Brain Dopamine–Serotonin Vesicular Transport Disease and Its Treatment

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SUMMARY

We describe a disease encompassing infantile-onset movement disorder (including severe parkinsonism and nonambulation), mood disturbance, autonomic instability, and developmental delay, and we describe evidence supporting its causation by a mutation in SLC18A2 (which encodes vesicular monoamine transporter 2 [VMAT2]). VMAT2 translocates dopamine and serotonin into synaptic vesicles and is essential for motor control, stable mood, and autonomic function. Treatment with levodopa was associated with worsening, whereas treatment with direct dopamine agonists was followed by immediate ambulation, near-complete correction of the movement disorder, and resumption of development.

KNOWN DISORDERS OF BIOGENIC AMINE NEUROMEDIATORS (DOPAMINE, norepinephrine, epinephrine, and serotonin) involve defects in nine enzymes1-9 and one transporter.10 Affected persons present in early childhood with symptoms referable to the affected neurotransmitter, and the disorders are diagnosed by measurement of neurotransmitter breakdown products in the cerebrospinal fluid (CSF). A deficiency in dopamine results in movement disorder; deficient norepinephrine or epinephrine causes autonomic dysfunction; and serotonin deficiency leads to sleep and psychiatric disturbances.2,3,6

We describe members of a family with symptoms of deficiencies in dopamine (dystonia, parkinsonism, and oculogyric crises), serotonin (sleep and mood disturbance), and epinephrine and norepinephrine (diaphoresis, temperature instability, ptosis, and postural hypotension), with no demonstrable deficiency of neurotransmitters in the CSF. Genome investigation revealed a mutation in the gene encoding VMAT2 that compromises transport of biogenic amines into synaptic vesicles, resulting in impairment of their synaptic transmission without detectable reductions in their amounts.

CASE REPORT

Eight children of an extended consanguineous Saudi Arabian family had similar clinical symptoms of a complex movement disorder that was inherited in an autosomal recessive fashion (Fig. 1A; and Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The parents were unaffected, but at least five had clinical depression.

The index patient, when she presented to us, was a 16-year-old girl with global developmental delay and abnormal movements. She had first been brought to medical attention at 4 months of age with hypotonia, loss of acquired head control, and paroxysmal stereotyped episodes of persistent eye deviation and crying.
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At 16 years of age, she had fatigue, excessive diaphoresis, profuse nasal and oropharyngeal secretions, noisy breathing, hypernasal speech, poor distal perfusion, cold hands and feet, disrupted sleep, hypotonia, dysarthria, and ataxia. There was no diurnal variation and no improvement but had slowed after presentation. The girl sat at 30 months, crawled at 4 years, and walked at 13 years. At 16 years of age, she had fatigue, excessive diaphoresis, profuse nasal and oropharyngeal secretions, noisy breathing, hypernasal speech, poor distal perfusion, cold hands and feet, disrupted sleep, hypotonia, dysarthria, and ataxia. There was no diurnal variation and no improvement but had slowed after presentation. The girl sat at 30 months, crawled at 4 years, and walked at 13 years.

Methods

GENETIC STUDIES

The study was approved by the research ethics board of the Hospital for Sick Children, and parents provided written informed consent. We genotyped more than 300,000 single-nucleotide polymorphisms (SNPs) in eight family members, V-3, V-4, V-6, V-7, V-8, V-9, V-10, and VI-2 (Fig. 1A) (with the use of an Illumina 300K SNP microarray), followed by homozygosity mapping to identify the homozygous loci shared by the affected children. A subset of 2500 SNPs with a minimal allele frequency greater than 0.4 in the population genotyped as part of the international HapMap study and with average spacing of approximately 1.0 Mb was selected for parametric linkage analysis. We used Sanger sequencing of candidate gene exons to identify the mutation, whole-exome sequencing (Agilent V4 50Mb capture kit and Illumina HiSeq 2000 sequencing) to rule out other mutations, and TaqMan genotyping to confirm the absence of the mutation in controls.

FUNCTIONAL ANALYSIS OF P387L-MUTANT VMAT2

We engineered a construct encoding VMAT2 that contained the P387L substitution, and we carried out an assay of vesicular serotonin uptake in a...
heterologous cell system (see the Methods section in the Supplementary Appendix). Transport mediated by VMAT2 was measured by incubating membrane preparations with tritiated serotonin, followed by rapid washing and filtration to retain vesicles with trapped substrate.

