

## ORTHOSTATIC INTOLERANCE AND TACHYCARDIA ASSOCIATED WITH NOREPINEPHRINE-TRANSPORTER DEFICIENCY

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### ABSTRACT

**Background** Orthostatic intolerance is a syndrome characterized by lightheadedness, fatigue, altered mentation, and syncope and associated with postural tachycardia and plasma norepinephrine concentrations that are disproportionately high in relation to sympathetic outflow. We tested the hypothesis that impaired functioning of the norepinephrine transporter contributes to the pathophysiologic mechanism of orthostatic intolerance.

**Methods** In a patient with orthostatic intolerance and her relatives, we measured postural blood pressure, heart rate, plasma catecholamines, and systemic norepinephrine spillover and clearance, and we sequenced the norepinephrine-transporter gene and evaluated its function.

**Results** The patient had a high mean plasma norepinephrine concentration while standing, as compared with the mean ( $\pm$ SD) concentration in normal subjects (923 vs.  $439 \pm 129$  pg per milliliter [ $5.46$  vs.  $2.59 \pm 0.76$  nmol per liter]), reduced systemic norepinephrine clearance ( $1.56$  vs.  $2.42 \pm 0.71$  liters per minute), impairment in the increase in the plasma norepinephrine concentration after the administration of tyramine ( $12$  vs.  $56 \pm 63$  pg per milliliter [ $0.07$  vs.  $0.33 \pm 0.37$  pmol per liter]), and a disproportionate increase in the concentration of plasma norepinephrine relative to that of dihydroxyphenylglycol. Analysis of the norepinephrine-transporter gene revealed that the proband was heterozygous for a mutation in exon 9 (encoding a change from guanine to cytosine at position 237) that resulted in more than a 98 percent loss of function as compared with that of the wild-type gene. Impairment of synaptic norepinephrine clearance may result in a syndrome characterized by excessive sympathetic activation in response to physiologic stimuli. The mutant allele in the proband's family segregated with the postural heart rate and abnormal plasma catecholamine homeostasis.

**Conclusions** Genetic or acquired deficits in norepinephrine inactivation may underlie hyperadrenergic states that lead to orthostatic intolerance. (N Engl J Med 2000;342:541-9.)

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**O**RTHOSTATIC intolerance is a syndrome characterized by adrenergic symptoms that occur when an upright posture is assumed: the heart rate increases by at least 30 beats per minute, without orthostatic hypotension.<sup>1</sup> Most patients with orthostatic intolerance are women between the ages of 20 and 50 years old.<sup>2</sup> This syn-

drome, first described by Da Costa<sup>3</sup> more than 100 years ago, has been called soldier's heart,<sup>4</sup> neurocirculatory asthenia,<sup>5</sup> and the mitral valve prolapse syndrome.<sup>6</sup> It is similar to the chronic fatigue syndrome in many respects.<sup>7</sup>

Most attempts to explain the physiologic and biochemical abnormalities associated with orthostatic intolerance have focused on an increased release of norepinephrine in response to the change from a supine to an upright position. An alternative explanation is that there is an abnormality in the clearance of norepinephrine from the synaptic cleft. The primary mechanism of inactivation of norepinephrine in the synapse is uptake into the neuron by the norepinephrine transporter. Approximately 80 to 90 percent of the norepinephrine released into many synapses is cleared by this mechanism, and the remaining 10 to 20 percent spills over into the circulation or extraneuronal tissue.<sup>8</sup> It is noteworthy that drugs that inhibit the norepinephrine transporter (e.g., cocaine, amphetamines, and tricyclic antidepressants) cause features typical of orthostatic intolerance (tachycardia, orthostatic symptoms, and high plasma catecholamine concentrations).

We evaluated a patient and her identical twin, both of whom had symptoms typical of orthostatic intolerance, and in both found clinical and laboratory signs of disordered uptake of norepinephrine. Because the norepinephrine transporter has a pivotal role in norepinephrine uptake at the synaptic cleft, we determined whether the impaired uptake of norepinephrine in these patients could have been caused by a mutation in the gene that encodes the norepinephrine transporter.

### METHODS

The proband was a 33-year-old woman with a 20-year history of exertional and orthostatic tachycardia, dyspnea, difficulty concentrating, and syncope. Her blood pressure had varied substantially after each of three cesarean sections, with values as high as 210/180 mm Hg. Treatment with beta-adrenergic-antagonist drugs, compression stockings, and fludrocortisone was ineffective. Implantation of a dual-chamber pacemaker decreased the frequency of the syncope, but the symptoms of orthostatic intolerance persisted. An echocardiogram revealed slight mitral regurgitation

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and possible mitral-valve prolapse. The proband's identical twin also had a history of mitral-valve prolapse as well as tachycardia and syncope that were worsened by exertion and an upright posture.

The study was approved by the Vanderbilt University investigational review board, and all subjects, including family members, gave written informed consent.

