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Molecular pathogenesis, experimental therapy and genetic counseling in hereditary sensory neuropathies

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Hereditary sensory and autonomic neuropathies (HSANs) represent a group of heritable peripheral nerve disorders usually taking a severe clinical course. HSAN-affected patients manifest with deep, poorly-healing ulcerations of the feet and hands. To date no definitive cure for HSANs has been developed and the molecular pathology of these disorders is complex. The aim of this review is therefore to present recent findings in terms of HSAN molecular pathogenesis. So far, mutations in 12 genes coding for different proteins have been reported in association with HSAN and the molecular pathogenesis has been elucidated in HSAN1a, HSAN4 and HSAN5. The genes involved in molecular pathogenesis of HSAN code for a wide spectrum of proteins from enzymes to specific nerve growth factors. As far as HSAN1a is concerned, the enhanced understanding has given rise to achievements in experimental therapy particularly in respect to disease models. Despite a rapid progress in studies on the molecular background of HSAN, numerous *loci* and genes remain still to be discovered.

Key words: Hereditary sensory and autonomic neuropathies, HSAN, HSN, molecular pathology

INTRODUCTION

Hereditary sensory and autonomic neuropathies (HSANs) constitute a heterogeneous group of slowly-progressing neurological disorders characterized by progressive loss of function that predominantly affects the peripheral sensory nerves. It results in depressed reflexes, distal sensory loss, altered pain and temperature perception and chronic ulcerations in feet and hands (Auer-Grumbach 2013). Degeneration of motor neurons is often accompanied by atrophy and weakness of distal limb muscles. Autonomic disturbance appears as syncope, gastroesophageal reflux, anhydrosis, constipation and heart rhythm disturbances. Motor involvement and hypoacusis may also be present. The onset is both juvenile and in adulthood. The progression is usually slow and the severity of the disease can vary even within a family (Auer-Grumbach et al. 2003). In EMG studies, patients with HSANs generally present axonal neuropathy, more severe in the lower than upper limbs. Compound motor actions-potential amplitudes are reduced, and motor nerve conduction velocities are slightly slowed (Dyck 1993). Studies of nerve biopsy specimens suggest another underlying demyelinating process (Auer-Grumbach et al. 2003). The incidence of HSANs has been estimated at about 1 in 25 000 (Axelrod and Gold-von Simson 2007, Auer-Grumbach 2008, 2013, Davidson et al. 2012).

CURRENT CLASSIFICATION OF HSANS AND THE GENES INVOLVED

HSANs are classified into types HSAN1-5 (under Dyck's classification), on the basis of age at onset, inheritance pattern, genes involved and additional features (nerve biopsy, electrophysiology, motor involvement, etc.) (Auer-Grumbach 2013, Rinaldi and Atul 2013). HSAN1 onset varies between the 2nd and 5th decade of life. The disease is mainly characterized by distal sensory loss in the upper and lower limbs and chronic skin ulcers, which can even lead to osteomyelitis and necrosis. Motor involvement can be present (Auer-Grumbach et al. 2003, Auer-Grumbach 2008).

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Table I

Classification of the hereditary sensory and autonomic neuropathies updated with newly discovered genes (on the basis of Dyck 1993, as modified by Auer-Grumbach 2013; OMIM)

Clinical type	Inheritance	Locus	Gene
HSN1A/HSAN1A	AD	9q22.1-q22.3	SPTLC1
HSN1B/HSAN1B	AD	3p24-p22	?
HSN1C	AD	14q24.3	SPTLC2, RAB7
HS1D	AD	14-q22.1	ATL1
HSN1E	AD	19p13.2	DNMT1
HSAN1F	AD	11q12.3-q13.1	ATL3
HSN2A/HSAN2A	AR	12p13.33	WNK1/HSN2
HMSN2B/HSAN2B	AR	5p15.1	FAM134B, RAB7/RAB7A
HSN2C	AR	2q37.3	KIF1A
HSAN2D	AR	2q24	SCN9A
HSAN3	AR	9q31.3	IKBKAP
HSAN4	AR	1q23.1	NTRK1
HSAN5	AR	1p13.2	NGFB
HSAN6	AR	6p12.1	DST
HSAN7	AD	3p22	SCN11A
HSN+spastic paraplegia	AR	5p15.2	CCT5
HSAN+diarrhea	AD	20p13	PRNP

(AD) autosomal dominant; (AR) autosomal recessive; (HSAN) hereditary sensory and autonomic neuropathy; (HSN) hereditary sensory neuropathy; (CMT2B) Charcot-Marie-Tooth type II B; (SPTLC1) serine palmitoyltransferase long chain subunit 1; (SPTLC2) serine palmitoyltransferase long chain subunit 2; (RAB7-RAB7A) a member of the RAS oncogene family; (ATL1) atlastin-1; (DNMT1) DNA methyltransferase 1; (ATL3) atlastin-3; (WNK1/HSN2) protein kinase, lysine-deficient 1; (FAM134B) family with sequence similarity 134, member B; (KIF1A) kinesin, heavy chain, member 1A; (SCN9A) sodium channel voltage-gated type IX, alpha subunit; (IKBKAP) inhibitor of kappa light polypeptide gene enhancer in b cells, kinase complex-associated protein; (DST) dystonin; (NTRK1) neurotrophic tyrosine kinase, receptor, type 1; (NGFB) nerve growth factor, beta subunit; (SCN11A) sodium channel voltage-gated type XI, alpha subunit; (CCT5) chaperonin containing T-complex polypeptide 1, subunit 5; (PRNP) prion protein.

HSAN2 symptomatology can be similar to that in HSAN1, but the disease usually develops before the 2nd decade of life. In HSAN2 there is little motor involvement and diffuse sensory loss is present.

Knowledge of HSAN2 clinics is still based on only a few families (Axelrod and Gold-von Simson 2007). However, as HSAN2 disease was detected recently in a patient from Poland, it points to a worldwide distribution of this subtype of HSAN (Potulska-Chromik et al. 2012).

