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A novel homozygous mutation in the *WNK1/HSN2* gene causing hereditary sensory neuropathy type 2

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Hereditary sensory and autonomic neuropathy type 2 is a rare disorder caused by recessive mutations in the *WNK1/HSN2* gene located on chromosome 12p13.33. Phenotype of the patients is characterized by severe sensory loss affecting all sensory modalities. We report a novel homozygous Lys179fsX182 (*HSN2*); Lys965fsX968 (*WNK1/HSN2*) mutation causing an early childhood onset hereditary sensory and autonomic neuropathy type 2, with acromutilations in upper and lower limbs, and autonomic dysfunction. To the best of our knowledge this is the first genetically proven case of hereditary sensory and autonomic neuropathy type 2 originating from East Europe.

Key words: sensory neuropathy, hereditary, autonomic, mutation, sympathetic skin response

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INTRODUCTION

Hereditary sensory neuropathies are a genetically and clinically heterogeneous group of disorders, primarily affecting the peripheral sensory system. Most patients present with sensory loss and insensitivity to pain, that result in chronic ulcerations or even extensive infections leading to amputations of fingers or toes.

Some childhood onset cases were attributed to mutations of the with-no-lysine(K)-1 (*WNK1*) gene, transmitted in an autosomal recessive mode. Large intronic deletions within the *WNK1* gene were first reported in families with pseudohypoaldosteronism type II (OMIM 145260).

Later studies demonstrated that mutations of *HSN2*, a nervous system–specific exon of the–*WNK1*, cause hereditary sensory neuropathy type II (HSAN2A; OMIM 201300). Since the first report 11 different mutations of the *WNK1/HSN2* gene were reported (Human Mutations Database). Most of the HSAN2 cases were observed in consanguineous families (autosomal recessive trait of inheritance); nevertheless, in some patients only one mutation in the *WNK1/HSN2* gene was detected.

For the molecular genetic purposes HSAN2 families have been identified in a genetically isolated Newfoundland population in the early 1990s. This isolate population is characterized by a multiplied founder effect for numerous disorders resulting from the immigration of the Protestant and Roman Catholic settlers to Newfoundland in the XIX century (Rahman *et al.*, 2003). The genetic linkage analysis studies were performed in two HSAN2 families. A large F1family was previously characterized at the clinical level by Ogryzlo and coworkers in 1946 (Ogryzlo, 1946).

In 2004 Lafreniere et coworkers mapped HSAN2 to the 12p13.33 region. As a result of an exhaustive search they identified an unknown open reading frame (ORF) located within intron 8 of the *WNK1* gene. In this ORF, encompassing one exon-long gene, they identified three homozygous mutations in the patients originating from Newfoundland (c. 594del A), Nova Scotia (c. 918-919 insA) and a nonsense mutation in a French Canadian patient (c. 943 C>T) (Lafreniere *et al.*, 2004).

The ORF of the gene responsible for the HSAN2 phenotype was designated as the *HSN2* gene (Lafreniere, MacDonald, Dube, MacFarlane, O'Driscoll, Brais, Meilleur, Brinkman, Dadivas, Pape, Platon, Radomski, Risler, Thompson, Guerra-Escobio, Davar, Breakefield, Pimstone, Green, Pryse-Phillips, Goldberg, Younghusband, Hayden, Sherrington, Rouleau, & Samuels, 2004). Later on, the *HSN2* gene has been shown not to be an autonomous gene since its transcript was detected in a longer-*WNK1/HSN2* isoform expressed in the neural tissue.

Especially high expression of the *WNK1/HSN2* isoforms was found in sensory components of the peripheral nervous system (Shekarabi *et al.*, 2008). Thus, mutations in the *WNK1* gene should be reported in the traditional nomenclature (*HSN2*) and in a new manner (*WNK1/HSN2*).

In the subsequent studies mutations in the *WNK1/HSN2* gene were identified also beyond the French Canadian and Newfoundland populations. The *WNK1/HSN2* gene mutations were found in Lebanese, Austrian, Italian, Belgian and Japanese HSAN2 affected patients (Auer-Grumbach *et al.*, 2006; Takagi *et al.*, 2006). To the best of our knowledge no mutations in the *WNK1/HSN2* gene have been reported it the Middle and East European population.

We report a novel mutation in the *WNK1/HSN2* gene resulting in the HSAN2 phenotype in our index case.

