Phosphoserine Aminotransferase Deficiency: A Novel Disorder of the Serine Biosynthesis Pathway

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We present the first two identified cases of phosphoserine aminotransferase deficiency. This disorder of serine biosynthesis has been identified in two siblings who showed low concentrations of serine and glycine in plasma and cerebrospinal fluid. Clinically, the index patient presented with intractable seizures, acquired microcephaly, hypertonia, and psychomotor retardation and died at age 7 mo despite supplementation with serine (500 mg/kg/d) and glycine (200 mg/kg/d) from age 11 wk. The younger sibling received treatment from birth, which led to a normal outcome at age 3 years. Measurement of phosphoserine aminotransferase activity in cultured fibroblasts in the index patient was inconclusive, but mutational analysis revealed compound heterozygosity for two mutations in the *PSAT1* gene—one frameshift mutation (c.delG107) and one missense mutation (c.299A→C [p.Asp100Ala])—in both siblings. Expression studies of the p.Asp100Ala mutant protein revealed a V_{max} of only 15% of that of the wild-type protein.

Disorders due to deficiency of the first and third enzymes of the three-enzyme serine biosynthesis pathway have previously been characterized. 3-Phosphoglycerate dehydrogenase (encoded by *PHGDH*) deficiency (MIM 601815) was the first serine-biosynthesis disorder to be reported (in 1996 by Jaeken and colleagues), and several patients have subsequently been identified.[1–3] This is a severe neurological disorder presenting typically with congenital microcephaly, seizures, psychomotor retardation, and spastic tetraparesis. Phosphoserine phosphatase (encoded by *PSPH*) deficiency (MIM 172480) has been identified to date in only one patient, and the clinical consequences of this enzyme deficiency are not clear because of the concomitant Williams syndrome in this patient.[4,5] Patients with these disorders have received treatment with supplemental serine and glycine, which had varying degrees of success.[1,2,6] Patients with a deficiency of phosphoserine aminotransferase (EC 2.6.1.52), the second enzyme in the pathway, have not been reported previously.

L-serine may be derived from four possible sources: dietary intake, degradation of protein and phospholipids, biosynthesis from the glycolytic-pathway intermediate 3-phosphoglycerate via the so-called phosphorylated pathway, or glycine.[7] The "nonphosphorylated pathway," involving D-glycerate and hydroxypyruvate as intermediates, is now known to be involved in serine degradation (see the "Discussion" section). Serine is a nonessential amino acid, and it is now clear that dietary sources are insufficient to meet all requirements.[1,2,8] The poor transport of serine across the blood-brain barrier means that the brain, in particular, appears to be reliant on local de novo synthesis from 3-phosphoglycerate.[9] Serine is required for both brain development and brain function; therefore, neurological symptoms predominate in the serine-biosynthesis disorders.[1–4,7] Glycine can be synthesized directly from serine, and vice versa, by the action of serine hydroxymethyl transferase, which explains why serine-deficiency disorders tend to also present with a low or low-normal glycine concentration.[1–3]

Subjects and Methods

Patients

This study was approved by the ethical committee of the Faculty of Medicine, Université Catholique de Louvain. Informed consent was obtained from the parents of the investigated family.

Patient 1 is a male, the second child of healthy, unrelated parents of British origin. He was born at term by cesarean delivery because of a low-lying placenta. He was healthy at birth, with head circumference and weight in the 9th percentile. At age 2 wk, he was admitted to the hospital with poor feeding and cyanotic episodes thought to be related to an upper respiratory tract infection. At age 7 wk, he was experiencing jerking movements and posturing. At age 9 wk, he presented with severe, intractable seizures and slight hypertonia. His head circumference was <0.4th percentile, whereas his weight was in the 2nd percentile. His seizures could not be controlled, despite multiple-anticonvulsants therapy including clonazepam, phenytoin, and paraldehyde, and he was transferred to the regional pediatric intensive care unit, received mechanical ventilation, and was sedated with thiopentone. Pyridoxine at 120 mg/d was given from age 10 wk but had no observable effect. Extensive biochemical investigations revealed no abnormalities, with the exception of low plasma and cerebrospinal fluid (CSF) concentrations of serine and glycine (table 1). Cranial imaging showed generalized atrophy, a hypoplastic