HSAN3, also known as familial dysautonomia (FD) or Riley-Day syndrome, is almost limited to the population of Ashkenazi Jews (1:3 600). HSAN3 has a very strong dysautonomic component, which can manifest in gastroesophageal reflux leading to a recurrent aspiration of food, postural hypotension and syncope. The disease is present at birth and appears as alacrimia (absence of tears), with emotional crying and feeding difficulties. HSAN4, also known as congenital insensivity to pain with anhidrosis (CIPA), starts in early infancy (Axelrod and Gold-von Simson 2007). A patient affected by the disease exhibits predominant loss of pain, which often leads to self-mutilation and episodes of hyperpyrexia due to anhidrosis. Patients can present with delayed developmental milestones, learning problems, hyperactivity and emotional lability during infancy. The disease can occur in all populations but is more common in Israeli Bedouins. Still less is known about HSAN5 symptomatology. In a few described cases the leading problem was separated sensory loss. No mental retardation has been detected in HSAN5 (Axelrod and Gold-von Simson 2007).

Recent genetic discoveries (*ATL3, PRNP*) emphasize the need to delineate a further genetic subgroup within Dyck's classification (Auer-Grumbach et al. 2013). Lately HSAN1F and HSAN with diarrhea have been described.

To date mutations in different genes, both dominant and recessive, have been shown to play a key role in HSAN pathogenesis. The genes involved code for different proteins from enzymes (*SPTLC1*) to nerve-specific growth factors (*NTRK1, NGFB*) (Auer-Grumbach et al. 2013) (Fig. 1).

Depending on the mode of inheritance, HSAN can be subdivided into two groups, involving dominant genes (*SPTLC1, SPTLC2*, *RAB7A, ATL1, ATL3, PRNP* and *DNMT1*) or recessive ones (*HSN2/WNK1, FAM134B, KIF1A, IKBKAP, NTRK1* and *NGFB*) (Table I). Thus far it is only in about 20 percent of cases that the gene responsible for a given disorder can be identified. In the remaining 80 percent of patients the mutated gene is still unknown (Rotthier et al. 2009). Ongoing studies are therefore aimed at discovering the new genes responsible for HSANs, and at revealing their functions.

HSAN1: *SPTLC1* MUTATION CAUSES CHANGES IN THE SPHINGOLIPID PATHWAY

Hereditary sensory and autonomic neuropathy type I (HSAN1) is an autosomal dominant condition and the most frequent HSAN subtype. Mutations in the *SPTLC1* and *SPTLC2* genes, which encode two of the three subunits of serine palmitoyltransferase (SPT), are causative for the disease (Bejaoui et al. 2001, Rotthier et al. 2010).

The widely expressed *SPTLC1* gene comprises 15 exons (approx. 85 kb), and is located on chromosome 9q21–q2. *SPTLC2,* the second gene for the SPT protein, is located on chromosome 14q24.3–q31 and consists of 12 exons (Rotthier et al. 2010).

Biochemistry

Serine C-palmitoylotransferase (SPT) is an enzyme classified as acyltransferase, which means that it transfers groups other than aminoacyl. It requires pyridoxal phosphate (PLP) as a cofactor. Human SPT is a heterodimer composed of two paralogous subunits: LCB1 and LBC2. LCB2 is responsible for the condensation reaction, but appears to be unstable unless associated with LCB1 (Gable et al. 2002). LBC3, an alternative to the LBC2 subunit, is expressed only in certain kinds of tissue (Hornemann et al. 2006). Some data suggest that SPT can be a larger component, probably an octamer. SPT is located on the endoplasmic reticulum (ER) (Gable et al. 2002). Serine C-palmitoylotransferase (SPT) is an enzyme that plays a key role in the initial and limiting step of sphingolipid synthesis. Sphingolipids (SLs) are composed of fatty acids and a polar head group, which derives from L-serine. They are an essential component of cell membranes, as well as playing a role in signal transferrance (sphingosine 1-phosphate) (Hanada 2003, Merrill 2011). SPT catalyzes a condensation reaction between palmitoyl-CoA and L-serine. The products are CoA, 3-dehydro-D-sphinganine, and CO_2. The final product of this meta-

bolic pathway is dihydroceramide (Merrill 2011). In HSAN1, gain-of-function mutations C133W, C133Y and V144D result in the formation of two atypical and neurotoxic sphingolipid metabolites (Penno et al. 2010). The mutations cause a shift in the substrate specificity of SPT, with the alternative substrates being either alanine or glycine. The result is the formation of deoxysphinganine (deoxy-SA) and deoxymethylsphinganine (deoxymethyl-SA), which both lack the hydroxyl group at C1. Thanks to this, deoxy-SLs can neither be condensated with phospho- and glycosphingolipids nor can they be made subject to degradation.

Mutations

To date, the seven identified disease-causing mutations in *SPTLC1* are p.C133W, p.C133Y, p.C133R, p.V144D, p.S331F, p.A352V and p.S331Y (Auer-Grumbach et al. 2013). The p.S331Y mutation has recently been associated with a distinct syndromic phenotype including severe and diffuse muscle wasting, hypotonia, prominent distal sensory disturbances, joint hypermobility, bilateral cataracts and considerable growth (Auer-Grumbach et al. 2013). The most frequent mutation is the single base substitution c.399T> G in exon 5 of the *SPTLC1* coding region (C133W). Most patients with this mutation share a common haplotype, which suggests a common ancestor (Nicholson et al. 2001).

In the case of *SPTLC2*, four different missense mutations (p.V359M, p.G382V, p.A182P and p.I504F) have been detected (Murphy et al. 2013a). Patients present with progressive distal sensory loss, distal muscle weakness, dysesthesia in hands and feet, and osteomyelitis. In HSAN1, no mutations of the third subunit have so far been found (Rotthier et al. 2010).

Experimental therapy

The aforementioned cellular accumulation to toxic levels of deoxysphingolipids (deoxy-SLs, dSL) is confirmed in HSAN1 patients who carry the C133W, the C133Y or V144D mutations, since elevated dSL levels are in fact confirmed in plasma. Tissue analysis of the sciatic nerves of the C133W mice in turn reveals the presence of highly-elevated levels of dSLs. Furthermore, *in vitro* studies on neuronal cultures show that dSDLs have a toxic effect on neurons, and interfere with the formation of neurits. Motoneurons seem to be less impaired by deoxy-SL accumulation than sensory neurons, and neurotoxicity is more pronounced in the case of deoxy-SA than in deoxymethyl-SA (Penno et al. 2010).

The recent findings on oral supplementation of L-alanine in C133W transgenic mice has left the way open for future experimental therapy.

