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Duplication of 7p11.2-p13, Including *GRB10,* in Silver-Russell Syndrome

David Monk,[1,2,*] Emma L Wakeling,[1,2,*] Virginia Proud,[3] Megan Hitchins,[1] Sayeda N. Abu-Amero,[1] Philip Stanier,[1] Michael A. Preece,[1,2] and Gudrun E Moore[1]

[1]Action Research Laboratory for the Molecular Biology of Fetal Development, Division of Paediatrics, Obstetrics and Gynaecology, Imperial College School of Medicine, Queen Charlotte's and Chelsea Hospital, and [2]Institute of Child Health, University College London, London; and [3]Division of Medical Genetics, Department of Pediatrics, Children's Hospital of the King's Daughters, Norfolk, VA

Summary

Silver-Russell syndrome (SRS) is characterized by pre- and postnatal growth failure and other dysmorphic features. The syndrome is genetically heterogenous, but maternal uniparental disomy of chromosome 7 has been demonstrated in ~7% of cases. This suggests that at least one gene on chromosome 7 is imprinted and involved in the pathogenesis of SRS. We have identified a de novo duplication of 7p11.2-p13 in a proband with features characteristic of SRS. FISH confirmed the presence of a tandem duplication encompassing the genes for growth factor receptor–binding protein 10 (*GRB10*) and insulin-like growth factor–binding proteins 1 and 3 (*IGFBP1* and *-3*) but not that for epidermal growth factor–receptor (*EGFR*). Microsatellite markers showed that the duplication was of maternal origin. These findings provide the first evidence that SRS may result from overexpression of a maternally expressed imprinted gene, rather than from absent expression of a paternally expressed gene. *GRB10* lies within the duplicated region and is a strong candidate, since it is a known growth supressor. Futhermore, the mouse homologue (*Grb10/Meg1*) is reported to be maternally expressed and maps to the imprinted region of proximal mouse chromosome 11 that demonstrates prenatal growth failure when it is maternally disomic. We have demonstrated that the *GRB10* genomic interval replicates asynchronously in human lymphocytes, suggestive of imprinting. An additional 36 SRS probands were investigated for duplication of *GRB10,* but none were found. However, it remains possible that *GRB10* and/or other genes within 7p11.2-p13 are responsible for some cases of SRS.

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Address for correspondence and reprints: D. Monk, Action Research Laboratory for the Molecular Biology of Fetal Development, Division of Paediatrics, Obstetrics and Gynaecology, Imperial College School of Medicine, Queen Charlotte's and Chelsea Hospital, Goldhawk Road, London W6 0XG, United Kingdom. E-mail: d.monk@ic.ac.uk

*The first two authors contributed equally to this work.

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Introduction

Silver-Russell syndrome (SRS [MIM 180860]) is a condition characterized by pre- and postnatal growth retardation, with relative sparing of cranial growth, triangular facies, and down-turned corners of the mouth. Fifth-finger clinodactyly and facial, limb, or truncal asymmetry are also frequently present (Silver et al. 1953; Russell 1954). Uniparental disomy (UPD) for maternal chromosome 7 (mUPD7) has been identified in ~7% of SRS cases investigated (Kotzot et al. 1995; Preece et al. 1997, 1999). No consistently isodisomic areas have been found in five patients with SRS with mUPD7 who were studied by our group (Preece et al. 1999). The phenotype associated with mUPD7 is therefore unlikely to be due to exposure of a recessive gene. Instead, one or more imprinted genes on chromosome 7 are thought to play a role in the pathogenesis of SRS. However, until now it has remained unclear whether the phenotype results from duplication of a maternally expressed gene or absence of a paternally expressed gene.

We report on a proband with a characteristic SRS phenotype and maternally derived duplication of 7p11.2-p13. The findings in this patient provide the first clear evidence that overexpression of a maternally expressed imprinted gene with growth-suppressing activity within this region is responsible for the SRS phenotype associated with mUPD7. It is also possible that smaller, cytogenetically undetectable duplications of such a gene may be responsible for the phenotype of growth restriction in non-UPD SRS probands.

GRB10 is a good candidate for this role. This gene, which codes for a growth-factor receptor–binding protein, maps to 7p11.2-p12 (Jerome et al. 1997). GRB10 binds both insulin and insulin-like growth–factor receptors via its SH2 domain, inhibiting their tyrosine kinase activity (Liu and Roth 1995; O'Neill et al. 1996). The gene therefore has growth-suppressing actions, and increased dosage would be consistent with a phenotype of growth failure. The mouse homologue (*Grb10/Meg1*) has recently been isolated in a systematic screen for maternally expressed, imprinted genes, by use of subtraction hybridization (Miyoshi et al. 1998). This gene is

located on mouse proximal chromosome 11. Maternal UPD (mUPD) for this region leads to prenatal growth failure, whereas paternal UPD (pUPD) for the same region leads to prenatal overgrowth (Cattanach and Kirk 1985). It has been suggested that *Grb10/Meg1* is responsible for these phenotypes via an imprinting effect (Miyoshi et al. 1998). Moreover, since the gene maps to human chromosome 7, it has also been proposed as a candidate for SRS (Miyoshi et al. 1998).

To investigate whether *GRB10* is subject to genomic imprinting in humans, we analyzed its imprinting status indirectly, by investigation of replication timing. *GRB10* was shown to replicate asynchronously, a common feature of most imprinted genes.

