Original Investigation

Mutations in VRK1 Associated With Complex Motor and Sensory Axonal Neuropathy Plus Microcephaly

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IMPORTANCE Patients with rare diseases and complex clinical presentations represent a challenge for clinical diagnostics. Genomic approaches are allowing the identification of novel variants in genes for very rare disorders, enabling a molecular diagnosis. Genomics is also revealing a phenotypic expansion whereby the full spectrum of clinical expression conveyed by mutant alleles at a locus can be better appreciated.

OBJECTIVE To elucidate the molecular cause of a complex neuropathy phenotype in 3 patients by applying genomic sequencing strategies.

DESIGN, SETTING, AND PARTICIPANTS Three affected individuals from 2 unrelated families presented with a complex neuropathy phenotype characterized by axonal sensorimotor neuropathy and microcephaly. They were recruited into the Centers for Mendelian Genomics research program to identify the molecular cause of their phenotype. Whole-genome, targeted whole-exome sequencing, and high-resolution single-nucleotide polymorphism arrays were performed in genetics clinics of tertiary care pediatric hospitals and biomedical research institutions.

MAIN OUTCOMES AND MEASURES Whole-genome and whole-exome sequencing identified the variants responsible for the patients’ clinical phenotype.

RESULTS We identified compound heterozygous alleles in 2 affected siblings from 1 family and a homozygous nonsense variant in the third unrelated patient in the vaccinia-related kinase 1 gene (VRK1). In the latter subject, we found a common haplotype on which the nonsense mutation occurred and that segregates in the Ashkenazi Jewish population.

CONCLUSIONS AND RELEVANCE We report the identification of disease-causing alleles in 3 children from 2 unrelated families with a previously uncharacterized complex axonal motor and sensory neuropathy accompanied by severe nonprogressive microcephaly and cerebral dysgenesis. Our data raise the question of whether VRK1 mutations disturb cell cycle progression and may result in apoptosis of cells in the nervous system. The application of unbiased genomic approaches allows the identification of potentially pathogenic mutations in unsuspected genes in highly genetically heterogeneous and uncharacterized neurological diseases.

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Hereditary motor and sensory neuropathies (HMSNs) represent a group of slowly progressive neurological diseases caused by dysfunction of the peripheral nerves with secondary muscle wasting and weakness usually presenting as a distal symmetric polyneuropathy. Hereditary motor and sensory neuropathies are genetically heterogeneous, with more than 40 disease-causing genes identified to date. Patients most often present with isolated nerve disease, but on rare occasions neuropathy is accompanied by additional clinical signs and symptoms yielding a complex neuropathy. The development of genome-wide analysis methods has enabled the identification of novel HMSN-associated genes and has revealed that some patients with atypical peripheral neuropathy have mutations in known HMSN genes.

We studied 3 patients with a severe, rapidly progressing distal symmetric polyneuropathy who also had microcephaly. Using genomic sequencing approaches, we identified likely pathogenic variants in the 3 affected individuals from 2 different families in the vaccinia-related kinase 1 gene (VRK1; GenBank NM_003384).

**Methods**

This research project was approved by the Baylor College of Medicine Institutional Review Board. Written informed consent was obtained from the parents of the subjects.

**Participants**

Two sisters (patients BAB3022 and BAB3280), aged 10 and 2 years, respectively, and a 9-year-old boy (patient BAB5311) from an unrelated family presented with delayed motor development and microcephaly with normal cognition. Patient BAB3022 was born after an uncomplicated full-term pregnancy; delayed motor development and microcephaly were noted on the 18-month well-child care visit. She began rolling over at 12 months, sat at 24 months, and was never able to walk without support. She became wheelchair bound at age 4 years, and 2 years later she developed limited use of her arms and hands. The patient experienced worsening scoliosis, compromising her respiratory function and requiring surgical intervention at age 10 years. Due to worsening respiratory function and decreased oral intake, tracheostomy and gastrostomy placement were implemented. She attends regular school and her cognitive testing did not reveal any intellectual deficits. Physical evaluations revealed nonprogressive microcephaly, hypotonia, progressive muscle wasting, diffuse weakness progressing from distal to proximal muscle groups, and loss of deep tendon reflexes. The patient did not have hypertonia, abnormal movements, or nystagmus.