In the study by Garofalo and coworkers (2011), transgenic C133W mice aged between three and nine months were supplied with a 10 percent L-serine-enriched diet. L-serine administration in the mice was found to contribute to dSL plasma concentrations and dSL levels in the sciatic nerve, liver, brain, and spine that were all lower than in untreated mice. The mice whose diet was supplemented with L-serine manifested an improved clinical course for their neuropathy: the animals were characterized by better rotarod scores and a greater diameter of both unmyelinated and myelinated axons. Mutant mice treated for 10 months scored significantly better on the rotarod than those treated for two months (P<0.01). Long-term effects on mechanical and thermal sensitivity showed the same trend, though differences did not reach statistical significance (Fig. 2).

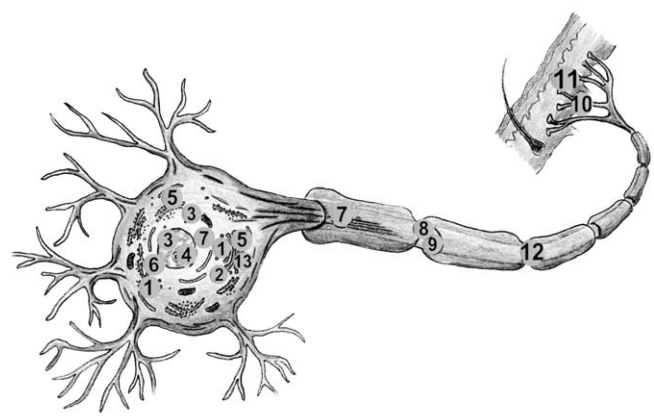
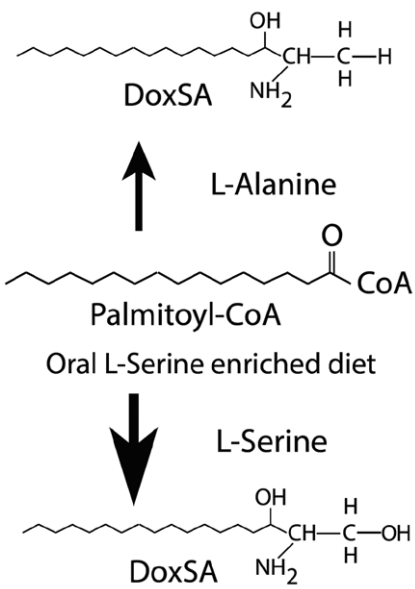


Fig. 1. Localization and function of the proteins encoded by the genes involved in HSAN pathogenesis. (1) SPTLC1-Golgi network-sphingolipid synthesis; (2) RAB7-Golgi network-vesicle trafficking; (3) IKBKAP-cytoplasm-elongator complex; (4) DNMT1-nucleus-methylation; (5) ATL1, ATL3-Golgi network-vesicle trafficking; (6) CCT5-cytoplasm-protein folding; (7) KIF1A-cytoplasm-anterograde axonal transportation; (8) SCN9A-transmembrane-potential building; (9) SCN11A-transmembrane-potential building; (10) NTRK1-receptor protein-signal transduction; (11) NGF-external cell environment-signal transduction; (12) WNK1-cell body; (13) FAM134B-Golgi network.

In contrast, other transgenic C133W mice aged between three and nine months had diets supplemented with L-alanine either long-term (for 10 months) or short-term (2 months). They were found to have higher dSL plasma concentrations, higher sensory thresholds and smaller axons in the sciatic nerves.

For the very first time an exceptional clinical therapy has been put into effect in the cases of 14 patients (aged 24–76 years) with the identified C133Y mutation in the *SPTLC1* gene. The patients were subdivided into two groups and administered a high dose (400 mg/kg) or a low dose (200 mg/kg) respectively of l-serine, daily for 10 weeks. Both groups showed reduced dose-dependant plasma deoxysphingolipid levels with a nadir six weeks into the therapy. Once the study was completed, plasma dSL levels began to rise again. Clinical improvement was not assessed systematically, but some patients reported a subjective increase in sensation (Garofalo et al. 2011). Oral l-serine supplementation seems to offer a promising future therapy for HSAN1-affected patients, but further clinical trials should be run, in order to investigate its effect.



C133W mouse	C133Y human
lower dSL plasma level	lower dSL plasma level
lower dSL tissue level	better sensation
better rotarod score	improvements of skin robustness

Fig. 2. Changes in metabolic pathways during oral serine supplementation (Garofalo et al. 2011). In a HSAN1-affected patient with a C133Y mutation and in C133W mice, palmitoyl-CoA is predominantly condensated with alanine – instead of serine – due to the gain-of-function mutation in the enzyme. The product is a toxic 1-deoxysphinganine, which is accumulated inside the cells to toxic levels. Following 10% serine supplementation of the diet there is a shift in the substrate. Palmitoyl-CoA is predominantly condensated with serine. The product is sphinganine, which is later converted to dihydroceramide.

HSAN1: EXOME SEQUENCING DETECTS A NOVEL MUTATION IN *ATL3*

A whole exome technique contributed to the discovery of a mutation in the *ATL3* (atlastin3) gene, responsible for a dominant condition termed HSAN1F. The mutation p.Tyr192Cys was identified in two families with sensory neuropathies characterized by loss of pain perception and destruction of the pedal skeleton (Kornak et al. 2014). Another missense mutation p. Pro338Arg was detected recently (Fischer et al. 2014).

Functional studies of the Atl3 protein have revealed that the protein is on the endoplasmatic reticulum. It is accumulated in the three-way junction, which connects tubules in the reticulum (Chen et al. 2013). The p.Tyr192Cys mutation leads to displacement of the Atl3 protein. In cells overexpresssing Atl3-Tyr192Cys, the reticulum structure is found to be significantly altered, and the three-way junction morphology is changed (Kornak et al. 2014).

HMSN2B: THE *RAB7* MUTATION AFFECTS VESICLE TRANSPORTATION

RAB7 is another gene identified as causative for HSAN2B (Auer-Grumbach 2008, Cogli et al. 2009). The symptoms of HMSN2B are a prominent motor involvement, sensory loss, foot ulceration and the need for amputations of toes due to infections. Muscle weakness, atrophy of the lower leg and later also the lower parts of the arm are the main characteristics of the disease (Manganelli et al. 2012). The onset is usually in adolescence or later in life so it can be easily mistaken for diabetes mellitus or toxic neuropathies. Cerebellar degeneration on MRI and lateral-gaze nystagmus can occur (Houlden et al. 2004).