As expected, duplication of 7p11.2-p13 in our proband was shown to result in duplication of *GRB10*. To investigate the possibility that submicroscopic duplications including *GRB10* are a more general cause of SRS, gene-dosage analysis of Southern blot hybridization was used to screen for duplication of the gene in a cohort of 36 other, non-UPD, SRS probands.

Patients and Methods

Duplication Proband (DP)

The proband (fig. 1) was a 5-year-old girl, born at 36 wk gestation, with birth weight 1.88 kg (>2 SD below mean). Her length at birth was 47.5 cm (>2 SD below mean). She was fed intermittently, via a gastrostomy tube, from age 2 years, because of prolonged feeding difficulties. At age 4 years, her height was 90.2 cm (>2 SD below mean). She was found to have a triangular face with a small chin, a relatively large down-turned mouth, frontal bossing, fifth-finger clinodactyly, slightly blue sclerae, and mild labial hypoplasia. There was no evidence of lateral asymmetry. At the time of assessment, she was noted to have mild developmental delay, persistent hypoglycemia, and increased sweating. Growth-hormone levels were normal. Treatment with growth hormone was started at age 5 years 1 mo, specifically in an attempt to modify her hypoglycemia as well as her height, which remained below the 3d centile (>2 SD below mean).

Her mother had had a birth weight of 2.93 kg (10th–25th centile). Her final height was just 142.1 cm (>2 SD below mean). She had a square face with a relatively large down-turned mouth and fifth-finger clinodactyly but no asymmetry. The proband's father was of average height and was phenotypically normal. The proband's sister was born with a weight of 3.34 kg (25th–50th centile). She did not have the characteristic facies associated with SRS, and her height at age 7 years was at the 5th–10th centile. Both maternal grandparents were small, with heights below the 3d centile, but neither had the characteristic SRS phenotype. Informed consent for these studies was obtained from the patient's parents and from the participating family members.

Figure 1 Proband with her mother

SRS Cohort

Thirty-six other SRS probands were included in the study, as described by Preece et al. (1997). Thirty fulfilled at least three of the following diagnostic criteria: low birth weight (>2 SD below mean); short stature at the time of diagnosis (>2 SD below mean); characteristic facial features; and facial, limb, or trunk asymmetry. The remaining six probands had retarded postnatal growth and typical facial features but slightly higher birth weights (2.58–3.11 kg). The cohort consisted of 17 females and 19 males, ages 0.83–34.3 years at the time of investigation. Classic facial features, as described by Russell (1954), were seen in 20, whereas 16 had a milder facial phenotype. Asymmetry was present in 17, clinodactyly in 26. In all cases, the karyotype was normal, and mUPD7 was excluded. For this study, ethical ap-

proval was obtained by the Joint Research Ethics Committee of Great Ormond Street Hospital and the Institute of Child Health (approval 1278).

Cytogenetic Analysis

Chromosome preparations from all six family members (DP, sister, parents, and maternal grandparents) were obtained from lymphoblastoid cell cultures treated, 2 h before harvest, with 10 μg ethidium bromide/ml and 0.02 μg colcemid/ml. Chromosome G banding was performed by use of a trypsin-Leishman technique, and metaphase images were captured with an Applied Imaging Cytovision system.

Preparation of Metaphase Chromosomes and Interphase Nuclei for FISH Analysis

Lymphoblastoid cell lines from all six family members were cultured by use of standard methods. Twenty-four hours after their feeding, cultures were exposed to colcemid for 1 h and were harvested according to standard protocol. This procedure produced metaphases in addition to interphase nuclei.

FISH Investigations in the DP and Other Family Members

PAC clones used in this study were from contigs mapping to 7p11.2-p13 (Human chromosome 7, Washington University, St. Louis, libraries RPC14, RPC15, and RPC16). PACs were obtained from the BACPAC resource Centre at Roswell Park Cancer Institute, Buffalo.

Standard miniprep DNA was prepared by nick translation with direct incorporation of either Spectrum Green or Spectrum Red dUTP (Vysis). FISH was performed as described by Harper and Delhanty (1996), with minor modifications. DNA from PAC dJ0020F22 was used as a control probe to confirm hybridization efficiency and chromosome number. This probe maps to chromosome 7q31.2 and contains exons 1–8 of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. For two-color FISH (as used in orientation analysis) the control probe was not included.

Slides were counterstained with DAPI vector shield and were examined by use of a Zeiss fluorescent Axioscope equipped with a triple-bandpass filter. Images were recorded and enhanced by use of both QUIPs M-FISH Smartcapture and IPLab software (Vysis).

FISH Analysis of Replication Timing

Nuclei were prepared from normal phytohemagglutinin-stimulated peripheral lymphocytes by use of standard cytogenetic techniques. Cultures were synchronized in S-phase by use of thymidine, as described elsewhere by Knoll et al. (1994). Replication timing was investigated by use of three contiguous PACs containing *GRB10* (dj0108E23, dj0898018, and dj0537P09) and was compared with that in both synchronous (*CFTR*-dj0020F22) and asynchronous (small nuclear ribonucleoprotein polypeptide N gene [SNRPN RPCI6 5E12]) controls.

Probes were prepared and hybridized in the same way as for the duplication analysis. Three distinct hybridization patterns on interphase nuclei were scored. Hybridization sequences that have not replicated (G1) appear as two single signals in the nucleus, and replicated sequences (G2) appear as two distinct pairs of signals. Asynchrony is scored as the percentage of nuclei that contain one single- and one double-hybridization pattern (G1/G2), corresponding to nuclei in which only one homologue has replicated. The replication pattern was scored only if the signals were not overlapping. For each probe, 100 interphase nuclei were scored.

