ELECTRONIC LETTER

Investigation of the *GRB2*, *GRB7*, and *CSH1* genes as candidates for the Silver-Russell syndrome (SRS) on chromosome 17q

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Silver-Russell syndrome (SRS) (MIM 180860) is characterised by intrauterine and postnatal growth restriction, in association with dysmorphic features most frequently including a small triangular facies, skeletal asymmetry, and fifth finger clinodactyly.[1–3] The genetic aetiology of SRS is heterogeneous. Maternal uniparental disomy for chromosome 7 (mUPD(7)) occurs in 7-10% of patients,[4 5] with strong evidence that disruption of imprinted gene expression, as opposed to mutation of a recessive gene, underlies the SRS phenotype in these cases.[6] A SRS-like phenotype has also been associated with ring chromosome 15 with accompanying deletion on 15q,[7 8] trisomy 18 mosaicism,[9] deletion on 18p,[10] and deletion of 8q11-q13.[11]

Three SRS cases have been described with disruptions involving distal 17q. These include two unrelated patients with severe SRS bearing reciprocal translocations, with the breakpoints originally assigned to 17q25. In the first case, the proband had an apparently balanced translocation (17;20)(q25;q13), inherited from her clinically normal father.[12] The second patient had a de novo translocation (1;17)(q31;q25).[13] The breakpoint in this latter case has recently been cloned and more accurately localised to 17q23.3-q24.[14] In the third case, a heterozygous deletion of the chorionic somatomammotrophin hormone 1 (*CSH1*) gene, which is located within the growth hormone and CSH gene cluster on 17q24.1, was identified in a patient with typical SRS. The deletion was inherited from the father, who appeared clinically normal, but had short stature.[15] CSH1, otherwise known as placental lactogen, is produced in the syncytiotrophoblast of the placenta and secreted into the maternal and fetal circulation. CSH1 is detectable in maternal serum from 6 weeks post conception, and levels increase linearly during gestation, peaking at about 30 weeks. CSH1 has been used as a marker for placental integrity during pregnancy, and low levels in the maternal serum have been associated with pathological conditions including intrauterine growth restriction (IUGR).[16] CSH1 may play a role in regulation of fetal growth and metabolism by stimulating insulin-like growth factor 1 (IGF-1) production by the fetus.[16 17]

Interestingly, the growth factor receptor binding protein (GRB) 2 and 7 genes map to 17q24-25[18] and 17q21-22,[19] respectively, near the translocation breakpoints. The GRB protein family, including GRB2, GRB7, GRB10, and GRB14, function in mitogenic signalling and are likely to be important in fetal growth. Each member contains a carboxy-terminal Src homology 2 (SH2) domain. GRBs 7, 10, and 14 are structurally very similar, with an additional pleckstrin homology (PH) domain.[20] The GRB proteins are important components of the insulin and IGF signal transduction pathways, interacting with various receptor tyrosine kinases and other tyrosine phosphorylated proteins via the SH2 domain.[21] *GRB10* has been implicated in SRS with the reports of two SRS patients with maternally inherited duplications of 7p11.2-p13 encompassing this gene.[22 23] *GRB10* is imprinted, showing paternal expression in human fetal brain[24 25] and a maternally transcribed isoform has been identified in skeletal muscle.[24] Although no sequence mutations of *GRB10* have been identified in 139 SRS patients, *GRB10* remains a candidate for this disorder through disruption of imprinted expression.[24–27] GRB2, in association with the guanine nucleotide exchange factor, Sos, interacts with the insulin receptor substrate 1, thus regulating Ras activation. GRB2 therefore plays a significant role in the regulation of the insulin signal transduction system.[28] GRB7 binds the Ret receptor,[29] the epidermal growth factor receptor,[30] and the insulin receptor.[31] Upregulation of both GRB2 and GRB7 has been implicated in cancer metastasis.[32 33]

One or more SRS genes must exist on distal chromosome 17q, most likely in the region 17q23-25. We investigated our cohort of SRS patients for genetic abnormalities involving the long arm of chromosome 17. Specifically, we focused on the three functional candidate genes, *CSH1*, *GRB2*, and *GRB7*, which map within or near the same chromosomal interval as the previous genetic defects on distal 17q associated with SRS.