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Table 1. Plasma and CSF Serine and Glycine Results for Patients 1 and 2 at Diagnosis

	Plasma[a] (μmol/liter)		CSF[a] (μmol/liter)	
Patient	Serine	Glycine	Serine	Glycine
1	51 (60–300)	121 (140–420)	18 (35–80)	<1 (3–10)
2	30 (50–350)	110 (200–600)	5 (35–80)	<1 (3–10)

NOTE.—Plasma and CSF amino acids were measured by ion-exchange chromatography with ninhydrin detection.

[a] The normal range is shown in parentheses.

cerebellar vermis, and poor white-matter development (fig. 1). An electroencephalogram was consistent with multifocal seizure activity. Treatment was begun with serine (500 mg/kg/d) and glycine (200 mg/kg/d) at age 11 wk, which normalized plasma and CSF concentrations. The clinical effect was limited, with only a marginal improvement in seizure control and responsiveness. Severe seizure episodes that required admission to the intensive care unit continued, the hypertonia worsened, and eventually the patient died at age 7 mo.

Patient 2 is the younger sister of patient 1. She was born at term by cesarean delivery because of the previous cesarean delivery. She was healthy at birth, with head circumference and weight in the 9th percentile. Prenatal monitoring had showed normal fetal development. However, a plasma sample taken at age 2 h revealed low concentrations of serine and glycine, which was also found in a CSF sample (table 1). Supplementation with serine (500 mg/kg/d) and glycine (200 mg/kg/d) was begun within the first 24 h of life. Cranial ultrasonography at age 3 wk and a magnetic resonance imaging (MRI) scan at age 4 mo showed no abnormalities. Her head circumference increased from the 9th percentile at birth to the 50th–75th percentile by age 18 wk, as did her weight. She experienced one apneic episode at age 2 wk but was otherwise asymptomatic and has remained asymptomatic. Her growth and development are normal at age 3 years.

Mutational Analysis

Genomic DNA was extracted from fibroblasts according to standard procedures. All exons of *PHGDH* (exons 1–12) and *PSAT1* (exons 1–9) and the coding exons of *PSPH* (exons 4–8) were PCR amplified using 0.2–0.5 μM of forward and reverse primers (Eurogentec) (table 2) with 75–300 ng of genomic DNA template. These (50 μl) reactions were performed using the indicated DNA polymerase in the recommended buffer, 0.2 mM nucleotide triphosphates and, where indicated, betaine (Sigma) (table 2). Biotools DNA polymerase was from Biotools, Optimase was from Transgenomic, and *Taq* polymerase was from Roche. The PCR amplifications started with an initial denaturation step at 95°C for 3–5 min, followed by 35 cycles for 0.5 min at 95°C, 0.5–1 min at the annealing temperature (table 2), and 1 min at 72°C. The final extension step at 72°C lasted 5–35 min. PCR products were purified and were sequenced directly on both strands by use of either an ABI 3100 Genetic Analyzer (Applied Biosystems) or a CEQ2000 sequencer (Beckman).

Table 2. Primers and PCR Conditions Used for Amplification of Exons of *PHGDH*, *PSAT1*, and *PSPH*

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Expression of Wild-Type and Mutated Phosphoserine Aminotransferase

Human recombinant wild-type phosphoserine aminotransferase (i.e., the full length cDNA, designated "PSATβ" by Baek et al.[10]) and PSAT-D100A were produced as fusion proteins with an N-terminal polyHis tag. The coding sequence (AY131232) was amplified by PCR with the use of *Pwo* DNA polymerase and two oligonucleotides (5′-GCCATATGGACGCCCCCAGGCAGG-3′, containing an *Nde*I restriction site, and 5′-CGGATCCTCATAGCTGATGCATCTCCAAAAAT-3′, containing a *Bam*HI restriction site; restriction sites are underlined) by use of human fibroblast cDNA as matrix. The amplified fragment was cloned into pBluescript restricted with *Eco*RV, to produce the plasmid pBS-PSAT. The coding sequence was excised from this plasmid by digestion with *Nde*I and *Bam*HI and was ligated into the *Nde*I and *Bam*HI sites of the expression vector pET15b, to produce the expression plas-

