Hereditary peripheral neuropathies diagnosed by next-generation sequencing

BACKGROUND Next-generation sequencing (NGS) is a genetic technique used to determine the order of nucleotides in DNA. The technique has proved to be more efficient than the traditional method, Sanger sequencing, for sequencing multiple genes. NGS is now being used to diagnose disorders in which multiple genes are involved. This study has examined whether next-generation sequencing produces a greater number of positive diagnoses than its traditional counterpart in patients with suspected hereditary peripheral neuropathy.

MATERIAL AND METHOD This study is a retrospective review of samples from 103 patients investigated for hereditary peripheral neuropathy, received by Telemark Hospital in the period 2012–14. After exclusion of duplication/deletion of PMP22, 96 samples were analysed by NGS with physical enrichment of 52 hereditary peripheral neuropathy genes.

RESULTS A genetic cause was identified in 35 patients (34 %) with peripheral neuropathy, of which 28 (27 %) were point mutations identified by NGS.

INTERPRETATION Of the pathogenic point mutations identified in this study, 12 were in genes that would previously have been analysed by Sanger sequencing in our department, whereas 16 were in genes that would not previously have been tested.

DNA sequencing is used to read the nucleotide sequence in all or part of an organism’s genetic material. The method «next-generation sequencing» (NGS) has revolutionised the speed and capacity of DNA sequencing, with the result that the human genome can now be sequenced in a few days, rather than the ten years required with traditional methods (Sanger sequencing) (1, 2).

NGS is the subject of some controversy. It is already used extensively in research, and is now being introduced into clinical genetic diagnostics. Two main variants are used: exome sequencing, which involves sequencing all coding genes (all exons) and gene panel sequencing, which involves sequencing selected genes, such as those responsible for a given disease.

Sanger sequencing was previously used to examine selected genes sequentially (2–4). However, this method imposes a limit on the number of genes that it is feasible to sequence. The much greater capacity of NGS makes it more efficient for diagnosing genetically heterogeneous diseases such as hereditary peripheral neuropathies, epilepsy and cardiomyopathies (4–6).

Charcot-Marie-Tooth disease (CMT) is the most common hereditary peripheral neuropathy, with a prevalence in Norway of 40–80 per 100,000 population (7, 8). The disease is also referred to as hereditary motor and sensory neuropathy (HMSN).

Clinical symptoms often begin distally in the legs, with motor signs such as paresis and atrophy, and sensory signs such as loss of sensitivity to vibration and touch. The disease develops gradually and may affect the arms later in the disease course. With further progression patients often develop walking difficulties, claw foot (pes cavus) and hammer toes. Severity and age of onset vary, but the disease is usually slowly progressive (9–11). Inheritance may be autosomal dominant, autosomal recessive or sex-linked.

Charcot-Marie-Tooth disease is further classified on the basis of nerve conduction velocity (NCV) in the median nerve: demyelinating disease (CMT1): NCV < 38 m/s; axonal disease (CMT2): NCV > 38 m/s and intermediate disease: NCV = 25–45 m/s (10, 11). Charcot-Marie-Tooth disease is closely related to several less common peripheral neuropathies: distal hereditary motor neuropathy (dHMN), hereditary sensory neuropathy (HSN) and hereditary sensory and autonomic neuropathy (HSAN). These diagnostic groups may be viewed as a continuum, both clinically and genetically (Fig. 1).

After completion of the human genome project and the introduction of NGS, the number of genes associated with Charcot-Marie-Tooth disease and other inherited peripheral neuropathies increased rapidly. Today there are around 90 genes associated with this disease group, as shown in Figure 2 (6, 12–14).

The most common cause of Charcot-Marie-Tooth disease is duplication (one additional copy) of the PMP22 gene, as diagnosed in 14 % of patients in families with clinical disease in eastern Akershus county, Norway (7). After PMP22 duplication, point mutations (single base changes in DNA) are the next most common cause of Charcot-
Marie-Tooth disease and other inherited peripheral neuropathies. In traditional clinical diagnostics, candidate genes are tested sequentially with Sanger sequencing. These candidate genes are selected by assessing clinical and neurophysiological features in the patient and the pattern of inheritance in the family (15, 16). Sanger sequencing is resource-intensive, and most clinical laboratories have only had the capacity to test a few of these genes (9, 12, 17). Matters are complicated further by genetic heterogeneity (one phenotype can be caused by different genotypes) and variable expressivity (one gene can produce different phenotypes).