PATIENTS AND METHODS

SRS patients

The patients studied were a subset of the 48 previously described, who have grossly normal karyotypes and in whom mUPD(7) had been excluded.[3 6] The study was approved by the Joint Research Ethics Committee of the Great Ormond Street Hospital for Sick Children and the Institute of Child Health (1278). Peripheral blood samples for DNA analyses and generation of lymphoblast cell lines were obtained with informed consent from the patients and their families. Lymphoblastoid cell lines for 36 patients, including two affected sibs, were obtained from the European collection of cell cultures (ECCAC) at Porton Down, Salisbury. The ECCAC numbers of the patients studied may be obtained from the authors.

Short tandem repeat (STR) typing of D17S254 near *CSH1*

Radioactive PCR of the D17S254 marker using previously designed primers[34] followed by autoradiography was performed in 44 SRS probands and their parents, as described by Eggermann *et al*.[15]

FISH analyses of *GRB2* and *GRB7*

PAC RP1-171G12 containing the *GRB2* gene and PAC RP1-37L10 containing the *GRB7* gene were used as FISH

Abbreviations: SRS, Silver-Russell syndrome; IGF-1, insulin-like growth factor 1; GRB, growth factor receptor binding protein; mUPD, maternal uniparental disomy; UTR, untranslated region

probes to detect structural rearrangements involving either gene in SRS. The PAC clones were identified from the gridded human PAC library (RPCI-1) using IMAGE partial cDNA clones corresponding to the 3′ untranslated region (UTR) of each gene as hybridisation probes (*GRB2* clone T283F07, GenBank accession AI631378 and *GRB7* clone TP65D02, GenBank accession AI804599). PCR and sequence analysis of a 3′ UTR exonic sequence confirmed that both PACs contained the 3′ UTR of the corresponding genes. FISH probes were prepared by nick translation of standard miniprep DNA from the genomic clones, with direct incorporation of Spectrum Red dUTP or Spectrum Green dUTP (Vysis). Slides of metaphase chromosomes and interphase nuclei from patient lymphoblastoid cells were prepared after exposure of the cell cultures to colcemid for three hours before harvesting. Dual coloured FISH analyses for detection of minor chromosomal anomalies were performed as previously described.[35] Either the *GRB2* probe (RP1-171G12) or the *GRB7* probe (RP1-37L10) labelled green was combined with a red labelled control probe (BAC RP2-601N13) containing the Charcot-Marie-Tooth (*CMT*) gene mapping to 17p11.2 on the opposite side of the centromere, and hybridised to the patient slides to identify small duplications or deletions. The *GRB2* and *GRB7* probes were then labelled red and green, respectively, and simultaneously hybridised to metaphase spreads to detect paracentric inversions of 17q21-25. Slides were counterstained with DAPI vector shield and examined using a Zeiss fluorescent Axioscope equipped with triple band pass filter.

Mutation screening of *GRB2*

The five coding exons of *GRB2*, including intron-exon boundaries, were PCR amplified from genomic DNA of SRS patients using previously designed primers.[36] The products were directly sequenced on an automated 377 DNA Sequencer (Perkin-Elmer). The sequences were compared to the published *GRB2* exonic sequences by BLAST analyses and compared to one another using Sequence Navigator to detect any sequence variants.

RESULTS

Owing to the previous finding of a case of SRS with a hemizygous deletion of *CSH1*, we screened 44 SRS patients for similar deletions of this gene by STR typing of the polymorphic tetranucleotide repeat marker D17S254,[34] which lies 1.4 kb upstream of *CSH1*. This marker was previously used by Eggermann *et al*[15] in their screen of *CSH1* in German SRS patients. In 41 pedigrees the SRS probands were heterozygous for D17S254, and the marker was informative for parental origin of the alleles. The remaining three patients were also heterozygous for D17S254, but the two bands were uninformative regarding origin of inheritance. Thus, no deletions of *CSH1*, similar to the one previously reported, were identified in our 44 patients, indicating that this is a rare event in association with SRS.