### Clinical Studies

The proband and her twin were admitted to the General Clinical Research Center at Vanderbilt University Medical Center. Use of all medications had been discontinued two weeks before admission. For three days, the women were given a caffeine-free, low-monoamine diet containing 150 mmol of sodium per day and 70 mmol of potassium per day. Urine was then collected for 24 hours for measurement of catecholamines and catecholamine metabolites. After an overnight fast, the women's blood pressure, heart rate, and plasma catecholamines were measured while they were supine and after 30 minutes of standing. Plasma and urine catecholamines were measured by high-performance liquid chromatography.<sup>9,10</sup> Testing of autonomic reflexes was performed as previously described.<sup>11</sup> In the proband, brachial blood pressure and plasma norepinephrine were measured before and at the time of maximal blood pressure after the injection of a 3-mg bolus of tyramine. Systemic spillover and clearance of norepinephrine before and during baroreflex-mediated sympathetic activation, induced by the administration of sodium nitroprusside (Ohmeda, Liberty Corner, N.J.), were measured in the proband during continuous monitoring of intraarterial blood pressure and heart rate, as follows. Tritiated norepinephrine was infused intravenously at a rate of 1  $\mu$ Ci per minute after the administration of a loading dose of 25  $\mu$ Ci per minute, as previously described,<sup>12,13</sup> and plasma norepinephrine and the specific activity of tritiated norepinephrine were measured.<sup>13</sup> Nitroprusside was then infused into a contralateral antecubital vein. When the systolic blood pressure had decreased by 20 mm Hg (at a rate of 1.2  $\mu$ g of nitroprusside per kilogram of body weight per minute), the measurements were repeated.

Blood pressure, heart rate, and plasma catecholamine concentrations in the proband and her twin sister were compared with those in 10 unrelated normal subjects recruited from a pool of normal volunteers; the sisters' plasma catecholamine responses after tyramine administration were compared with those in 9 unrelated normal subjects; and their norepinephrine spillover and clearance were compared with those in 8 unrelated normal subjects.

Blood samples were obtained from the proband, her nine siblings, and her mother for DNA analysis. In the mother and all the siblings except for one sister, blood pressure and heart rate were determined in the supine position and after 5 minutes of standing, and plasma catecholamines were measured after the subjects had been supine for 20 minutes and then after they had been standing for 30 minutes.

### Detection of Mutations

Amplification and sequencing were performed at the Vanderbilt Center for Molecular Neuroscience DNA Sequencing Core. Genomic DNA was isolated from venous blood with the PureGene DNA Extraction Kit (Gentra Systems, Minneapolis), and the exons of the human norepinephrine-transporter gene (*SLC6A2*; McKusick no. 163970<sup>14</sup>) were amplified with the use of the polymerase chain reaction and sense and antisense primers (the sequences of oligonucleotides used for the exonic polymerase chain reaction and the conditions for amplification are available on request). The amplified products (60 ng per aliquot) were directly sequenced with fluorescent dideoxynucleotide chain terminators (AmpliTaQ FS, Perkin Elmer Applied Biosystems, Foster City, Calif.) on an automated DNA sequencer (ABI 310, Perkin Elmer Applied Biosystems).

#### Functional Analysis of the Identified Mutation

DNA encoding a human norepinephrine transporter with alanine replaced by proline at position 457 (Ala457Pro) was created

with the use of a kit (QuikChange Site-Directed Mutagenesis Kit, Stratagene, La Jolla, Calif.) with the oligonucleotides 5'CCTTC-AGTACTTTCCTTCTCC<sup>CCCTGTTCTGCATAACCAAG3'</sup> and 5'CTTGGTTATGCAGAACAGGGG<sup>GAGAAGGAAAGTACTGA-AGG3'</sup>. The underlined bases are those that were introduced to create a mutation in which guanine was replaced by cytosine at position 237 (G237C) or to introduce a *ScaI* restriction site that could be used to identify mutant plasmids. Amplified DNA was cloned into a construct (pcDNA3, Invitrogen, Carlsbad, Calif.) containing wild-type human norepinephrine-transporter complementary DNA (cDNA) in which a silent mutation, or one that does not result in a change in amino acid (in this case, leucine at position 438), had previously been introduced to create a unique *AflIII* restriction site and thus facilitate subcloning of the mutated sequence back into the wild-type construct. The subcloned region was sequenced with the norepinephrine-transporter oligonucleotides 5'CATTCT-GGGCTGTGTGT3' and 5'GTGGTTGTGGTCAGCATCATC3'. DNA from multiple isolates of mutant clones was purified (Qiagen, Santa Clarita, Calif.) to test the effect of the Ala457Pro mutation on transporter activity.

Wild-type norepinephrine transporter, norepinephrine transporter with the Ala457Pro mutation, and pcDNA3 plasmids were transiently transfected into Chinese-hamster-ovary cells (American Type Culture Collection, Rockville, Md.) with the use of lipofectamine. The cells were assayed for tritiated norepinephrine-transporter activity (20 nmol per liter) 72 hours after transfection, as previously described.<sup>15</sup>

#### Genotyping of Ala457Pro Alleles

Allele-specific oligonucleotide hybridization was used to test for the presence of the Ala457Pro mutation, with hybridization of 5'CCTTCTC<sup>CCCTGTT3'</sup> to the wild-type allele and hybridization of 5'CCTTCTC<sup>CCCTGTT3'</sup> to the mutant allele. The underlined bases are those used to identify the single-nucleotide polymorphism in the mutant allele. All samples of genomic DNA were coded before genotype analysis in order to preserve the anonymity of the subjects. Genotypes were then used to associate genotypes with phenotypes.

