted five years ago to our Department for a 2-year history of gait disorder.

On admission her cranial nerves were normal. The motor function was normal in the upper limbs. The deep tendon reflexes in the upper limbs were hypoactive and the abdominal reflexes were absent. There was bilateral foot drop and pes cavus. She had moderate weakness and wasting of the anterior tibial and peroneal muscles, with bilateral foot drop. The knee and ankle jerks were absent. The plantar responses were extensor. There was a distal sensory loss for all modalities (vibration > proprioception) in the lower limbs. She also presented mild slowing of fine coordinated movements and a wide-based unsteady gait. The results of nerve conduction studies showed demyelinating neuropathy with uniform slowing of conduction velocities. Motor conduction velocity of her median nerve was 22.3 m/s. Somatosensory evoked potentials revealed prolonged latency from the right median nerve and lack of any response from the right tibial nerve. The right sural nerve biopsy showed demyelination and remyelination with the form of "onion" bulb formation, there were no inflammatory infiltrates (Fig. 1). The MRI T2-weighted sequences showed both abnormal hyperintense pathological focal lesion in white matter and brain atrophy. The level of very long chain fatty acid was not elevated.

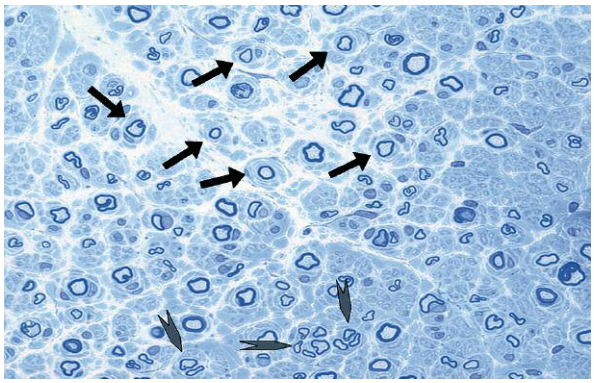

Fig. 1. Sural nerve biopsy in the proband III:3. On semithin cross epon section onion bulbs of different size were visible (arrows) as well as scanty clusters of regenerated fibers (arrowheads). There were no features of inflammation.

The latencies of visual evoked responses and brainstem auditory evoked responses were prolonged. Fundus and anterior segment of the eyes were normal.

On the psychological examination she scored 57 (71) 85 points in Wechsler Intelligence Scale (WAIS-R). She presented a tendency to decline of her cognitive function.

The family history showed the pedigree of only three consecutive generations. Although the mother and brother of the patient did not complain of any difficulty in walking, on neurological examination they presented bilateral *pes cavus* and a slight distal sensory loss in lower limbs. The ankle jerks were absent. In both cases, nerve conduction studies showed demyelinating neuropathy with uniformly slowed conduction velocity. The patient's brother's MRI scan was completely normal.

At that time the preliminary diagnosis of demyelinating CMT1 with coexisting central nervous system involvement was made.

Four years later the patient was readmitted to our Department because of progression of her gait disorder and incontinence of the urine.

Neurological examination revealed prominent gait ataxia, intention tremor in the upper and lower limbs. She had, similar to the previously reported, moderate weakness and wasting of the anterior tibial and peroneal muscles and the toe extensors. She had also wide-based unsteady gait and neurogenic bladder.

The laboratory tests were again irrelevant. The results of the electrophysiological studies did not show

any significant difference from the previously obtained ones. The MRI T2-weighted sequences showed much more pronounced cortical atrophy and hyperintense pathological demyelinating lesions in brain white matter with the corpus callosum atrophy (Fig. 2). The demyelinating lesions were also present in the cervical part of the spinal cord. Visual evoked responses and brainstem auditory evoked responses were more prolonged comparing to the previously obtained results. Her psychological examination did not show any further decline of her cognitive function.

CSF parameters were within normal limits, however detailed analysis detected oligoclonal bands.

Dominant clinical features e.g. progressive cerebellar ataxia and neurogenic bladder as well as results of MRI scan of the brain and spinal cord strongly suggested diagnosis of multiple sclerosis. After exclusion of similar condition resembling MS, according to the McDonald criteria, primary progressive multiple sclerosis (PPMS) was diagnosed [14].