**RESULTS**

**MUTATION IDENTIFICATION**

Homozygosity mapping identified a single homozygous 3.2-Mb interval on chromosome 10q in the region of 10q25.3-26.11 that was shared by five affected family members but not by unaffected members (Fig. 1B). Parametric linkage analysis revealed a significant logarithm of odds (lod) score of 4.1 in this region. Another locus, on chromosome 3, yielded a significant lod score of 3.1 but did not correspond to a region of shared homozygosity. We sequenced exons and exon–intron boundaries of eight genes known to have neuronal functions and observed a novel variant (c.1160C→T) in exon 13 (Fig. 1C), which is predicted to result in a substitution of leucine for proline at position 387 (p.P387L) in VMAT2. The variant was homozygous in affected family members but not in 78 unaffected members, 26 of whom carried the variant in the heterozygous state.

We also performed whole-exome sequencing in the proband, which independently identified the SLC18A2 change and revealed no other novel nonsynonymous variant in the linked region of shared homozygosity. SLC18A2 c.1160C→T is not present in data sets of sequenced genomes, including the 1000-genome database. In addition, screening for SLC18A2, one of the most extensively studied candidate genes for involvement in Parkinson's disease, was previously performed in 704 healthy persons of diverse ethnic backgrounds and 452 patients with Parkinson's disease,\(^{11-13}\) none of whom had the c.1160C→T change. Collectively, these results suggest that SLC18A2 c.1160C→T is the causative defect in this family.

**FUNCTIONAL CHARACTERIZATION OF P387L-MUTANT VMAT2**

SLC18A2 encodes the VMAT2 protein located in membranes of monoamine synaptic vesicles (Fig. 1D, 2A, and 2B). Proline residues adjacent to transmembrane segments have major structural effects and are overrepresented among residues subject to disease-causing substitutions.\(^{15}\) The proline residue in the 387 position (Pro387) of the VMAT2 protein is adjacent to a transmembrane segment (Fig. 1D). Sequence alignment shows that Pro387 is highly conserved through evolution and thus suggests that its substitution is likely to be deleterious. It is also conserved in the paralogous protein VMAT1 and in the Caenorhabditis elegans CAT-1 protein — the single vesicular monoamine transporter in nematodes (Fig. 1E).\(^{16}\) The residue is not conserved in the vesicular acetylcholine transporter, which maintains 39% identity with VMAT2; this finding implies that Pro387 may have a specific role in monoamine transport.

To determine the effect of the P387L mutation on VMAT2 transport activity, we transiently and separately expressed nonmutant and mutant human VMAT2 in COS-7 cells. Immunoblot analysis of membrane preparations confirmed equivalent levels of mature glycosylated VMAT2 in parallel transfections, suggesting that there was no major defect in protein processing. However, P387L-mutant VMAT2 showed dramatically decreased activity as compared with nonmutant VMAT2 (Fig. 2C). Use of the specific VMAT inhibitor reserpine confirmed that P387L-mutant VMAT2 still exhibited some weakly measurable uptake (Fig. 2D). Thus, the P387L mutation results in severe, but not complete, loss of function.

**TREATMENT**

Defective monoamine loading into synaptic vesicles, and therefore neurotransmission, was consistent with symptoms of monoamine deficiency in affected members of the family, despite their normal levels of brain monoamine. With this insight, we gave the proband a direct dopamine-receptor agonist (pramipexole), which resulted, within 1 week, in dramatic and sustained disappearance of parkinsonism and dystonic attacks and improvement in other symptoms (Table 1). We then provided treatment to the younger siblings, who also had improvement. It seemed that the younger the affected child, the more substantial the recovery (Table 1). The affected children are now in their 32nd month of treatment, with continuing benefits and minimal side effects (slight overactivity and weight loss).
DISCUSSION

