

# Paternally inherited deletion of CSH1 in a patient with Silver-Russell syndrome

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## Abstract

**In a continuing study on the aetiology of Silver-Russell syndrome (SRS), we detected a patient with a heterozygous deletion in the growth hormone gene cluster (17q22-q24). The deletion of the chorionic somatomammotrophin hormone 1 (CSH1) gene was inherited from the patient's father. The patient shows typical symptoms of SRS. Though deletions of CSH1 have been reported without any phenotypic consequences, the heterozygous deletion might be involved in the aetiology of SRS in the case presented here. Apart from other observations in SRS, like maternal uniparental disomy 7, changes in the genomic region 17q22-qter might be responsible for the expression of this syndrome for at least some of the patients, leading to the heterogeneity of SRS.**

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Silver-Russell syndrome (SRS), a malformation syndrome characterised by pre- and post-natal growth retardation, has been described in more than 380 patients, most of them being sporadic cases.<sup>1</sup> The aetiology is not yet known. Even though a few patients with chromosomal abnormalities have been described, there is no uniform pattern of genetic abnormalities, apart from two SRS patients where the chromosomal region 17q25 was involved in chromosomal rearrangements.<sup>2,3</sup> Additionally, several patients with features similar to SRS and partial monosomy for distal 15q have been reported.<sup>4</sup> Studies published previously describe maternal uniparental disomy for the entire chromosome 7 in almost 10% of the patients.<sup>5-8</sup>

The human growth hormone (GH) gene cluster is localised in the vicinity of the breakpoint 17q25 (17q22-q24). It consists of two GH genes and three genes encoding chorionic somatomammotrophin hormones (CSH), the arrangement of which in the GH gene cluster has been defined as 5'GH-N (normal) - CSH-L (like) - CSH1 - GH-V (variant) - CSH2.<sup>9</sup> Only GH-N, CSH1, and CSH2 are thought to be expressed. CSHs are expressed by the placental syncytiotrophoblast and therefore their genetic determination is a function of the fetal genome. For the two functional chorionic CSHs, a gene dosage effect in the case of deletions of one of the genes has been

described, a situation somewhat comparable to that in the  $\alpha$  thalassaemias.<sup>10</sup>

To evaluate the number of SRS patients with genomic variations in the GH cluster, we screened 54 SRS families with a short tandem repeat marker (STR) located in this cluster. Further characterisation of the GH gene cluster was performed with a specific cDNA probe.

## Case report

The patient was born at 30 weeks of gestation by caesarean section. Severe growth retardation was detected in the 23rd week by ultrasound. The operation was performed because of oligohydramnios and an acute deterioration of CTG. At birth, the infant had symmetrical growth retardation with a weight of 640 g ( $-3.9$  SD<sup>11</sup>), a length of 32.5 cm ( $-3.4$  SD), and a head circumference of 22.7 cm. The placenta was small with infarctions. The patient was the second child of a healthy mother. The first child was born prematurely after 28 weeks and died 10 days postnatally. The mother's height is within the normal range (161 cm,  $-0.6$  SD), whereas the father is short (163 cm,  $-2.2$  SD). The patient's motor development was slightly delayed and in childhood he had behavioural difficulties with hyperactivity. Myopia was diagnosed at the age of 2½ years and is not progressive. In infancy, the patient exhibited typical dysmorphic features of SRS with a large forehead and a small, asymmetrical face. Throughout infancy and childhood, severe growth failure continued. At the age of 7½ years he had a height of 108.9 cm ( $-3.1$  SD) and a weight of 14.7 kg ( $-2.8$  SD); bone age was 5½ years (Greulich and Pyle). Studies performed at this age showed a normal male karyotype, normal GH secretion, and normal values for serum IGF-I, IGFBP-3, and thyroid hormones.

## DNA studies

Genomic DNA was extracted from peripheral lymphocytes of the patient and his parents according to standard protocols.<sup>13</sup> A cell line is not available.

