Genomic characterisation of C7orf10 in Silver-Russell syndrome patients

E Meyer, H A Wollmann, T Eggermann

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tter-Russell syndrome (SRS) describes a uniform
malformation syndrome characterised by intrauterine
and postnatal growth retardation (IUGR/PGR), asymme-
try 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 pheno-
type.

SRS patients with chromosomal aberrations are rare. How-
ever, five SRS patients have been described carrying rear-
rangements in 7p. From the findings in these patients, a
central role of chromosomal bands 7p12-p14 can be deline-
ated. Nakabayashi et al. have shown in two of these patients
that the breakpoints on 7p14 were localised 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
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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 by performing a BLAST search in public
databases with the cDNA AK021870
information.

Informed consent was obtained by performing a BLAST search in public
databases with the cDNA AK021870 as a probe. Thereby, we
identified the chromosome 7 working draft sequence segment
gi18567765 containing the C7orf10 gene.

This information was used to choose intronic primers for
the amplification of the 15 exons and the intron-exon
boundaries of the C7orf10 gene by standard PCR. Primer
information and PCR conditions are listed in table 1. In the
case of exon 1, standard PCR conditions were changed in that
1.5 mmol/l MgCl2 was replaced by 1.5 mmol/l MgSO4.
Additionally, the PCR enhancer system (2×) provided by Inv-
itrogen was used. For amplification of exons 2 and 6, 5% for-
mamide was added to the mixture. Fragment sizes were
between 200-300 bp. Analysis of these fragments was
performed by SSCP as published previously. Unusual SSCP
patterns were directly sequenced using the BigDye Termina-
Sequence Kit (Applied Biosystems).

To analyse genomic duplications or deletions in the C7orf10
gene, we used a TaqMan assay approach. Probe and primers were
designed using the PrimerExpress™ software (Applied Biosys-
tems, Weiterstadt). We designed a TaqMan probe specific
for exon 14 of C7orf10, since in this fragment we did
not detect any variants which might influence the amplifica-
tion efficiency. This exon is localised proximally to the break-
point in the SRS patients reported by Monk et al. and Nakaba-
yashi et al. The TaqMan probe (probe c7orf10-Ex14
tgctccgatgcgaggacgtc, primers: c7orf10-Ex14TF-ctttt-
gcgttcggattcctac, c7orf10-Ex14TR-agccaatctctccacagt) contained a FAM reporter dye connected to the 5’ end. Probes
and primers were purchased from Operon. As a reference
locus, we used exon 3 of FVIII; primer and probe information
was published by Wilke et al.

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
FVIII reference locus were prepared and run in parallel.

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
FVIII reference locus. For females, this ratio was multiplied by
a factor of 2.
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 at cDNA position 1217, corresponding to a silent mutation (L243L). In the first SSCP fragment of exon 15, we detected a C>G transversion at cDNA position 652, representing a silent mutation (R218R).

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. Since these fragments span a large chromosomal region, cryptic duplications or deletions between these regions might remain undetected.

Table 1

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Primer sequences (5'→3')</th>
<th>Nucleotide position (gi18567765)</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>F: cccctggtctgcaccgccatct</td>
<td>2.499184-2.499040</td>
<td>40°C*</td>
</tr>
<tr>
<td></td>
<td>R: cccctggtctgcaccgccatct</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>2.453216-2.453162</td>
<td>40°C†</td>
</tr>
<tr>
<td></td>
<td>R: gaaagggaaaaagacacaaccac</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>2.452207-2.452131</td>
<td>50°C</td>
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<tr>
<td></td>
<td>R: gaaagggaaaaagacacaaccac</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 4</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>2.454668-2.445586</td>
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<tr>
<td></td>
<td>R: gaaagggaaaaagacacaaccac</td>
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<td>Exon 5</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>2.444615-2.444572</td>
<td>50°C</td>
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<tr>
<td></td>
<td>R: gaaagggaaaaagacacaaccac</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 6</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>2.439126-2.439104</td>
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<td>R: gaaagggaaaaagacacaaccac</td>
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<tr>
<td>Exon 7</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>2.395100-2.395007</td>
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<td></td>
<td>R: gaaagggaaaaagacacaaccac</td>
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<td>Exon 8</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>2.352830-2.358083</td>
<td>50°C</td>
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<tr>
<td></td>
<td>R: gaaagggaaaaagacacaaccac</td>
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<tr>
<td>Exon 9</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>2.315983-2.315885</td>
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<td>R: gaaagggaaaaagacacaaccac</td>
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<td>Exon 10</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>2.183456-2.183383</td>
<td>50°C</td>
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<td>Exon 11</td>
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<td>2.1732.173541</td>
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<td>Exon 13</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>1.948687-1.948606</td>
<td>50°C</td>
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<tr>
<td></td>
<td>R: gaaagggaaaaagacacaaccac</td>
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<td>Exon 14</td>
<td>F: cccccccgtagcacccgtagacct</td>
<td>1.883308-1.883243</td>
<td>57°C</td>
</tr>
<tr>
<td></td>
<td>R: gaaagggaaaaagacacaaccac</td>
<td></td>
<td></td>
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<td>Exon 15</td>
<td>Fragment 1</td>
<td>1.772428-1.771995</td>
<td>50°C</td>
</tr>
<tr>
<td></td>
<td>Fragment 2</td>
<td>1.772428-1.771995</td>
<td>50°C</td>
</tr>
</tbody>
</table>

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

Table 2

<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></td>
<td>c.642G/G</td>
<td>19.6% (9)</td>
<td>3.5%/2</td>
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<tr>
<td></td>
<td>G/A</td>
<td>30.4% (14)</td>
<td>53.4%/31</td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>50% (23)</td>
<td>43.1%/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>C/G</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>

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REFERENCES