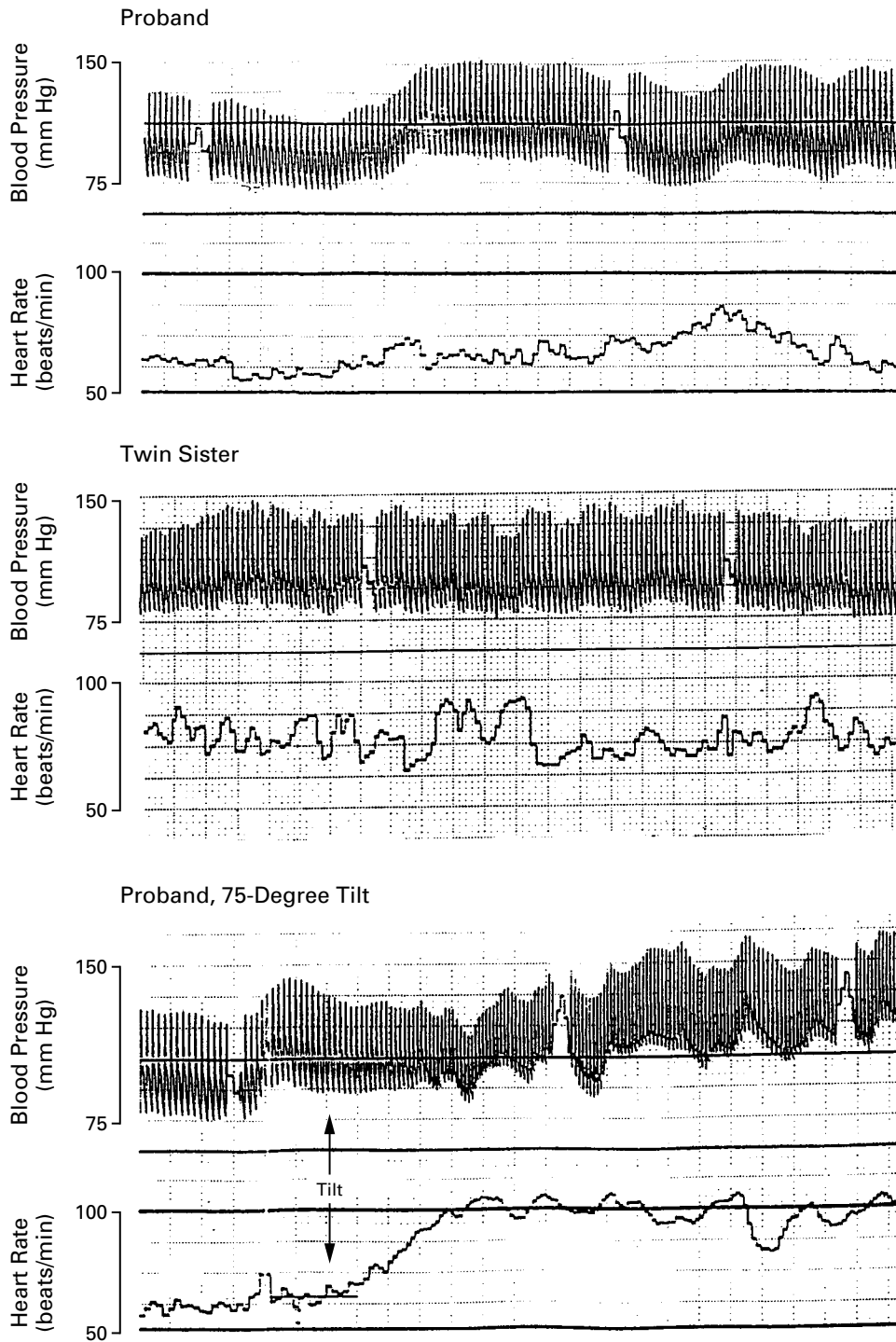
#### Statistical Analysis

Paired and unpaired t-tests were used to compare clinical findings between the groups of subjects and within each group before and after exposure to various stimuli. Data were analyzed with Prism software (GraphPad Software, San Diego, Calif.). All P values are two-sided.

## RESULTS

### Autonomic Responses

The proband and her twin sister had normal autonomic reflexes, but their blood pressure and heart rate were variable (Fig. 1). Their blood pressure, heart rate, and plasma catecholamine concentrations in the supine and upright positions and those in the unrelated normal subjects<sup>13</sup> are shown in Table 1. In the proband and her twin, the plasma concentrations of dihydroxyphenylglycol, an intraneuronal metabolite of norepinephrine,<sup>9</sup> were low in relation to the plasma norepinephrine concentrations (ratio of dihydroxyphenylglycol to norepinephrine in the supine position, 3.06 in the proband and 2.41 in her twin, vs. 5.52 in the normal subjects; ratio in the upright position, 1.05 in the proband and 1.17 in her twin, vs. 3.44 in the normal subjects). Urinary excretion of norepinephrine was high in both the proband and her twin (435 and 125  $\mu$ g per 24 hours [2.57 and



**Figure 1.** Continuous Recordings of Blood Pressure and Heart Rate in the Proband and Her Twin Sister. Beat-by-beat recordings of blood pressure as determined by photoplethysmography and continuous recordings of heart rate show spontaneous increases of up to 50 mm Hg in blood pressure and 25 beats per minute in heart rate in the proband and her twin sister. In the proband, a 75-degree tilt increased the variation in blood pressure and heart rate.