To identify variations in the GH gene cluster, short tandem repeat typing (STR) of the D17S254 locus<sup>14</sup> was carried out. D17S254 is localised in the GH cluster, 1.4 kb upstream of CSH1 and 11.6 kb downstream of CSH-L. Additionally, we typed the following STRs to confirm biparental origin of the chromosomes 17: (17pter)-D17S1866-D17S926-D17S1606-D17S254-D17S948-D17S802-(17qter) (table 1). Data and PCR conditions can be obtained

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Table 1 Results of STR typing in the patient heterozygous for the CSH1 deletion and his parents. The order of the loci corresponds to the map published by Dib *et al*<sup>3</sup>

Locus	Allelic status of father	Mother	Patient	Informativity*
D17S1866	3-4	1-2	1-4	Biparental
D17S926	1-1	1-2	1-1	—
D17S1606	1-3	1-2	1-3	Heterozygosity
D17S254	1†	2-3	3	Hemizygosity
D17S948	2-3	1-3	1-3	Heterozygosity
D17S802	1-2	3-4	2-4	Biparental

\*Informativity in respect to the patient's allelic status.  
 †The father's monoallelic status in D17S254 might be interpreted as hemizygosity.

from the Genome Database. To exclude the possibility of allelic non-amplification<sup>15</sup> of D17S254, we designed a new pair of primers spanning the polymorphic region. Primer sequences can be requested from the authors. STR PCR products were analysed by sequencing gel electrophoresis and bands were visualised by silver staining.

For further analysis of the observed hemizygosity in the GH gene cluster in one of our patients, we used the GH cDNA cGH800 probe.<sup>16</sup> The probe contains nearly the full length cDNA of the human growth hormone and is homologous to the five genes in the GH cluster. Four µg of genomic DNA of the patient and his parents as well as of healthy controls was digested with restriction endonucleases *Bam*HI or *Hind*III at 4 U/µg DNA for six hours. Digested DNA was subjected to electrophoresis on a 0.7% agarose gel. Transfer to Hybond N<sup>+</sup> nylon membranes (Amersham) was performed by alkaline blotting and 25 ng of the non-linearised cGH800/pSPT18 plasmid was labelled with <sup>32</sup>P-dCTP using a random priming DNA labelling kit (Amersham). Hybridisation was performed at 42°C overnight. After stringent washing at 60°C, filters were autoradiographed overnight with x ray film at -70°C.

**Results and discussion**

In this study we screened SRS patients for genomic variations in the GH cluster (17q23-24). By typing 54 SRS families with the STR D17S254, we observed hemizygosity in one out of 46 informative cases. Analysis of the patient's DNA showed only one maternal allele (fig 1) but no paternal allele. Allelic non-amplification, as described by Koorey *et al*,<sup>15</sup> could be excluded. Biparental inheritance of the homologous chromosomes 17 was shown by analysing further STRs on chromosome 17 (table 1). Paternity was assessed by typing five STRs from chromosomes other than chromosome 17.

To identify a possible deletion in the GH gene cluster of the hemizygous patient, we hybridised the DNA of the patient and parents as well as that of healthy controls with a cDNA probe for the GH gene. The DNA of the SRS family and those of the controls displayed the pattern expected after *Bam*HI digestion: 8.3 kb (CS-L), 6.8 kb (CSH2), 5.3 kb (CSH1), 3.8 kb (GH-N), and 3 kb +1.2 kb (GH-V) (fig 2). The sizes of all bands were normal, but both the patient's DNA and that of his father showed

weaker intensities of the 5.3 kb band (CSH1). We conclude that the patient and his father are heterozygous for a deletion of CSH1. Digestion with *Hind*III confirmed this finding (not shown): the 14.8 kb band corresponding to CSH1 was weaker in the patient and his father than in controls. The other two bands, 21.3 and 25 kb, representing GH-V-CSH2 and GH-N-CSH-L, respectively, were normal.

Taking the results of these tests together, we conclude that the deletion in the case presented here is within the 5.3 kb fragment of CSH1 and includes the coding region of CSH1 as well as the cAMP and the PMA responsive elements (-1102 to -1096).