CASE REPORT

Our proband is a 12.5-years old girl, born at term after uncomplicated pregnancy. Her family history was

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Abbreviations: EMG, electromyography; *HSN2*, a nervous system-specific exon of the *WNK1*; HSNA2, hereditary sensory and autonomic neuropathy type 2; ORF, open reading frame, OMIM, Online Mendelian Inheritance; in Man, PubMed, database of references and abstracts on life sciences and biomedical topics; SSR, sympathetic skin responses; *WNK1*, with-no-lysine(K)-1 gene

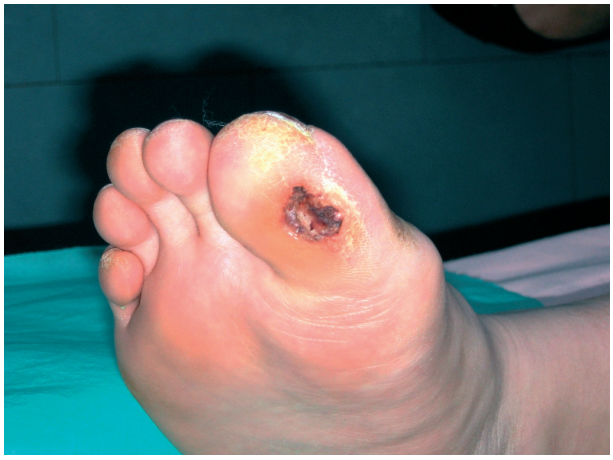

Figure 1. Trophic changes on the right toe of the proband.

not relevant, she has two older and one younger healthy sisters. Her parents are not related, however, their families have been living in the same small village for several generations.

Her developmental milestones were normal. She had some swallowing difficulties noted in the first year of life. Since her early childhood the parents noticed that she did not cry when hurt. She was prone to burns and unnoticed injuries. When she was 5, she had a painless fracture of one of her metatarsal bones. At that time a neuropathy with insensitivity to pain was suspected. Since the age of 8 she had recurrent fever episodes, not related to infection.

She was first seen at our department a year ago. She was in a good condition. Examination revealed sensory loss in her upper extremities up to elbows, and in the lower extremities up to her knees. All sensory modalities were affected. She had areflexia in her lower extremities.

No muscle weakness or atrophy was present. She had ulceration and swelling of her right foot. The nails of fingers and toes were dysplastic. Her blood pressure was normal; there were no clinical symptoms of dysautonomia. Her mental development was also normal. A year later her sensory loss progressed. She continued to have trophic changes on her right foot (Fig. 1).

Electrophysiological studies revealed absent sensory nerve action potentials. Motor nerve conduction studies and concentric needle EMG results were within normal limits. Auditory sympathetic skin responses (SSR) were absent. SSR with electrical stimulation elicited responses with prolonged latencies to her palms, while no responses were recordable in the soles. Measurement of the R-R interval in her electrocardiogram gave a normal result.

Neurological exam and EMG of both her parents were normal.

DNA analysis methods and results. The blood sample was taken from the proband (III:7). The family members participating in this study gave their informed consent. The study was approved by the local ethics committee at the Warsaw Medical University; No. of approval 120/2008.

The whole genomic DNA was isolated from white blood cells by the desalting procedure. In the proband (III:7), first, a duplication and deletion of the peripheral myelin protein 22 gene (*PMP22*) were excluded in the real-time PCR (Q-PCR) approach (Aarskog & Vedeler, 2000).

A sequence of the *WNK1* gene corresponding to the *HSN2* region was amplified in the PCR reaction with designed 5 pairs of primers. The *WNK/HSN2* region was directly sequenced using the BigDyeTM Terminator Version 1.1 Ready Reaction Cycle Sequencing kit on the ABI 3730/xl genetic analyzer (Applied Biosystem).

Two systems of mutation's nomenclature have been used. For the traditional/historical *HSN2* nomenclature the reference sequences i.e. NM_213655.2 (mRNA) and NC_000012.11 (genomic sequence) were applied. For the new *WNK1/HSN2* gene nomenclature two reference sequences NM_213655.3 (mRNA) and NG_007984.2 (genomic sequence) were used.

The *PMP22* gene dosage analysis revealed a normal dosage of the *PMP22* gene.

Direct sequencing of the *WNK1/HSN2* gene revealed a homozygous two -nucleotide deletion 539_540ΔAG (NM-213655.2-HSN2)/ 2897_2898ΔAG (NM-213655.3-WNK1/HSN2), leading to a frame-shift mutation resulting in a premature stop codon (by conceptual translation) at position 182 of the HSN2 protein (Lys179fsX182) or Lys965fsX968 (WNK1/HSN2) (Fig. 2).

