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The human *MAGEL2* gene and its mouse homologue are paternally expressed and mapped to the Prader–Willi region

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Prader–Willi syndrome (PWS) is a complex neurogenetic disorder. The phenotype is likely to be a contiguous gene syndrome involving genes which are paternally expressed only, located in the human 15q11–q13 region. Four mouse models of PWS have been reported but these do not definitively allow the delineation of the critical region and the associated genes involved in the aetiology of PWS. Moreover, targeted mutagenesis of mouse homologues of the human candidate PWS genes does not appear to result in any of the features of PWS. Therefore, the isolation of new genes in this region remains crucial for a better understanding of the molecular basis of PWS. In this manuscript, we report the characterization of *MAGEL2* and its mouse homologue *Magel2*. These are located in the human 15q11–q13 and mouse 7C regions, in close proximity to *NDN*/*Ndn*. By northern blot analysis we did not detect any expression of *MAGEL2*/*Magel2* but by RT–PCR analysis, specific expression was detected in fetal and adult brain and in placenta. Both genes are intronless with tandem direct repeat sequences contained within a CpG island in the 5′-untranscribed region. The transcripts encode putative proteins that are homologous to the MAGE proteins and NDN. Moreover, *MAGEL2*/*Magel2* are expressed only from the paternal allele in brain, suggesting a potential role in the aetiology of PWS and its mouse model, respectively.

INTRODUCTION

Prader–Willi syndrome (PWS) and Angelman syndrome (AS) are distinct genetic diseases which result, respectively, from a deficiency of paternal gene expression and of maternal gene expression within the human 15q11–q13 chromosomal region. These diseases are classical examples of genomic imprinting, a process by which a subset of autosomal genes is differentially expressed in relation to the parent-of-origin allele (1).

PWS is a neurogenetic disorder which occurs once in ~10 000–15 000 births. Its clinical criteria are transient severe hypotonia in the newborn period, subsequent hyperphagia leading to obesity, developmental delay and mental retardation, behaviour disorders, hypogonadism, short stature and a dysmorphic appearance (2). The predominant cytogenetic defects in this syndrome are 15q11–q13 deletions of paternal origin in 70–80% of cases and maternal uniparental disomy for chromosome 15 in 20–30% of cases (1,3). A few PWS patients (1%) have a biparental origin of the 15q11–q13 region but with a mutation, a microdeletion including the first exon of *SNRPN*, which causes an imprinting defect. This region has been termed an imprinting centre (IC), which not only appears to function to regulate *in cis* chromatin structure, DNA methylation and gene expression throughout a 2 Mb imprinted domain within 15q11–q13 but which also has been hypothesized to play a role in resetting of the imprint in the germline of the opposite sex (4). Moreover, some PWS patients with an imprinting mutation and no detectable mutation in the IC have recently been reported (5).

The molecular and cytogenetic findings in PWS strongly suggest that the PWS phenotype is caused by a deficiency of a paternally expressed gene(s). Four imprinted genes, in the critical region and expressed exclusively from the paternal allele, have been isolated (Fig. 1a) (for a review see ref. 3). *SNURF-SNRPN* (6,7), *ZNF127* (8) and *NDN* (9,10) encode for proteins whereas *IPW* is spliced and polyadenylated and could play a functional role as an RNA (11). *PAR1* and *PAR5* are paternally expressed sequences which remain poorly characterized (12). Each of these genes, except for *PAR-1* and *PAR-5*, has a known homologue in the mouse (7,13–16). Mice deficient for *Snrpn* (17), *Snurf* (18), *Ipw* (cited in ref. 15), *Zfp127* (15) and *Ndn* (19) have been independently created but no biological role can be ascribed to any one of these genes in the PWS mouse model. However, no behavioural studies have been done for mice deficient in these genes.

We now report the characterization of a novel human gene, *MAGEL2*, close to the *NDN* locus in the PWS critical region. We have shown that *MAGEL2* is imprinted and expressed from the paternal allele in human brain and fibroblasts. We have characterized its murine homologue, *Magel2*, localized in a region

