LETTER TO JMG

Characterisation of the growth regulating gene *IMP3*, a candidate for Silver-Russell syndrome

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J Med Genet 2002;39:575–581

Silver-Russell syndrome (SRS) is characterised by pre- and postnatal growth restriction in association with other clinically recognised dysmorphic features such as triangular facies, asymmetry, and fifth finger clinodactyly.^{1–3} Since the major diagnostic features involve reduced growth, it is tempting to postulate that altered expression of a protein within a growth factor cascade may be causative.

There have been numerous documented defects of genes coding for proteins in the insulin-like growth factor (IGF) signalling pathways, whether the receptors, ligands, or signal modulators, which result in a SRS-like phenotype. Probands with ring chromosomes or deletions involving the 15q26-qter region present with growth failure and SRS-like features.^{4–11} It has been proposed that the phenotypes are the result of hemizyosity at the IGF1 receptor (*IGF1R*) gene. We have shown, however, that hemizyosity at this locus is not a common cause of SRS.^{9–11} In addition, it has been well documented that maternal uniparental disomy (UPD) of chromosome 7 is present in approximately 10% of SRS cases^{13–16} and no consistent regions of isodisomy have been shown for the full length of the chromosome.¹⁷ This suggests that there are imprinted genes on chromosome 7, which when disrupted are responsible for the phenotype. Recently two independently reported candidate gene regions on chromosome 7 containing imprinted genes defined by cytogenetic disruptions in SRS probands have been reported. Two unrelated probands with maternally transmitted duplications of 7p11.2-p13 define the first region.^{10–18} Recently, a number of other cytogenetic disruptions including balanced translocations and inversions within this region have been described in association with the SRS or SRS-like phenotype¹⁹ (Monk *et al*, manuscript submitted). This candidate gene region contains the growth related genes insulin-like growth factor binding proteins 1 and 3 (*IGFBPs*) and growth factor binding protein 10 (*GRB10*). The latter has been shown to be imprinted, and it is conceivable that overexpression of this gene could give rise to the phenotype in these cases. However, all of the above genes are excluded from playing a major role in the aetiology through both imprinting and mutation analyses.^{11–20–25} The second SRS candidate region is defined by a single SRS case that resulted

from segmental uniparental isodisomy for 7q32-qter. This second region is known to contain two imprinted genes, *MEST* and *CoPg2*.²⁶

The human 7p11.2-p13 region is orthologous to the imprinted region on mouse proximal chromosome 11. Mice with maternal duplication of the region proximal to the T41Ad reciprocal translocations breakpoint (MatDp(prox11)) present with prenatal growth failure, a situation similar to the observed growth restriction in the duplication and mUPD(7) SRS cases.²⁷ In addition to the mouse chromosome 11 imprinted region, human chromosome 7 has homology with multiple mouse chromosomal regions displaying imprinted phenotypes.

Mouse proximal chromosome 6 harbours two imprinted regions and has homology with human 7q32-qter, 7q21, and 7p15. Mice with maternal duplications of chromosome 6 proximal to the T(4;6)77H reciprocal translocation breakpoint in 6A3.2 (MatDp(prox6)) define a domain that is responsible for early embryonic lethality, whereas the region between the T77H and T(6;13)6Ad breakpoints is associated with growth retardation when maternally duplicated. This second region contains the imprinted gene cluster of *Mest*, *Copg2*, *Copg2AS*, *Mit1/Lb9* (fig 1).²⁸

In this report, we describe the mapping, expression analysis, and imprinting status for the novel functional candidate gene *IGF2* mRNA binding protein 3 (*IMP3*). *IMP3* is an interesting candidate for SRS since it maps to human chromosome 7, mouse chromosome 6, and is involved with *IGF2* regulation of prenatal growth.