**TABLE 1.** BLOOD PRESSURE, HEART RATE, AND PLASMA CATECHOLAMINE CONCENTRATIONS IN THE PROBAND, HER TWIN SISTER, AND 10 UNRELATED NORMAL SUBJECTS.\*

SUBJECT	SYSTOLIC/DIASTOLIC BLOOD PRESSURE		HEART RATE		PLASMA NOREPINEPHRINE		PLASMA EPINEPHRINE		PLASMA DHPG		RATIO OF DHPG TO NOREPINEPHRINE	
	SUPINE	UPRIGHT	SUPINE	UPRIGHT	SUPINE	UPRIGHT	SUPINE	UPRIGHT	SUPINE	UPRIGHT	SUPINE	UPRIGHT
	mm Hg		beats/min		picograms per milliliter							
Proband	120/74	110/77	80	109	269	923	11	23	824	968	3.06	1.05
Twin sister	132/74	156/95	79	131	199	911	22	116	480	1068	2.41	1.17
Normal subjects	108±6/63±6	106±10/67±10	65±6	83±13	200±63	439±129	25±10	44±41	1104±365	1415±256	5.52	3.44

\*Plus-minus values are means ±SD. Plasma concentrations of norepinephrine, epinephrine, and dihydroxyphenylglycol (DHPG) were measured once in the proband, her twin sister, and the normal subjects. To convert values for plasma norepinephrine to nanomoles per liter, multiply by 0.005911. To convert values for plasma epinephrine to picomoles per liter, multiply by 5.46.

0.74 μmol per 24 hours], respectively; normal value, <90 μg per 24 hours [0.53 μmol per 24 hours]).

#### Response to Tyramine

After it is taken up into neurons by the norepinephrine transporter, tyramine has a hypertensive effect caused by the release of norepinephrine.<sup>16,17</sup> In the normal subjects, the mean (±SD) systolic blood pressure increased by 19±2 mm Hg and the mean plasma norepinephrine concentration increased by 56±21 pg per milliliter (0.33±0.12 nmol per liter) in response to 3 mg of tyramine. In the proband, the same dose of tyramine increased systolic blood pressure by 18 mm Hg but increased the plasma norepinephrine concentration by only 12 pg per milliliter (0.07 nmol per milliliter).

#### Spillover and Clearance of Systemic Norepinephrine

The arterial plasma norepinephrine concentration at rest was slightly higher in the proband than in the normal subjects (280 pg per milliliter [1.66 nmol per liter] vs. 204±51 pg per milliliter [1.21±0.30 nmol per liter]). This increase was due primarily to a decrease in the rate of removal of norepinephrine from the circulation (norepinephrine clearance): although the rate at which norepinephrine entered the circulation (norepinephrine spillover) was lower in the proband than in the normal subjects (436 vs. 514±277 ng per minute [2.58 vs. 3.04±1.64 nmol per minute]), norepinephrine clearance in the proband was less than half that in the normal subjects (1.56 vs. 2.42±0.71 liters per minute). During the infusion of nitroprusside, norepinephrine spillover increased to 1072 ng per minute (6.34 nmol per minute) in the proband but only to 745±212 ng per minute (4.40±1.25 nmol per minute) in the normal subjects. Norepinephrine clearance did not change appreciably after the nitroprusside infusion in either the proband (1.76 liters per minute) or the normal subjects (2.31±0.68 liters per minute).

#### Identification of a Functional Missense Mutation in the Norepinephrine-Transporter Gene

The combination in the proband of a low ratio of plasma dihydroxyphenylglycol to norepinephrine, a blunted response of plasma norepinephrine to tyramine, and a decreased plasma norepinephrine clearance suggested a potential defect in the norepinephrine transporter. The presence of similar findings in her twin sister suggested a genetic origin.

Direct sequence analysis of the norepinephrine-transporter gene in the proband revealed no divergence from previously published sequences<sup>14,18</sup> in exons 1 through 8 and exons 10 through 15. However, two novel polymorphisms were identified in exon 9: a silent polymorphism in which cytosine was replaced