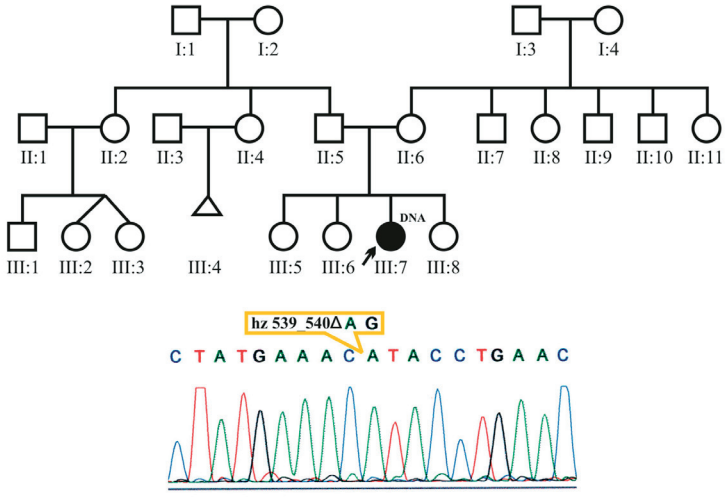

Figure 2. Pedigree chart of three-generational family with HSAN II (upper panel). The proband III:7 is indicated with an arrow. A homozygous two nucleotide AG deletion hz539-540ΔAG (*HSN2*) 2897_2898ΔAG (*WNK1/HSN2*) was identified in the proband III:7 in the *HSN2/WNK1* gene (lower panel).

DISCUSSION

To the best of our knowledge we report the first *WNK1/HSN2* mutation in a HSAN2 affected patient originating from Eastern European population. An earlier report described two Polish patients with recessive hereditary sensory neuropathy, but no molecular studies were conducted in them (Jedrzejowska & Milczarek, 1976).

Given the rules of conceptual translation, this new homozygous Lys179fsX182/Lys965fsX968 mutation in the *WNK1/HSN2* gene results in a nonfunctional protein.

All mutations of the *WNK1/HSN2* gene reported to date have been shown to result in the frame shift or codon

stop and to be loss-of-function mutations (Coen *et al.*, 2006; Rotthier *et al.*, 2009; Shekarabi *et al.*, 2008).

There is no possibility to make reliable phenotype-genotype correlations for *WNK1/HSN2* mutations since the vast majority of them represent private mutations occurring in single families or even in single patients.

Only the S307fsX319 mutation found in a French Canadian was detected in 13 families due to a founder effect in an isolate population (Roddier *et al.*, 2005).

Interestingly, all the reported French Canadian patients living in the southern part of the Quebec province in Canada presented a relatively homogeneous phenotype. All of them had first HSAN symptoms in the first decade of life. All of them developed foot, toe, finger or hand infections in the first decade of life. Necrosis was observed in the patients starting from the beginning of the second decade. The patients in the second, third or fourth decade had undergone leg amputations, which indicates slowly progressive nature of HSAN2 (Roddier *et al.*, 2005). Our patient presented with congenital sensory loss leading to multiple injuries and a painless bone fracture. Although she did not have prominent autonomic disturbances, such as hyperhidrosis, urinary incontinence or slow pupillary reaction to light, her sympathetic skin response results were grossly abnormal. Abnormalities in SSR in hereditary sensory neuropathies are attributed to anhidrosis or hyperkeratotic skin, and are a constant feature of HSAN IV. In contrast, SSR are preserved in HSAN III (Hilz *et al.*, 1999; Hilz & Dutsch, 2006). Interestingly, our patient also reported several episodes of fever, a symptom not frequent in HSAN II (Rotthier *et al.*, 2009).

There is no doubt that the Lys179fsX182 (HSN2); Lys965fsX968 (WNK1/HSN2) variant detected in our study is a pathogenic mutation causative for the HSAN2 phenotype observed in the proband. We decided not to perform sequencing analysis of the whole *WNK1* gene due to the prior detection of a homozygous, deleterious Lys179fsX182 mutation in the HSN2region. The whole sequence of *WNK1* should be analyzed only in the HSAN2 affected patients, in whom no mutations have been found in the HSN2 region.

Our case report expands the list of mutations of *WNK1/HSN2* causing hereditary sensory and autonomic neuropathy type II. It also proves the presence of this rare disease in East-Europeans.

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