Tetranucleotide Repeat–Marker Analysis

Six tetranucleotide microsatellite repeat markers located around *GRB10* (*D7S1818, D7S3069, D7S1833, D7S1830, D7S1831,* and *D7S1800*) were studied by use of radioactive PCR. Amplifications were carried out in a 25-μl reaction volume containing 50 ng DNA, 1 × reaction buffer, 2 mM dNTP mix, 1.5 mM $MgCl_2$, 50 ng each primer, 0.1 μl $\alpha[^{32}P]$-dCTP (ICN), and 0.75 U Bio*Taq* polymerase (Bioline). Reaction conditions for each set of primers were denaturation at 94°C for 4 min, followed by 20 cycles at 94°C (annealing temperature), 72°C for 1 min, and 72°C for 5 min. Annealing temperatures were 55°C for *D7S1818*, 56°C for *D7S3069*, 54°C for *D7S1833*, 58°C for *D7S1830*, 57°C for *D7S1831*, and 56°C for *D7S1800*.

Six microliters of PCR-reaction product were denatured and electrophoresed on a 6% denaturing polyacrylamide gel (National Diagnostic). Incorporation of radioactivity in each band was quantified by volumetric analysis by use of a PhosphorImager (model 400; Molecular Dynamics).

Quantitative Analysis of Southern Blot Hybridization

Six micrograms of total genomic DNA were digested with 30 U of *Xba*I (Promega) for 6 h at 37°C and electrophoresed on 0.8% agarose gels overnight. Southern blotting and hybridization were carried out by use of standard methods (Sambrook et al. 1982). Filters were probed simultaneously with a cDNA fragment of *GRB10* and an *Xba*I fragment, located in the DiGeorge syndrome region on chromosome 22q, which was used as a control probe. The 679-bp cDNA fragment of *GRB10* was generated by use of PCR primers designed

to amplify nucleotides 1014–1692 (Genbank accession number AF001534). The 6.9-kb control probe A6121-1 was obtained by digestion of cosmid A6121 with *Xba*I. Hybridization of the digested cosmid to human placental DNA confirmed that this fragment did not contain repetitive sequences.

For each filter, incorporation of radioactivity in each band was measured as for the tetranucleotide analysis. After the first reading, filters were stripped and reprobed. The average of two readings for each proband was taken. DNA from six normal individuals was interspersed, on each filter, with DNA from 10 SRS probands. The mean readings for *GRB10* and A6121-1 bands for all normal individuals on a filter were represented as "GN" and "CN," respectively. The readings for *GRB10* and control bands for each proband were represented as "GP" and "CP," respectively.

The ratio of GP/CP in each proband to the mean value of GN/CN in normal individuals was calculated by use of the formula, (GP × CN)/(GN × CP). When there is one copy of *GRB10* for each copy of A6121-1, the ratio will approximate 1. A duplication of the gene will result in three copies of *GRB10* for two copies of the control—and, hence, a ratio of 1.5. Since the *GRB10* probe used hybridized to two *Xba*I fragments, separate ratios were calculated for upper and lower *GRB10* bands.

Results

Cytogenetic Analysis

Cytogenetic examination of chromosomes from the DP with SRS showed duplication of the region 7p11.2-p13 (fig. 2). Her mother, father, sister, and both maternal grandparents were all shown to have normal karyotypes.

Identification of 7p11.2-p13 Duplication by FISH

Figure 3*A* shows the duplicated signals for a PAC containing the *GRB10* gene (dJ0108E23) in the DP. Identical FISH analysis did not detect a similar duplication in the additional family members or in five patients with non-mUPD SRS for whom cell lines were available (data not shown).

Characterization of the Extent of the Duplication

Probes proximal and distal to the *GRB10* gene, covering a region of ~16 cM, were mapped by FISH onto interphase and metaphase nuclei from the DP. Results of the interphase FISH experiments using 17 PACs are shown in figures 3*B* and 4. The centromeric breakpoint maps between the PACs containing *GRB10* (dJ0108E23) and *EGFR* (dJ069I12). The telomeric breakpoint for the duplication was found to be between

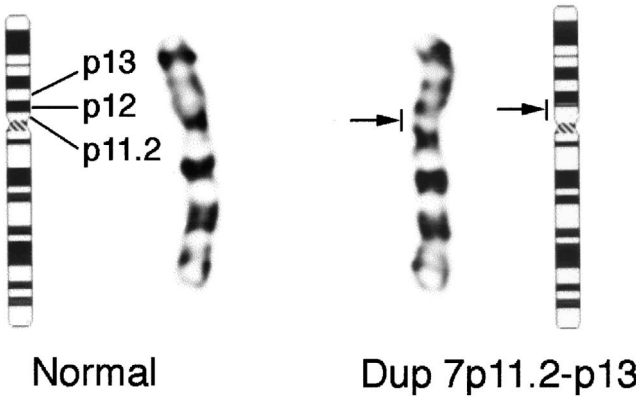

Figure 2 Partial karyotype with ideogram showing duplication of chromosome 7p11.2-p13, indicated by arrow; the normal homologue is on the left.

PACs dJ01178G13 and dJ0570D02. This defines an interval of ~10 cM (cM distances based on information from the Genetic Location Database).

Orientation of the Duplication

Two-color interphase FISH was used to orient the duplication in a head-to-tail direction, as shown in figure 3*C*. PACs dJ0108E23 and dJ0647J21 were labeled with Spectrum Red and Specrum Green dUTP, respectively, and hybridized onto interphase nuclei from the DP. Nuclei showed four signals together, in a red-green-red-green order, indicating a tandem duplication, and a pair of signals elsewhere in the nucleus, one red and one green, which were from the normal chromosome. PACs dJ0817I18 and dJ0673M15 were also hybridized together, and the same pattern was observed.