The mutation in SLCL8A2 that we describe here is expected to affect monoamine neurotransmission and thus result in a phenotype that has overlap with all monoamine disorders. Because movement disorder is conspicuous among symptoms of monoamine disturbance, the clinical picture of the disease that we describe is closest to that of diseases affecting dopamine — chiefly, deficien-
cies in dopamine transporter, tetrahydrobiopterin, tyrosine hydroxylase, and aromatic l-amino acid decarboxylase (AADC) (Fig. 2A and 2B). The phenotype of the affected siblings has particular similarity to AADC deficiency in that it improves with direct dopamine agonism but not with levodopa, although the siblings had greater improvement than that typically observed in those with AADC deficiency treated with dopamine agonists, and rather than having a lack of response to levodopa, the siblings had a worsening of symptoms. Two other features that distinguish the disease we describe here from AADC deficiency are the lack of improvement with the AADC enzyme cofactor vitamin B₆ and the absence of worsening in the evening, which in AADC deficiency is the result of neurotransmitter depletion due to insufficient production.¹⁴,⁷,⁸

The standard diagnostic test in patients with suspected diseases of monoamine metabolism is the measurement of monoamine metabolites in the CSF. Because each specific defect results in a particular metabolite profile, this single test specifies the disease.²,³,⁶ Analysis of monoamines or their metabolites in urine is not reliable for the diagnosis of monoamine neurotransmitter diseases,²,³,⁶ except for one — AADC deficiency — in which increased 3-O-methyldopa with decreased vanillylmandelic acid (Fig. 2A) in the proper clinical context is highly suggestive and generally confirmed by mutation analysis.¹,⁴,⁷,⁸ In the present condition, the urine shows abnormalities because VMAT2 also functions at sites outside the central nervous system, including the peripheral nervous system, adrenal medulla, and platelets.¹⁷ Our detection of abnormalities in the urine but not the CSF may reflect differences in monoamine and metabolite stabilities, processing, and normal ranges between the brain and the periphery. In any case, it appears that AADC and VMAT2 deficiencies, which are metabolically and clinically similar disorders, could be screened for by urine testing and then confirmed by gene sequencing, thus obviating the need for a lumbar puncture.

Direct characterization of the mutant VMAT2 protein in this study revealed a severe detriment of vesicular transporter function, which could be due to poor incorporation of the transporter into vesicle membranes or to loss of activity. Proline-to-leucine substitutions are generally considered to be deleterious to organismal fitness,¹⁸,¹⁹ on
the basis of analyses of amino acid substitutions in evolutionarily conserved proteins, and to be damaging to protein function, owing to a physicochemical difference. Proline places unique constraints on the flexibility of the peptide backbone, particularly with respect to insertions of adjacent transmembrane segments.22

A complete knockout of Slc18a2 in mice results in a lack of exocytotic monoamine neurotransmission; the mutant animals feed poorly and die within days after birth.21,22 By contrast, mice that express just 5% native Vmat2 levels live to adulthood and have minor age-related motor deficits over time.23 The phenotypic spectrum of Vmat2 deficiency in mice is therefore broad and consistent with a requirement for large decreases in protein function to cause severe motor symptoms.

We found that the motor phenotype was correctable and that the extent of correction appeared to depend on the stage of the disease. If true, this dependency could be due to irreversibly perturbed reorganization of dopamine pathways in brains subjected to chronic deficiencies in monoamine neurotransmission during active brain development. Although the improvement in the patients in this study was striking, it was not complete, probably because of monoamine deficiency during development and also because of ongoing deficiencies of the non-dopamine amines and impairment in regulated release of dopamine.

Heterozygous mice with a single Slc18a2 allele have no motor phenotype but do have a depressive behavioral phenotype.24 We found a very high rate of depression among the parents of our patients (all five of the five parents interviewed reported depression). This is also seen in parents of patients with AADC deficiency and is thought to be caused by clinically significant reductions in serotonin in these persons with hemizygous defects in the serotonin pathway.1,4,7,8 To what extent mutations in the genes encoding AADC and VMAT2 may contribute to common depression and its heritability remains to be seen.

The initial selection of treatment of the affected children on the basis of clinical phenotype alone (parkinsonism) led to severe, immediate worsening of the movement disorder. This was probably caused by the known toxicity of elevated levels of dopamine, in particular to dopaminergic neurons.25 Subsequent identification of the underlying pathophysiology allowed the rational selection of an appropriate treatment. A related severe disorder, sepiapterin reductase deficiency (see Fig. 2A), was recently diagnosed by means of whole-genome sequencing in 14-year-old fraternal twins; this previously known disorder had been undiagnosed (and therefore untreated) for many years because of the difficulties in obtaining a precise diagnosis for rare diseases.26 Diagnosis allowed treatment of the disorder, which led to recovery in those children.

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