ONLINE MUTATION REPORT

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

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Silver-Russell syndrome[1 2] 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.[3] 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.[4] Additionally, Hannula *et al*[5] 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,[6] paternally expressed gene 10 (*PEG10*) and epsilon-sarcoglycan (*SGCE*) in 7q21,[7] and the mesoderm specific transcript (*MEST*) in 7q32.[8] 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.[9–12]

Recently, Nakabayashi *et al*[13] 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*[7] 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.[13] It exists as a 4.2 kb transcript in many fetal and adult tissues.

Key points

- Owing to its putative role as regulator of MEST expression, the transcript *MESTIT1* is a strong candidate gene for Silver-Russell syndrome in 7q32.
- We screened the two exons of *MESTIT1* for genomic variants by SSCP.
- It can be excluded that genomic variants in *MESTIT1* are involved in the aetiology of Silver-Russell syndrome.

Although mutations in the *MEST* gene itself could not be identified in three independent studies[9 12] (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*.[3] 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 Primers used for SSCP screening of *MESTIT1* in our study population

Fragment	Nucleotide position	Primer F-R	Fragment size (bp)
MESTIT1-1	142451-142800*	aagcacctgctcctggttga cttcttcaccaggtactcct	350
MESTIT1-2	142756-143105*	tcctctaggtgtgggatagt gtgacagagcgagacttcat	350
MESTIT1-3	143033-143440*	gtagcttcagggatatattgg actgagggcaaggcaaattg	408
MESTIT1-4	143368-143708*	gtttaatttggttgagagcg tctagagggattgggtggct	341
MESTIT1-5	143656-144006*	ggacagagaatttgtcccat gctcggaacgctaagtgtta	351
MESTIT1-6	143953-144308*	acatctgcgttcagttccca ctactgtgactgcagactga	356
MESTIT1-7	144231-144585*	ttctgtgagttctaaccagc tcacaacagtggtatgggat	355
MESTIT1-8	144541-144900*	tgaatctgtggtaccatctc cttccagaaaggagcttcct	360
MESTIT1-9	144853-145210*	taccttgggtcaaattctcc cctgaagacaacaggaattc	358
MESTIT1-10	145155-145435*	gtctgaagggaggatttctc atggaaactttgagtcctac	281
MESTIT1-11	2170-2566†	aaccagacccctgcagaagtg aggcacaagaaagagggagga	397

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

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

Polymorphism	Primers*	Restriction enzyme	Allele frequencies† in SRS patients and controls	
c.126G>A	MESTIT11-F MESTIT11-R	*Eag*I	G: 88 A: 4	G: 98 A: 2
c.580G>A	MESTIT19-F MESTIT19-R	*Msc*I	G: 84 A: 8	G: 95 A: 5
c.2487T>A	MESTIT13-F MESTIT13-R	*Tsp*509I	T: 87 A: 5	T: 85 A: 15

*Primer sequences correspond to those listed in table 1. †Numbers of chromosomes.

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.[14] 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 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.[14]

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 transversion was observed at nucleotide 2487, which affects the restriction site of *Tsp*509I. Two G to A transitions were observed at nucleotide 580 and nucleotide 126; for these variants, restriction assays using *Msc*I and *Eag*I were established.

The allelic distribution of these novel polymorphic variants were similar in SRS patients and in 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.[13] 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 *PAX4*,[8–17] *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-qter, 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.

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