Microsatellites

Six polymorphic markers located around *GRB10* were studied. Haplotypes for each marker were examined in the proband and parents, so that the parental origin of the duplication could be determined. Of the six markers, two (*D7S1818* and *D7S3069*) were informative for the origin of the duplication. Allele dosage was determined by use of the PhosphorImager. In both cases, measurements showed that the maternal inherited band was more intense than the paternal band, indicating that the duplication was of maternal origin (fig. 5).

Investigation of GRB10 *Dosage in SRS*

Confirmation of *GRB10* duplication in the DP was obtained by gene-dosage analysis of Southern blot hybridization. At the same time, evidence of duplication in other family members and in 36 other, non-mUPD7,

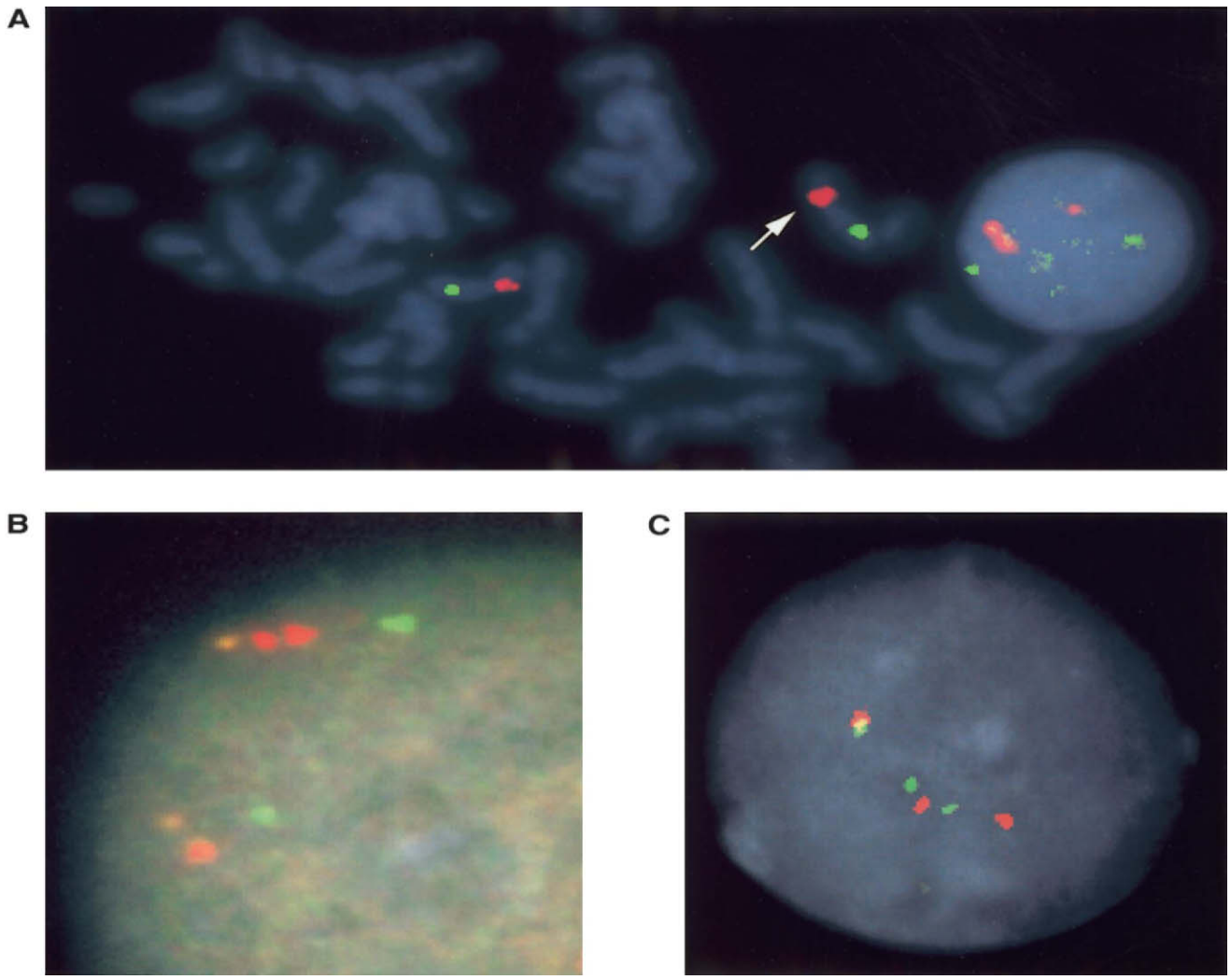

Figure 3 *A,* FISH photomicrograph of nuclei from the DP, hybridized with the *GRB10* containing PAC dJ0108E23 (*red*) and the control dJ0020F22 (*green*). Three red hybridization signals were clearly visible in all interphase nuclei, indicative of a duplication, compared with the two control signals (*green*). In the metaphase spreads, an increase in dJ0108E23 signal intensity was observed on one homologue, indicated by the arrow, confirming a duplication. *B,* Interphase nucleus from the DP, hybridized with dJ0108E23 (*red*), dJ069I12 (*orange*), and dJ0570D02 (*green*). The orange-red-red-green pattern indicates that the dJ069I12 and dJ0570D02 PACs are not contained within the duplication, unlike the double signal from the dJ0108E23 PAC. The adjacent orange, red, and green signals indicate the normal chromosome 7. *C,* Interphase nucleus from the DP, hybridized with dJ0108E23 (*red*) and dJ0647J21 (*green*). The red-green-red-green pattern suggests a duplication in a tandem arrangement. The adjacent red and green signals indicate the normal chromosome 7.