Material and methods

All members of this CMT-affected family (the proband, her brother and her parents) gave informed consent and the study was approved by the local Ethics Committee. Additionally, the proband agreed to the publication of her medical records.

First, a duplication of the *Peripheral Myelin Protein 22* gene (*PMP22*) was excluded using the Real Time PCR (RT-PCR) approach. A relative dosage (RQ) of the *PMP22* gene in the proband, her brother and mother was estimated at III:3 – 0.920, III:1 – 0.825, II:5 – 0.870. In our experience, the *PMP22* gene dosage in normal individuals (two copies of the *PMP22* gene) ranges from 0.700 to 1.090.

In addition, the three coding exons 2-4 of the *LITAF* gene were amplified by PCR (primer sequences previously described by Street *et al.* [18]). The promoter sequence of the *TNF-α* gene was amplified with a pair of primers 5'-TATGAGTCTCCGGGTCAGAAT-3' and 5'-TCTCGGTTTCTTCTCCATCG-3', designed using *PRIMER3* software.

The PCR products were directly sequenced using BigDye™ Terminator Version 1.1 Ready Reaction Cycle Sequencing kit on the ABI 3730/xl Genetic analyzer (Applied Biosystems). The *LITAF* gene sequence was analyzed by comparing with reference sequences NM_004862.3 (transcript variant 1) and the *TNF-α* promoter sequence was analyzed by comparing with reference sequences NC_000006.11 in the Basic Local Alignment Search Tool (Blast NCBI – http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

A direct sequencing of the *LITAF* gene in the proband (III:3) revealed a heterozygous G to A transversion at the nucleotide 334 resulting in a substitution of glycine to serine arginine at codon 112 of the *LITAF* gene. Direct sequencing of the *LITAF* gene performed in the brother of the proband (III:1) and the mother of the proband (II:5) has also revealed Gly112Ser mutation (Fig. 3).

Sequencing analysis of the promoter region of the *TNF-α* gene revealed in the proband (III:3) a heterozygous sequence variant –308G>A which was absent in DNA samples of III:1 and II:5. This sequence variant was previously detected and designated as rs1800692.

Discussion

Our proband presented with symptoms attributable to two coexisting disorders – Charcot-Marie-Tooth type 1C disease with primary progressive multiple sclerosis (PPMS), while her brother and mother, who carry only the LITAF gene mutation, presented with mild peripheral neuropathy symptoms only.

Although our patient may represent the random coincidence of both disorders (an overlapping syndrome), a possible role of *LITAF* and *TNF-α* genes mutations in their development cannot be excluded.

The *LITAF* gene was identified as a transcription factor of the tumor necrosis factor α gene many years before its characteristics as a gene responsible for CMT1C disease. In the experimental approach, inhibition of the LITAF mRNA expression resulted in the reduction of the TNF-α transcription [11]. The Gly112Ser mutation in the *LITAF* gene detected in the CMT family may act in our patient at least in two independent ways. In the peripheral nerve, the mutated *LITAF* gene causes a typical course of mild peripheral demyelinating neuropathy seen in our proband's affected family members, and was previously described in other CMT1C patients harbouring Gly112Ser mutation [1,8,16,18].

In CMT1X disease caused by mutations in the *GJB1* gene numerous patients with transient demyelinating central nervous system changes were demonstrated [7]. Similarly to the *LITAF* gene, the *GJB1* gene is also expressed in the central nervous system [7]. Some CMTX1-affected patients displayed demyelination in