IMP3 is a member of a closely related mRNA binding protein family with a molecular mass of 64 kDa, with an overall sequence identity of 59% with other family members. *IMP3* sequence is identical to the Koc (KH domain containing protein overexpressed in cancer) protein sequence derived from a pancreatic tumour cDNA screen²⁹ and is thought to have an important role in the differentiation process during early human embryogenesis.³⁰

The *IMP* proteins contain two functional RNA recognition motifs (RRM) and four hnRNP K homology domains which suggests an involvement with RNA stability or transcriptional regulation.³¹ Members of the *IMP* protein family have been shown to have multiple attachment sites within the 5' UTR of *IGF2* leader 3 mRNA and the 3' UTR of *H19* mRNA.³² *H19* is a maternally expressed gene that is physically linked to the paternally expressed *IGF2* gene. *H19* RNA is not translated but is thought to be involved in *IGF2* regulation since their imprinted expression is mechanistically linked. This link makes up the basis for the enhancer competition theory for the 11p15.5 imprinting cluster, disruptions of which are responsible for a proportion of the overgrowth phenotype seen in Beckwith-Wiedemann syndrome (BWS).³³

It is speculated that the *IMP* family of proteins act as factors that are involved in the mechanical coupling of *IGF2/H19* post-translational regulation.³² *IMP1* has been shown to be involved in such a regulation, via interactions with the 6 Kb *IGF2* leader 3 mRNA, but not the constitutively translated 4.8

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Figure 1 Human-mouse map showing regions of homology with the imprinted region on mouse proximal 6. The chromosome 6 breakpoints T77H, T6Ad, and T7Ca are shown in relation to known imprinted genes. *Imp3* maps distal to the T77H breakpoint, within the imprinted region associated with growth retardation.

Kb *IGF2* leader 4 mRNA. This regulation allows for rapid adjustment of *IGF2* at critical developmental stages via a *trans* acting mechanism.³¹ Disruptions of *IMP3* expression, therefore, may be involved in the growth phenotypes seen in the mouse chromosome 6 partial disomies and Silver-Russell syndrome.

MATERIALS AND METHODS

Control lymphocyte preparations

PHA stimulated human lymphocytes were separated from peripheral blood using Histopaque 1077 (Sigma) and cultured, harvested, and fixed by conventional techniques.

Monochromosomal somatic cell hybrids

Somatic cell hybrids produced by electrofusion of mouse E2 cells and human lymphocytes to produce cells retaining a single human chromosome were acquired from GMP Genetics Inc¹⁰ and have been described elsewhere (Monk *et al*, manuscript submitted). Cell lines E2(P11) and E2(M19) carry a single human chromosome 7 of defined parental origin for which imprinted expression at the *MEST* loci has previously been shown. Standard cell culture techniques were used to propagate the hybrid cell lines according to the supplier's specific recommendations.

Mouse proximal chromosome 6 reciprocal translocations

Standard protocols of intercrossing mice heterozygous for T(4;6)77H with a chromosome 6 breakpoint in G band 6A3.2 were used to generate mice with maternal (MatDp(dist 6))

and paternal (PatDp(dist 6)) partial disomies for distal chromosome 6.^{28,35} All animal studies were carried out under the guidance issued by the Medical Research Council "Responsibility in the Use of Animals for Medical Research" (July 1993) and the Home Office Project Licence Number 30/1518.

Metaphase spreads from mice heterozygous for T(4;6)77H were generated from bone marrow samples using standard methods. Snap frozen tissue samples from both maternal (MatDp(dist 6)) and paternal (PatDp(dist 6)) lines were used for expression analyses.

FISH

Slides were dehydrated through a 70%, 90%, 100% ethanol series and denatured in 70% formamide/2 × SSC for two minutes, and rehydrated. Probes were prepared from standard miniprep DNA using nick translation to incorporate either SpectrumGreen or SpectrumRed (Vysis). FISH was performed as described by Monk *et al*¹² with minor modifications. Slides were counterstained with DAPI and examined using a Zeiss fluorescent Axioscope equipped with a triple bandpass filter. Images were recorded and enhanced using both QUIPS M-FISH and SmartCapture IPLab software (Vysis).

Genomic library screens

All genomic clones used for FISH probes not generated by library screening came from Kirsch *et al*¹³ and Korenberg *et al*.¹⁴ Identification of the human *IMP3* containing BAC clone RG271G13 resulted from BLAST research using the complete human *IMP3/Koc1* cDNA AF117108 as a query sequence.