The *RAB7* gene consists of six exons and demonstrates linkage to the 3q12-q22 locus (Cogli et al. 2009). The Rab7 protein is a member of the Rab proteins family, which form part of the Ras small GTPase

superfamily. These proteins play a regulating role by cycling between an active (GTP-bound) and an inactive (GDP-bound) condition (Stenmark and Olkkonen 2001, Zerial and McBride 2001, Yang et al. 2004). There are two separate forms of Rab7 protein in humans: Rab7a and Rab7b (a close homologue). These participate in different metabolic processes (Progida et al. 2010): while Rab7b is in the lysosome and is involved in monocytic differentiation, lysosomal degradation of TLR4 and TLR9, as well as transport from the endosomes to the trans-Golgi network (Chavrier et al. 1990), Rab7a is involved in HMSN2B pathogenesis, and is located in the late endosome compartment and in lysosomes. Rab7a plays a crucial role in endocytosis and in vesicular trafficking (Cogli et al. 2010).

When Rab7 was inhibited, growth factor-deprived cells maintained their mitochondrial membrane potential and displayed prolonged, growth factor-independent, nutrient-dependent cell survival.

Biochemistry

The Rab7a protein plays a key role in the endocytic pathway. In normal cells cargoes are internalized from the membranes and transported to the early endosomes. There they are sorted to different compartments: the recycling endosome, the late endosome, the Golgi apparatus and the lysosome (Van Der Sluijs et al. 1991, Bucci et al. 1992). Such Rab proteins as Rab4, Rab5, Rab22 and Rab25 (Soldati et al. 1995, Wang et al. 2000, Rink et al. 2005) are associated with the early endosome. Rab7 and Rab9 (Bucci et al. 2000) in turn interact with late endosomes. To ensure proper maturation of the endosome the sequenced action of Rab5 and Rab7 is required (Soldati et al. 1995). The dissociation of Rab5 and recruitment of Rab7 is provided by HOPS (homotypic fusion and protein sorting; class C Vps protein). The HOPS complex is created by Vps11, Vps16, Vps18,Vps33, Vps39 and Vps41. Vps39 binds to Rab7 and converts it to the GTP bound active form. The active form of Rab7 can recruit the downstream effector. One of the best known downstream effectors of Rab7 is RILP (Rab7-interacting lysosomal protein). It takes part in late endosomal/lysosomal formation, as well as interacting with endocellular movement. The motor ability of the late endosome/lysosome is a consequence of Rab7-RILP-dynein-dynectin interaction (Jordens et al. 2001). Other downstream effectors of Rab7 are Retromers, Rabring7 and TrkA (Fig. 3). They are responsible for regulating the retrograde transport from endosomes to the *trans*-Golgi network. Through an interaction with TrkA (tropomyosin receptor tyrosine kinase A), Rab7 controls the endosomal trafficking and signalling of nerve growth factor (NGF). Ngf contributes to neurite outgrowth (Saxena et al. 2005). Rab7 proapoptotic function performed by limiting cell-autonomous nutrient uptake was also described (Edinger et al. 2003). Inhibition of *RAB7* led to a prolonged, growth factor-independent, nutrient-dependent cell survival (Edinger et al. 2003).

Mutations

Thus far, four missense mutations (L129, K157, N161 and V162) have been identified as responsible for HMSN2B. All of these occur within highly-conserved domains in close proximity to the GTP-binding pocket (Cherry et al. 2013). Several studies have proposed a neuron-specific gain-of-function mechanism of the

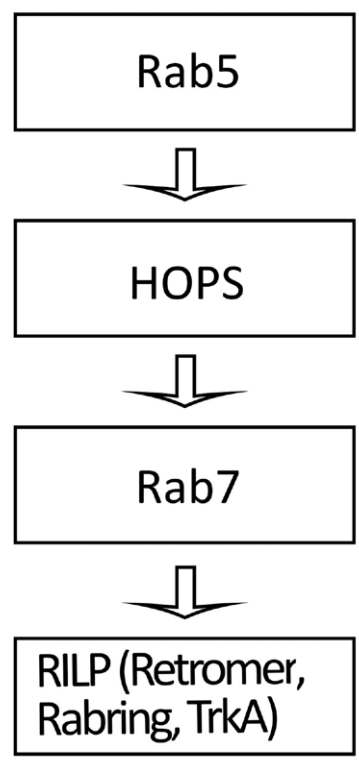


Fig. 3. The sequence of factors required for proper endosome maturation. Rab5 and Rab7 regulate maturation of the endosome. Rab7 is located in the early endosome. HOPS proteins assist in the dissociation of Rab5 and in the recruitment of Rab7. Downstream effectors of Rab7 are RILP, Retromer, Rabring and TrkA.

HSAN1C alleles, with a view to explaining the dominant neuronal phenotype of this gene (Spinosa et al. 2008). However, recent discoveries make it clear that it is rather dose-dependent loss-mutation that is responsible for HMSN2B. With a second *RAB7* allele retaining 5–50 percent of function, activity may be sufficient to provide for normal functioning in most cells (Zhang et al. 2009). However, over the years, some defects can slowly accumulate in the cells most sensitive to Rab7-dependent endolysosomal degradation (Markgraf et al. 2007, Cherry et al. 2013).

Animal model

Lately a *Drosophila melanogaster* model of HMSN2B was constructed. The L129F mutation was inserted with *in vitro* mutagenesis. In transgenic Rab7 flies, larval pain and temperature perception, motor performance and axonal transport were assessed. Larvae with the mutant *RAB7* gene exhibited decreased motor performance, as well as reduced temperature reception and nociception. When the mutant protein was expressed in the motor neurons, the *Drosophila melanogaster* larvae presented with a crawling defect. Upon examination, altered vesicle transportation was identified. More Rab7-positive vesicles were present in the mutant when compared with the wild-type axons. Moreover, mutant vesicles spent less time on average in the stationary phase. The animal model will contribute to a better understanding of the pathomechanism underlying CMT2B. The transgenic *Drosophila melanogaster* can be used in the future in pre-clinical trials of pharmacological agents (Janssena et al. 2014).