GRB2 and *GRB7* have been more accurately localised to 17q25.1 and 17q21.1, respectively, according to the current International Human Genome Project working draft (http://genome.cse.ucsc.edu/). We investigated these two genes for any minor structural abnormalities including duplications, deletions, or paracentric inversions in a group of 36 patients for whom lymphoblastoid cell lines were available, by dual colour FISH analyses. Normal hybridisation patterns for both the *GRB2* and *GRB7* genes were observed both in metaphase and interphase nuclei in all 36 patients screened. The red *CMT* signal was visualised on the short arm of chromosome 17 and the green *GRB2* or *GRB7* signal seen on the distal end of the long arm of chromosome 17, on both homologues, within single metaphases for each patient tested. At interphase, two signals for the *CMT* and *GRB2* or *GRB7* probes were identified within single nuclei, confirming that no duplications or deletions of these genes were present using this more sensitive technique. In addition, the *GRB2* and *GRB7* signals, when combined, were present in the correct order with respect to the centromere in metaphase chromosomes, indicating that there were no paracentric inversions in the cases screened either. These data exclude any small structural rearrangements involving either *GRB2* or *GRB7* on distal chromosome 17 in these 36 SRS patients.

Since *GRB2* maps within the SRS candidate region of 17q23-25, as defined by the two translocation breakpoints,[12 13 14] this gene is a more likely positional candidate for SRS than *GRB7*, which maps further proximal in 17q21.1. We therefore screened half of the SRS patients who had been analysed by FISH for sequence mutations of *GRB2*. In total, 19 SRS cases, including two affected sibs, were screened for pathological mutations by sequencing. No pathogenic mutations of *GRB2* were identified, indicating that this gene is not associated with the disease phenotype in these patients. No variation from the published exon sequences was identified in any of the probands tested, showing that this gene is highly conserved.

DISCUSSION

We have investigated our cohort of SRS patients for defects of the terminal region of chromosome 17q, which may be responsible for the clinical phenotype at three candidate loci, *CSH1*, *GRB2*, and *GRB7*. These genes were not only investigated as individual SRS candidates in this study, but also served as markers for disruptions involving the region 17q21.1-25.1. No pathological changes were identified in any of the SRS patients screened, suggesting that defects of distal 17q are rare in association with SRS.

CSH1 at 17q24.1 maps within the 17q23-25 SRS candidate region and, furthermore, has previously been implicated in SRS with the finding of a hemizygous deletion of this gene in one patient.[15] Recently, a second SRS case with a hemizygous deletion of *CSH1* was identified (T Eggermann, personal communication). We ruled out hemizygosity of a locus closely linked to *CSH1* in 44 SRS patients. Since the polymorphic marker used to analyse *CSH1* is located 1.4 kb upstream, any small deletions or mutations within *CSH1* would have remained undetected. Including this study, just two patients with *CSH1* deletions including the D17S254 marker have been identified in 106 patients analysed[15] (T Eggermann, personal communication). The effect of hemizygosity of *CSH1* on the pathogenesis of SRS is questionable as homozygous or compound heterozygous deletions of the gene, or deficiency of CSH1 in maternal serum during pregnancy, have been reported with no detrimental effect on fetal growth, and apparently normal phenotypes at birth.[37–40] Although low maternal CSH1 levels in pregnancy have been associated with diabetes, pre-eclampsia, and IUGR,[16] reduced CSH1 is unlikely to have been the primary defect in these cases. Other factors, such as infarction of the placenta impairing CSH1 secretion, may have contributed more to the pathological pregnancy. CSH1 has also been proposed to play a role in fetal nutrition during maternal fasting.[41] It is possible that hemizygosity of *CSH1*, in concert with other genetic or environmental factors during pregnancy, may be responsible for the SRS phenotype in the single case described.

GRB2 and *GRB7* were analysed in our SRS patient group because of their functional relationship with *GRB10* and their proximity to the 17q23-25 translocation breakpoints. However, no structural rearrangements involving either gene was identified. Neither were any sequence mutations identified in *GRB2*, the stronger positional candidate of the two genes, in 19 of our SRS patients. In a similar study, no mutations of *GRB2* were identified in 10 German SRS patients.[42] The combined data indicate that *GRB2* is unlikely to play a significant aetiological role in SRS. *GRB7* was not analysed in further detail as this member of the gene family maps outside the 17q23-25 SRS region.

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A genomic contig has recently been constructed across 17q23-q24, and the translocation (1;17)(q31;q23.3-q24) breakpoint cloned.[14] Analysis of genes in this region, and the effect of the translocations on their expression, will greatly aid in determining which factors in growth regulation are disrupted in the SRS phenotype seen in these cases. Although this disorder is genetically heterogeneous, a common growth pathway may be disturbed in a large proportion of cases.

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