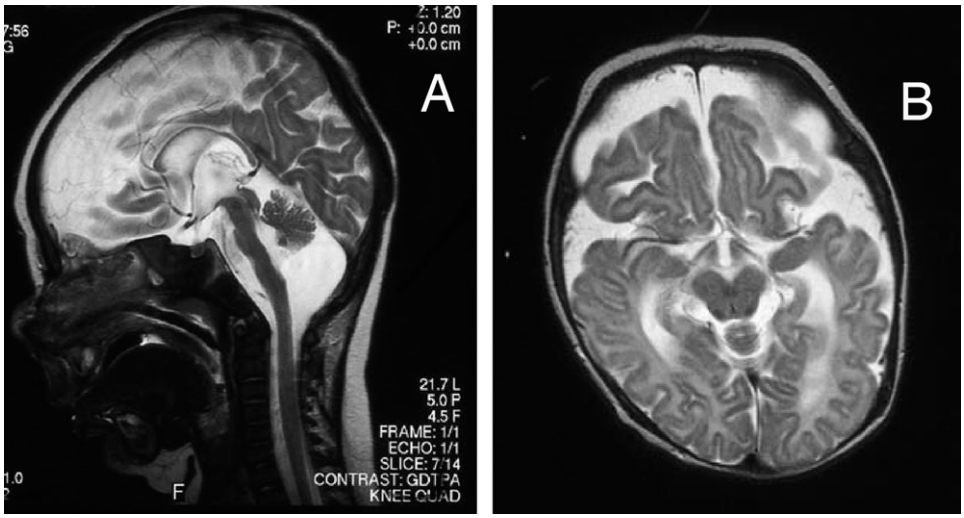

Figure 1. MRI scans from patient 1 showing generalized atrophy and a hypoplastic cerebellar vermis

Table 3. Enzymatic Activities in Fibroblast Extracts from Patient 1 and Controls

Sample	3-Phosphoglycerate Dehydrogenase (nmol/min/mg protein)	Phosphoserine Aminotransferase (nmol/min/mg protein)	Phosphoserine Phosphatase (nmol/min/mg protein)
Controls	29.5 ± 2.7[a]	2.0 ± 0.3[b]	1.7 ± 0.2[c]
Patient 1	70	0.9	2.4

[a] Mean ± SEM for 15 control subjects.
[b] Mean ± SEM for 9 control subjects.
[c] Mean ± SEM for 14 control subjects.

mid pET15-PSAT. The Asp100Ala point mutation was introduced into pBS-PSAT by site-directed mutagenesis with the use of the Quick Change kit (Stratagene). An *Nde*I restriction-enzyme site was also introduced into the DNA, providing a rapid method to screen for this mutation. The forward primer used for mutagenesis was 5′-GCAGGAAGGTGTGCGG*CATATG*TGGTGACAGGAG-3′ (the nucleotide change is in italics and the resulting *Nde*I site is underlined). The reverse primer was complementary to the forward primer. An *Nhe*I-*Bam*HI fragment containing the mutation was exchanged for the same restriction fragment in the wild-type expression vector (pET15-PSAT). To produce the recombinant enzyme, *Escherichia coli* BL21 cells[11] were transformed with the expression vector and were grown in 0.5 liter of LB medium at 37°C until absorbance at 600 nm reached 0.6 U. The culture was then quenched for 30 min on ice. Expression of the recombinant protein was induced by addition of 1 mM isopropyl thiogalactoside for 16 h at 18°C. Cells were collected by centrifugation and were lysed in 10 ml of 100 mM Tris (pH 8) containing 5 μg/ml leupeptin, 5 μg/ml antipain, 0.25 mM phenylmethylsulfonyl fluoride, 25 mM KCl, and 1 mg/ml lysozyme. The lysate was centrifuged at 20,000 *g* for 30 min at 4°C. The protein was purified over a 1-ml His-trapFF affinity column.