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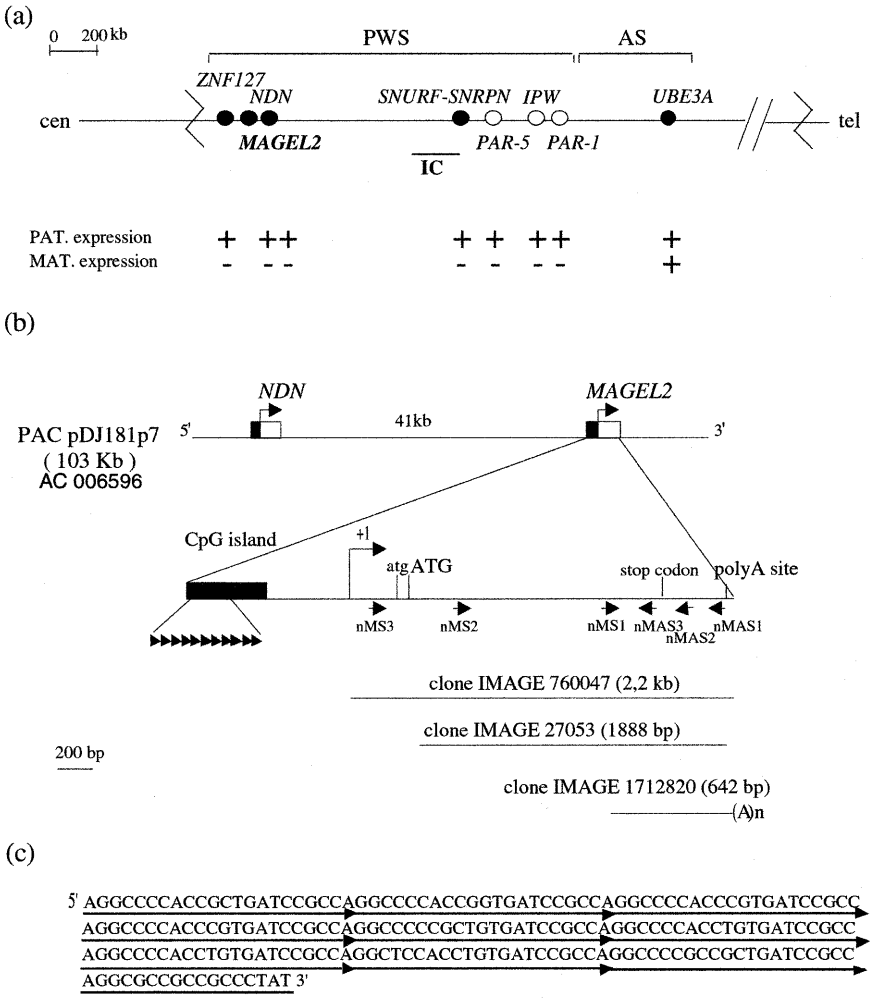

Figure 1. Mapping and structural features of *MAGEL2*. (**a**) Physical map of the 15q11–q12 region including the PWS and AS critical region, the typical 4 Mb deletion breakpoints (vertical zigzag lines), genes encoding proteins (black circles), genes encoding transcripts only (empty circles) and the imprinting centre (IC). Parent-of-origin gene expression is indicated. (**b**) Transcriptional map of the PAC pDJ181p7. Two potential transcripts, *NDN* and *MAGEL2* (empty square), with the same transcriptional orientation (arrows), have been identified. CpG islands (black boxes) are located upstream of each transcript and genomic features corresponding to the *MAGEL2* transcript are indicated. Primers used for genomic and cDNA sequencing and for RT–PCR are indicated. The three IMAGE cDNA clones which have been sequenced are represented. (**c**) Sequence of the CpG island with 9.5 tandem copies of a 221 bp tandem repeat, located 638 bp upstream to the ATG.

(7C) of conserved synteny to the human PWS region, on murine BACs which contain *Ndn*. We demonstrate that *Magel2* is expressed only from the paternal allele in brain. The human and mouse *Magel2* genes have a similar genomic organization. They encode putative proteins of 500 and 490 amino acids, respectively, which have an overall similarity of 77%. These proteins are highly homologous and belong to what we refer to as the MAGE–NDN gene family.

The novel imprinted *MAGEL2* gene and its mouse homologue may play a role in the aetiology of PWS and its mouse model, respectively.

RESULTS

Localization and identification of the human *MAGEL2* gene

The 103 863 bp sequence derived from the human PAC pDJ181p7 (AC006596) was analysed using the NIX software (HGMP-RC), a bioinformatic tool which integrates the output of different programs in a single graphical interface. This PAC maps to the 15q11–q12 PWS chromosomal region and contains the STS WI-30582 marker corresponding to the *NDN* gene. The genomic sequence of the PAC was analysed with a view to identifying potential transcribed sequences, sequence homology with other genomic or cDNA sequences and CpG islands.

