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

D Monk, L Bentley, C Beechey, M Hitchins, J Peters, M A Preece, P Stanier, G E Moore

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. 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. It has been proposed that the phenotypes are the result of hemizygosity at this locus is not a common cause of SRS. In addition, it has been well documented that maternal uniparental disomy (UPD) of chromosome 7 is present in approximately 10% of SRS cases and no consistent regions of isodisomy have been shown for the full length of the chromosome. 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. 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 phenotype (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 (GFBP10). 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. 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.

The human 7p11.2-p13 region is orthologous to the 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. 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)177H 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 associated with growth retardation when maternally duplicated. This second region contains the imprinted gene cluster of Mest, Copg2, Copg2AS, Mih1/Lkb9 (fig 1).

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 screen and is thought to have an important role in the differentiation process during early human embryogenesis.

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. 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. H19 is a maternally expressed gene that is physically linked to the paternal expressed IGF2 gene. H19 RNA is not translated but is thought to be involved in IGF2 regulation since their imprinting 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). 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. 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
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 Inc 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 6.38 39 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 miniprepped DNA using nick translation to incorporate either SpectrumGreen or SpectrumRed (Vysis). FISH was performed as described by Monk et al40 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 al41 and Korenberg et al.42 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- CATCAGATGCGGTCATCTTG, EX4 R- ACTGTACATTCACATCATCACG, 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 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- CACCCATGGGAAATCCATGGCA; R- TCTAGACGGCAGGTGCTCACC, annealing temperature 59°C) confirmed the presence of cDNA. MEST lymphocyte specific isoform expression primers (PEG33- ATGGGAAACCCGCGCCATTTGG; PEG34- ATAGTGATGTGGTCTCGGTTTGACTCTG, PEG36- ATGTTGATAACGCCGCTCAATGG, annealing temperature 58°C), 16IMP3 fetal gene primers (EX2 F- AGAAGCACCAGATGCTAAAG; EX3 R- AACTCTGCCAGCAGCAAG, annealing temperature 56°C), and human specific IMP3 primers for monochromosomal somatic cell hybrid analysis (IMP3F7- CCTCTTGCTGTCAGAGTT; IMP3R8- ACAAAGGAAGTGGACAGGC, 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 IMAP1998D031297 mouse sequence (EMBL: MAA65936) that had 93% overall homology to the human IMP3 cDNA clone AF117108 (mImp3 F- GTCTATGAAATGATATGCTC; mImp3 R- CACCTCTCTTAAAGGGAAGTGCAGAGC, annealing temperature 56°C), Hprt control primer at 37°C. Random hexamers were used to confirm the presence of cDNA from mouse samples (Hprt F- GTCTATGAAAATGATATGCTC; Hprt R- CACCTCTCTTTAGGACTAAC, 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. Major structural abnormalities, trisomy mosaicism, and mUPD(7) had 93% overall homology to the human IMP3 cDNA clone AF117108 (mImp3 F- GTCTATGAAATGATATGCTC; mImp3 R- CACCTCTCTTAAAGGGAAGTGCAGAGC, annealing temperature 56°C), Hprt control primer at 37°C. Random hexamers were used to confirm the presence of cDNA from mouse samples (Hprt F- GTCTATGAAAATGATATGCTC; Hprt R- CACCTCTCTCTTTAGGACTAAC, annealing temperature 55°C).

### 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/). 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.
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 6q26-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.

Table 1 Table showing the intron/exon boundaries for IMP3

<table>
<thead>
<tr>
<th>Exon</th>
<th>Size (bp)</th>
<th>Acceptor site intron-EXON</th>
<th>Donor site EXON-intron</th>
<th>Intron Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>309</td>
<td>ggtgagcagTGAAAATAGA</td>
<td>1</td>
<td>26474</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>cttcctagGTGCTGGATA</td>
<td>2</td>
<td>40427</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>tctcttagAGATCGGAAA</td>
<td>3</td>
<td>57033</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>cttcctagGTGCTGGATA</td>
<td>4</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td>cttcctagGTGACACTGTA</td>
<td>5</td>
<td>9946</td>
</tr>
<tr>
<td>6</td>
<td>282</td>
<td>ccattcagAGACTAGAC</td>
<td>6</td>
<td>3579</td>
</tr>
<tr>
<td>7</td>
<td>135</td>
<td>ccattcagAGACTAGAC</td>
<td>7</td>
<td>1547</td>
</tr>
<tr>
<td>8</td>
<td>124</td>
<td>cttcctagGTGACACTGGA</td>
<td>8</td>
<td>2811</td>
</tr>
<tr>
<td>9</td>
<td>137</td>
<td>cttcctagGTGACACTGGA</td>
<td>9</td>
<td>1527</td>
</tr>
<tr>
<td>10</td>
<td>125</td>
<td>acgtctcagCTCAAGGCC</td>
<td>10</td>
<td>22801</td>
</tr>
<tr>
<td>11</td>
<td>116</td>
<td>ttctcagagCAATCGGAG</td>
<td>11</td>
<td>1415</td>
</tr>
<tr>
<td>12</td>
<td>74</td>
<td>ttcgcctgcagGCTGAGGGGA</td>
<td>12</td>
<td>3984</td>
</tr>
<tr>
<td>13</td>
<td>136</td>
<td>ttcgcctgcagGCTGAGGGGA</td>
<td>13</td>
<td>669</td>
</tr>
<tr>
<td>14</td>
<td>117</td>
<td>ttcgcctgcagGCTGAGGGGA</td>
<td>14</td>
<td>269</td>
</tr>
<tr>
<td>15 (3′ UTR)</td>
<td>2248</td>
<td>cttcctagGTGACACTGGA</td>
<td>15</td>
<td>200</td>
</tr>
</tbody>
</table>

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
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 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 of the samples.

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.

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±11cm which is within the candidate region defined by the mapping of chromosomal disruptions in numerous SRS and SRS-like probands" (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.26 Murine Imp3 has previously been reported to be 4.4 kb in placenta 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. 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.27 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 3’ UTRS.28 This post-transcriptional regulation of Igf2 leader 3 has been shown to coincide with the highest expression levels of Imp3, when studied by northern blotting.29 Both Mori et al30 and Nielsen et al31 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 transfecteds 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.32

Since IMP3 has been proposed to have growth suppressing activities, the imprinting status of human IMP3 was investigated 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 mechanisms 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 development 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).

**Authors’ affiliations**

D Monk, L Bentley, M Hitchins, P Stanier, G E Moore, Institute of Reproductive and Developmental Biology, Imperial College School of Medicine, Hammersmith Campus, Du Cane Road, London W12 ONN, UK

C Beechey, J Peters, Mammalian Genetics Unit, Medical Research Council, Harwell, Didcot, Oxfordshire, UK

D Monk, M A Preece, Institute of Child Health, University College London, London, UK

Correspondence to: Dr D Monk, Institute of Reproductive and Developmental Biology, Imperial College School of Medicine, Hammersmith Campus, Du Cane Road, London W6 0XG, UK; d.monk@ic.ac.uk

**REFERENCES**


www.jmedgenet.com