Extensive clinical evaluation included brain magnetic resonance imaging (MRI) at ages 1 and 4 years, showing microcephaly with a simplified gyral pattern but no evidence of other intracranial abnormalities (Figure 1A). Electrophysiological studies showed evidence of severe axonal motor and sensory peripheral neuropathy with severely decreased amplitudes; some sensory and motor nerves were nonresponsive (Table). Axonal etiology was further supported by histopathological studies of the sural nerve that showed mild to moderate reduction of the number of large myelinated fibers and a reduced number of unmyelinated axons (Figure 1C). Muscle biopsy showed marked variation in fiber size and shape and large-group atrophy with increased connective tissue that was suggestive of neurogenic myopathy (Figure 1D). The patient underwent extensive genetic laboratory evaluation, and findings were normal on metabolic, cytogenetic, and molecular studies. Despite numerous efforts, the cause of the neurological problems remained unexplained.

The affected sibling, patient BAB3280, presented with microcephaly in utero (Figure 1B). Her motor development was delayed; she was unable to sit or walk without support at age 20 months. Intellectual development was concordant with expected milestones. She manifested mild hypotonia but had normal muscle bulk, antigravity or greater power in all extremities, and normal deep tendon reflexes. No abnormal movements were appreciated during physical examination. Brain MRI showed microcephaly and a simplified gyral pattern but no other cranial abnormalities (Figure 1A). The electrophysiological studies found evidence for axonal motor and sensory neuropathy as in her older sibling (Table). Family history was negative for neurological disorders, and the parents denied consanguinity. The affected girls have another sister who at age 5 years is normocephalic with normal motor and intellectual development and normal findings on physical examination. The presence of clinical symptoms in 2 affected children suggested a genetic cause with a potential autosomal recessive mode of inheritance.

Patient BAB5311 was a fraternal twin, conceived by in vitro fertilization and born at 34.5 weeks of pregnancy. Prenatal surveillance ultrasonography revealed microcephaly at 30 weeks' gestation and decreased fetal movements. The patient began to noticeably deviate from his developmental milestones at age 4 months. He began to sit at 8 months, walked at 18 months, and remained ambulatory until 6 years. Clinical evaluation at age 9 years revealed severe nonprogressive microcephaly (6 SDs below mean; Figure 2B), worsening hypotonia, muscle atrophy, tongue atrophy, decreased deep tendon reflexes, and preserved sensation and cognition. Medical history was also positive for sleeping problems, tremor, hypophonia, and dysarthric speech. Similar to patient BAB3022, the patient has had worsening scoliosis and required gastric feeding tube placement. Brain MRI showed microcephaly, simplified gyral pattern, normal pons and cerebellar hemispheres, and underdeveloped cerebellar vermis (Figure 2A). The electrophysiological studies revealed motor and sensory axonal neuropathy (Table). Neither the parents nor the twin sister have any evidence of neurological problems based on self-report of lack of neuropathy symptoms and objective clinical examination (Figure 2C).

**Clinical and Electrophysiological Assessments**

The clinical evaluation was performed by several independent neurologists and medical geneticists. The MRI scans of all affected individuals were evaluated by a pediatric neuroradiologist. Electron microscopy of a sural nerve biopsy specimen and hematoxylin-eosin staining of a muscle biopsy specimen of patient BAB3022 were evaluated by a neuropathologist.
Neurophysiological studies were carried out in the affected individuals; motor nerve conduction velocities and sensory nerve conduction velocities were determined.