In two Norwegian studies of patients with clinical Charcot-Marie-Tooth disease, one based on families in eastern Akershus and the other on a clinical population in Northern Norway, Sanger sequencing was used to test for point mutations in the six and seven genes, respectively, with presumed highest frequency (GJB1, MPZ, MFN2, PMP22, LITAF, EGR2, NEFL). Point mutations were identified in 11% and 8% of patients respectively (7, 18). International studies have reported a somewhat higher proportion of point mutations in these genes, 16–20% (19–21). We believe there is a need for a more efficient assay, with the capacity to test more of the genes implicated in neuropathy. A specific genetic diagnosis can provide patients and their relatives with information about prognosis and recurrence risk and may be relevant to future gene-specific therapies (17, 22).

In a recent study, the families from eastern Akershus were tested for point mutations in 51 genes associated with hereditary neuropathy via gene-panel NGS. The discovery rate for point mutations increased from 11% with Sanger sequencing in a previous study (7) to 30% using NGS (6). Internationally, University College London Hospitals and Aarhus University Hospital are among those now offering NGS as part of clinical diagnostics for hereditary peripheral neuropathies. However, most research to date has described single families; there have been few large studies of the usefulness of this approach for patients.

We have now reviewed the results of gene-panel NGS for the first 103 patients tested in our clinic for suspected hereditary peripheral neuropathy. The aim was to examine whether NGS provides a greater number of positive diagnoses than its traditional counterpart in a clinical population of patients with suspected hereditary disease.

**Material and method**

This quality assurance study is based on a retrospective review of material obtained through standard practice at the Section of Medical Genetics, Telemark Hospital. The material includes specimens from all index patients with suspected hereditary peripheral neuropathy received between 1 March 2012 and 1 May 2014, a total of 103 patients.

Samples were received after consultations with the departmental geneticist and neurologist (n = 47) or were sent in by 44 different external parties (n = 56). The latter were distributed as follows: neurological departments (n = 27), neurologists in private practice (n = 8), general practitioners (n = 8), genetics departments (n = 5), paediatric departments (n = 5), other (n = 3). CMT1 was suspected in ten patients, CMT2 in 16, CMTX (sex-linked) in three, unknown Charcot-Marie-Tooth disease in 44 and neuropathy in 30.

Most patients lived in Eastern Norway, in the counties Vestfold (n = 21), Oslo (n = 18), Akershus (n = 12), Buskerud (n = 12), Telemark (n = 12), Østfold (n = 6), Oppland (n = 5) and Hedmark (n = 3). A total of 14 patients lived in other parts of Norway. Patients from our previous study (6) could potentially be part of this sample, but we believe that this applies to very few, if any.

The data were anonymised, and the study was approved by the Norwegian Social Science Data Services (NSD), which granted exemption from the need to obtain informed consent. The method used was standard diagnostic practice at the hospital over the period concerned, and the approval of the regional ethics committee was therefore not sought for this study. Patients have access to information about the method via the results sent to the requisitioning doctor, and on the hospital’s website.

All patients were first tested for duplication/deletion of PMP22, if not already done, using Multiplex Ligation Probe Amplification (MLPA). Patients with negative results underwent further testing with NGS. Prior to sample work-up, a gene panel comprising 52 genes associated with peripheral neuropathy was designed for physical enrichment and NGS. The gene list is shown in Fig. 2. DNA was extracted from blood samples and the sample work-up was performed according to a standard protocol from Illumina (Illumina Inc, San Diego, CA). The laboratory methods and bioinformatic analysis have been described in more detail previously (6).

Variants were classified as follows: class 5 – clearly pathogenic, class 4 – likely to be pathogenic, class 3 – unknown significance, class 2 – unlikely to be pathogenic, class 1 – clearly not pathogenic. The classification of variants is partly a manual process, based on the study of normal frequencies, conservation in other organisms, data from internal controls and previously published literature. Variants assigned to classes 4 and 5 are reported to the patient as likely and clearly pathogenic respectively. Class 3 variants are not reported with full nomenclature, but as variants of unknown significance. In the case of class 3 variants, samples will often be requested from family members in an attempt to clarify the variant’s significance. Patients with class 3–5 variants are advised to undergo genetic counselling, in which their clinical signs and symptoms are considered alongside the genetic data and an effort is made to clarify variants of unknown significance. Class 1 and 2 variants are not reported to the patient.