The mouse PAC RP21 639M11 resulted from a screen of the mouse RPC21 library, using an intronic PCR product probe

generated from the BAC RG271G13 template (EX3 F-CATCAGAGTGCCGTCCTTTG; EX4 R-ACTTGATCATTCTCAT CAGG, 55°C) to avoid pseudogene contamination. Positive clones were confirmed by further PCR and sequencing of the product.

Expression analysis

Total RNA was isolated from fetal tissues (Research Ethics Committees of the Royal Postgraduate Medical School (96/4955)) and somatic hybrid cells using the Trizol extraction technique (Life Technologies BRL).

For cDNA synthesis, 2 µg denatured total RNA from each sample was used to produce cDNA in 20 µl reactions with 1 × RT buffer, 40 U M-MLV reverse transcriptase (Promega), 1 unit Rnase inhibitor (Promega), 1 mmol/l each dNTP, and 0.2 µg primer at 37°C. Random hexamers were used to produce cDNA for analysis of all genes. A duplicate set of samples was processed, with the RT omitted to detect any genomic DNA contamination of the RNA. For each sample, PCR using *GAPDH* primers (F-CCACCCATGGCAAATCCATGGCA; R-TCTAGACGGCAGGTCAGGTCCACC, annealing temperature 59°C) confirmed the presence of cDNA. *MEST* lymphocyte specific isoform expression primers (PEG33- ATGGGA TAACGCGGCCATGGTG; PEG34- ATAGTGATGTGGTCTCG GTTTGTCACCTG, PEG36-AGTGGATAACGCGGCCATGGTG, annealing temperature 58°C),¹⁵ *IMP3* fetal expression primers (EX2 F-AGAAGCACCAGATGCTAAAG; EX3 R-AACTCT GCCAGCAGCAAAGG, annealing temperature 56°C), and human specific *IMP3* primers for monochromosomal somatic cell hybrid analysis (IMP3'F-CCTTTGCTGCTGGCAGAGTT; IMP3'R-AACAAAGGGAAGTGCAGAGC, annealing temperature 56°C) were designed to amplify between exons, across at least one intron, to detect amplification of genomic contamination or antisense RNA on the basis of product size. Mouse *Imp3/Koc1* primers were designed within regions of homology between species crossing numerous introns. Primer sequences were based on the Koc IMAGE clone IMAGp998D031297 mouse sequence (EMBL: MAAA63596) that had 93% overall homology to the human *IMP3* cDNA clone AF117108 (mImp3 F-GTCTTATGAAAATGATATTGCTTC; mImp3 R-CACCTCTTC TTTAGGACTAAC, annealing temperature 56°C). *Hprt* control primers were used to confirm the presence of cDNA from mouse samples (*Hprt* F- GTCTTATGAAAATGATATTGCTTC; *Hprt* R- CACCTCTTC TTTAGGACTAAC, annealing temperature 55°C).

Mutation analysis in SRS cohort

Twenty-five classical SRS patients were included in the study; these patients were a subset of 53 previously described by Preece *et al.*¹⁶ Major structural abnormalities, trisomy mosaicism, and mUPD(7) had been ruled out by microsatellite repeat marker analysis. Peripheral blood samples for DNA analysis were obtained with patients' informed consent (approved by the Joint Research Ethics Committee of the Great Ormond Street Hospital and the Institute of Child Health-1278).

The ORF of *IMP3* was PCR amplified from cDNA to avoid pseudogene contamination in the 25 SRS patients using overlapping primer pairs (IMP3F1-CTGGATCCATCCATCACCCTG, IMP3R1-CAGTTTCCGAGTCAGTGTTTAC, annealing temperature 58°C; IMP3F2-CTAGTCCAGTATGGAGTGGTG, IMP3R2-GTAATCGACTTCTCAGCAGC, annealing temperature 60°C; IMP3F3-CCAGTCTAAAATCGATGTCC, IMP3R3-CATGATCTC CTCCTCAGCTTTG, annealing temperature 58°C; IMP3F4-CCCACTATTACAGTTAAAGGC, IMP3R4-CTTGAAGTGGCT TCTGGTG, annealing temperature 58°C; IMP3F5-TTGCT CCAGCAGAAGCACCAG, IMP3R5-CTGGTCTCAGAAACA ACTG, annealing temperature 55°C). The PCR products were directly sequenced. SRS patient sequences were analysed for mutation and polymorphisms using the Sequence Navigator