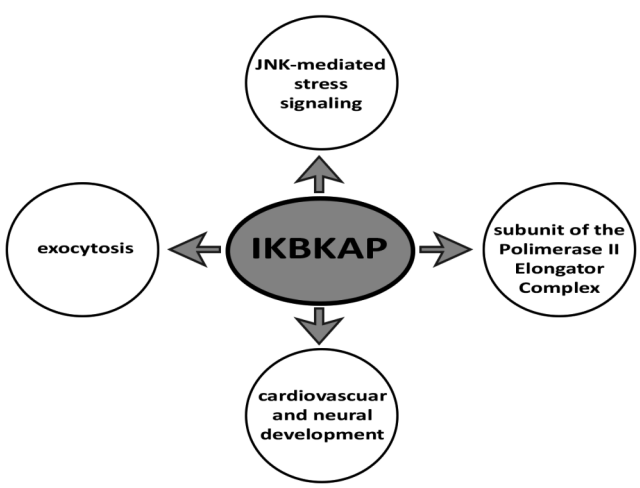


Fig. 4. The pleiotropic role of Ikbkap. The best known process in which Ikbkap is engaged is elongation. Moreover, Ikbkap contributes to exocytosis, cell migration and the organization of the microtubules. Its role in cardiovascular and neural development still requires further investigation.

HSAN2: *HSN2* – AN EXON WITHIN THE INTRON OF *WNK1*

Hereditary sensory and autonomic neuropathy type II (HSAN2) is a rare neuropathy caused by recessive mutations in exon *HSN2* of the *WNK1* gene (Kurth 2010). Phenotype in the patients is characterized by an early-onset reduced sensation to pain, temperature, and touch (Sherakabi et al. 2008). Onset is at birth, often before puberty. Trophic changes can be present in both the upper and lower limbs. The patients can exhibit a loss of tendon reflex, fractures of hands, feet, limbs and Charcot joints. Autonomic disturbance, such as gastroesophageal reflux, hypotension, hyperhydrosis or anhidrosis can be present as well (Gemignani and Marbini 2001, Axelrod and Gold-von Simson 2007).

Wnk1 [with-no-lysine(K) 1; WNK lysine deficient protein kinase 1] is an enzyme encoded by the *WNK1* gene in humans. *WNK1* spans 160 kb, contains 28 exons and has a complex transcriptional regulation (isoform, splice variants) (Wilson et al. 2001). The gene *WNK1/HSN2* is located on 12p13.33 (Lafreniere et al. 2004). *HSN2* is an alternative *WNK1*-spliced exon located within the intron of the *WNK1* gene. *WNK1/HSN2* is expressed in nervous tissues, in both the cell body and axons, while *WNK1* is only expressed in the cell body (not in the axons). Neuronal *WNK1/HSN2* seems to be stronger in the sensory neurons than in motor neurons. The Wnk1 protein is absent from axonal fibers of the sciatic nerve (Shekarabi et al. 2008).

The *WNK1* gene has two distinct promoters: P1 and P2. Data gathered so far suggest that the transcription of *WNK1/HSN2* occurs through the second promoter. An alternatively-spliced *HSN* exone is nestled between exons 8 and 9 of the *WNK1* gene. The *WNK1/HSN2* nervous system isoforms appear to include either *HSN2* alone or *HSN2* together with the other exon (putative exon 8B). Putative exon 8B can be spliced in some transcripts from brain and spinal cord (Shekarabi et al. 2008).

Biochemistry

Wnk1 is a large size (~230 kD) protein, lacking the lysine which is normally present in strand 3, subdomain II in protein kinases (Delaloy et al. 2006, McCormik and Ellison 2011). The Wnk1 protein contains an N-terminal kinase domain, an autoinhibitory domain and a long C-terminal tail, and can form tetramers. Wnk1 belongs to the Wnk (With No lysine (K)) family and the serine/threonine protein kinases superfamily, and is engaged in regulating ion permeability of epithelia (McCormik and Ellison 2011). Wnk1 is prevalently located in the polarized epithelia of the liver and kidney, as well as in the developing brain.

The Wnk1 protein interacts with the ERK5 MAP kinase pathway. The MAP kinase (MAPK) cascades are involved in many signal transduction pathways, including cell-cycle regulation, transcription, apopto-