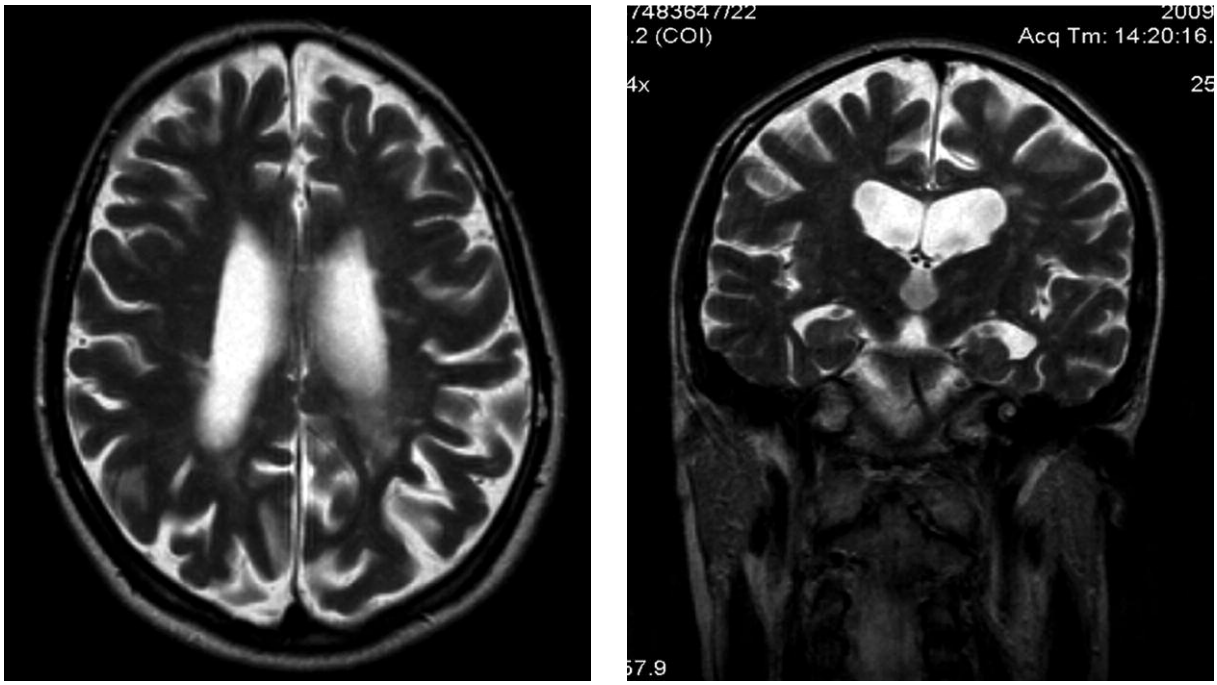

Fig. 2. MRI T2-weighted images in the proband III:3.
Axial and coronal MRI brain images demonstrate subcortical and periventricular bilateral white matter lesions with pronounced corpus callosum and cortical atrophy.