**Classification of sequence variants is**
The neuron

Endosomal sorting and cell signalling
DNM2 – CMT2, ICMT
FBXO38* – dHMN
GNB4* – ICMT
HAX1D10* – CMT and foot deformity
IKBAP – HSAN
IFRD1* – HSMSN and ataxia
NGB – HSAN
NTRK1 – HSAN
RAB7 – CMT2
SDX10 – PCWH
TUBB3* – CFFOM
WNK1 – HSAN

Nuclear processes
AARS – CMT2
DNMT1* – HSAN
gars – CMT2, dHMN
HARS* – PN
HINT1* – CMT2
IGHMBP2 – ICMT
KARS* – ICMT
LMNA – CMT2
MARZ* – CMT2
MED25 – CMT2
PRPS1 – CMT
PRP51 – CMTX
PLEKHS5 – ICMT, dHMN
SETX – dHMN
TGFP* – HSMSN proximal
tDP1* – SCA and HMSN
VRK1* – HSMSN and microcephaly
YARS – ICMT

ER and Golgi apparatus
ATL1 – HSN
ATL3* – HSAN
BSEL2 – dHMN
FAM134B – ICMT
REEP1 – dHMN
VAPB* – dHMN

Mitochondria
AIFM1* – CMTX
dHKT10* – CMT2
GDAP1 – CMT2, CMT4, ICMT
MFN2 – CMT2
PDKP1 – CMTX
POLG – CMT2, dHMN

Axonal transport
BICD2* – dHMN
dHNU1H1* – CMT2
HSNB1 – CMT2, dHMN
KIF1A* – HSAN
KIF1B – CMT2
KIF5A* – HSP and PN
MYH14* – PNMRH
NEFL – CMT1, CMT2

Ion channels
ATP7A – dHMN
SCN11A* – HSAN
SCN9A* – HSAN
SCL1A2A – HSMSN
TRPV6 – CMT2, dHMN

Cell division
SEPT9 – HNA

Toxins
SOD1 – CMT2
SPTLC1 – HSN
SPTLC2* – HSAN

Synaptic transmission
DCTN1 – dHMN
SLC5A7* – dHMN

The neuron

Cytoskeleton
DST* – HSAN
gAN – gAN

Proteasome and protein aggregation
CCT5* – HSAN
dNB2* – HMSN
dHSB1 – CMT2, dHMN
dHSB2 – dHMN
dHSB2 – CMT2, dHMN
LRSAM1* – CMT2
dPRNP* – HSAN
dTRIM2* – CMT2

Myelin
MF2 – CMT1, CMT2, ICMT
PMP22 – CMT1, HNPP

Transcription and mRNA processing
CTDP1 – CCFDN
EGR2 – CMT1

Endosomal sorting and cell signalling
AHRGEF10* – PN and low NCV
CFFDN – CMT2, ICMT
FIQ4 – CMT4
LITAF – CMT1
MTMR2 – CMT4
NDRG1 – CMT4
SBF1* – CMT4
SFB2 – CMT4
SH2TC2 – CMT4

Mitochondria
GDAP1 – CMT2, CMT4, ICMT
HK1 – CMT4
SURF1* – CMT4

Ion channels
GJB1 – CMTX
GJB3* – SN and deafness

Figure 2 Genes associated with hereditary peripheral neuropathy, their associated phenotypes and presumed pathogenic mechanisms [6, 12–14]. Genes marked with an asterisk are not part of the gene panel used in the current study, but were included in the diagnostic gene panel in our department from autumn 2014. Colour codes for phenotype as in Figure 1. DNMs and GADPs are listed twice since they have roles in both neurons and Schwann cells. Abbreviations: CCFDN = congenital cataracts, facial dysmorphism, and neuropathy; CFFOM = congenital fibrosis of the extraocular muscles; CMT = Charcot-Marie-Tooth disease; CMTX = Charcot-Marie-Tooth disease type X (sex-linked); dHMN = distal hereditary motor neuropathy; ER = endoplasmic reticulum; gAN = giant axonal neuropathy; HSAN = hereditary sensory neuropathy; HNA = hereditary neuralgic amyotrophy; HNPP = hereditary neuropathy with liability to pressure paresis; HSAN = hereditary sensory and autonomic neuropathy; HSFN = hereditary sensory neuropathy; HSP = hereditary spastic paraplegia; ICMTA = intermediate Charcot-Marie-Tooth disease; NCV = nerve conduction velocity; PCWH = peripheral demyelinating neuropathy, central demyelination, Waardenburg syndrome and Hirschsprung disease; PN = peripheral neuropathy (for genes not specified in another class); PNMRH = peripheral neuropathy, myopathy, hoarseness and hearing loss; SCA = spinocerebellar ataxia; SN = sensory neuropathy (for genes not specified in another class).
All variants in classes 4 and 5 were verified by Sanger sequencing. Family members were often tested with Sanger sequencing for variants in classes 3–5.