SRS probands was carried out. Filters with *Xba*I digests of DNA from both normal individuals and SRS probands were hybridized simultaneously with test and control probes *GRB10* and A6121-1. The *GRB10* cDNA probe gave two distinct bands, of ~11.0 and 5.0 kb. The primers used to generate this probe must span an intron including an *Xba*I site, since cDNA sequence of the probe was known to contain no *Xba*I restriction sites. The control probe A6121-1 hybridized to a single, intermediate-size 6.9-kb band (fig. 6).

The ratios of *GRB10*'s upper- and lower-band intensities to the A6121-1–band values in probands, normalized against the mean ratios for normal controls, are shown in figure 7*A* and *B*. Both sets of ratios followed a normal distribution, with almost all values being close to 1.00. Mean proband ratios were calculated by excluding the readings for the DP. The mean value obtained for the upper *GRB10* band was 1.04 (SD, 0.16), and that for the lower *GRB10* band was 1.00 (SD, 0.13). With the exception of the DP and one other SRS proband, the range of values for the upper band was 0.74–1.28, and that for the lower band was 0.73–1.29. Ratios generally fell within or just outside the 95% confidence limits.

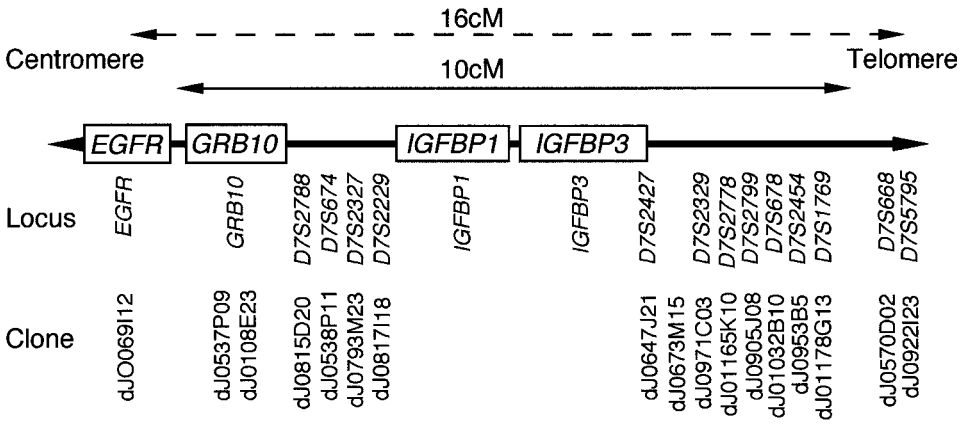

Figure 4 Physical map of 7p11.2-p14, showing the extent of the duplication as detected by interphase FISH. The duplication was analyzed by use of 17 PAC clones from a 16-cM region containing the 10-cM duplication.

DNA from the proband with the duplication is shown on the Southern blot in figure 6. Ratios, of 1.85 and 1.44, for the upper and lower bands, respectively, clearly demonstrate an increased *GRB10* dosage associated with duplication of the gene.

GRB10 dosage was also investigated in the family of the DP. DNA from the DP, her sister, her mother, her father, her maternal grandmother, her maternal grandfather, and four controls was digested with *Xba*I and hybridized with *GRB10* and A6121-1 probes, as described above. As expected, her karyotypically normal relatives all had ratios close to 1.00. Ratios for the upper and lower bands were 0.89–0.97 and 0.86–0.94, respectively. In contrast, the DP again had high ratios of 1.33 and 1.34 for the upper and lower *GRB10* bands, respectively, consistent with increased gene dosage (data not shown).

Proband 1 had a normal ratio of 1.09 for the lower *GRB10* band. However, a low ratio of 0.63 for the upper band suggested hemizygosity. Southern blot hybridization in this proband gave an additional band, of ~15 kb (data not shown). Investigation of parental DNA showed that this additional band was inherited from the phenotypically normal father (fig. 6, *arrow*). The most likely explanation of the findings in proband 1, therefore, is that she is heterozygous for a rare *Xba*I RFLP.

Replication Timing of GRB10

GRB10 was found to replicate asynchronously in human control lymphocytes, as determined by the frequency of nuclei displaying the asynchronous G1/G2 pattern. In general, most genes replicate on homologous chromosomes in a synchronous manner, with <10% of nuclei showing the asynchronous replication pattern. In contrast, all imprinted genes display the asynchronous G1/G2 pattern in 25%–40% of nuclei. The level of asynchrony for the three overlapping PACs containing *GRB10* showed a level of asynchrony similar to that of the *SNRPN* asynchronous control probe (table 1). Synchrony was observed at the *CFTR* locus on chromosome 7, as expected.

Discussion

Reports of mUPD7 in ~7% of SRS probands have led to speculation that one or more imprinted genes on chromosome 7 play a role in this condition (Kotzot et al. 1995; Preece et al. 1997, 1999). However, the location of the SRS gene(s) on chromosome 7 is still unknown. Probands with karyotypic abnormalities of this chromosome are therefore of great interest. Here we have presented the first report of a tandem 7p11.2-p13 duplication in a child with characteristic features of SRS. Arguably, this finding is, to date, the most useful cytogenetic clue to the location of the SRS gene(s) on chromosome 7.