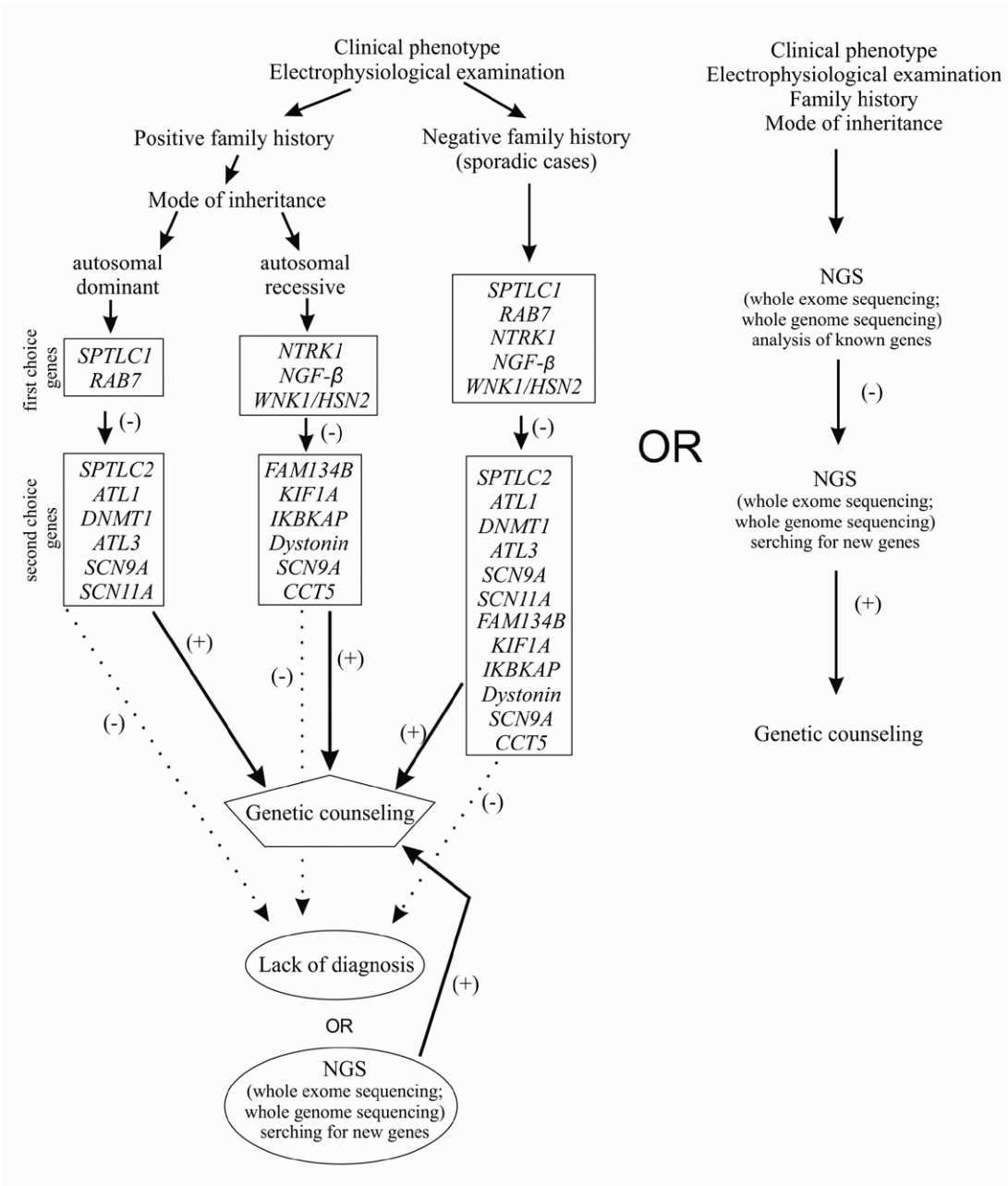


Fig. 5. Algorithm for genetic testing in HSAN patients

sis, and proliferation (Rinehart et al. 2011). Wnk1 protein plays a significant role in the regulation of ionic transport through the plasma membrane. It activates the serum- and glucocorticoid-inducible protein kinase (SGK1), subsequently leading to sodium-channel activation in the epithelium (Heise et al. 2010). Wnk1, together with Wnk4, clathrin and AP-1 (activator protein 1) participates in the formation of vesicles of the trans-Golgi network. Wnk1 and Wnk4 stimulate clathrin dependent endocytosis of renal outer medullar potassium 1 (ROMK1) (Wang et al. 2000, Wang et al. 2008). Wnk1/Hsn2 interacts with synaptotagmin 2 (SYT2), which acts as a calcium sensor in neurons and neuroendocrine cells, regulating both endo- and exocytosis. Wnk1/Hsn2 binds and phosphorylates synaptotagmin 2, within its calcium-binding C2 domains (Wang et al. 2000). The role of Wnk1/Hsn2 in the central nervous system is still uncertain and demands further investigation.

Mutations

11 mutations in *WNK1* have thus far been discovered (the Human Mutation Database OMIM). Two founder mutations in the *WNK1* gene (918insA and Q315X) have been found in patients from the southern part of Quebec (Roddier et al. 2005). The mutations segregate with a phenotype characterized by disturbances of sensation in terms of pain, temperature and position.

Animal model

An animal model of HSAN2 on *Danio rerio* embryos was constructed (Bercier et al. 2013). A strong expression of *WNK1/HSN2* in neuromasts of the peripheral lateral line (PLL) system was observed. After knocking down *WNK1/HSN2* in embryos by using antisense morpholino oligonucleotides, PLL developed improperly. An interaction between the Wnk1 kinase and neuronal potassium chloride cotransporter KCC2 was reported suggesting a novel pathway, where a loss-of-function mutation in Wnk1 induces an overexpression of KCC2.

HSAN2B

HSAN2B is caused by mutations in the FAM134B gene (five different mutations have been reported so far) (Kurth et al. 2009, Ilgaz Aydinlar et al. 2014). The gene function has not been discovered yet. FAM134B was overexpressed in a significant number of esophageal squamous cell carcinoma (ESCC) cell lines. FAM134B plays a role also in colorectal cancer (Kasem et al. 2014). Overexpression of the gene in cells caused an increase in growth rate, colony formation in soft agar, and foci formation in confluent cultures (Tang et al. 2007).

HSAN2C

Hereditary sensory neuropathy type II C (HSN2C) is caused by a mutation in the *KIF1A* gene. Mutations in the same gene were also reported in the hereditary spastic paraplegia (SPG 30). Clinical manifestation of HSAN2C include an early onset distal sensory loss leading to ulceration and to distal muscle weakness (Riviere et al. 2011). *KIF1A* is a member of the kinesin family. It is responsible for an anterograde transport of synaptophysin, synaptotagmin and Rab3A along axonal microtubules. An animal model of the disease presented with motor and sensory disturbances, a reduction in the density of synaptic vesicles in nerve terminals and accumulation of clear vesicles in cell bodies of the neurons (Yonekawa et al. 1998).

HSAN3: SPLICE SITE MUTATIONS IN THE *IKBKAP* GENE

The *IKBKAP* gene covers a 68 kb genomic sequence, includes 37 exons and is located on the long (q) arm of chromosome 9 at position 31. Ikbkap (I-κ-B kinase complex associated protein), also called ELP-1 (elongation protein1), is a protein encoded by the *IKBKAP* gene. It is a well conserved 150 kDa eukaryotic protein, which does not belong to any protein family and lacks domain structure. Almost 90 percent of the Ikbkap protein is located in the cytozol (Cuajungco et al. 2001, Holmberg et al. 2002, Johansen et al. 2008).

Mutation in the *IKABKAP* gene is responsible for familial dysautonomia (FD) (Anderson et al. 2001). FD, also known as Riley-Day syndrome or hereditary sensory and autonomic neuropathy type III (HSAN3), is a recessive neuropathy (Klebanoff et al. 1980, Silveira et al. 2012) almost exclusively affecting Ashkenazi Jews. FD manifests in alacrimia, depressed

tendon reflexes, decreased pain and temperature perception and gastrointestinal dysmotility. Other symptoms include poor development and progressive degeneration of the sensory and autonomous nervous system. The course of disease is fatal and only 50 percent of affected patients reach the age of 40.

Biochemistry

The expression of the mutated and normal Ikbkap protein varies from tissue to tissue. The most affected are sensory and autonomous neurons in which the mutant and shorter *IKBKAP* mRNA is mainly produced (Slaughenhaupt et al. 2001, Naumanen et al. 2008).