The method is accredited in accordance with ISO 15189.

Results
The study material included 55 women (53 %) and 48 men (47 %). The average age was 48 years (SD 20) at sample receipt.

Prior to NGS, seven patients (7 %) were diagnosed with duplication or deletion of PMP22 via MLPA. The remaining 96 patients underwent NGS of 52 peripheral neuropathy genes. All 96 samples met accredited quality-control standards. On average, 99.1 % (SD 0.9) of all relevant nucleotides had a coverage of more than 30x.

Among the 96 samples that underwent NGS, nine variants (9 %) were classified as clearly pathogenic, 19 (18 %) as likely to be pathogenic and ten (10 %) as being of unknown significance. Patients were informed about any clearly pathogenic and likely pathogenic variants. A total of 35 patients (34 %) thus received a genetic diagnosis, distributed among 15 different genes. In all, 28 (27 %) patients had point mutations identified by NGS (Table 2). One patient had pathogenic variants in two genes.

Discussion
A genetic cause of suspected hereditary peripheral neuropathy was identified in 35 of the 103 patients in this study. Seven had duplication/deletion of PMP22, while clearly/likely pathogenic point mutations were identified with NGS in 28 (27 %). By comparison, our former approach of Sanger sequencing seven genes (due to capacity constraints), would have detected pathogenic variants. A total of 35 patients (34 %) thus received a genetic diagnosis, distributed among 15 different genes. In all, 28 (27 %) patients had point mutations identified by NGS (Table 2).

Table 2 Variants classified as certain or likely pathogens, detected by next-generation sequencing of samples sent to the Section of Medical Genetics, Telemark Hospital, due to suspected hereditary peripheral neuropathy (N = 103). Results are presented alongside those from other Norwegian studies. Note that the studies include different populations and have different designs. For example, the earlier studies included patients with suspected Charcot-Marie-Tooth disease, whereas the present study includes patients with suspected hereditary peripheral neuropathies in general. The studies cannot therefore be compared directly. No point mutations were detected in the following genes, which have therefore been excluded from the table: AIP7A, CDP1, DCTN1, EGR2, FAM134B, FIG4, GAN, GARS, GDA1, HK1, HSPB3, HSPB8, IKBKAP, LITAF, MED25, MTM1, NDRG1, NSF, NTRK1, PLEKHGS, PMP22, POLG, PRPS1, PRX, RAB7, SBF2, SEPT9, SLC12A6, S0X10, SPTLC1, TRPV4, WNK1, YARS

