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Phosphoserine phosphatase deficiency in a patient with Williams syndrome

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Abstract

Decreased serine levels were found in plasma and cerebrospinal fluid (CSF) of a boy with pre- and postnatal growth retardation, moderate psychomotor retardation, and facial dysmorphism suggestive of Williams syndrome. Fluorescence in situ hybridisation with an elastin gene probe indicated the presence of a submicroscopic 7q11.23 deletion, confirming this diagnosis. Further investigation showed that the phosphoserine phosphatase (EC 3.1.3.3.) activity in lymphoblasts and fibroblasts amounted to about 25% of normal values. Oral serine normalised the plasma and CSF levels of this amino acid and seemed to have some clinical effect. These data suggest that the elastin gene and the phosphoserine phosphatase gene might be closely linked. This seems to be the first report of phosphoserine phosphatase deficiency.

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Serine is a non-essential, key amino acid, synthesised de novo from 3-phosphoglycerate, a glycolytic intermediate. As shown in fig 1, the latter is converted to serine through the successive action of 3-phosphoglycerate dehydrogenase (EC 1.1.1.95), 3-phosphoserine aminotransferase (EC 2.6.1.52), and 3-phosphoserine phosphatase (EC 3.1.3.3.).[1–3] This amino acid can also be synthesised from glycine. Serine is not only a building block for protein synthesis but also a precursor of a number of compounds including amino acids (glycine and cysteine), phospholipids (phosphatidylserine and sphingomyelin), and glycolipids. It is also a major source of methylene tetrahydrofolate and of other one carbon donors required for the synthesis of purines and thymidine.[4] We previously reported decreased serine levels in the cerebrospinal fluid of two brothers with congenital microcephaly, profound psychomotor retardation, epilepsy, and growth retardation. Fibroblasts of both patients showed decreased levels of 3-phosphoglycerate dehydrogenase.[5] In this paper we report on a boy with Williams syndrome and with decreased levels of serine in plasma and CSF. It was found that this patient, in addition to a defect in the elastin locus typical of Williams syndrome,[6–9] had a partial defect in the last step of serine biosynthesis, namely that catalysed by 3-phosphoserine phosphatase.

Patient and methods

The patient, a Belgian boy, was born by caesarian section after a 37 week pregnancy, with birth weight 1760 g, length 42.5 cm, and head circumference 30 cm. He had early feeding difficulties associated with gastro-oesophageal reflux and oesophagitis. He also had facial features suggestive of Williams syndrome including puffy eyelids, broad forehead, bitemporal narrowing, wide mouth, full cheeks, and micrognathia. There was also grade II hypospadias. Length and weight evolved approximately parallel with, and below, the 3rd centile (at 22 months, length was 75 cm (3rd centile=80 cm) and weight 8.4 kg (3rd centile=10 kg)) while the head showed a slight catch up growth before the age of 4 months and between the ages of 12 and 22 months (43.5 cm at 1 year, 3rd centile=45 cm and 46 cm at 22 months, 3rd centile=46.6 cm). Psychomotor development was slow. Plasma amino acid analysis by ion exchange chromatography and fluorescence detection showed decreased to low-normal fasting serine levels (53-80 µmol/l, n=4 between the ages of 5 and 12 months; normal range 70-187). One to two hours after feeding, serine levels were normal. CSF serine was decreased (18 µmol/l at the age of 1 year; control range 27-57). Plasma and CSF phosphoserine and glycine levels were normal.

Chromosome studies were performed on a peripheral blood lymphocyte culture with G banding and fluorescent in situ hybridisation using the elastin Williams syndrome chromosome region (WSCR) probe, with D7S42 chromosome control probe.

Oral L-serine was given to the patient from the age of 1 year up to the present age of 22 months. The initial dosage of 200 mg/kg per day was increased to 300 mg/kg per day at the age of 15 months (divided into three doses per day).