Figure 2 (A) The location of *IMP3* by FISH on DAPI stained human lymphocyte metaphase spreads. The *IMP3* containing BAC RG 271G13 (green) maps to 7p15, flanked by the known marker probes RP4 540D02 (orange) at 7p14 and RG 261H24 (red) at 7p21. (B) Mouse chromosome 6 ideogram with the breakpoint T(4:6)77H shown at A3.2. Pseudo G banded chromosomes 6, 4', and 6' are illustrated with *Imp3* hybridisation signals on the normal chromosome 6 at B2.3 and on the long 4' translocation product below the T77H breakpoint. No signals were found on the small 6' translocation product.

program, and through direct comparison to the published *IMP3* cDNA sequence by BLAST analysis.

RESULTS

Mapping of *IMP3*

The chromosomal localisation of *IMP3* has not previously been reported. The cDNA sequence, however, originally appeared in the working draft sequence (<http://genome.cse.ucsc.edu/>) at cytogenetic location 7p11.2 ± 11 cM. This sequence was contained within the BAC clone RG 271G13, which was shown to contain the last 4 exons and 3' UTR. FISH analysis with this clone indicates a much more distal localisation at 7p15 (fig 2A) which is corroborated by a recent build of the working draft sequence (Aug 2001 freeze). Since regions of mouse chromosome 6 are known to have homology with this region (<http://www.mgu.har.mrc.ac.uk/imprinting>) (fig 1), dual FISH on normal mouse metaphase spreads using the *Imp3* containing mouse PAC RP21 639M11 and the known mouse chromosome 6 marker RG 412F6, confirmed its localisation to distal chromosome 6 (data not shown). The *Imp3* PAC probe resulted from a mouse RP21 library screen using a ~700 bp human intronic PCR product probe generated from the human BAC RG 271G13 template to avoid pseudogene contamination.

Fig 2B shows a FISH photomicrograph of pseudo G banded metaphase chromosomes from a mouse heterozygous for T(4:6)77H. No hybridisation signals were seen on the small 6' chromosome; however, consistent signals were seen half way down the larger 4' chromosome in 19 cells. The FISH signals were localised to band B2.3 on the normal chromosome 6. These results show that *Imp3* is located distal to the breakpoint of T77H and therefore outside the redefined early embryonic lethality imprinting region, but remains a candidate for the growth retardation imprinted phenotype.

Table 1 Table showing the intron/exon boundaries for *IMP3*

Exon	Size (bp)	Acceptor site intron-EXON	Donor site EXON-intron	Intron	Size (bp)
1	309		GCGCTTTCAGgtgggccg	1	26474
2	61	gtgtccgcagGTAAATAGA	AAAGGCCAAAGgtgagctgt	2	40427
3	55	tctctgcagGATTCGAAA	ACAGTGGGAGgtaagaattt	3	57033
4	51	ctctgatagGTGCTGGATA	TGTGAGCAAGgtacatgtct	4	91
5	64	cttctctagTGAACACTGA	AAGCTAGACAgtaagcagct	5	9946
6	282	caacttcagAGCACTAGAC	CCCAGTCTAAgtgagatgc	6	3579
7	135	ccactatcagAATCGATGTC	ATATAAAATTgtgagtaag	7	1547
8	124	ctatttacagCACAGAAGAG	CGATATCTCCgtgagtgctc	8	2114
9	137	cattctgcagATTGCAGGAA	TTCTATGAATgttagtttg	9	1527
10	125	actgttgcagCTTCAAGCAC	GCAGTTTGAAGtaagataac	10	22801
11	116	tttctaacagCAATCAGAAA	TTCAATTAAGgtactgttct	11	1415
12	74	tctgttacagATTGCTCCAG	TCAGTTCAAGgtatgtgtc	12	3984
13	136	tcatctgcagGCTCAGGGGA	AAAACGGCAAgtaactcagc	13	669
14	117	tcttttcagGTGAATGAAC	TGCCAGGCAAgtggtctcta	14	269
15 (3'UTR)	2248	cttttctagGTGCCCCAGA	CATCATTAGgtgcagcttg		