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<tr>
<td></td>
<td>N = 81</td>
<td>Per cent</td>
<td>number</td>
</tr>
<tr>
<td>PMP22 duplication³</td>
<td>14 (11)</td>
<td>6 (26)²</td>
<td>14 (11)</td>
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<tr>
<td>PMP22 deletion³</td>
<td>– –</td>
<td>– –</td>
<td>– –</td>
</tr>
<tr>
<td>GJB1</td>
<td>6 (5)³</td>
<td>5 (20)</td>
<td>7 (6)</td>
</tr>
<tr>
<td>SH3TC2</td>
<td>– –</td>
<td>– –</td>
<td>5 (4)</td>
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<tr>
<td>MFN2</td>
<td>4 (3)³</td>
<td>2 (7)²</td>
<td>5 (4)</td>
</tr>
<tr>
<td>SOD1</td>
<td>– –</td>
<td>– –</td>
<td>1 (1)</td>
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<tr>
<td>HSPB1</td>
<td>– –</td>
<td>– –</td>
<td>1 (1)</td>
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<tr>
<td>MP2</td>
<td>1 (1)</td>
<td>1 (6)²</td>
<td>1 (1)</td>
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<tr>
<td>DMN2</td>
<td>– –</td>
<td>– –</td>
<td>1 (1)</td>
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<tr>
<td>LMNA</td>
<td>– –</td>
<td>– –</td>
<td>2 (2)²</td>
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<tr>
<td>AARS/ATL1⁴</td>
<td>– –</td>
<td>– –</td>
<td>0 (0)</td>
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<tr>
<td>ATL1</td>
<td>– –</td>
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<td>0 (0)</td>
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<tr>
<td>ARHGEF10</td>
<td>– –</td>
<td>– –</td>
<td>1 (1)</td>
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<tr>
<td>BSCL2</td>
<td>– –</td>
<td>– –</td>
<td>0 (0)</td>
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<tr>
<td>DYNC1H1</td>
<td>– –</td>
<td>– –</td>
<td>1 (1)</td>
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<tr>
<td>FGD4</td>
<td>– –</td>
<td>– –</td>
<td>0 (0)</td>
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<tr>
<td>IGHMBP2</td>
<td>– –</td>
<td>– –</td>
<td>0 (0)</td>
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<tr>
<td>KIF1B</td>
<td>– –</td>
<td>– –</td>
<td>1 (1)</td>
</tr>
<tr>
<td>MP2/MFN2⁵</td>
<td>– 0 (1)</td>
<td>1 (1)</td>
<td>0 (0)</td>
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<tr>
<td>NEFL</td>
<td>– 0 (0)</td>
<td>0 (0)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>REEP1/SED⁶</td>
<td>– –</td>
<td>– –</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Total mutations</td>
<td>25 (20)</td>
<td>14 (60)³</td>
<td>44 (36)</td>
</tr>
<tr>
<td>Total point mutations</td>
<td>11 (9)</td>
<td>8 (33)³</td>
<td>30 (24)</td>
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</table>

¹ Only variants judged to be certain or likely pathogens are listed
² Study based on the same patients as Braathen et al. 2011
³ Duplication or deletion studied with Multiplex Ligation Probe Amplification (MLPA)
⁴ Value may be artificially low, as additional patients may have been tested for PMP22-duplication/deletion prior to referral and thus not included in the study
⁵ Number has been altered compared to original article after communication with the author (G. J. Braathen)
⁶ Pathogenic variants found in two different genes
most cases been diagnosed during earlier assessment in a neurology department.

There are currently two centres in Norway that test for point mutations associated with hereditary peripheral neuropathies – the University Hospital of North Norway and Telemark Hospital. We therefore cannot exclude the possibility that the proportion of point mutations detected in those genes that are «traditionally» tested is artificially low in this study (12%). Patients who tested positive for mutations in these genes may have been excluded at an earlier stage, such that we instead received samples from those with negative results in previous genetic testing. On the other hand, the percentage of point mutations in the seven genes traditionally tested was higher (12%) in this study than in the study from Northern Norway (8%) (18). Both of these studies recruited patients from a clinical population (18). However, patients in the eastern Akershus study were recruited from the general population (6). The studies in which NGS was performed are therefore not directly comparable.

Genes that are typically regarded as rare causes of hereditary peripheral neuropathy and are not therefore Sanger sequenced (e.g. SH3TC2, HSPB1, and SOD1) were relatively common in both this group of patients and those from eastern Akershus (6), as shown in Table 2. Other genes have been routinely Sanger sequenced (e.g. EGR2 and LITAF), but have not yet been shown to contain pathogenic mutations – either in Norwegian (Table 2) or Spanish (19) studies. Mutations were detected in a wide range of genes in both our current and previous studies (6); as more patients are tested, this range is likely to expand further. This increases the benefits, in our view, of using NGS. For analyses performed with Sanger sequencing, the discovery rate in Norwegian studies is lower than in international studies (19–21). The reason for this is not apparent, but it is possible that currently undiscovered genes may play a larger role in Scandinavia, but it is possible that currently undiscovered genes may play a larger role in Scandinavia, or that foreign patient populations are more highly selected.

The number of genes associated with heterogeneous diseases has increased rapidly following the introduction of NGS. The gene panel used in this study therefore does not contain all 90 of the genes associated with peripheral neuropathies today. A gene panel for NGS can easily be updated to a newer version, however.