Serine biosynthesis was investigated in fibroblasts and lymphoblasts. The source of reagents and the enzymatic assays have been described elsewhere.[5] Phosphoserine phosphatase was also assayed through the release of [^{32}P]Pi from [^{32}P]phosphoserine. The assay mixture contained 25 mmol/l Mes buffer, pH 6.5, 0.1 mmol/l phosphoserine, 20 000 cpm [^{32}P]phosphoserine, 1 mmol/l $MgCl_2$, 1 mmol/l di-thiothreitol, and 5 µl of extract in a final

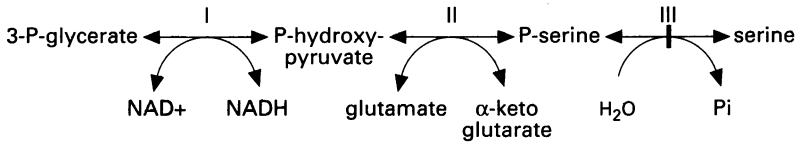

Figure 1 The pathway of de novo serine biosynthesis. I: 3-phosphoglycerate dehydrogenase; II: 3-phosphoserine aminotransferase; III: 3-phosphoserine phosphatase. Vertical block denotes the enzymatic defect in the patient.

volume of 0.1 ml. The incubations were carried out at 30°C for 10 to 30 minutes and were arrested by addition of 250 µl of 5% thrichloroacetic acid and 5 µl of 10 mmol/l Pi. [^{32}P]Pi was extracted according to McClard.[10] Fibroblasts (passages 3-11) were cultured as previously described.[5] Lymphoblasts (passages 2-5) were cultured in Iscove's Modified Eagle Medium supplemented with 10% fetal calf serum, GlutaMAX I, 0.24 mmol/l asparagine, 0.55 mmol/l arginine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cells in suspension were collected by centrifugation (1000 × *g*, 10 minutes) and washed twice in Mg^{2+}/Ca^{2+} free phosphate buffered saline. Cell pellets were either disrupted immediately for enzyme assays or stored at −80°C for up to three months without loss of activity. Cell extracts were prepared as previously described.[5]

Results

Prometaphase chromosome studies showed an apparently normal 46,XY G banded karyotype but fluorescent in situ hybridisation showed a submicroscopic 7q11.23 deletion in all 10 cells examined.

During treatment with oral serine, a slight catch up of head growth was noted; whereas before treatment, from the ages of 4 to 12 months, head circumference remained at 1.5 cm below the 3rd centile, after 10 months of oral serine, head circumference was only 0.6 cm below the 3rd centile. There was no catching up of length and weight. Under treatment with serine at 200 mg/kg per day (divided into three doses) fasting CSF serine level was 23 µmol/l (control range 27-57) before the morning serine dose, while under 300 mg/kg per day a low normal value of 29 µmol/l was obtained.

Activity of 3-phosphoserine phosphatase amounted to only about 25% of the activity of the mean control value both in fibroblasts (0.36 and 0.50 mU/mg protein; controls (n=14), mean 1.67, SD 0.18) and in lymphoblasts (0.35 and 0.39 mU/mg protein; controls (n=11), mean 1.34, SD 0.09), whereas the activities of 3-phosphoglycerate dehydrogenase and of 3-phosphoserine aminotransferase were normal (table 1). The Km of phosphoserine phosphatase (determined on double reciprocal plots) was about 10 µmol/l both in the patient and in the controls. There was, however, a significant difference in the inhibition exerted by serine. The release of [^{14}C]serine from [^{14}C]serine 3-phosphate was partly inhibited by cold serine when it was catalysed by the enzyme from controls but not when it was catalysed by the enzyme of the patient (fig 2A). When the enzymatic activity was measured through the liberation of [^{32}P]Pi from [^{32}P]phosphoserine, the activity was inhibited in all cases, though relatively less with the patient's enzyme than with the enzyme from controls (fig 2B).