Genomic structure

BLAST two sequence analysis using the *IMP3* full length cDNA sequence (AF117108) against sequenced genomic clones GS 117B4 (AC021876) and RG 271G13 (AC005082) was used to define the intron/exon boundaries. From the draft sequences, *IMP3* was determined to contain 15 exons spanning a minimum of 175 950 bp of genomic DNA. All identified intron/exon boundaries adhere to the consensus acceptor and donor splicing sequences (table 1). The transcript contains an open reading frame (ORF) of 1739 bp, a 2.2 kb 3' UTR which includes a polyadenylation signal (AATAAA) 26 bp from the polyA tail, and a 267 bp 5' UTR (fig 3). Initial RT-PCR showed the presence of pseudogenes, since RT- contained identical sized products to RT+ and genomic controls (data not shown), even though the PCR primers were designed between exons crossing numerous introns. Database searches showed three intronless pseudogenes of varying length compared to the mRNA sequence located on human chromosomes 6 q26-q27 (AL023775), chromosome 4 (AC080045), and chromosome 18 (AC090245). Further BLAST 2 sequence analysis showed that the pseudogenes were ~95% homologous to AF117108, resulting from ancestral mRNA integration. All subsequent expression primers were designed carefully to *IMP3* mRNA specific regions.

Expression of *IMP3* in first and second trimester fetal tissue

Normal human fetal tissue from both first (8 week) and second (14 week) trimesters were used (fig 4). In addition, expression was also observed in adult lymphocytes and fibroblasts (data not shown). Expression of *IMP3* was investigated by reverse transcriptase PCR (RT-PCR) and northern analysis. PCR products of 175 bp were seen in all tissues analysed indicating the correct size without DNA or pseudogene contamination. This suggests that *IMP3* is expressed ubiquitously during fetal development. Since the PCR cycle number used was outside the linear range, no conclusions on relative expression levels between tissues can be drawn. Northern analysis on total RNA showed the presence of a single transcript of ~4.4 kb size in placenta and limb (data not shown). It is known that the full length human *IMP3* mRNA is 4206 bp, so the remaining ~200 bp within the primary transcript must represent the polyA tail.

Imprinting analysis of the human *IMP3* gene

Expression levels of *IMP3* in E2 somatic cell hybrids retaining individual chromosomes 7 was examined by RT-PCR, using identical cDNA pools that showed monoallelic expression for *MEST* isoform 1. *IMP3* was expressed in both sets of hybrid cell

Figure 3 Schematic diagram showing the genomic structure, mRNA splicing, and protein domains for the human *IMP3* gene. The genomic organisation of *IMP3* is contained within genomic clones GS 117B4 and RG 271G13. A computer predicted ORF map using DNA Strider is shown, where vertical lines are Met residues and long vertical lines are stop codons. Within mRNA AF117108 there is an ORF of 1736 bp, flanked by a 267 bp 5' UTR and a 2.2 kb 3' UTR. The positions of the functional protein domains are also shown.

Figure 4 Gel electrophoresis of RT-PCR products. Panel (A) shows the ubiquitous expression of the 175 bp *IMP3* product in a range of human fetal tissues, and (B) the 598 bp *GAPDH* controls. Pl = placenta, Li = liver, B = brain, H = heart, Sk = skin, K = kidney, I = intestine, Lu = lung, Ey = eye.

lines. These findings strongly suggest that *IMP3* is biallelically expressed (fig 5). Using a A→G intragenic polymorphism at base pair 1598 of the cDNA (AF117108) found during the mutation screen, the imprinting status of *IMP3* within a range of fetal tissues was investigated. The A→G polymorphism creates a *MspA1* restriction site. A screen of cDNA from five fetuses for heterozygosity, as detected by the presence of both

Figure 5 (A) The expression of *MEST* isoforms 1 and 2 in control lymphocytes, E2(P11), and E2(M19) monochromosomal somatic cell lines analysed by means of RT-PCR. On the left side of the gel, the 370 bp products resulting from isoform 1 specific primers PEG33-PEG34 can clearly be seen in the lymphocyte control and E2(P11) only. The 312 bp non-imprinted isoform 2 product (PEG36-PEG34) is expressed from both parental alleles and control lymphocytes, as shown on the right side of the gel. Therefore, *MEST* imprinting is maintained within the hybrids. (B) The biallelic expression of *IMP3*. The 535 bp RT-PCR product can be observed in the control lymphocyte (Ly), E2(P11), and E2(M19) lanes, clearly showing equal expression from each chromosome 7 allele in each hybrid.