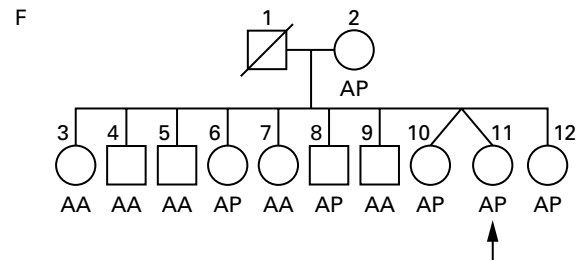
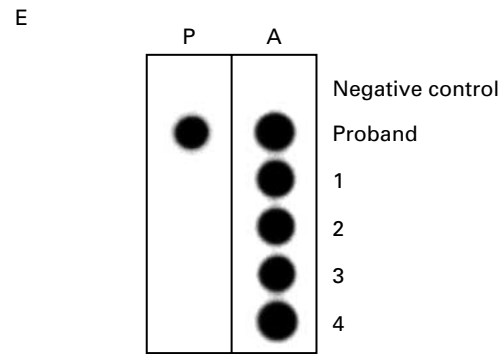
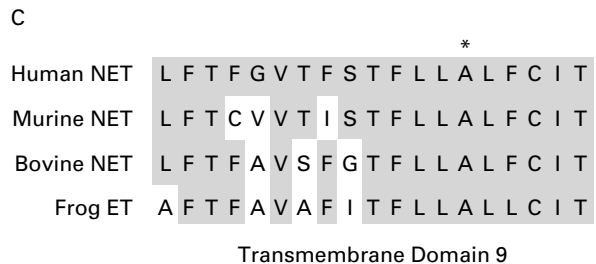
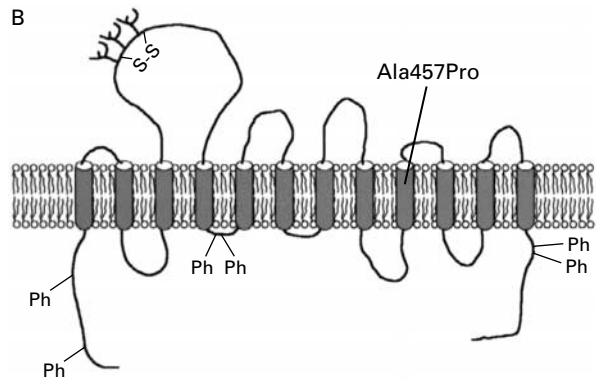
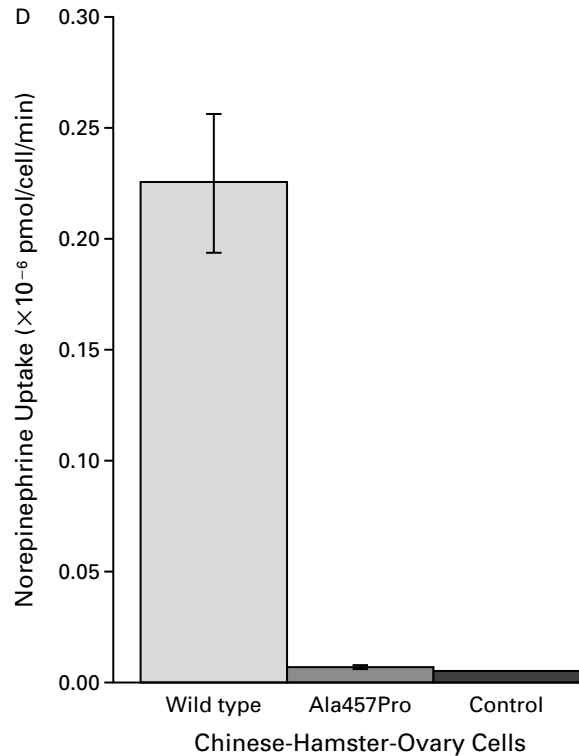
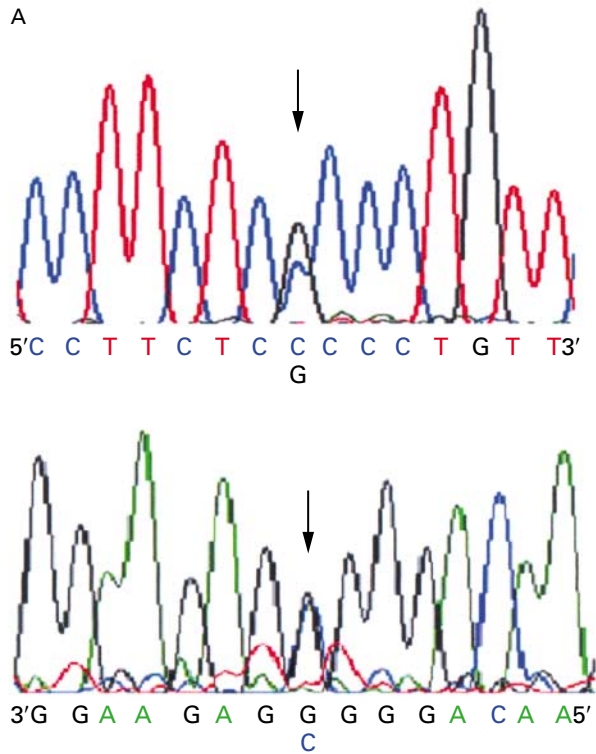
**Figure 2 (facing page).** Evaluation of the Norepinephrine-Transporter (NET) Mutation.

DNA sequencing of the norepinephrine-transporter gene (Panel A) identified both cytosine (C) and guanine (G) (arrows) at position 237 of exon 9 in both the sense (top) and antisense (bottom) DNA strands, indicating heterozygosity at this locus. This change from cytosine to guanine results in a change from alanine to proline at amino acid position 457 (Ala457Pro). The Ala457Pro mutation is in transmembrane domain 9 of the norepinephrine transporter (Panel B). S-S denotes a disulfide bond, and Ph canonical sites for protein phosphorylation. Transmembrane domain 9 is highly conserved among the related murine and bovine norepinephrine transporters and the frog epinephrine transporter (ET) (Panel C). Shading indicates areas of homology among species. The asterisk denotes the site of mutation. As compared with the wild-type norepinephrine transporter, the transporter with the Ala457Pro mutation results in impairment of mean norepinephrine uptake in transiently transfected Chinese-hamster-ovary cells (Panel D). I bars indicate 1 SD. Panel E is a schematic diagram of the hybridization study and shows the presence of the mutant (P, for proline) and wild-type (A, for alanine) alleles. Panel F shows a pedigree of the proband. P (for proline) denotes the mutant allele and A (for alanine) the wild-type allele. Circles denote female family members, and squares male family members. The slash denotes a deceased family member, and the arrow indicates the proband.

by adenine at position 154 (C154A) and a missense mutation in which guanine was replaced by cytosine at position 237 (G237C) (position numbers refer to the GenBank sequence for exon 9). The proband was heterozygous for both the C154A and the G237C polymorphisms (Fig. 2A). The G237C mutation re-

sults in a change from alanine to proline (Ala457Pro) within a highly conserved region of transmembrane domain 9 (Fig. 2B and 2C).