Ikbkap has been shown to have a pleiotropic role in both the peripheral and central nervous systems (Hunnicutt et al. 2012) (Fig. 4). Ikbkap protein is associated with neuronal development, but further investigation is still required to establish its function. Numerous studies indicate that Ikbkap is a subunit of the RNA Polymerase II Elongator Complex (IKAP/ELP1, ELP2 and ELP3), which promotes the elongation of many genes (Hawkes et al. 2002). Ikap also associates with Jnk, and enhances Jnk-mediated stress signaling. Ikap may also play a role in cell motility and/or migration, by organizing actin and microtubules. Alterations in the levels of Ikap, through both gain and loss of function, affect neuronal polarity, differentiation and survival. Ikap contributes to the maintenance of neural-precursor proliferation, and prevents precocious differentiation into neurons (Naumanen et al. 2008).

Mutations

FD is caused by a point, loss-of-function mutation (Axelrod 2004, Axelrod and Gold von Simson 2007). Although three mutations in the *IKBKAP* gene have been discovered, the mutation in 99.5 percent of patients is in a splice acceptor site in intron 20 (IVS20+6T>C mutation). The splicing site is weakened, which results in a skipping of the 20th intron and a reduction in amounts of the Ikbkap protein (Slaughenhaupt et al. 2001). The other mutation is R696P, thus far detected in four Ashkenazi patients. There is an exclusive non-Jewish *IKBKAP* mutation identified in one patient in exon 26, P914L (Leyne et al. 2003).

Animal model

An animal model of FD which mimics the molecular and pathological features of the disease has been created to better understand the pathogenesis of HSAN3 (Dietrich et al. 2011, 2012). The mouse *IKBKAP* gene was targeted by homologous recombination. Homozygosity for either mutation led to developmental delay, cardiovascular or brain malformations, and early embryonic lethality. *IKBKAP* is found to be essential for the expression of specific genes involved in cardiac morphogenesis, and cardiac failure is the likely cause of abnormal vascular development and embryonic lethality. The transgenic animals can be used in pre-clinical trials of pharmacological agents.

Experimental therapy

First clinical trials with oral kinetin treatment have been carried out (Axelrod et al. 2011). Eight individuals with FD diagnosis took part in the relevant 28-day study, the kinetin being found to correct the FD-splicing defect in FD patients, and to raise the level of normal *IKBKAP* mRNA. Normal splicing of *IKBKAP* increased in six of the eight subjects after eight days. The kinetin was well tolerated and safe. Recently, phosphatidylserine has been shown to increase total *IKBKAP* expression, though its effects on *IKBKAP* levels in FD patients will need to be assessed in further studies.

HSAN4 AND HSAN5: CHANGES IN PAIN SIGNALING PATHWAYS

HSAN4 and HSAN5 are autosomal recessive conditions caused by the mutations in Nerve growth factor (*NGF*) gene and its receptor *NTRK1*. HSAN4 (congenital insensitivity to pain with anhidrosis, CIPA) is characterized by a profound loss of pain sensitivity, leading to injuries, self-mutilation and osteomyelitis, anhidrosis and mental retardation. Israeli-Bedouin are the population most affected by the disease. Our knowledge of HSAN5 is based on few cases. The patient presents loss of pain perception and impaired temperature sensitivity, ulcers, and in some cases self-mutilation. The autonomic involvement is variable (Axelrod and Gold von Simson 2007). In some cases a mild mental retardation has been identified (Carvalho et al. 2011).

Biochemistry

Ngf is a member of the neurotrophin family. It is synthetized as a pro- neurotrophin, which is later cleaved by proteases. Ngf plays an important role in the growth, maintenance, and survival of neurons, as well as in cell signaling. Together with neurotrophin-3 (Nt3) and glial cell line-derived neurotrophic factor (Gdnf), Ngf takes part in the regeneration of damaged axons. Ngf is in turn involved in retrograde transportation. The protein interacts with two receptors: p75 NTR and Trka (Pattarawarapan and Burgess 2003). Interaction with p75 NTR leads to either proapoprotic or prosurvival actions. Binding with Ntrka induces the downstream signaling cascade: the Ras-mitogenactivated protein kinase (MAPK) pathway, the phosphatidylinositol-3-kinase (PI3K) pathway and phospholipase activation. Through the Trka receptor, Ngf plays a role in sympathetic axon growth (Kuruvilla et al. 2004). Mutation in the *NGF/TRKA* gene leads to a loss of pain perception. Knockout mice models have been constructed for *NGF, TRKA* and *p75*. In all three cases nociceptive function was found to be impaired (Lee et al. 1992, Crowley et al. 1994, Smeyne et al. 1994).

The *NTRK1* gene, also known as *TRKA,* is located on chromosome 1 (1q21-q22) and contains 17 exons (Greco et al. 1996). It codes for the high-affinity tyrosine kinase receptor I for Neurotrophic Growth Factor (Ngf). A single transmembrane domain divides the Trka protein into extracellular (NGF-binding) and intracellular domains (responsible for signaling) (Wiesmann et al. 1999).

Twelve different mutations (substitutions, insertions and deletions) have been identified in patients with CIPA (OMIM). The *NTRK1* gene codes for a ubiquitously expressed transduction factor. The heterodimeric transcriptionally active form is composed of p50/p65 (Kaltschmidt et al. 1993). The Ntrk1 receptor is located in different cellular compartments. While in cytoplasm it is inactive, under the influence of multiple stimuli it migrates to the nucleus and there switches to the active form. Ntrk1 participates in signal transduction by activating the Ikb kinases complex. In nervous system development, it takes part in nociception. Its expression is altered in many neurodegenerative diseases, such as Alzheimer's, Huntington's and Parkinson's disease (Memet 2006).

Mutations

The human nerve growth factor beta (*NGFB*) gene is located on chromosome 1p13.2. The gene has been identified as causative for HSAN5 within individuals homozygous for a point mutation in *NGFB*. Thus far, two HSANV5-causative mutations (R211W and 681delGG) have been identified in the *NGFB* gene (OMIM). Mature Ngf is a 26 kDa protein composed of two identical 13 kDa monomers (McDonald et al. 1991).

Experimental therapy

While Ngf /Trka signaling is involved in pain sensation, it is a potential target in analgetic therapy.

Some experimental therapies interacting with Ngf signaling in cases of both HSAN4 and HSAN5 have been put into practice lately, e.g. exogenous Ngf administration (Apfel 2002), NGF-antibodies (Holmes 2012), and a genetically modified autologous fibroblast which expresses Ngf (Tuszynski et al. 2005).