Figure 6 (A) A schematic *IMP3* cDNA amplified with primers IMP3F4/IMP3R4 across the *MspA1* polymorphism in exon 12, and its restriction fragment sizes for the *MspA1*+ and *MspA1*- alleles. (B) The clear biallelic expression of *IMP3* in a range of 13 week fetal tissues, represented by one cut and one uncut allele.

the 420 bp undigested *MspA1* PCR product and the *MspA1* digested fragments of 355 bp and 65 bp, showed one heterozygous fetus. Both digested and undigested PCR products were observed in all tissues investigated, indicating expression from both parental alleles (fig 6).

Expression of *Imp3* in adult mouse tissue

Normal adult mouse tissues were analysed. Expression of *Imp3* by RT-PCR showed a 385 bp product in brain, liver, heart, gut, spleen, kidney, lung, ovary, and tongue (data not shown). RT-PCR was performed on liver, kidney, and brain samples from adult mice with maternal partial disomy (MatDp(distal 6)) and paternal disomy (PatDp(distal 6)) for the region distal to the T(4:6)77H breakpoint. Clear evidence of biallelic expression was obtained (fig 7). Control *Hprt* cDNA products were seen for all RT+ samples.

Mutation screen in SRS cohort

Twenty-five SRS patients, in whom cytogenetic abnormalities and mUPD(7) had been excluded, were analysed for disease causing mutations. No mutations were detected in any

Figure 7 Expression of *Imp3* in adult tissues from mice with paternal or maternal duplications of chromosome 6 distal to the T77H breakpoint. (A) The lack of *Mest* expression in MatDp.dist 6. (B) The biallelic expression of *Imp3* with expression in MatDp.dist 6, PatDp.dist 6, and normal sibs. (C) *Hprt* controls on the same tissues are shown. Identical results were seen for both liver and kidney samples.

patient. However, a novel polymorphism was identified at nucleotide 1598 of cDNA AF117108. This base change does not result in an amino acid change since both GCA and GCG code for alanine. Seven heterozygous patients were identified, indicating that *IMP3* is expressed from both parental alleles in lymphocytes, verifying the imprinting data obtained from the monochromosomal somatic cell hybrids. This polymorphism was subsequently used to analyse the expression of *IMP3* in a variety of human fetal tissues.

DISCUSSION

The *IGF2* mRNA binding protein 3 gene is an excellent candidate for SRS based on its functional involvement in the regulation of the imprinted *IGF2* leader 3 mRNA transcript and its localisation on human chromosome 7. Initially, *IMP3* was given the cytogenetic location 7p11.2 ± 11 cM which is within the candidate region defined by the mapping of chromosomal disruptions in numerous SRS and SRS-like probands¹⁰ (Monk *et al*, manuscript submitted). However, subsequent FISH analyses using the genomic clone RG 271G13 mapped the gene to 7p15, a region that shares homology with the imprinted region of mouse proximal chromosome 6. In the current study, expression analyses showed transcription of *IMP3* in a wide range of human fetal tissues from as early as 8 weeks' gestation using RT-PCR. With the recent reports of isoform specific imprinting,^{24 25 38} northern blot analysis was used to examine whether *IMP3* has multiple splice isoforms. A ~4.4 kb single transcript was seen in both placenta and limb. These results are similar to those previously published.²⁹ Murine *Imp3* has previously been reported to be ~4.4 kb in placenta³⁰ indicating that the transcript is conserved between species. *Imp3* expression has been detected in various embryonic tissues and whole mouse embryos, with highest expression at E10, then decreasing until birth.^{30 31} However, expression data in adult mouse are limited. This study has shown *Imp3* mRNA was detectable in a range of adult mouse tissues. Overall the results of these expression studies support the view that the *IMP3* product has an important role to play in normal human and mouse fetal growth and development.