**Genomic Sequencing**

Patient BAB3022 underwent whole-genome sequencing on the SOLiD sequencing platform (Life Technologies Corp) at ap-
proximately 30 times the average depth of coverage. Mapping to the reference human genome sequence and variant calling were performed using the SOLiD software tools and Corona Lite bioinformatic suite. Patient BAB3280 underwent whole-exome capture using the Baylor College of Medicine Human Genome Sequencing Center VCRome version 2.1 design (Roche NimbleGen, Inc) followed by sequencing on the HiSeq platform (Illumina, Inc) at approximately 100 times the average depth of coverage through the Centers for Mendelian Genomics research initiative. Sequence data were aligned and mapped to the human genome reference sequence (hg19) using the Mercury in-house bioinformatic pipeline. Variants were called using the ATLAS3 and SAMtools4 suites and annotated with an in-house–developed annotation pipeline that uses ANNOVAR5 and additional tools and databases. Patient BAB5311 underwent whole-exome sequencing in a clinical molecular laboratory. Raw sequence data (fastq files) were transferred to Baylor College of Medicine and analyzed using the bioinformatic approaches previously described for patient BAB3280.

### Confirmation of Variants

Candidate variants in all the affected individuals were confirmed by Sanger sequencing after polymerase chain reaction amplification of the region containing the respective variants using specific primers. Segregation of the variants was tested in the 2 sets of parents and available unaffected siblings.

### Single-Nucleotide Polymorphism Array Genotyping

High-resolution single-nucleotide polymorphism arrays were performed for patients BAB5311, BAB5320, and BAB5321 using the Illumina HumanOmni2.5 BeadChip according to the manufacturer’s protocols. Analysis was performed using the genotyping application of the Illumina GenomeStudio software.

### Results

For sisters BAB3022 and BAB3280, analysis of the sequence data under a recessive model resulted in identification of 2 likely disease-causing variants in VRK1: chr14:96 391 443 (G>A) (c.G706A; p.V236M) and chr14:96 382 234 (G>A) (c.G266A; p.R89Q). No disease-associated variants were noted in any known HMSN-associated genes. Both variants were found in the affected sisters as compound heterozygous alleles that were inherited from nonconsanguineous heterozygous carrier parents (Figure 1E). The unaffected sister did not inherit either VRK1 mutation. Both variants are novel, not previously observed or reported in any database to our knowledge, and predicted to be damaging to protein function by independent bioinformatic algorithms.

Patient BAB5311 was found to have a homozygous nonsense mutation in VRK1: chr14:97 342 370 (C>T) (c.C1072T; p.R358X). This variant is a reported disease-causing mutation (rs137853063) as it had been previously identified in a patient with atypical pontocerebellar hypoplasia (PCH) type 1A (OMIM #607596). Currently, the frequency of this variant is thought to be approximately 0.007% in the general population and 0.0116% in the population of European descent. Both parents, who are self-reported to be of Ashkenazi Jewish ancestry but not related, and the unaffected twin sister of the pa-

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### Table. Electrophysiological Studies Showing Evidence for Axonal Motor and Sensory Peripheral Neuropathy