A girl with characteristic features of SRS and 47XX,UPD(7)mat+r(7)pat/46XX,UPD(7)mat mosaicism was recently described by Miyoshi et al. (1999). Microsatellite-marker analysis showed biparental inheritance at four centromeric loci, leading the authors to conclude that "if the putative SRS gene is imprinted, it can be ruled out from 7p13-q11" (p. 326). However, the data can only be used to infer exclusion of a paternally—but not of a maternally—expressed gene from this region. Moreover, the trisomic region is likely to be considerably smaller than 7p13-q11 (Wakeling et al., in press-*a*). To our knowledge, karyotypic abnormalities of chromosome 7 have not been reported in any further probands with characteristic features of SRS.

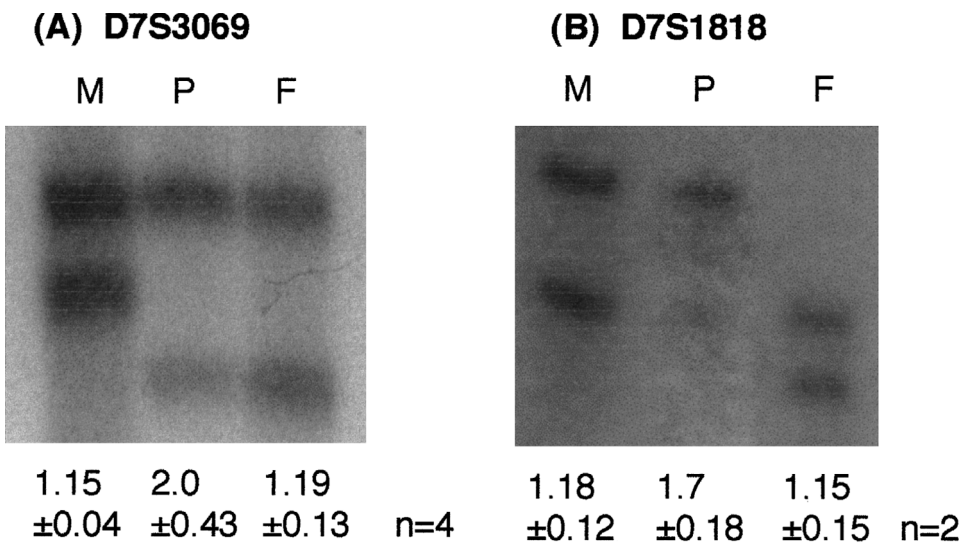

Figure 5 Tetranucleotide-repeat markers for loci *D7S3069* (*A*) and *D7S1818* (*B*). Signal intensity was measured by use of a PhosphorImager. The relative intensity of the two bands is expressed as a ratio of upper to lower signals (±SE) in the mother (lanes M), proband (lanes P), and father (lanes F). For both markers, the maternally inherited band in the proband was of increased intensity.

In addition to the proband described by Miyoshi et al. (1999), at least five other reports of trisomy for a region overlapping 7p11.2-p13 have been published. Four of these cases involved much larger segments of duplicated chromosomal material. Three had duplications of the entire short arm of chromosome 7 (Carnevale et al. 1978; Odell et al. 1987; Zerres et al. 1989); two of these three were due to familial translocations. Another child had duplications of both 7p13-q21 and 5q35-qter (Wahlström et al. 1976). In all four cases, the extent of the duplication was much greater than that found in our proband, and, as expected, the phenotypes described were markedly more severe. Since other autosomes were involved in some of these patients, it is also difficult to attribute specific features of their phenotypes to duplication of chromosome 7.

Interestingly, Schaefer et al. (1995) described a 10-mo-old child with a familial inverted duplication of 7p12.2-p13, some of whose features were reminiscent of SRS. She had feeding difficulties, failure to thrive, bilateral fifth-finger clinodactyly, and developmental/speech delay. However, her birth weight was thought to be normal (25th centile), her facial features were not characteristic of SRS, and she showed significant "catch-up" growth after feeding via a gastrostomy tube was initiated. At the time of the report, her height was 81 cm (3d–10th centile), and her weight was 9.9 kg (just below the 3d centile). Furthermore, although her brother, mother, and grandmother were all cognitively delayed and had the same inverted duplication of chromosome 7, none are described as having growth retardation. The trisomic region in this family overlaps that in our proband. Phenotypic differences, in particular the presence/absence of growth failure, may result from differences in extent, position, and/or orientation of the two duplications, and a more detailed comparison is planned.

In the proband described in this report, microsatellite-marker analysis was used to demonstrate the maternal origin of the duplication. This finding is consistent with

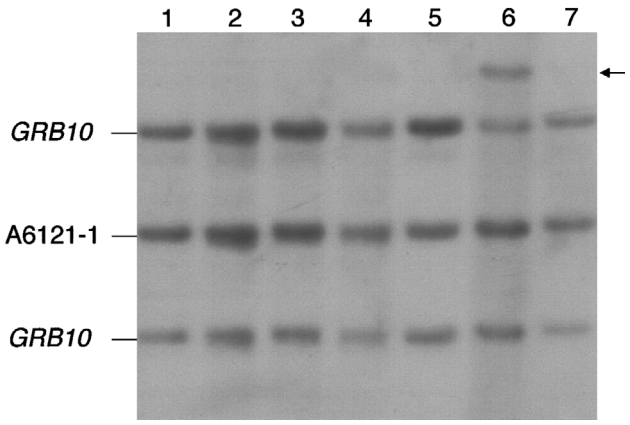

Figure 6 Southern blot hybridization of DNA from normal controls (lanes 1, 4, and 7) and SRS probands (lanes 2, 3, 5, and 6), digested with *Xba*I. The positions of the upper and lower *GRB10* bands and of the A6121-1 band are indicated on the left. DNA from the proband with the 7p11.2-p13 duplication and increased *GRB10* dosage is shown in lane 5. DNA from proband 1 with *Xba*I RFLP (*arrow*) and with hemizygosity for the upper *GRB10* band is shown in lane 6.

