ELECTRONIC LETTER

Genomic characterisation of *C7orf10* in Silver-Russell syndrome patients

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Silver-Russell syndrome (SRS) describes a uniform malformation 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.¹ 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. However, five SRS patients have been described carrying rearrangements in 7p.²⁻⁶ From the findings in these patients, a central role of chromosomal bands 7p12-p14 can be delineated. Nakabayashi *et al*⁶ 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 skeletal muscle and liver. According to Nakabayashi *et al*,⁶ 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*⁷ 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 in water to a concentration of 20 ng/μl and stored at room temperature overnight before use.

Information on the genomic structure of the *C7orf10* gene was obtained by performing a BLAST search in public databases with the cDNA AK021870⁸ 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.⁸ 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 Invitrogen was used. For amplification of exons 2 and 6, 5% for-

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.

mamide was added to the mixture. Fragment sizes were between 200-300 bp. Analysis of these fragments was performed by SSCP as published previously.⁸ Unusual SSCP patterns were directly sequenced using the BigDye Termination Sequencing 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 Biosystems, 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 amplification efficiency. This exon is localised proximally to the breakpoint in the SRS patients reported by Monk *et al*⁵ and Nakabayashi *et al*.⁶ The *TaqMan* probe (probe c7orf10-Ex14 tggcctctgtatggagatggagcatc, primers: c7orf10-Ex14-F gttttt-gccttttcagggtattacaca, c7orf10-Ex14-R acggaatcttccccacagtt) 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*.⁹

PCR was carried out using an ABI Prism 7000 sequence detection system and 96 well MicroAmp optical plates. PCR was performed as described previously.¹⁰ 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*.⁹ 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.

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

Fragment	Primer sequences (5'→3')	Nucleotide position (gi18567765)	Annealing temperature
Exon 1	F: cccttggtctgaccgacagact R: ccttagccaccagaccgaatct	2.499184-2.499040	40°C*
Exon 2	F: agaatacctgtagctctgt R: gaaggaggaaaacccaatcac	2.453216-2.453162	40°C†
Exon 3	F: tatgagcgtgctgtgtgac R: gagtaatacctttacctggtc	2.452207-2.452131	50°C
Exon 4	F: acagagcaagactccatctc R: caagccacaatgatataaac	2.445668-2.445586	50°C
Exon 5	F: tgggcttctgtgtttgggc R: tgcacaacgtacagatcaccca	2.444615-2.444572	50°C
Exon 6	F: cctggcaaacgtgtgcaat R: aagccagcaaaagggttctt	2.439126-2.439104	40°C†
Exon 7	F: acatgggttttagcacacccct R: atccctatgggttagtcagag	2.395100-2.395007	45°C
Exon 8	F: cttcttttcaaccaacct R: aggtataaccagacacagt	2.358230-2.358083	50°C
Exon 9	F: ctaactgatggacagggtt R: caaccctagactgtgtagt	2.315983-2.315885	50°
Exon 10	F: cttttgtgtctgtcaaca R: actgaacttccctggcttt	2.183456-2.183383	50°C
Exon 11	F: ataggacctgaacactagcac R: caccagataaatgcttatcac	2.173-2.173541	50°C
Exon 12	F: aggtgtgtttacattctcc R: tggacagaaaacagagggcgtt	2.136458-2.136355	50°C
Exon 13	F: gatcatgtgtgtactggcag R: gattatccttctggctgca	1.948687-1.948606	50°C
Exon 14	F: ctgtccatgccttgcaattga R: gagctcataagctacctgtag	1.883308-1.883243	57°C
Exon 15			
Fragment 1	F: aacaggcttctgtcttctt R: agaagggtccaagcaaatgt	1.772428-1.771995	50°C
Fragment 2	F: cctcataacctgacccgaat R: gtaagtagaataaggagtgca		50°C

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

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 transition at cDNA position 652 was detected, representing a silent mutation (L243L). In the first SSCP fragment of exon 15, we detected a C>G transversion at cDNA position 1217, corresponding to the published SNP rs1053953. Both variants were detected in similar frequencies in SRS patients and controls (table 2). In addition, we detected the previously published SNPs rs699484 at nucleotide position 1772165 and rs1053953 at position 1772000.

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

Exon	Nucleotide exchange	Frequencies in SRS	Frequencies in controls
8	c.642G/G	19.6% (9)	3.5%/2
	G/A	30.4% (14)	53.4%/31
	A/A	50% (23)	43.1%/25
15	c.1217G/G*	34.8% (16)	41.4% (24)
	G/C	45.7% (21)	46.6% (27)
	C/C	19.5% (9)	12% (7)

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

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 aetiology 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 and other chromosomes could be involved in SRS.¹ 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.¹¹

With respect to the SRS candidate region in 7p, more than 90 SRS patients have been screened for duplications and genomic rearrangements,^{5-12,13} 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 *GRB10*, *IGFBP1*, and *IGFBP3*. Since these fragments span a large chromosomal region, cryptic duplications or deletions between these regions might remain undetected.

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