The technical quality of NGS in this study, with > 99% coverage at a depth of 30x, was almost as good as that previously reported for Sanger sequencing (accuracy > 99.9%) (24). However, NGS is significantly faster at sequencing large numbers of genes. Another advantage in our view is that multiple variants are considered simultaneously in an overall picture, making it easier to detect pathogenic variants in other genes. In contrast to exome sequencing, there is no possibility of making incidental findings in gene-panel NGS because only disease-relevant genes are studied.

It was previously thought that around 90% of the genetic defects in Charcot-Marie-Tooth disease are present in only four genes (PMP22 duplication/deletion or point mutations in GJB1, MPZ, and MFN2) (18–20). As a result, these genes were usually analysed first if no other information was provided, as recommended by Norwegian guidelines (15, 16). However, in recent Norwegian studies, including this one, this picture no longer appears correct now that more of the genes associated with peripheral neuropathy are being tested (6). Moreover, mutations in genes considered to be rare causes of Charcot-Marie-Tooth disease have been detected more frequently than mutations in some of the supposedly most common genes. Since populations and selection methods differ between studies, however, it can be difficult to compare results and draw firm conclusions.

Nevertheless, the fact that so many more mutations are discovered when more genes are tested surely suggests that there is a need to reconsider current practice. There is now a consensus among international experts on peripheral neuropathies that the most profitable strategy for genetic testing is NGS, after exclusion of PMP22 duplication/deletion and possibly Sanger sequencing of GJB1 (12, 22).

We wish to thank Hilde Tveitan Hilmarsen and Anne Signe Be for verifying variants by Sanger sequencing.

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References


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Table 1  Recommended criteria for classification of variants. If a variant satisfies criteria belonging to different classes, it is generally assigned to the lowest class. It is important to emphasise that these criteria are for guidance only, and that interpreting genetic variants involves a considerable degree of judgement. None of the criteria in the table below should therefore be considered absolute requirements. Table adapted from Høyer et al. (6)

<table>
<thead>
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<th>Class</th>
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| 1     | Clearly neutral variant | 1. Present in $\geq 1\%$ of dbSNP137, 1000 genomes and/or Exome Sequencing Project (ESP)  
2. and/or present in $\geq 4$ internal controls$^1$  
3. Described as benign in multiple high-quality published reports |
| 2     | Likely neutral variant | 1. Present in 0.1–1.0 $\%$ of dbSNP137, 1000 genomes and/or ESP  
2. and/or present in 2–3 unrelated internal controls  
3. and/or described as benign in the literature  
4. and/or found in other phenotypes in multiple patients at the Department of Medical Genetics, Telemark Hospital  
5. and/or loss/gain of splice sites predicted by 5/5 predictors (Splice-SiteFinder-Like, MaxEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder) (applies only to variants in introns) |
| 3     | Variant of unknown significance (VUS) | 1. Present in $\leq 0.1\%$ of dbSNP137, 1000 genomes and/or ESP  
2. and/or present in $\leq 1$ internal controls  
3. Divergent predictions from variant prediction databases/software: SIFT, Polyphen, Align GVGD and Mutation Taster (applies only to non-synonymous variants in coding regions) |
| 4     | Likely pathogenic variant | 1. Reported as pathogenic in one study, with the same genotype and phenotype  
2. and/or functional studies available showing a physical effect of the mutation  
3. and/or proximity to other reported pathogenic mutations with similar degree of amino acid conservation that correlates with phenotype and zygosity. Also, predicted pathogenic in 2 of 4 variant prediction databases/software: SIFT, Polyphen, Align GVGD and Mutation Taster (applies only to non-synonymous variants in coding regions)  
4. and/or stop codon mutations $\geq 50$ bp upstream of the last exon-intron boundary in coding regions  
5. and/or frameshift insertions/deletions  
6. and/or loss/gain of splice sites predicted by multiple predictors (Splice-SiteFinder-Like, MaxEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder)  
7. and/or found in multiple patients with similar phenotypes by Section of Medical Genetics |
| 5     | Clearly pathogenic variant | 1. Reported as pathogenic in multiple studies, and with an appropriate phenotype and the same genotype as that reported previously. Established causal relationship with phenotype  
2. Multiple functional studies available showing a physical effect of the mutation. Disease may be due to loss or gain of function |

$^1$ The internal controls used in this analysis undergo neurological examination to exclude peripheral neuropathy, and they are not related to individuals with peripheral neuropathy.