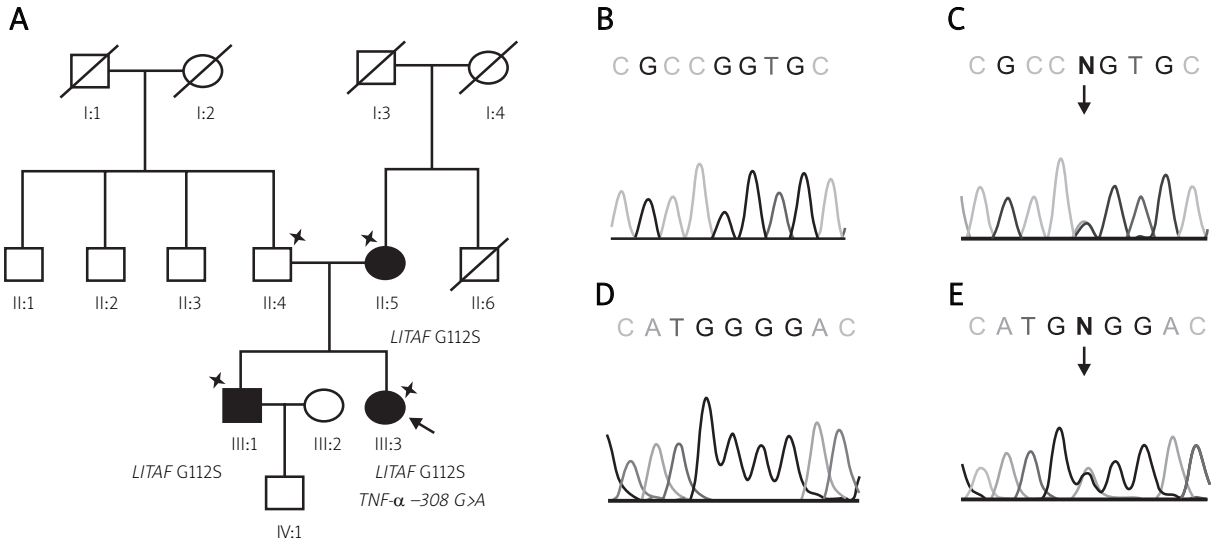

Fig. 3. A) A four-generation pedigree of a CMT family with an autosomal dominant mode of inheritance. The arrow indicates the proband. DNA available for analysis is marked with asterisks. The affected individuals are represented by black symbols and the unaffected family members are indicated by open symbols, **B)** wild type *LITAF* sequence, **C)** heterozygous transversion 334G>A in the *LITAF* gene (Gly112Ser) found in II:5, III:1 and III:3, **D)** wild type *TNF-α* promoter sequence, **E)** heterozygous sequence variant –308G>A in *TNF-α* promoter region found in III:3.

the white matter of the cerebral hemispheres visible in MRI investigation [12]. Parman *et al.* reported on CMTX1/CMT1X family in which peripheral neuropathy coexisted with multiple sclerosis in the proband. The patient harbouring the R164W mutation in the *GJB1* gene had also disseminated white matter cerebral –cerebellar brain stem and spinal lesions typical of MS [13].

In the most frequent form of CMT2 – CMT2A disease, some mutations in the Mitofusin-2 gene have been reported to segregate with periventricular and subcortical hyperintense lesions in the central nervous system [2]. Brain white matter lesions observed in five CMT1A Italian family members were recognized as central demyelination due to genetic neuropathy, and did not meet the diagnostic criteria for multiple sclerosis [15].

In the currently reported family, the symptoms of CNS involvement were observed only in our proband, but not her mother or brother. His MRI was also normal.

Our proband carried not only the *LITAF* mutation, but also a polymorphism in the promoter sequence of the *TNF-α* gene. We hypothesize that in the central nervous system, the Gly112Ser mutation in the *LITAF* gene may have triggered the inflammatory reaction leading to PPMS, by influencing expression of the *TNF-α* gene. The 308G>A polymorphism was detected in the promoter sequence of the *TNF-α* gene. The *TNF-α* gene promoter region polymorphism was previously investigated in different MS populations with conflicting results. Although the –308G>A polymorphism has been shown to occur in similar frequencies in the MS-affected patients and in the control groups, its functional relevance has not been proven [9]. In the MS-affected patients harbouring the –308G>A polymorphism, a significantly higher *TNF-α* mRNA expression level was detected [9]. Functional relevance of the –308G>A polymorphism was also confirmed by an *in vitro* approach, in which high transcriptional activity of the *TNF-α* gene harbouring –308G>A allele was found [20].

The possible *LITAF* gene mutation influence on development of immune reaction in the peripheral nervous system has been recently suggested. The Ile92Val polymorphism in the *LITAF* gene was previously found in a family with demyelinating motor and sensory polyneuropathy with conduction block responsive to prednisone treatment [17]. The *TNF-α* gene was not tested in this family. A clinical course resembling acquired neuropathy, and electrophysiological pattern of motor and sensory neuropathy with conduction block was also reported in a German family with (c.430G>A p.V144M) mutation in the *LITAF* gene [4].

Recognition of this genetic-inflammatory association is difficult and based only on the clinical case reports.

We conclude that coexistence of demyelinating peripheral neuropathy and primary progressive multiple sclerosis is not necessarily coincidental. Central and peripheral involvement in our proband can result from interplay of mutations of two genes related in function.

Acknowledgements

This study was supported by a grant of the Polish Ministry of Science and Higher Education (NN 402 27 63 36) to AK.

Conflict of the interest: none.