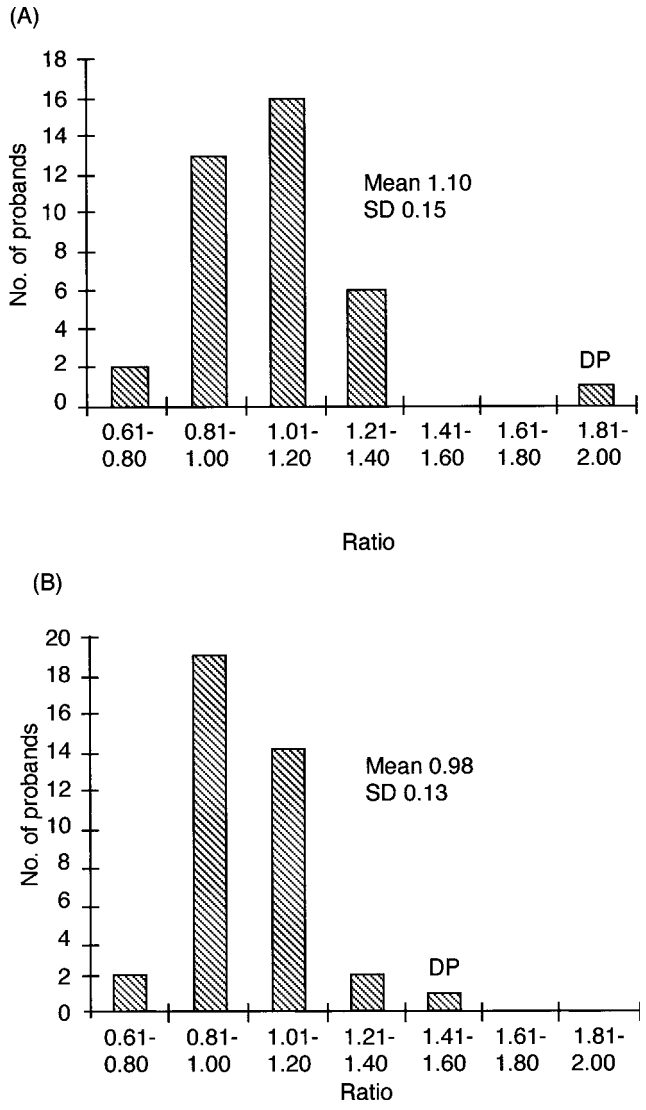

Figure 7 Histograms representing the ratio of *GRB10* signal to control for 37 probands, as determined by quantitative analysis of Southern blot hybridization. *A*, Upper *GRB10* band. *B*, Lower *GRB10* band. SD and mean for the sample are given, for both sets of ratios. Ratios for DP lie well above the normal range, confirming increased dosage of *GRB10*. No other SRS probands have similarly increased ratios.

the hypothesis that an imprinted gene is responsible for the phenotype in those patients with mUPD7. Moreover, it provides the first evidence that duplication of a maternally expressed gene, rather than absence of a paternally expressed gene, is involved. Overexpression of a maternally expressed, imprinted gene with growth-suppressing activity, located within the region 7p11.2-p13, could account for the growth failure seen in both our proband with duplication 7p11.2-p13 and in those with mUPD7.

The duplicated region 7p11.2-p13 is homologous to an imprinted region on mouse proximal chromosome 11. mUPD for this region in mice results in prenatal growth failure. Conversely, pUPD results in prenatal overgrowth (Cattanach and Kirk 1985). Within the 10-cM duplicated region lie three genes with growth-related functions that have been proposed elsewhere as candidates for SRS: *IGFBP1*, *IGFBP3,* and *GRB10* (Kotzot et al. 1995; Preece et al. 1997; Miyoshi et al. 1998). A recent study failed to find any evidence for imprinting of either *IGFBP1* or *IGFBP3,* and these two genes are therefore unlikely to play a major role in the SRS phenotype (Wakeling et al., in press-*b*). *GRB10*, however, is a strong candidate, for two main reasons. First, the gene is known to have growth-suppressing actions (Liu and Roth 1995; O'Neill et al. 1996), and, second, its homologue in the mouse (*Grb10/Meg1*) was recently isolated in a systematic screen for maternally expressed, imprinted genes, by use of subtraction hybridization (Miyoshi et al. 1998).

Although the imprinting status of *GRB10* in humans remains to be determined, it is tempting to speculate that it is also expressed from the maternal allele alone. FISH studies of normal human lymphocytes have demonstrated asynchronous replication of the *GRB10* gene, at a frequency similar to that for imprinted genes from the Prader-Willi syndrome region (Kitsberg et al. 1993; Knoll et al. 1994; Gunaratne et al. 1995; White et al. 1996). This provides the first experimental evidence that *GRB10* is located within an imprinted region, suggesting that this gene is also imprinted in humans. Further work using expressed polymorphisms to look at the imprinting status of *GRB10* is currently in progress.