Enzyme Assays

Enzyme assays were performed on fibroblasts by use of methods described elsewhere.[1] The activity of recombinant wild-type and mutated phosphoserine aminotransferase was measured spectrophotometrically at 340 nm by measuring the disappearance of reduced nicotinamide adenine dinucleotide (NADH) in an assay mixture (1 ml) containing, unless otherwise indicated, 25 mM HEPES (pH 7.1), 5 μM pyridoxal phosphate, 1 mM $MgCl_2$, 0.5 mM α-ketoglutarate, 25 mM KCl, 150 μM NADH, and 10 μg/ml recombinant rat liver 3-phosphoglycerate dehydrogenase.[12] The reaction was run at 30°C and was initiated by the addition of 0.5 mM phosphoserine.

Results

3-Phosphoglycerate dehydrogenase and phosphoserine phosphatase activity in fibroblasts of patient 1 were not decreased compared with that in controls (table 3). Phosphoserine aminotransferase activity was low in comparison with that in controls but not sufficiently to conclude that there is a deficiency disorder. At this stage, because of the failure of enzyme assays to reveal a diagnosis despite clear biochemical and clinical indication of a serine/glycine deficiency disorder, the decision was made to begin mutational analysis. No mutations were identified in the *PHGDH* and *PSPH* genes, whereas both affected siblings were found to be compound heterozygotes for mutations in the *PSAT1* gene. They have one frameshift mutation, c.delG107 in exon 2, and one missense mutation, c.299A→C (p.Asp100Ala) in exon 4 (fig. 2). Analysis of DNA from the parents indicated that the frameshift mutation was inherited from the father and that the missense

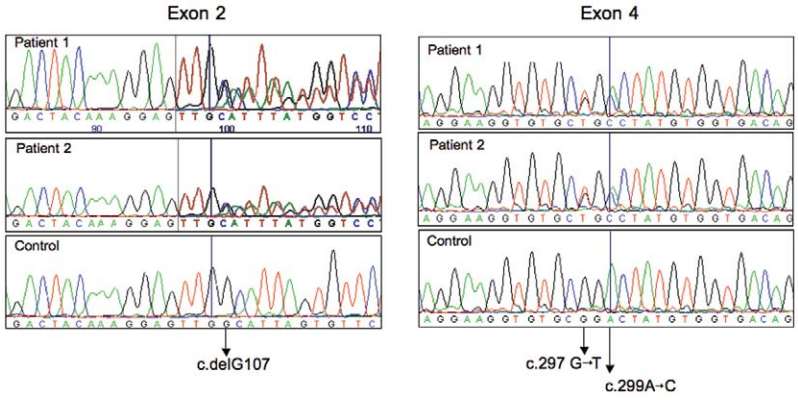

Figure 2. Mutational analysis of patients 1 and 2. A heterozygous 1-bp deletion in exon 2 and a heterozygous A→C transversion in exon 4, leading to the replacement of the Asp at position 100 with an Ala, were found in the *PSAT1* gene. Both patients were also heterozygous for a silent polymorphism in exon 4. In the panels corresponding to exon 2, only the wild-type sequence is shown below each chromatogram.

Figure 3. Alignment of *Homo sapiens* (Hsap) and *E. coli* (Eco1) phosphoserine aminotransferase. Identical residues are indicated in black. The effect of the mutations found in the patients on the amino acid sequence is indicated above the alignment. The asparagine residue (Ser138) that interacts with the side chain of Asp100 in *E. coli* phosphoserine aminotransferase is highlighted in gray.

mutation was inherited from the mother. The missense mutation was found next to a known silent polymorphism in exon 4 (c.297G→T [p.Ala99Ala]; *rs3739474*), which both children inherited from their mother. The missense mutation was not found in 119 controls by denaturing high-performance liquid chromatography and sequencing, whereas the polymorphism was found with a G allele frequency of 0.65 and was consistent with Hardy-Weinberg equilibrium.