References

1. Bennett CL, Shirk AJ, Huynh HM, Street VA, Nelis E, Van Maldergem L, De Jonghe P, Jordanova A, Guergueltcheva V, Tournev I, Van Den Bergh P, Seeman P, Mazanec R, Prochazka T, Kremensky I, Haberlova J, Weiss MD, Timmerman V, Bird TD, Chance PF. SIMPLE mutation in demyelinating neuropathy and distribution in sciatic nerve. Ann Neurol 2004; 55: 713-720.
2. Chung KW, Kim SB, Park KD, Choi KG, Lee JH, Eun HW, Suh JS, Hwang JH, Kim WK, Seo BC, Kim SH, Son IH, Kim SM, Sunwoo IN, Choi BO. Early onset severe and late-onset mild Charcot-Marie-Tooth disease with mitofusin 2 (MFN2) mutations. Brain 2006; 129: 2103-2118.
3. Favorova OO, Favorov AV, Boiko AN, Andreewski TV, Sudomoina MA, Alekseenkov AD, Kulakova OG, Gusev EI, Parmigiani G, Ochs MF. Three allele combinations associated with multiple sclerosis. BMC Med Genet 2006; 26: 63.
4. Gerding WM, Koetting J, Epplen JT, Neusch C. Hereditary motor and sensory neuropathy caused by a novel mutation in LITAF. Neuromuscul Disord 2009; 19: 701-703.
5. Gray OM, Abdeen H, McDonnell GV, Patterson CC, Graham CA, Hawkins SA. An investigation of susceptibility loci in benign, aggressive and primary progressive multiple sclerosis in Northern Irish population. Mult Scler 2009; 15: 299-303.
6. Inherited Peripheral Neuropathies Mutation Database. http://www.molgen.ua.ac.be/CMTMutations/
7. Kleopa KA, Scherer SS. Molecular genetics of X-linked Charcot-Marie-Tooth disease. Neuromolecular Med 2006; 8: 107-122.
8. Latour P, Gonnaud PM, Ollagnon E, Perelman S, Stojkovic T, Stoll C, Vial C, Ziegler F, Vandenberghe A, Maire I. SIMPLE mutation analysis in dominant demyelinating Charcot-Marie-Tooth disease: three novel mutations. J Peripher Nerv Syst 2006; 11: 148-155.
9. Mäurer M, Kruse N, Giess R, Marini C, Carolei A. Gene polymorphism at position -308 of the tumor necrosis factor alpha promotor

is not associated with disease progression in multiple sclerosis patients. J Neurol 1999; 246: 949-954.
10. Meggouh F, de Visser M, Arts WF De Coo RI, van Schaik IN, Baas F. Early onset neuropathy in a compound form of Charcot-Marie-Tooth disease. Ann Neurol 2005; 57: 589-591.
11. Myokai F, Takashiba S, Lebo R, Amar S. A novel lipopolysaccharide-induced transcription factor regulating tumor necrosis factor alpha gene expression: molecular cloning, sequencing, characterization, and chromosomal assignment. Proc Natl Acad Sci U S A 1999; 96: 4518-4523.
12. Panas M, Karadimas C, Avramopoulos D, Vassilopoulos D. Central nervous system involvement in four patients with Charcot-Marie-Tooth disease with connexin 32 extracellular mutations. J Neurol Neurosurg Psychiatry 1998; 65: 947-948.
13. Parman Y. Hereditary neuropathies. Curr Opin Neurol 2007; 20: 542-547.
14. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald Criteria'. Ann Neurol 2005; 58: 840-846.
15. Sacco S, Totaro R, Bastianello S, Marini C, Carolei A. Brain white matter lesions in an italian family with charcot-marie-tooth disease. Eur Neurol 2004; 51: 168-171.
16. Saifi GM, Szigeti K, Wiszniewski W, Shy ME, Krajewski K, Hausmanowa-Petrusewicz I, Kochanski A, Reeser S, Mancias P, Butler I, Lupski JR. SIMPLE mutations in Charcot-Marie-Tooth disease and the potential role of its protein product in protein degradation. Hum Mutat 2005; 2: 372-383.
17. Scelsa SN. Familial, demyelinating sensory and motor polyneuropathy with conduction block. Muscle Nerve 2010; 41: 558-562.
18. Street VA, Bennett CL, Goldy JD, Shirk AJ, Kleopa KA, Tempel BL, Lipe HP, Scherer SS, Bird TD, Chance PF. Mutation of a putative protein degradation gene LITAF/SIMPLE in Charcot-Marie-Tooth disease 1C. Neurology 2003; 60: 22-26.
19. Street VA, Goldy JD, Golden AS, Tempel BL, Bird TD, Chance PF. Mapping of Charcot-Marie-Tooth disease type 1C to chromosome 16p identifies a novel locus for demyelinating neuropathies. Am J Hum Genet 2002; 70: 244-250.
20. Wilson AG, Symons JA, McDowell TL. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 1997; 94: 3195-3199.