OTHER GENES INVOLVED IN HSAN AND RARE SUBTYPES OF HSAN

New molecular techniques, including next-generation sequencing, have accelerated the discovery of new genes responsible for HSAN. Up to 20 HSAN genes have been identified altogether. They perform different molecular functions from DNA methylation (*DNMT1*) (Klein et al. 2011), through potential building (*SCN9A*) (Drenth and Waxman 2007) and to protein folding (*CCT5*) (Nikawa and Kimura 2012). Proteins encoded by them are located in various cellular compartments of the neural tissue (the cytosol, nucleus, ER and Golgi apparatus). Functional studies of mutation have contributed to a better understanding of the role of the proteins responsible for HSAN. In the case of many mutations, an animal model of the disease has been constructed. Based on clinical diagnosis and genes involved, further subtypes of HSAN were delineated (i.e. HSAN6 and HSAN7). HSAN6 was diagnosed only in one Ashkenazi Jewish family and associated with a severe phenotype: neonatal hypotonia, respiratory and feeding difficulties. The mutation is located in *DST* gene, coding for dystonin, which is responsible for proper cytoskeletal formation and vesical transportation (Edvardson et al. 2012, Hu et al. 2009). HSAN7

Table II

Other genes (not fully described in the text above) involved in HSAN pathogenesis

Gene	Molecular function	Location	Protein interacting partners	HSN subtype	Reference
ATL1	ER and Golgi apparatus morphogenesis, vesicular trafficking	Tubular ER, Golgi apparatus	ER-shaping proteins, DP1/ Yop1p, SPG31, spastin	HSN1D	(Rismanchi et al. 2008, Hu et al. 2009, Guelly et al. 2011)
DNMT1	DNA methylation	Post-mitotic neurons	MeCP2, HDAC1, HDAC2, DMAP1	HASN1E	(Klein et al. 2011, Qin et al. 2011)
FAM134B	Unknown	Dorsal root ganglia	Unknown	HSAN2B	(Kurth et al. 2009, Murphy et al. 2012)
KIF1A	neurogenesis, anterograde axonal transportation, vesical transportation	Neural tissue	BDNF	HSN2C	(Duncan 2006, Riviere et al. 2011, Kondo et al. 2012)
SCN9A	Potential building, (sodium channel)	Small –diameter peripheral neuron axons, dorsal root ganglion, sympathetic ganglion	unknown	HSAN2D	(Drenth and Waxman 2007, Faber et al. 2012)
DST	Cytoskeletal formation, vesical transportation	Central nervous system, muscle, skin	Dynactin-1	HSAN6	(Edvardson 2012)
SCN11A	Potential building (sodium channel)	Dorsal root ganglion, sympathetic ganglion neurons	BDNF, NTRK2	HSAN7 Absent pain	(Blum et al. 2002, Leipold et al. 2013, Zhang et al. 2013)
CCT5	Actin and tubulin folding	ER, cytosol	ATP (Kalisman 2013), CCT-alpha (TCP1) and CCT-zeta (CCT6A)	HSN+spastic paraplegia	(Liou and Willison 1997, Bouhouche et al. 2006, Nikawa and Kimura 2012)
PRNP	Signal transduction	Plasma membrane	unknown	HSAN+Diarrhea	(Mouillet-Richard et al. 1999, Vanik and Surewicz 2002, Mead et al. 2013)

was found in two unrelated patients and manifests with inability to experience pain, self-mutilations and multiple painless fractures. *SCN11A,* coding for a sodium channel, was identified as a gene underlying the disease (Leipold et al. 2013, Zhang et al. 2013). In cases of rare subtypes of HSAN, our knowledge of the phenotype caused by a mutation is often based only on a few affected individuals or families (as with *CCT5, ATL3, DST, PRNP* and *SCN11A*). The table below (Table II) presents the molecular function, localization and interaction of the *ATL1, DNMT1, FAM134B, KIF1A, SCN9A, DST, SCN11A, PRNP* and *CCT5* proteins.

MOLECULAR DIAGNOSTICS AND GENETIC COUNSELING IN HSAN

In recent years, many new genes responsible for hereditary sensory neuropathies have been identified, and much new information about gene function and pathomechanisms of these disorders has been gathered. In spite of this expanding knowledge of inherited peripheral neuropathies, most HSAN cases are still inextricable on a molecular basis (Murphy et al. 2013b). Molecular diagnosis plays an essential role in establishing an appropriate diagnosis, as well as allowing for prognostic information, genetic counseling and prenatal diagnosis, and possibly future preimplantation genetic diagnosis. Aspects of medical management can be determined, and, in some cases, information could be provided on the possible risk with drug supplementation, giving a chance for treatment, as in the case of L-serine supplementation in patients with the *SPTLC1* gene mutation.

Until recently, molecular diagnoses were based on mode of inheritance, specific clinical phenotype and electrophysiological examination. In the majority of laboratories, analyses are performed on selected HSAN genes, mainly on those that are most prevalent. The analysis of rare genes analysis is unlikely to be offered in routine investigations (Fig. 5). In HSAN the mutations described most often are accumulated in the *NTRK1* and *RAB7* genes (Rotthier et al. 2009). Unfortunately, gene screening only reveals disease mutations in single families, leaving many cases unresolved.

In addition, the steadily increasing number of HSAN genes involved in HSAN consumes more and more time and entails ever-greater costs for traditional Sanger sequencing analysis. In many laboratories, funding and personnel capabilities are exceeded.

Moreover, molecular diagnosis is hampered by a lack of mutation "hot spots", the occurrence of many so-called "private" mutations (found only in single families) and the dispersal of mutations across the whole gene sequence, and not merely the main functional gene domains. Therefore, requests for DNA analysis need to take this limitation into account.

CONCLUSIONS

Thus a new player is coming on to the diagnostic scene – next-generation sequencing. At present it is still too expensive for use in routine testing, but a steady advance of technology is ensuring that the costs of each analysis are reducing. This method, in disorders like hereditary neuropathies that are characterized by an enormous amount of genes associated with the diseases, provides the opportunity for the analysis of all known genes for a relatively low price in comparison with Sanger sequencing of the same number of genes. While the NGS method generates a vast amount of data irrelevant to diagnostic needs, the targeted next-generation sequencing is an efficient tool for genetic screening in HSAN, also helping in the selection of patients for genome-wide approaches and the discovery of new genes (Schabhüttl et al. 2014).

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