Multiple sequence alignments show that Asp100 is well conserved. From the three-dimensional structure of *E. coli* phosphoserine aminotransferase,[13] which shares 46% sequence identity with its human homologue (fig. 3), it turns out that the residue in the equivalent position in the bacterial sequence (Asp93) is buried, with its side chain hydrogen bonded to the nitrogen and the side-chain hydroxyl group of Ser138 (fig. 4). It is likely that, in the human sequence, Asp100 forms bonds with the asparagine (Asn143) similar to those between Asp 93 and Ser138 of the *E. coli* sequence. Replacement of Asp100 by an alanine will disrupt the hydrogen bonds that associate Asp100 with the neighboring loop.

To test the effect of the Asp100Ala mutation, we prepared bacterial expression vectors that encode wild-type and mutated human phosphoserine aminotransferases as fusion proteins with an N-terminal His tag. Both the wild-type protein and the Asp100Ala mutant were expressed in *E. coli*. Extracts were prepared, and the recombinant proteins were purified by metal-affinity chromatography. These experiments showed that the yield of soluble phosphoserine aminotransferase was consistently much lower (by ~10-fold) for the mutant protein than for the wild-type protein (fig. 5). This was observed irrespective of the temperature at which the protein was expressed (18°C or 37°C). The kinetic properties of both purified proteins were determined. The main difference found was that the V_{max} of the purified mutated protein (expressed per mg of purified protein) was ~15% of the V_{max} of the purified wild-type protein (V_{max} = 1.35 μmol/min/mg of protein). No difference in K_m for phosphoserine (5 μM in both cases) was noticed.

Discussion

The first two individuals with phosphoserine aminotransferase deficiency have been identified and described. This disorder is characterized biochemically by low plasma and CSF concentrations of serine and glycine and clinically by intractable seizures, acquired microcephaly, hypertonia, and psychomotor retardation. Outcome is poor once the individual becomes symptomatic, but the younger sibling of the index patient has received successful treatment with serine and glycine supplementation that began at birth.

The biochemical and clinical features of phosphoserine aminotransferase deficiency are similar to those of 3-phosphoglycerate dehydrogenase deficiency. Importantly, in keeping with the other serine/glycine deficiency disorders, these two patients have a partial enzyme deficiency. This is not unexpected, given that, in the mouse *Phgdh* knockout model, complete enzyme deficiency is incompatible with life at the fetal stage.[14]

Although the clinical presentation of patient 1 is very similar to that of patients with 3-phosphoglycerate dehydrogenase deficiency, there are some important differences. First, neither patient 1 nor patient 2 (who did not receive treatment until birth) displayed congenital microcephaly. On first consideration, this may suggest a less severe disorder with a greater bioavailability of serine and glycine, despite the equally low plasma and CSF levels. However, once patient 1 became symptomatic, he displayed symptoms that can be considered significantly

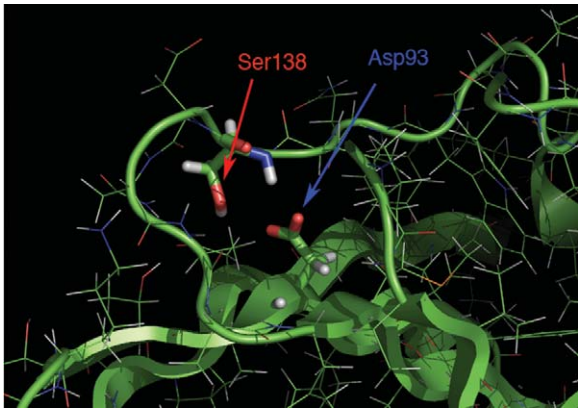

Figure 4. Model of the position of Asp93 (equivalent of Asp100) and the neighboring residue Ser138 (equivalent of Asn143) in *E. coli* phosphoserine aminotransferase. The image was drawn with PyMOL software by use of the *E. coli* phosphoserine aminotransferase coordinates (Protein Data Bank code 1BJN).[10]

