Genomic characterisation of C7orf10 in Silver-Russell syndrome patients

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ilver-Russell syndrome (SRS) describes a uniform
omalformation syndrome characterised by intrauterine
and postnatal growth retardation (IUGR/PGR), asymmetry
of the head and limbs, a small triangular face, and other
less constant features. The majority of the 400 cases described
so far occurred sporadically, but some familial cases have been
reported. A subset of 7-10% of SRS patients shows maternal
uniparental disomy (mUPD) of chromosome 7, thereby
implying that imprinted gene(s) on this chromosome play a
key role in the aetiology of the disease. Mutations in this gene
or imprinting mutations may contribute to the SRS phenotype.

SRS patients with chromosomal aberrations are rare. How-
ever, five SRS patients have been described carrying rearran-
gements in 7p. From the findings in these patients, a
central role of chromosomal bands 7p12-14 can be deline-
ated. Nakabayashi et al. have shown in two of these patients
that the breakpoints on 7p14 were located within the same
gene, C7orf10.

C7orf10 consists of 15 exons and spans more than 700 kb
of DNA. Northern blot analyses showed that C7orf10 is mainly
expressed in kidney and expression could also be observed in
skeletal muscle and liver. According to Nakabayashi et al.,
the deduced protein contains a CAIB-BAIF domain. Enzymes
with this domain have diverse function, such as carnitine
dehydratase and fatty-acid CoA racemase. So far, the
physiological function of the C7orf10 transcript is unknown.

We decided to screen our SRS patients for mutations and
genomic rearrangements of the coding region of C7orf10, in
particular because of its genomic localisation within a region
affected by chromosomal rearrangements.

MATERIAL AND METHODS

Our study population consisted of 45 SRS patients in whom
diagnosis was ascertained according to Wollmann et al. and in
whom cytogenetic abnormalities and uniparental disomy 7
have been excluded. Fifty-eight healthy probands of German
origin were investigated as controls.

DNA was isolated from blood samples by a simple salting
out procedure and photometric measuring of DNA was
performed on a Gene Quant II (Pharmacia). DNA was diluted
out procedure and photometric measuring of DNA was

Data evaluation was carried out using the ABI7000SDS
software as described by Wilke et al. Separate standard curves
were generated for the test and the reference loci. Using these
curves, the starting gene copy number relative to the reference
subject were determined for each well, and the mean of the
relative starting copy numbers of the test locus and the

PCR was carried out using an ABI Prism 7000 sequence
detection system and 96 well MicroAmp optical plates. PCR
was performed as described previously. All reactions of the
same run were prepared from the same master mix. Each well
of the 96 well plate contained either 50 ng of sample DNA, or
125 ng, 25 ng, or 5 ng of standard DNA, respectively. Each test
sample and each amount of standard DNA was amplified in
two different wells. Reactions for the C7orf10 test locus and the

Data evaluation was carried out using the ABI7000SDS
software as described by Wilke et al. Separate standard curves
were generated for the test and the reference loci. Using these
curves, the starting gene copy number relative to the reference
subject were determined for each well, and the mean of the
relative starting gene copy number values in different wells
was calculated for each test sample and each locus. For males,
the absolute copy number of the SMN1 test locus per haploid
genomic equivalent was calculated by the ratio of the means of
the relative starting copy numbers of the test locus and the

For females, this ratio was multiplied by a factor of 2.

Key points

• Owing to its genomic localisation in 7p14, C7orf10 is a
  strong candidate gene for Silver-Russell syndrome (SRS).

• Its coding region was therefore screened for genomic
  variants by SSCP and real time PCR in a cohort of 45 SRS
  patients.

• We thereby excluded that mutations in C7orf10 play a
  major role in the aetiology of the disease.
RESULTS AND DISCUSSION

Several reports on chromosomal and molecular findings in SRS patients suggest that a genomic rearrangement in the short arm of chromosome 7 is involved in SRS. Since C7orf10 is disrupted by chromosomal breakages in 7p in at least two SRS patients, this factor is an attractive candidate for SRS.

We therefore screened our SRS patients for mutations of the coding region as well as for duplications/deletions. By SSCP analysis, we detected two abnormal patterns in exons 8 and 15 of the C7orf10 coding sequence. In the case of exon 8, a G>A exchange was detected, corresponding to the published SNP rs1053953. Both variants were detected in similar frequencies in SRS patients and controls, corresponding to a copy number of two.