Heterologous expression of the wild-type norepinephrine-transporter gene and human cDNA encoding the Ala457Pro mutation revealed that norepi-



nephrine transport was greatly diminished by the Ala457Pro mutation. In Chinese-hamster-ovary cells that had been transiently transfected with cDNA encoding the human norepinephrine transporter, uptake of tritiated norepinephrine was 10 times that in vector-transfected cells, whereas in cells transfected with cDNA encoding the Ala457Pro mutation in the norepinephrine transporter, the uptake was 2 percent or less (Fig. 2D). Multiple clones were tested, and in another cell line (LLC-PK1, provided by M. Hahn), all the clones were devoid of transport activity (data not shown).

#### Segregation of the Ala457Pro Mutation with a Phenotype

The proband's mother and eight of the proband's siblings were genotyped by allele-specific oligonucleotide hybridization. The mother and four siblings (including the twin sister) were heterozygous for the mutant allele (Fig. 2E). Their heart rates while supine were slightly but not significantly greater than those of their family members with the wild-type genotype, but their heart rates while standing were significantly higher (Fig. 3A and 3B). Likewise, plasma norepinephrine concentrations in the supine position were somewhat higher and plasma norepinephrine concentrations in the upright position were significantly higher in the heterozygous family members than in those with the normal genotype (Fig. 3C and 3D). Finally, the ratios of dihydroxyphenylglycol to norepinephrine in plasma in both the supine and upright positions were significantly lower in those who were heterozygous for the mutation than in the other family members (Fig. 3E and 3F).