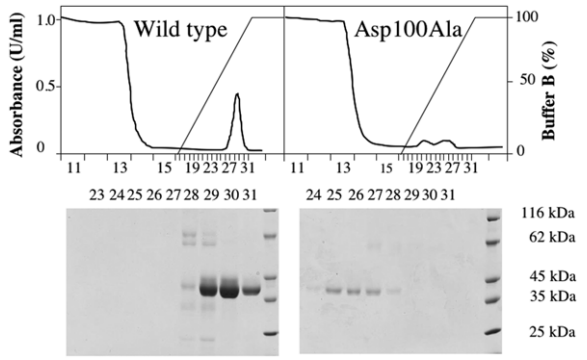

Figure 5. Purification of recombinant human wild-type and Asp100Ala mutant phosphoserine aminotransferase by chromatography on metal-affinity column. Bacterial extracts were loaded onto a 1-ml His-trapFF affinity column (not shown), which was washed with buffer. Phosphoserine aminotransferase was eluted with a linear imidazole gradient. Fractions were collected as indicated. Samples from the indicated fractions were analyzed by SDS-PAGE.

more severe than those displayed by the patients with 3-phosphoglycerate dehydrogenase deficiency. With congenital microcephaly and severe developmental delay, the dehydrogenase-deficient patients may not display overt seizure activity until ages 6 mo to 1 year, and long-term survival is the norm.[6] In addition, they display a much greater clinical response to serine and glycine supplementation, with seizures being much better controlled or even abolished. Patient 1, however, showed little response to treatment with serine and glycine supplementation, despite normalization of plasma and CSF levels, and died at age 7 mo. The severity of the outcome in this patient is possibly due to brain damage suffered during a seizure episode early in the clinical course.

The experience with patient 2 confirms the observation of de Koning et al. that serine-deficiency disorders can be treated successfully if treatment is begun before symptoms appear.[15] In the case of patients with 3-phosphoglycerate dehydrogenase deficiency, this requires prenatal treatment. Given that the patients with phosphoserine transaminase deficiency appear to be just as deficient in serine and glycine as are the patients with 3-phosphoglycerate dehydrogenase deficiency, the fact that neither patient was overtly symptomatic at birth suggests that the transaminase deficiency was better tolerated during prenatal life than was the dehydrogenase deficiency. Another possibility is that, because of other genetic or external dietary factors, there was a better supply of serine and glycine to the fetus from this mother, although, given the poor transportation of serine and glycine across the blood-brain barrier and the supraphysiological doses of serine and glycine required to treat this condition, it is hard to envisage a mechanism that would make such a substantial difference in the availability of these amino acids to the fetal brain.[9,15,16] Until additional, unrelated patients are identified, it is not possible to know whether rapidly acquired, rather than congenital, microcephaly is the norm in phosphoserine aminotransferase deficiency. It is clear from patient 1 that, very shortly after birth, at a time when brain development is still a very active process, the supply of serine and glycine became insufficient to meet requirements, and irreversible damage was sustained.

Previous studies have indicated that 3-phosphoglycerate dehydrogenase and phosphoserine aminotransferase both catalyze reactions that are close to thermodynamic equilibrium.[17] It is therefore unlikely that 3-phosphohydroxypyruvate accumulates in phosphoserine aminotransferase deficiency, particularly because 3-phosphoglycerate is easily converted to pyruvate (and hence to other metabolites) by glycolysis. The lack of serine, the end product of the pathway, appears, therefore, to be the major mechanism for causation of the clinical features in this disorder, as further indicated by the normal development of patient 2. This is in contrast to disorders of amino acid catabolic pathways. They typically lead to accumulation of an amino acid, an intermediate of the catabolic pathway, or a product thereof, which, in many instances (e.g., phenylalanine in phenylketonuria[18] and succinylacetone in hypertyrosinemia type I[19]) exert toxic effects. The mechanisms by which serine deficiency leads to pathological consequences are unknown, but they probably involve decreased synthesis of serine-derived compounds, such as membrane lipids (phospholipids or ceramides) or the neuromodulators glycine or D-serine. D-serine, which is formed through isomerization of L-serine by serine racemase, is known to be decreased in the CSF of children with 3-phosphoglycerate dehydrogenase deficiency.[8] It is likely that this is also the case for patients with phosphoserine aminotransferase deficiency, though this point is not specifically addressed in the present study. Alternatively, reduced availability of serine may result in deficiency of 1-carbon groups that are essential for purine and pyrimidine metabolism.[7]