Using a real time PCR approach, we excluded duplications or deletions of exon 14 of the C7orf10 gene; the ratio of C7orf10/FVIII ranged between 0.8 and 1.3 in both patients and controls, corresponding to a copy number of two. On the whole, our results do not indicate a relevant role of C7orf10 in the etiology of SRS. Of course, we cannot exclude that mutations in C7orf10 are present in a relatively small number of SRS cases, since SRS is heterogeneous. However, multiple studies suggest that several loci on chromosome 7 or other chromosomes could be involved in SRS. It is possible that SRS is a contiguous gene syndrome or the result of mutations in several components of one metabolic pathway resulting in the same phenotype, as suggested for the GH/IGF axis.

With respect to the SRS candidate region in 7p, more than 90 SRS patients have been screened for duplications and genomic rearrangements, but no evidence for a central role of duplications in 7p was obtained. The patients in these studies were screened by microsatellites, fluorescence in situ hybridisation (FISH), or quantitative approaches aimed at certain genes or regions, that is, the genes for IGFBP1 and IGFBP3. Since these fragments span a large chromosomal region, cryptic duplications or deletions between these regions might remain undetected.

Table 1 Primers and PCR conditions used for mutation analysis in C7orf10

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Primer sequences (5′→3′)</th>
<th>Nucleotide position (g18567765)</th>
<th>Annealing temperature</th>
</tr>
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<tbody>
<tr>
<td>Exon 1</td>
<td>F: ccttggtctgaccgacagacta R: ccttgccacacagactaatc</td>
<td>2.4991842.499040</td>
<td>40°C*</td>
</tr>
<tr>
<td>Exon 2</td>
<td>F: agaatcactggtcccttgt R: gctggtcccttgtctgcc</td>
<td>2.4522072.452131</td>
<td>50°C</td>
</tr>
<tr>
<td>Exon 3</td>
<td>F: ccttggtctgaccgacagacta R: ccttgccacacagactaatc</td>
<td>2.4456682.445586</td>
<td>50°C</td>
</tr>
<tr>
<td>Exon 4</td>
<td>F: cctcataacctcggtccgaat R: gctggtcccttgtctgcc</td>
<td>2.4391262.439104</td>
<td>40°C†</td>
</tr>
<tr>
<td>Exon 5</td>
<td>F: acagggttggtgccacccta R: cctcataacctcggtccgaat</td>
<td>2.3951002.395007</td>
<td>45°C</td>
</tr>
<tr>
<td>Exon 6</td>
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<td>Exon 7</td>
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<td>50°C</td>
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<td>Exon 8</td>
<td>F: actgagctgcctgtcaact R: cctcataacctcggtccgaat</td>
<td>2.1834562.183383</td>
<td>50°C</td>
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<td>F: acagggttggtgccacccta R: cctcataacctcggtccgaat</td>
<td>2.1732.173541</td>
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<td>2.1364582.136355</td>
<td>50°C</td>
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<tr>
<td>Exon 11</td>
<td>F: acagggttggtgccacccta R: cctcataacctcggtccgaat</td>
<td>2.1364582.136355</td>
<td>50°C</td>
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<td>Exon 12</td>
<td>F: acagggttggtgccacccta R: cctcataacctcggtccgaat</td>
<td>1.9486871.948606</td>
<td>50°C</td>
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<td>Exon 13</td>
<td>F: acagggttggtgccacccta R: cctcataacctcggtccgaat</td>
<td>1.8833081.883243</td>
<td>57°C</td>
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<td>Exon 14</td>
<td>F: acagggttggtgccacccta R: cctcataacctcggtccgaat</td>
<td>1.7724281.771995</td>
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<td>Exon 15</td>
<td>Fragment 1 F: acagggttggtgccacccta R: cctcataacctcggtccgaat</td>
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<td>50°C</td>
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<td>Fragment 2 F: acagggttggtgccacccta R: cctcataacctcggtccgaat</td>
<td>1.7724281.771995</td>
<td>50°C</td>
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</tbody>
</table>

* MgSO₄ instead of MgCl₂, enhancer from Invitrogen. † 5% formamide.

Table 2 Genomic variants and their frequencies detected in C7orf10 detected by SSCP

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide exchange</th>
<th>Frequencies in SRS</th>
<th>Frequencies in controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>c.642G/G</td>
<td>19.6% (13)</td>
<td>3.5% (1)</td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>30.4% (14)</td>
<td>53.3% (23)</td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>50% (23)</td>
<td>43.7% (25)</td>
</tr>
<tr>
<td>15</td>
<td>c.1217G/G*</td>
<td>34.8% (16)</td>
<td>41.4% (24)</td>
</tr>
<tr>
<td></td>
<td>G/C</td>
<td>45.7% (21)</td>
<td>46.6% (27)</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>19.5% (9)</td>
<td>12% (7)</td>
</tr>
</tbody>
</table>

*The polymorphism in exon 15 has been described previously as SNP rs1053953.
REFERENCES