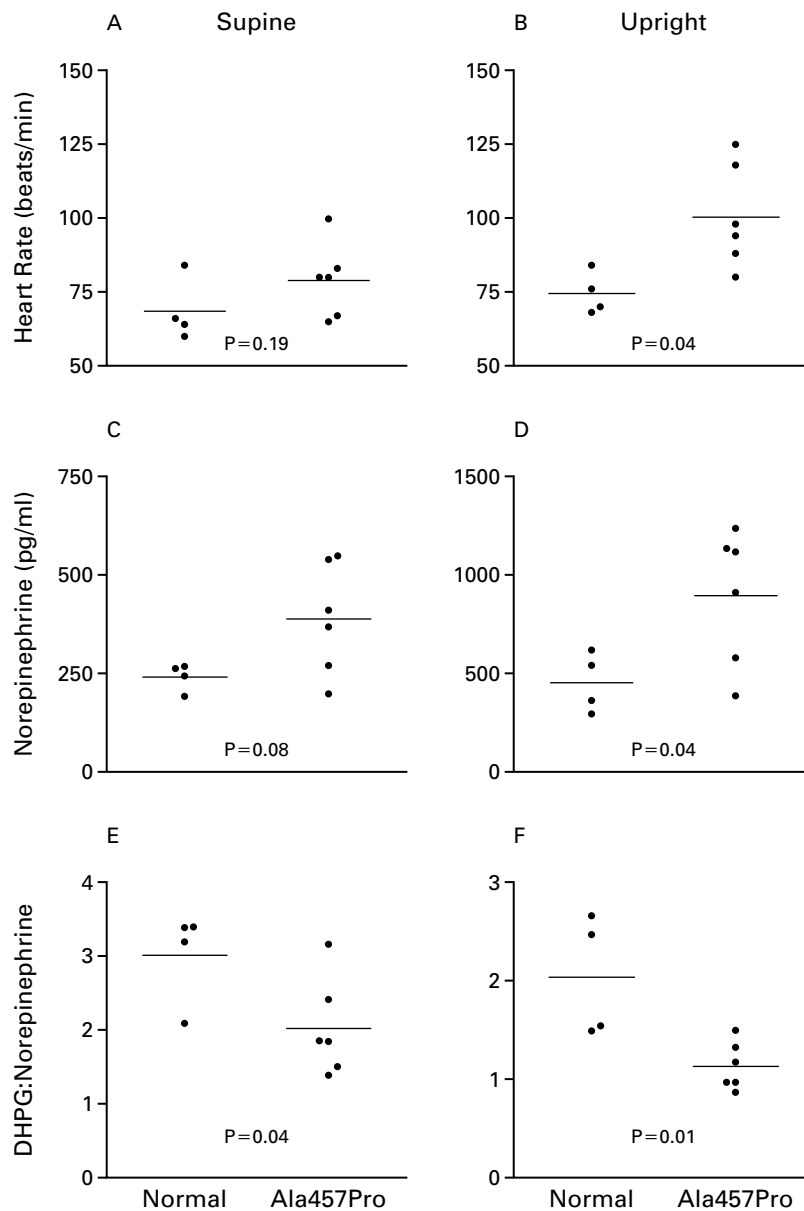
#### DISCUSSION

The deficiency in norepinephrine transport in the proband and several members of her family was the result of a functional mutation in the gene encoding the norepinephrine transporter. Previously detected coding polymorphisms in this gene have no reported effect on norepinephrine transport.<sup>19</sup> In contrast, the Ala457Pro mutation renders the transporter nonfunctional and segregates with altered heart-rate regulation and altered norepinephrine metabolism. The proband had a defect in norepinephrine uptake. Her heart rate while supine was about 10 beats per minute faster than the mean value for age-matched normal subjects<sup>20</sup> and rose substantially when she stood. This change in heart rate was accompanied by an increase in the plasma norepinephrine concentration to almost four times its value in the supine position. These changes may have been caused by either an increase in the release (spillover) of norepinephrine or a decrease in its clearance.<sup>8,21</sup> The blunting of the increase in plasma norepinephrine in the proband in response to the administration of tyramine and her low systemic norepinephrine clearance suggest that the uptake of norepinephrine was impaired. The ab-

normal relation between plasma concentrations of dihydroxyphenylglycol and norepinephrine provides further evidence of impaired norepinephrine uptake. Some of the norepinephrine taken up into neurons by the norepinephrine transporter reaches the vesicles, where it is stored for later release, but much of it is converted to dihydroxyphenylglycol by monoamine oxidase.<sup>8</sup> The dihydroxyphenylglycol then enters the circulation and can serve as a marker of norepinephrine uptake and monoamine oxidase activity<sup>9</sup> (Fig. 4). The relatively low ratios of dihydroxyphenylglycol to norepinephrine in plasma in the proband and her twin sister indicate that norepinephrine transport in these women was impaired.

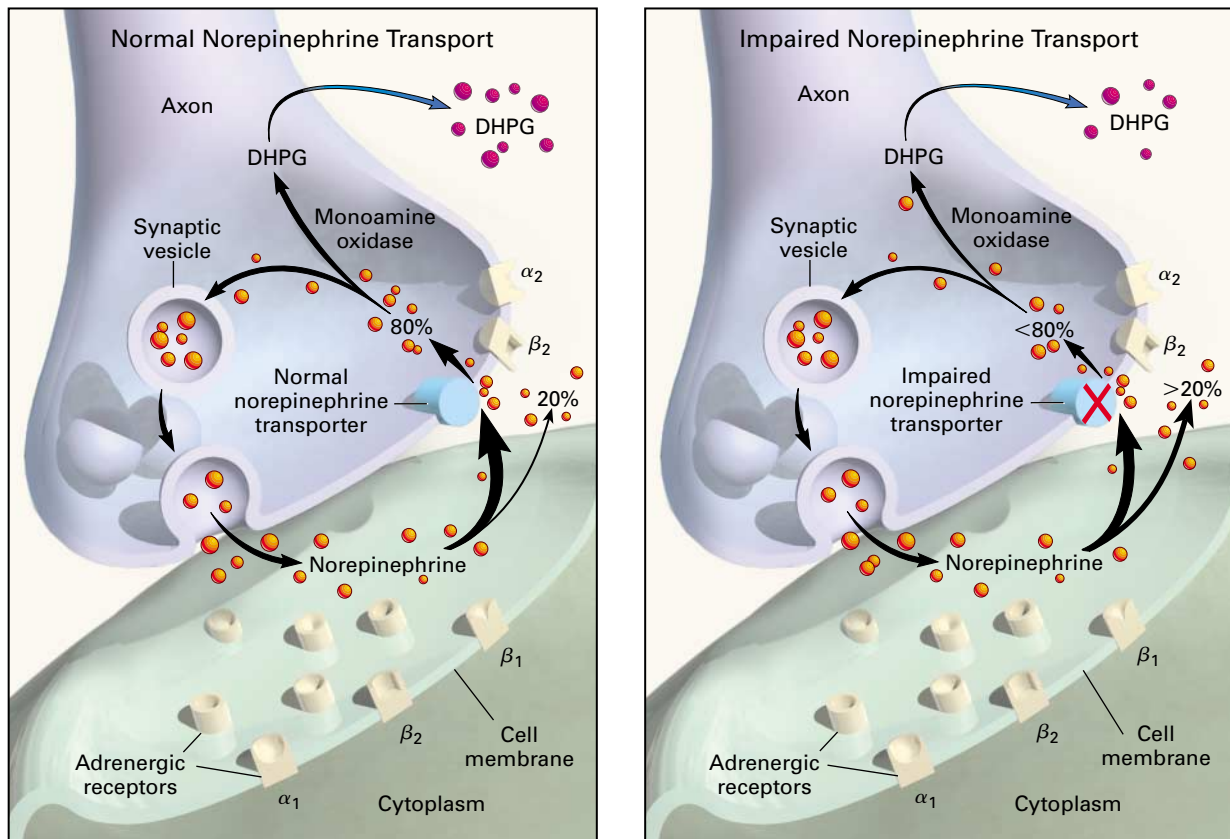
These findings in both the proband and her identical twin strongly suggested the presence of an abnormality in the norepinephrine-transporter gene, which has been mapped to chromosome 16q.<sup>22</sup> Analysis of this gene in the proband revealed a missense mutation that resulted in the replacement of an alanine residue with a proline residue in a highly conserved transmembrane region of the transporter. Because the substitution of proline disrupts alpha-helical secondary structures that are supported by alanine residues, this mutation may disrupt the transport of norepinephrine. Functional analysis of the proband's mutant norepinephrine transporter demonstrated that it had 2 percent or less of the activity of the transporter encoded by the wild-type gene.