These cases highlight the problem of identifying patients with serine/glycine-deficiency disorders and the importance of making the diagnosis, given that subsequent affected siblings can receive very effective treatment. The plasma serine and glycine concentrations of patient 1 were only marginally below the lower limit of the reference range and could easily have been overlooked. This emphasizes the importance of measuring serine and glycine in CSF, as well as in plasma.[1] Enzymatic confirmation of a diagnosis, once a serine-biosynthesis disorder is suspected, is also problematic. Assays of all three enzymes in the pathway in patient 1 did not reveal a clear enzyme deficiency, necessitating mutational analysis to uncover the diagnosis. The decrease in the phosphoserine aminotransferase activity found in fibroblasts of patient 1 was only ~50%, whereas a much larger decrease would be expected from the molecular studies, given that the patient was compound heterozygous for a null mutation and an amino acid substitution that decreases the enzymatic ac-

tivity of recombinant phosphoserine aminotransferase by at least about sixfold. These findings suggest that the enzymatic assay used in fibroblasts is not entirely specific for phosphoserine aminotransferase or that compensatory mechanisms lead to increased expression of the mutated enzyme. It is therefore advisable to look for mutations in the genes encoding the three enzymes of the serine-biosynthesis pathway in patients with serine deficiency.

It is now generally accepted that serine biosynthesis from 3-phosphoglycerate proceeds via the phosphorylated pathway, involving 3-phosphohydroxypyruvate and phosphoserine as intermediates, and that the nonphosphorylated pathway, involving hydroxypyruvate and D-glycerate as intermediates, is used for serine degradation in humans.[20] Some species use serine dehydratase to degrade serine. Phosphoserine phosphatase catalyzes an irreversible reaction, and there is no kinase that acts on free serine, which indicates that the phosphorylated pathway can serve only to make serine. Reciprocally, the existence of a kinase that converts D-glycerate to 2-phosphoglycerate and the finding that D-glycerate dehydrogenase shows an ~200-fold higher affinity for NADP (which is essentially present in reduced form) than for NAD[21] make it likely that the nonphosphorylated pathway proceeds unidirectionally from serine to 2-phosphoglycerate. Inborn errors of serine metabolism strengthen this view, because a deficiency in any of the three enzymes of the phosphorylated pathway leads to serine and glycine deficiency. Furthermore, deficiency of D-glycerate dehydrogenase[22] leads to the accumulation of L-glycerate, a reduction product of hydroxypyruvate made by L-lactate dehydrogenase, and deficiency of D-glycerate kinase[23] leads to accumulation of D-glycerate. These accumulations make sense only if one considers that the nonphosphorylated pathway serves to degrade serine.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for 3-phosphoglycerate dehydrogenase deficiency and phosphoserine phosphatase deficiency)
PyMOL software, http://pymol.sourceforge.net/