The expression of *Igf2* is regulated at both the transcriptional and post-transcriptional levels reflected in the array of mRNAs transcribed from the *Igf2* gene. Mouse *Igf2* leader 3 and leader 4 mRNA are transcribed by their respective promoters and have the same coding regions.^{40 41} Mouse *Igf2* leader 3 mRNA, transcribed from the P2 promoter in humans, is expressed by E9.5 and by E12.5 the protein synthesis is repressed. This is in contrast to the constitutively transcribed *Igf2* leader 4 mRNA. The distinct translational behaviour of the two *Igf2* mRNAs is likely to reflect the presence of specific *trans* acting factors that bind and interact with the specific 5' UTRs.⁴⁰ This post-transcriptional repression of *Igf2* leader 3 has been shown to coincide with the highest expression levels of *Imp3*, when studied by northern blotting.³⁰ Both Mori *et al*³⁹ and Nielsen *et al*³¹ have shown that *Imp3* inhibits translation of chimeric *Igf2* leader 3-luciferase transcripts, suggesting that *Imp3* is the *trans* acting element regulating the temporal and spatial expression of IGFII protein within critical stages of development. IGFII is known to influence cell proliferation and differentiation. Recent studies have shown that *Imp3* inhibits the IGFII dependent cellular proliferation and differentiation. IGFII expressing P19 undifferentiated neuronal cells were transfected with flag tagged *Imp3* and induced to differentiate into neurones with retinoic acid treatment. In this in vitro model, cells transfected with *Imp3* failed to differentiate, and the nucleus of the transfectants were not apoptotic. These results suggest that *Imp3* is inhibiting differentiation and proliferation by repressing the translation of the mouse *Igf2* leader 3 mRNA, resulting in inhibition of IGFII signalling.³⁹

Since *IMP3* has been proposed to have growth suppressing activities, the imprinting status of human *IMP3* was investi-

gated in both human and mouse. Through its function and mapping, *IMP3* was an obvious candidate for a role in the SRS phenotype associated with mUPD(7). An initial analysis using monochromosomal somatic cell hybrids clearly showed biallelic expression. A more extensive imprinting screen involving multiple fetal tissues was undertaken to discount tissue specific imprinting. Using the A→G polymorphism in exon 12 found during the mutation screen, biallelic expression was observed in placenta and all fetal tissues examined. Concordant results were found using the T(4:6)77H MatDp.dist 6 and PatDp.dist 6 mice, as biallelic expression was observed in adult brain, kidney, and liver.

No sequence mutations were identified in the *IMP3* coding region in 25 SRS cases screened. Since the gene had been shown not to exhibit monoallelic expression, epigenetic mutations were not investigated. Collectively, the biallelic expression of *IMP3* and the lack of any coding mutations makes the involvement of this gene in SRS unlikely. It therefore seems likely that the causal gene or genes will reside in one or both of the candidate regions defined by the chromosome 7 cytogenetic disruption at 7p11.2-p13 or 7q32-qter, both of which are known to harbour imprinted genes and share homology with imprinted mouse regions.

The identification of any novel monoallelically expressed genes within the mouse chromosome 11 and chromosome 6 imprinted regions will result in excellent human orthologous candidate genes for SRS. Since the SRS phenotype is so heterogeneous, no single gene may be responsible for all the features of SRS in any one person. It is also plausible that the similar growth restricted phenotypes may in fact result from disruptions of different components of a single endocrinological or biochemical pathway. Identification of the gene or genes causing SRS would aid considerably in the identification of the other genes involved in growth restriction on other chromosomes by providing information on growth and developmental pathways affected.

ACKNOWLEDGEMENTS

We would like to thank Susan Price for clinical assessment of the majority of SRS patients in the main SRS cohort. We are also grateful to Mark Harrison for technical assistance with breeding the mice. This work was supported by funding from Children Nationwide (DM), the Wellcome Trust (LB), and the Dunhill Medical Trust (MH).

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