Searching for genomic variants in the MESTIT1 transcript in Silver-Russell syndrome patients

E Meyer, H A Wollmann, T Eggermann

J Med Genet 2003;40:e65 (http://www.jmedgenet.com/cgi/content/full/40/5/e65)

Silver-Russell syndrome 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. 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. Additionally, Hannula et al 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, paternally expressed gene 10 (PEG10) and epsilon-sarcoglycan (SGCE) in 7q21, and the mesoderm specific transcript (MEST) in 7q32. 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.

Recently, Nakabayashi et al 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 isoforms and is called MESTIT1 (MEST intronic transcript 1). MESTIT1 is composed of two exons separated by an intron of 874 bp. Nakabayashi et al showed that the transcript MESTIT1 is paternally expressed in fetal tissues and fibroblasts and that it is transcribed in the opposite direction to MEST without any significant open reading frame. It exists as a 4.2 kb transcript in many fetal and adult tissues.

Although mutations in the MEST gene itself could not be identified in three independent studies (S Mergenthaler, personal communication), it is conceivable that genomic disturbances of MESTIT1 result in altered expression of MEST and thereby cause the SRS phenotype. Therefore, genomic alterations of 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. 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>
<thead>
<tr>
<th>Table 1 Primers used for SSCP screening of MESTIT1 in our study population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fragment</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>MESTIT1-1</td>
</tr>
<tr>
<td>MESTIT1-2</td>
</tr>
<tr>
<td>MESTIT1-3</td>
</tr>
<tr>
<td>MESTIT1-4</td>
</tr>
<tr>
<td>MESTIT1-5</td>
</tr>
<tr>
<td>MESTIT1-6</td>
</tr>
<tr>
<td>MESTIT1-7</td>
</tr>
<tr>
<td>MESTIT1-8</td>
</tr>
<tr>
<td>MESTIT1-9</td>
</tr>
<tr>
<td>MESTIT1-10</td>
</tr>
<tr>
<td>MESTIT1-11</td>
</tr>
</tbody>
</table>

The nucleotide position corresponds to that in *AC007938 and †AB045582.
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.15 PCR reactions always included 5% for-mamide 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 characterised by direct sequencing of PCR products using the Big Dye Terminator Cycle Sequencing System (ABI, Weiterstadt, Germany).

To allow rapid genotyping of MESTIT1, PCR based restriction fragment length polymorphism assays for the novel variants in the transcript were carried out (table 2) using the protocols described previously.16

**RESULTS**

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 MscI and EagI were established.

The allelic distribution of these novel polymorphic variants were similar in SRS patients and controls.

**DISCUSSION**

MESTIT1 has been proposed as a candidate for SRS because of its chromosomal localisation (7q32) and its putative role in the regulation of MEST expression.15 However, we did not detect any clinically relevant changes in MESTIT1. Some genomic variants may have been missed since the sensitivity of SSCP is less than 100%.15 However, the polymorphisms in MESTIT1 show the same distribution in patients and controls excluding the possibility of allelic association.

To sum up, polymorphisms in MESTIT1 are unlikely to play an important role in SRS. In addition to MEST/PEG1, COPG2, and PAH3,12,17 MESTIT1 is a further transcript in 7q32 that has been excluded as a gene causing SRS. However, the identification of an imprinting cluster in 7q32 defined by the genes above as well as the finding of maternal UPD(7) in nearly 10% of SRS patients, among them a patient with a UPD restricted to 7q31-pter, makes the delineation of SRS as another imprinting syndrome still highly probable.

Further molecular investigations on the imprinted region in 7q32 will be necessary to estimate the contribution of chromosome 7 disturbances to the aetiology of SRS.

**ACKNOWLEDGEMENTS**

We thank all the SRS families for participating in this study. The study was supported by Pharmacia GmbH, Germany.

**Authors’ affiliations**

E Meyer, T Eggermann, Institute of Human Genetics, University Hospital, Aachen, Germany

H A Wollmann, Children’s Hospital, University of Tübingen, Germany

Correspondence to: Dr T Eggermann, Institute of Human Genetics, RWTH Aachen, Pauwelsstrasse 30, D-52074 Aachen, Germany; teggermann@ukaachen.de

**REFERENCES**


**Table 2** Polymorphisms detected in MESTIT1 and their frequencies in SRS patients and controls. Restriction assays were established for the three variants

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primers*</th>
<th>Restriction enzyme</th>
<th>Allele frequencies† in SRS patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.580G&gt;A</td>
<td>MESTIT19-F, MESTIT19-R</td>
<td>MscI</td>
<td>G: 84, A: 8; G: 95, A: 5</td>
</tr>
<tr>
<td>c.2487T&gt;A</td>
<td>MESTIT13-F, MESTIT13-R</td>
<td>Tsp509I</td>
<td>T: 87, T: 85; A: 15, A: 14</td>
</tr>
</tbody>
</table>

*Primer sequences correspond to those listed in table 1. †Numbers of chromosomes.
Searching for genomic variants in the *MESTIT1* transcript in Silver-Russell syndrome patients

E Meyer, H A Wollmann and T Eggermann

*J Med Genet* 2003 40: e65
doi: 10.1136/jmg.40.5.e65

Updated information and services can be found at:
http://jmg.bmj.com/content/40/5/e65

*These include:*

**References**
This article cites 17 articles, 7 of which you can access for free at:
http://jmg.bmj.com/content/40/5/e65#BBL

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**
Articles on similar topics can be found in the following collections

- Molecular genetics (1254)
- Drugs: endocrine system (107)
- Epidemiology (630)
- JMG Online mutation reports (168)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/