References

1. Jaeken J, Detheux M, Van Maldergem L, Foulon M, Carchon H, Van Schaftingen E (1996) 3-Phosphoglycerate dehydrogenase deficiency: an inborn error of serine biosynthesis. Arch Dis Child 74:542–545
2. de Koning TJ, Duran M, Dorland L, Gooskens R, Van Schaftingen E, Jaeken J, Blau N, Berger R, Poll-The BT (1998) Beneficial effects of L-serine and glycine in the management of seizures in 3-phosphoglycerate dehydrogenase deficiency. Ann Neurol 44:261–265
3. Pineda M, Vilaseca MA, Artuch R, Santos S, Sau I, Aracil A, Van Schaftingen E, Jaeken J (2000) 3-Phosphoglycerate dehydrogenase deficiency in a patient with West syndrome. Dev Med Child Neurol 42:629–633
4. Jaeken J, Detheux M, Fryns J-P, Collet J-F, Alliet P, Van Schaftingen E (1997) Phosphoserine phosphatase deficiency in a patient with Williams syndrome. J Med Genet 34:594–596
5. Veiga-da-Cunha M, Collet J-F, Prieur B, Jaeken J, Peeraer Y, Rabbijns A, Van Schaftingen E (2004) Mutations responsible for 3-phosphoserine phosphatase deficiency. Eur J Hum Genet 12:163–166
6. de Koning T, Duran M, Van Maldergem, Pineda M, Dorland L, Gooskens R, Jaeken J, Poll-The BT (2002) Congenital microcephaly and seizure due to 3-phosphoglycerate dehydrogenase deficiency: outcome of treatment with amino acids. J Inherit Metab Dis 25:119–125
7. de Koning T, Snell K, Duran M, Berger R, Poll-The BT, Surtees R (2003) L-serine in disease and development. Biochem J 371: 653–661
8. Fuchs SA, Dorland L, de Sain-van der Velden MG, Hendriks M, Klomp LW, Berger R, de Koning TJ (2006) D-serine in the developing human central nervous system. Ann Neurol 60: 476–480
9. Smith Q, Momma S, Aoyagi M, Rappoport S (1987) Kinetics of neutral amino acid transport across the blood-brain barrier. J Neurochem 49:1651–1658
10. Baek JY, Jun do Y, Taub D, Kim YH (2003) Characterization of human phosphoserine aminotransferase involved in the phosphorylated pathway of L-serine biosynthesis. Biochem J 373:191–200
11. Studier FW, Moffatt BA (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J Mol Biol 189:113–130
12. Achouri Y, Rider MH, Van Schaftingen E, Robbi M (1997) Cloning, sequencing and expression of rat liver 3-phosphoglycerate dehydrogenase. Biochem J 323:365–370
13. Hester G, Stark W, Moser M, Kallen J, Markovic-Housley Z, Jansonius JN (1999) Crystal structure of phosphoserine aminotransferase from *Escherichia coli* at 2.3 Å resolution: comparison of the unligated enzyme and a complex with α-methyl-L-glutamate. J Mol Biol 286:829–850
14. Yoshida K, Furuya S, Osuka S, Mitoma J, Shinoda Y, Watanabe M, Azuma N, Tanaka H, Hashikawa T, Itohara S, et al (2004) Targeted disruption of the mouse 3-phosphoglycerate dehydrogenase gene causes severe neurodevelopmental defects and results in embryonic lethality. J Biol Chem 279:3573–3577
15. de Koning T, Klomp L, Van Oppen A, Beemer F, Dorland L, Van den Berg I, Berger R (2004) Prenatal and early postnatal treatment in 3-phosphoglycerate dehydrogenase deficiency. Lancet 364:2221–2222
16. Ronzoni S, Marconi A, Cetin I, Paolini C, Teng C, Pardi G, Battaglia F (1999) Umbilical amino acid uptake at increasing maternal amino acid concentrations: effect of a maternal amino acid infusate. Am J Obstet Gynecol 181:477–483
17. Lund K, Merrill DK, Guynn RW (1985) The reactions of the phosphorylated pathway of L-serine biosynthesis: thermodynamic relationships in rabbit liver *in vivo*. Arch Biochem Biophys 237:186–196
18. Scriver CR, Kaufman S (2001) Hyperphenylalaninemia: phe-

nylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 1667–1724
19. Mitchell GA, Grompe M, Lambert M, Tanguay RM (2001) Hypertyrosinemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 1777–1805
20. Snell K (1986) The duality of pathways for serine synthesis is a fallacy. Trends Biochem Sci 11:241–243
21. Van Schaftingen E, Draye JP, Van Hoof F (1989) Coenzyme specificity of mammalian liver D-glycerate dehydrogenase. Eur J Biochem 186:355–359
22. Danpure C (2001) Primary hyperoxaluria. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 3323–3356
23. Van Schaftingen E (1989) D-glycerate kinase deficiency as a cause of D-glyceric aciduria. FEBS Lett 243:127–131