Alterations in the GH gene cluster have not so far been described in SRS patients. Inactivation of placental CSH and GH genes causes an abnormal biochemical phenotype, but it does not cause disease.<sup>17,18</sup> CSH concentrations in maternal serum increase linearly until about the 30th week and the rate of secretion is greater than that of any protein hormone. Reduced CSH secretion has been detected in different pathological conditions associated with abnormal fetal growth, including diabetes, pre-eclampsia, erythroblastosis, and intrauterine growth retardation.<sup>19</sup> In the mother the increase of serum IGF-I during pregnancy, which is a prerequisite for adequate nutrient supply to the fetus and normal fetal growth,<sup>20</sup> is at least in part the result of the increasing CSH concentrations. In pregnancies associated with fetal homozygosity for deletion of the CSH genes, maternal IGF-I concentrations decline to values normally associated with hypopituitarism. Because of this, Grumbach *et al*<sup>21</sup>

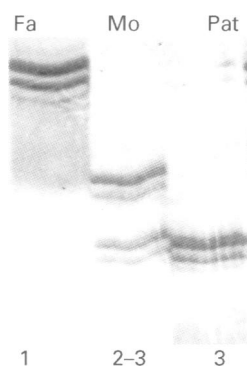


Figure 1 Typing of the STR D17S254 shows hemizygosity of the patient. The patient has inherited only one maternal allele (3) and no paternal allele was detectable. The father's "homozygosity" at this locus (allele 1) corresponds to the hemizygosity at this locus observed by using a GH-cDNA probe. The slight band shift between the maternal and patient's PCR product is an artefact. Fa=father, Mo=mother, Pat=patient.

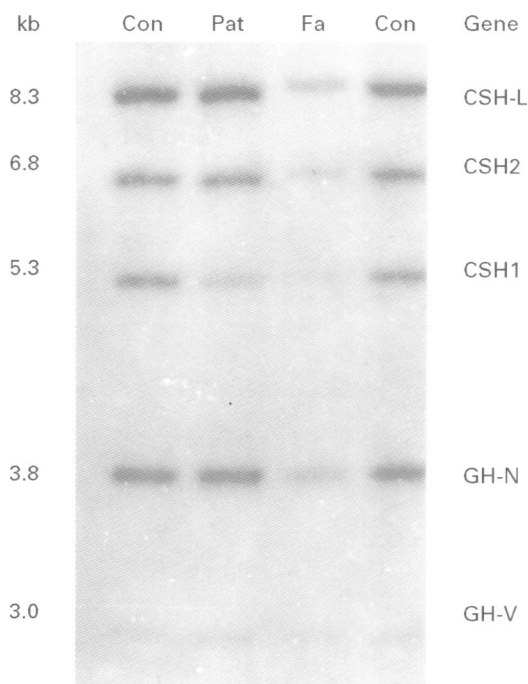


Figure 2 Autoradiograph of DNA digested with *Bam*HI and hybridised with <sup>32</sup>P-dCTP-GH cDNA probe from the SR patient, his father, and two healthy controls. Note the weaker intensities of the CSH1 band (5.3 kb) in the patient and his father. Band intensities were compared by eye in the patient, his father, and nine controls. The 1.2 kb band of GH-V cannot be seen on the autoradiograph. Con=control, Pat=patient, Fa=father.

suggested a role for CSH in supplying of nutrients to the fetus during maternal fasting.

Although no direct effect of CSH deficiency on fetal growth has been shown so far,<sup>17 18</sup> recent experiments indicate that CSH may indirectly affect fetal tissue growth.<sup>19</sup> CSH stimulates amino acid transport and uptake and IGF production and there are diabetogenic and anabolic effects of CSH in different fetal tissues mediated by specific CSH receptors.<sup>19</sup>

Additionally, it has been shown that in pathological pregnancies with intrauterine growth retardation CSH concentrations are reduced.<sup>22</sup> In summary, CSH plays a pivotal role during pregnancy, but whether there is a relation between the heterozygous deletion in our patient and the observed intrauterine growth retardation remains unclear.

The paternally inherited heterozygous deletion of CSH1 we found might be an interesting finding in respect to the aetiology of intrauterine growth restriction and Silver-Russell syndrome, which to our present knowledge seems to be heterogeneous. Further analyses will show whether there are candidate genes or candidate gene clusters in the vicinity of the breakpoint 17q25 and whether CSH1 or functionally related factors are involved in the aetiology of SRS.

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