The pathophysiologic features of orthostatic intolerance have been thought to be due to a primary<sup>23</sup> or secondary<sup>23-26</sup> activation of sympathetic outflow. A deficiency of the norepinephrine transporter may explain several of the clinical findings in patients with this disorder: the high heart rate in the supine position, the high plasma concentration of norepinephrine in association with the relatively low plasma concentration of dihydroxyphenylglycol, the impaired response of norepinephrine to tyramine,<sup>13</sup> the reduced systemic clearance of norepinephrine,<sup>13</sup> and the disparity between the changes in heart rate and plasma norepinephrine concentrations and the change in muscle sympathetic-nerve activity in the upright position.<sup>27</sup> In many patients with orthostatic intolerance, the disproportionately greater increase in heart rate than in diastolic pressure in the upright posture<sup>13</sup> may also be explained by a deficiency of the norepinephrine transporter. The noradrenergic synaptic clefts in the heart are approximately three times as narrow as the synaptic clefts in the vasculature,<sup>28</sup> making the removal of norepinephrine from the synapses in the heart far more dependent on the activity of the norepinephrine transporter than is removal from synapses in the vascular beds.<sup>29</sup> Therefore, a decrease in transporter activity could result in a disproportionately greater effect on heart rate than on blood pressure, as is observed in patients with orthostatic intolerance.



**Figure 3.** Heart Rate and Plasma Catecholamine Concentrations in the Proband and Her Family in the Supine and Upright Positions.

Heart rate, plasma concentrations of norepinephrine, and the ratio of its intraneuronal metabolite, dihydroxyphenylglycol (DHPG), to norepinephrine were determined in the proband and nine family members (three family members with the Ala457Pro mutation and six with the normal genotype). In the supine position, the heart rate in the family members with the mutation was similar to that in those without the mutation. However, in the upright position, the heart rate and plasma norepinephrine concentrations were significantly higher in those with the mutation than in those without it. Plasma norepinephrine concentrations in the supine position were somewhat higher in the family members with the mutation than in the others. The ratio of the plasma DHPG concentration to the plasma norepinephrine concentration was significantly lower in both the supine and the upright positions in those with the mutation, a finding consistent with impairment of norepinephrine uptake. Each point represents one subject, and the horizontal lines represent the mean values. To convert plasma norepinephrine values to nanomoles per liter, multiply by 0.005911. P values are for the comparison between family members with the Ala457Pro mutation and those with the normal genotype.



**Figure 4.** Neuronal Metabolism of Norepinephrine in Persons with Norepinephrine-Transporter Deficiency.

Under normal conditions, norepinephrine is released from vesicles in the neuron into the synaptic space, where it can interact with presynaptic and postsynaptic  $\alpha$ - and  $\beta$ -adrenergic receptors. Approximately 80 percent of the norepinephrine in the synaptic space is taken up by the norepinephrine transporter into the neuron that released it, and approximately 20 percent spills over into the circulation. Norepinephrine taken up by the neuron that released it is preferentially converted to dihydroxyphenylglycol (DHPG) by monoamine oxidase; some is repackaged into the synaptic vesicles. DHPG diffuses out of the neuron into the circulation. In persons with norepinephrine-transporter deficiency, the release of norepinephrine into the synaptic space is normal, but because of the decreased activity of the norepinephrine transporter, less than 80 percent of norepinephrine is taken up into the neuron that released it, so that the spillover into the circulation is greater than 20 percent. In addition, more norepinephrine is available for interaction with the adrenergic receptors in the synaptic space. Because of the decreased uptake of norepinephrine, the production of DHPG is decreased. The reduced DHPG concentration in the neuron results in lower concentrations of this metabolite in the plasma and, consequently, a ratio of plasma DHPG to plasma norepinephrine that is lower than normal.

Impairment of the norepinephrine transporter in the central nervous system may also contribute to the phenotype. Norepinephrine released in the brain can either increase<sup>30,31</sup> or decrease<sup>32</sup> sympathetic outflow. Because acute pharmacologic blockade of the norepinephrine transporter causes a decrease in sympathetic outflow,<sup>33</sup> one would expect a decrease in sympathetic tone with a deficiency of the norepinephrine transporter. Yet in the proband and in many other patients with orthostatic intolerance, central sympathetic tone seems to be increased.<sup>34,35</sup> This paradoxical increase may represent the sum of the effects of partial inhibition of norepinephrine transport at multiple sites in the central nervous system.

The Ala457Pro mutation does not explain all cas-

es of orthostatic intolerance. The mutation was not present in any of 254 unrelated persons, including normal subjects, patients with hypertension, and other patients with orthostatic intolerance (data not shown). Furthermore, in the current study, family members who had the mutation also had some of the physiologic and biochemical abnormalities detected in the proband and her twin sister, but none had the full-blown syndrome. Thus, other genetic or environmental factors must have contributed to the phenotype in the two affected women.

In conclusion, the identification of defective norepinephrine transport in patients with orthostatic intolerance suggests a previously unrecognized mechanism in some patients with this clinical problem.

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