Table 1 Activities of the enzymes of the serine biosynthesis in fibroblasts and lymphoblasts of the patient and controls (x [SD] mU/mg protein)

	3-phosphoglycerate dehydrogenase	*3-phosphoserine aminotransferase*	*3-phosphoserine phosphatase*
Fibroblasts			
Controls	29.49 [2.65] (15)	1.98 [0.28] (9)	1.67 [0.18] (14)
Patient	26.28 [1.36] (3)	3.14 [0.21] (3)	0.36; 0.50
Lymphoblasts			
Controls	ND	0.88 [0.23] (11)	1.34 [0.09] (11)
Patient	ND	0.84 [0.34] (3)	0.35; 0.39

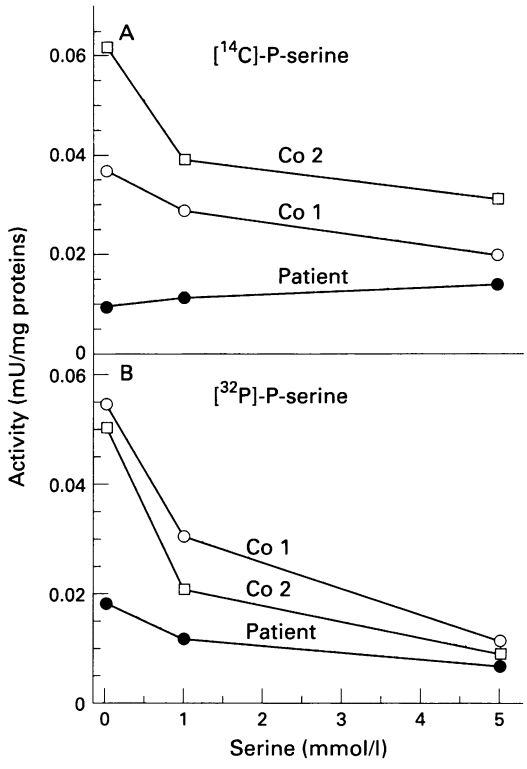

Figure 2 Effect of serine on phosphoserine phosphatase activity in extracts of lymphoblasts from two controls and the patient. The enzymatic activity was measured in lymphoblast extracts by the release of radiolabelled serine (A) or Pi (B) from [^{14}C] or [^{32}P]phosphoserine.

Discussion

The present patient had moderately but significantly decreased levels of serine in CSF and low to low-normal fasting levels of plasma serine. These findings suggested a defect in the biosynthesis of serine. This was confirmed by the finding in cells of the patient of markedly decreased activity of 3-phosphoserine phosphatase, the enzyme catalysing the third and rate limiting step of serine synthesis from the glycolytic intermediate, 3-phosphoglycerate (fig 1). Since this step is rate limiting for the pathway,[11-13] the decrease in phosphoserine phosphatase activity is expected to result in a commensurate decrease in the formation of this amino acid. However, the clinical picture was suggestive of Williams syndrome. This diagnosis was substantiated by finding a deletion in the elastin gene region of chromosome 7 (7q11.23).[9] The association of Williams syndrome and 3-phosphoserine phosphatase deficiency in the same patient

suggests a relationship between them, the most probable being that the 3-phosphoserine phosphatase gene is closely linked to the elastin gene and therefore involved in the deletion. Studies with interspecific cell hybrids have indeed indicated that human 3-phosphoserine phosphatase is encoded by chromosome 7.[14] The more precise assignment to region 7p15[15 16] could argue against a deletion involving both genes. It should be noted, however, that this assignment was based on gene dosage studies[15 16] and that these have to be interpreted with caution since the activity of phosphoserine phosphatase was found by Minelli *et al*[17] not to be reduced in cells with monosomy 7.

The fact that the enzymatic activity is reduced by more than 50% (actually to about 25%) and the finding that it has modified kinetic properties compared to the controls indicate that the second copy of the gene is mutated. An alternative explanation would be that the residual activity is contributed by the product of a different gene. Moro-Furlani *et al*[12] found, however, that in most tissues phosphoserine phosphatase consisted of one single major isozyme; the minor isozymes found in some samples of postmortem tissue were probably the result of post-translational modification.

Whether the moderate decrease of serine levels in the present patient has clinical consequences remains to be determined. That there might be an effect is suggested by the slight catch up of the head growth. This treatment was able to normalise CSF and probably also brain serine levels. As previously pointed out for 3-phosphoglycerate dehydrogenase deficiency,[5] this synthesis defect is unusual among the amino acid defects, which, in the large majority, are catabolic defects.[18]

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