As predicted, duplication of *GRB10* was demonstrated by both FISH and gene-dosage analysis in our proband. However, in a well-defined cohort of 36 other, non-mUPD7, SRS probands, no evidence could be found for duplication of *GRB10*. Gene dosage for all but one proband (who had a rare RFLP) was normal. In addition, in those patients whose cell lines were available, FISH analysis was used to exclude duplication of the gene. It seems, therefore, that submicroscopic duplication of *GRB10* is not a common cause of the SRS phenotype.

If it is assumed that *GRB10* is imprinted and maternally expressed, mechanisms other than duplication of the gene may be causing its overexpression in SRS probands. In Beckwith-Wiedemann syndrome (BWS), paternal 11p15 duplications are seen in <1% probands (Li et al. 1997). Biallelic expression of *IGF2* is the most common molecular abnormality in patients with BWS without cytogenetic abnormalities. By analogy, overexpression of *GRB10* in non-mUPD7 SRS probands could result from loss of imprinting. It would be interesting to compare the level of *GRB10* expression, both in mUPD SRS probands and in non-mUPD7 SRS probands, with

Table 1

Collated Results Obtained from FISH Analysis of Allele-Specific Replication Timing in Normal Lymphocytes

Case and Replication Pattern	Mean ± SD No. of Nuclei				
	GRB10			CFTR	SNRPN
	dj0108E23	dj0898018	dj0537P09	dj0020F22	RPCI6 5E12
Control 1 lymphocytes:					
G1 (2 signals)	67.3 ± 5.79	69 ± 3.64	66.9 ± 5.21	88.86 ± 3.02	64 ± 4.85
G1/G2 (3 signals)	27.9 ± 3.22	27.44 ± 2.9	29.2 ± 3.97	9.2 ± 3.74	29.9 ± 6.58
G2 (4 signals)	4.8 ± 2.05	3.56 ± .74	4 ± 2.76	1.94 ± 1.86	6.1 ± 3.78
Control 2 lymphocytes:					
G1 (2 signals)	64.7 ± 2.62	62.14 ± 2.68	62.46 ± 6.5	86.7 ± 3.48	65.2 ± 2.9
G1/G2 (3 signals)	29.1 ± 2.59	30.9 ± 3.17	31.64 ± 4.06	9.98 ± 2.53	30.7 ± 1.87
G2 (4 signals)	6.2 ± 1.98	6.96 ± 1.86	5.9 ± 3.29	3.32 ± 3.12	4.7 ± 1.91

Note.—For each trial, ~100 nuclei were counted on each hybridized slide. A total of five trials for each probe were analyzed in each case for each replication pattern; means and SDs were calculated on the same sample.

that in normal controls. Overexpression of *GRB10* in mUPD7 probands would be indicative of its maternal-specific expression in humans. Overexpression of the gene in non-UPD probands would provide evidence for loss of *GRB10* imprinting in SRS and would suggest direct involvement of this gene in the disease phenotype.

GRB10 dosage was investigated because it had already been identified as a good candidate for SRS. However, other genes may be involved. Since many imprinted genes have been found to lie within clusters, it may be the case that *GRB10* lies within a cluster of imprinted genes, all or some of which are duplicated in our proband. One or more of these may be involved in SRS, in addition to or instead of *GRB10*. Further investigation of this region is underway.

Another explanation for the phenotype in this proband is overexpression of a nonimprinted gene within the region of trisomy. It is also theoretically possible that the phenotype in mUPD7 probands is due to mosaicism for trisomy 7 and increased dosage of a nonimprinted gene. However, no evidence for low-level mosaicism was found in two mUPD7 SRS probands when parental-allele inheritance in fibroblast and lymphoblast DNA was investigated by Southern blot hybridization (authors' unpublished data). Neither of these patients was asymmetric. One other asymmetric patient without mUPD7 was also similarly investigated for the presence of trisomy 7 mosaicism, but no evidence for this was seen. More-extensive studies using fibroblasts from many affected patients would be needed to investigate the possibility that some asymmetric cases of SRS are the result of mosaicism for either trisomy 7 or duplication 7p11.2-p13.

SRS is likely to be genetically heterogeneous. Most cases are sporadic, although some familial cases have been described (Duncan et al. 1990). Associations of SRS with abnormalities of chromosomes 8, 15, 17, and 18 have also been reported (Chauvel et al. 1975; Midro et al. 1993; Schinzel et al. 1994; Rogan et al. 1996). It may be that different genes on one growth-related pathway are involved via several distinct mechanisms. A gene(s) that is involved in the SRS phenotype and associated with duplication 7p11.2-p13 may share a common growth-regulatory axis with genes on other chromosomes implicated in the disorder.

The findings in this proband have important implications for continuing research into the molecular basis for SRS. The duplication in this proband was difficult to detect cytogenetically; therefore, if duplications within the region 7p11.2-p13 are the cause of other cases of SRS, they may be submicroscopic. We suggest that similar cases may now come to light if investigating clinicians request careful scrutiny of this region when sending SRS samples for karyotyping. It seems likely that the candidate gene(s) for this condition will lie within 7p11.2-p13. Recognition of additional probands with karyotypic abnormalities of this region would strengthen this hypothesis and would allow further definition of the SRS critical region on chromosome 7.

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Electronic-Database Information

The accession number and URLs for data in this article are as follows:

Genetic Location Database, http://cedar.genetics.soton.ac.uk/public_html/

Human chromosome 7, Washington University, St Louis, http://www.genetics.wustl.edu

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Silver-Russell syndrome [MIM 180860])

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