<table>
<thead>
<tr>
<th>NCS</th>
<th>Patient No.</th>
<th>Age 4 y</th>
<th>Age 10 y</th>
<th>Age 20 mo</th>
<th>Age 8 y</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right median</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity, m/s</td>
<td>BAB3022</td>
<td>53 (&gt;38.2)</td>
<td>NR</td>
<td>46.5 (&gt;39.2)</td>
<td>69 (&gt;45)</td>
</tr>
<tr>
<td>NP amplitude, μV</td>
<td>BAB3280 at Age 20 mo</td>
<td>2 (3.2-13.8)</td>
<td>NR</td>
<td>1.6 (3.7-11.6)</td>
<td>0.02 (3.0-14.3)</td>
</tr>
<tr>
<td>Right ulnar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity, m/s</td>
<td>BAB3022</td>
<td>NA</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>NP amplitude, μV</td>
<td>BAB3280 at Age 20 mo</td>
<td>NA</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Right sural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity, m/s</td>
<td>BAB3022</td>
<td>NA</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>NP amplitude, μV</td>
<td>BAB3280 at Age 20 mo</td>
<td>NA</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Motor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right median</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity, m/s</td>
<td>BAB3022</td>
<td>38.1 (&gt;50)</td>
<td>30.2 (&gt;46.5)</td>
<td>45 (&gt;50)</td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>BAB3280 at Age 20 mo</td>
<td>11.4 (13-76)</td>
<td>32.2 (14-82)</td>
<td>25 (13-76)</td>
<td></td>
</tr>
<tr>
<td>Right common peroneal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity, m/s</td>
<td>BAB3022</td>
<td>46.5 (&gt;50)</td>
<td>NA</td>
<td>43 (&gt;50)</td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>BAB3280 at Age 20 mo</td>
<td>2.3 (7-60)</td>
<td>NA</td>
<td>23 (7-60)</td>
<td></td>
</tr>
<tr>
<td>Right tibial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity, m/s</td>
<td>BAB3022</td>
<td>NA</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>BAB3280 at Age 20 mo</td>
<td>NA</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; NCS, nerve conduction study; NP, negative peak; NR, nonrecordable.

*Reference ranges are in parentheses.*
Patient were confirmed to be heterozygous carriers for this mutation. We performed high-resolution single-nucleotide polymorphism arrays on the affected patient and both parents to narrow a potential common haplotype region in which the mutation occurred. We confirmed by single-nucleotide polymorphism array data that the parents are not related, and we were able to narrow the common haplotype to a homozygous 500 673–base pair (g.chr14:97 072 050-97 572 723) genomic interval including only \( VRK1 \) and harboring the founder p.R358X mutation (Figure 3).

Discussion

Whole-exome sequencing is a powerful research and diagnostic tool that allows identification of sequence variants in genetically and clinically heterogeneous conditions.\(^8\)\(^9\) Hereditary motor and sensory neuropathies represent a genetically heterogeneous group of disorders, with more than 40 disease-associated genes to date.\(^3\)\(^4\) All affected individuals in this study manifested an autosomal recessive axonal neuropathy associated with microcephaly and cerebral dysgenesis. The latter finding is inconsistent with mutations in known HMSN-associated genes. The patients did not meet clinical criteria for any known form of HMSN or other hereditary neurodevelopmental disorder, and extensive molecular investigations that included sequencing of known HMSN genes failed to delineate the molecular cause of the disease. Other possible disorders that may include neuropathy accompanied by microcephaly, such as some congenital disorders of glycosylation, were unlikely based on the lack of additional clinical features and normal findings on biochemical analyses.

Genomic studies identified many sequence variants in these 2 sisters (10 366 nonsynonymous variants in patient BAB3022 and 10 555 in patient BAB3280), of which they shared novel compound heterozygous mutations in 3 genes. However, only the compound heterozygous \( VRK1 \) mutations both could explain the observed phenotype and were consistent...
with mendelian expectations. It is a challenge to identify potential disease-causing variants in the myriad of variants produced by genomic sequencing approaches. Interpretation of candidate variants can be aided by prediction algorithms of the variant’s effect on the function and structure of the normal protein and the frequencies of rare and polymorphic variants in other individuals not affected by the observed phenotype. However, these data are most informative when applied in the context of a genetic model that considers the family history and overlapping clinical presentation between affected individuals.

