Silver-Russell syndrome\(^4\) \(^5\) is a malformation syndrome characterised by a severe reduction in weight and length at birth, short stature in later life, asymmetry of the head and limbs, and other less constant abnormalities.\(^5\) Typical craniofacial abnormalities include a relatively large, prominent forehead and a small triangular face. The aetiology of the disease is heterogeneous. However, in approximately 7-10% of cases maternal uniparental disomy (UPD) of chromosome 7 can be detected.\(^1\) Additionally, Hannula et al.\(^1\) have reported a SRS patient with a segmental maternal UPD(7) restricted to 7q31-qter. The finding of maternal UPD(7) in SRS indicates that either mutations in imprinted genes on chromosome 7 or imprinting mutations are responsible for the SRS phenotype in at least some patients.

So far, three imprinted loci have been identified on chromosome 7: growth factor receptor protein 10 (GRB10) in 7p12,\(^1\) paternally expressed gene 10 (PEG10) and epsilon-sarcoglycan (SGCE) in 7q21,\(^1\) and the mesoderm specific transcript (MEST) in 7q32.\(^1\) Owing to their role in human growth, their genomic localisation, and their imprinting status, GRB10 and MEST have been exhaustively studied by several groups for mutations in SRS patients. There is no evidence for a major role of these genes in the aetiology of the disease.\(^3\)\(^4\)

Recently, Nakabayashi et al.\(^3\) identified a non-coding RNA that might be involved in the regulation of MEST expression during development. The corresponding DNA sequence is localised in the intron of one of the two MEST isofoms and is called \textit{MESTIT1} (MEST intronic transcript 1). \textit{MESTIT1} is composed of two exons separated by an intron of 874 bp. Nakabayashi et al.\(^3\) showed that the transcript \textit{MESTIT1} is paternally expressed in fetal tissues and fibroblasts and that it is transcribed in the opposite direction to \textit{MEST} without any significant open reading frame.\(^3\) It exists as a 4.2 kb transcript in many fetal and adult tissues.

Although mutations in the \textit{MEST} gene itself could not be identified in three independent studies\(^9\)\^-\(^12\) (S Mergenthaler, personal communication), it is conceivable that genomic disturbances of \textit{MESTIT1} result in altered expression of \textit{MEST} and thereby cause the SRS phenotype. Therefore, genomic alterations of \textit{MESTIT1} might be involved in the aetiology of SRS.

### MATERIAL AND METHODS

We studied 46 patients with clinical features of SRS according to Wollmann et al.\(^1\) In this cohort, chromosomal aberrations and maternal UPD(7) had been previously excluded. As controls, we screened more than 50 German probands of normal growth. The study was approved by the ethical committee of the University Hospital of Aachen.

### Table 1

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Nucleotide position</th>
<th>Primer F-R</th>
<th>Fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MESTIT1-1</td>
<td>142451-142800*</td>
<td>aagcacttgctcttgtagta</td>
<td>350</td>
</tr>
<tr>
<td>MESTIT1-2</td>
<td>142756-143105*</td>
<td>cttacctccgcttctctct</td>
<td>350</td>
</tr>
<tr>
<td>MESTIT1-3</td>
<td>143033-143440*</td>
<td>gtagcagggcagctgacctat</td>
<td>408</td>
</tr>
<tr>
<td>MESTIT1-4</td>
<td>143368-143708*</td>
<td>acgctcggagcaaagagtgttg</td>
<td>341</td>
</tr>
<tr>
<td>MESTIT1-5</td>
<td>143656-144006*</td>
<td>gctacgagagatggcccat</td>
<td>351</td>
</tr>
<tr>
<td>MESTIT1-6</td>
<td>143953-144308*</td>
<td>aaccttcgtcgtcaaccccta</td>
<td>355</td>
</tr>
<tr>
<td>MESTIT1-7</td>
<td>144231-144585*</td>
<td>ctaaccatctggctaggaatgg</td>
<td>360</td>
</tr>
<tr>
<td>MESTIT1-8</td>
<td>144541-144900*</td>
<td>tctctgtgctggctacccccc</td>
<td>358</td>
</tr>
<tr>
<td>MESTIT1-9</td>
<td>144853-145210*</td>
<td>gctacgagacacagggatgt</td>
<td>281</td>
</tr>
<tr>
<td>MESTIT1-10</td>
<td>145155-145435*</td>
<td>aagcagagacacagagggagga</td>
<td>397</td>
</tr>
</tbody>
</table>

The nucleotide position corresponds to that in *AC007938 and †AB045582.

### Key points

- Owing to its putative role as regulator of MEST expression, the transcript \textit{MESTIT1} is a strong candidate gene for Silver-Russell syndrome in 7q32.
- We screened the two exons of \textit{MESTIT1} for genomic variants by SSCP.
- We can exclude that genomic variants in \textit{MESTIT1} are involved in the aetiology of Silver-Russell syndrome.
Genomic DNA was extracted from peripheral lymphocytes by standard techniques. The genomic DNA sequence coding the MESTIT1 transcript was screened by single strand conformation polymorphism analysis (SSCP); the two corresponding DNA segments were divided into 11 fragments to allow a reliable detection rate (table 1). Information on primer sequences are listed in table 1. PCR and SSCP were performed as described recently.13 PCR reactions always included 5% formamide and 10% glycerol and annealing temperature was 50°C for all fragments. To demonstrate the sensitivity of the SSCP and to characterise unusual SSCP patterns, PCR products were characterized by direct sequencing of PCR products using the Big Dye Terminator Cycle Sequencing System (ABI, Wettertard, Germany).

By screening the genomic fragment encoding MESTIT1, we identified three new polymorphisms (table 2). At the nucleotide position of the EST AF482998, an A to T transition was observed at nucleotide 2487, which affects the restriction site of Tsp509I. Two G to A transitions were observed at nucleotide 580 and nucleotide 126; for these variants, restriction assays using MdiI and EagI were established. The allelic distribution of these novel polymorphic variants were similar in SRS patients and controls. Restriction assays were established for the three variants.

**REFERENCES**