The homozygous nonsense mutation in VRK1, identified in patient BAB5311, had been previously reported in only 1 consanguineous Israeli family with progressive microcephaly of prenatal onset, PCH, neurogenic muscle atrophy, and mild intellectual disability.6 Affected individuals in the family presented with early-onset ataxia and hyperreflexia followed by progressive muscle wasting and areflexia. The electrophysiological studies showed evidence of motor sensory neuropathy, but no further characterization was provided. Interestingly, this variant seems to have occurred as a founder variant in the Ashkenazi Jewish population, as both parents of our patient BAB5311 are of Ashkenazi Jewish ancestry and share the identical mutation and surrounding haplotype found in the family described by Renbaum et al.6

The PCHs are classified into categories PCH1 through PCH8 based on clinical findings observed on radiologic brain and spine imaging studies (ie, cerebellar and pontine hypoplasia), neurological signs and symptoms, and the natural history of the disease. Currently, classification of PCH does not consider underlying genetic causes such as mutations in genes like TSEN54, in which defects result in different PCH types.11 The original classification of PCH distinguished PCH1 from other forms of PCH based on early onset of respiratory distress, hypotonia, weak tendons, and central and peripheral motor dysfunction (the latter thought to occur due to anterior horn cell degeneration) and death occurring within the first few months of life.12 14 Conversely, PCH2 through PCH8 present with progressive microcephaly and additional neurological findings including extrapyramidal dyskinesia, dystonia, and epilepsy, without motor or anterior horn cell involvement and degeneration.12,13
Concordant with the previous report of the family with mutations in VRK1, patients BAB3022, BAB3280, and BAB5311 share many clinical features with the described patients (microcephaly, peripheral neuropathy with secondary muscle atrophy), but their presentation is quite distinct owing to the lack of observed PCH on MRI, lack of central nervous system neurological symptoms (ataxia, hypertonia), and normal cognitive function. Additionally, some features are shared by our cases and those that have been previously reported (microcephaly, hypertonia, motor neuron disease), the phenotype observed in our patients with mutations in VRK1 do not conform within the clinical definition of PCHA as our patients do not have PCH and do have normal intellectual development.

The vaccinia-related kinase 1 gene encodes a serine/threonine kinase that is crucial for cell cycle progression and cell division and is proposed to be involved in nervous system development and maintenance. It is an early response gene that directly phosphorylates important transcription factors such as p53, c-Jun, and activating transcription factor 2 (ATF2). The gene is ubiquitously expressed in human tissues, with higher levels of expression in mitotically active cells. Null alleles of VRK1 result in embryonic lethality in Caenorhabditis elegans and Drosophila melanogaster, while hypomorphic alleles lead to sterility in mice due to the inability of germ cells to proliferate and differentiate.

The mutations observed in the 2 sisters of the first family, p.V236M and p.R89Q, localize to the kinase domain of the protein and are considered to be damaging by bioinformatic prediction algorithms. It is challenging to explain why VRK1 mutations result in pathology restricted to the nervous system. Nevertheless, mutations in many different genes involved in DNA replication, DNA damage response, and DNA single-strand break repair do result in neurological phenotypes. Moreover, mutations in genes whose proteins are important for DNA replication and cell cycle progression can result in syndromic microcephaly.

The VRK1 protein and p53 directly interact by phosphorylating each other in an autoregulatory loop. The VRK1 uniquely phosphorylates p53 at Thr18, stabilizing and activating it to interact with other transcription factors and preventing the binding of mouse double minute 2 homolog (MDM2). It has been proposed that phosphorylation of p53 in response to oxidative stress is connected to the kinase function of cleavage and polyadenylation factor 1 subunit 1 (CLP1). Disruption of the latter was shown recently to result in fatal deterioration of motor function due to progressive loss of motor neurons potentially resulting in abnormal apoptosis. We hypothesize that a similar mechanism of abnormal neural apoptosis may explain the neurological phenotype observed in patients with VRK1 mutations. Some evidence of this process was found on brain MRI studies for patient BAB5311 in which enlarged Virchow-Robin/perivascular spaces were observed in addition to progressive volume loss (Figure 2A). More experimental studies are required to better characterize the role of VRK1 in the nervous system and elucidate the biological consequences of mutations in human subjects. In conclusion, VRK1 may be a novel HMSN locus that can be associated with a complex peripheral neuropathy phenotype: autosomal recessive axonal motor sensory neuropathy and microcephaly.