The NIX program identified the known *NDN* gene [open reading frame (ORF) from 30539 to 31501] as well as a potential intronless new transcript (ORF from 71918 to 73507) (Fig. 1b). Three human expressed sequence tags (ESTs) on the coding strand (GenBank accession nos HS1251679, HS87688 and HS1214174) and one EST (GenBank accession no. AI129312) on the reverse strand have been identified with sequences identical to the genomic sequence associated with this new putative transcript. We retrieved an EST cluster (HS141496) from Unigene-*Homo sapiens* and from UniEST at TigemNet. These clusters contain nine ESTs, two of which

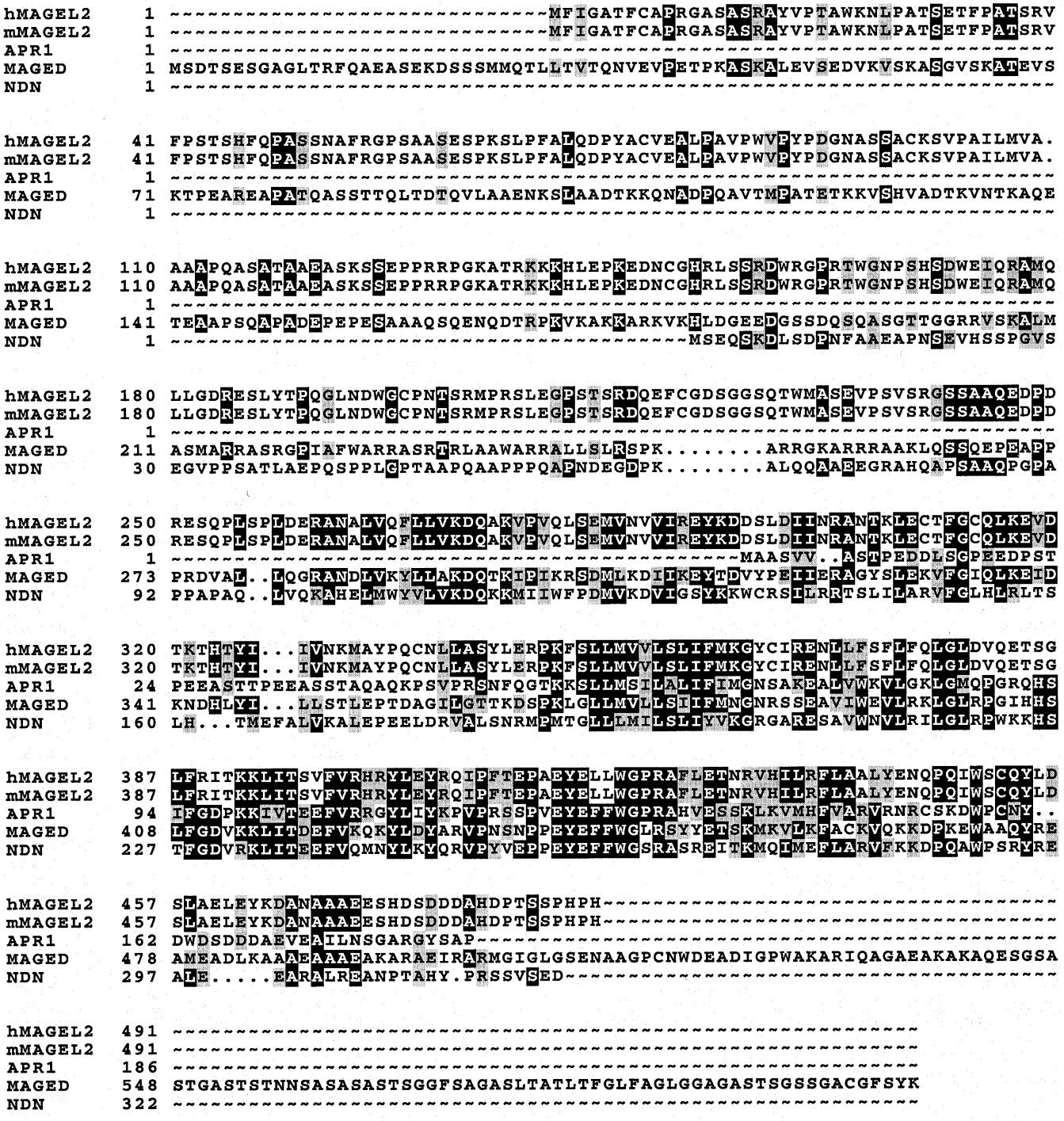

Figure 2. Alignment of human and mouse MAGEL2 protein sequences with human homologous proteins. Identical amino acids are shaded dark grey; conserved amino acids are shaded light grey. An amino acid is shaded only if present in three of the five proteins aligned. MAGE D, human MAGE D protein; NDN, human necdin protein; APR-1, human apoptosis-related protein 1.

have a poly(A) tail (data not shown). Therefore, a 642 bp contig sequence corresponding to the 3′-end has been established. We have completely sequenced the IMAGE clones 760047, 27053 and 171820 corresponding to the ESTs HS1251679, HS87688 and AI129312, respectively. We have obtained a 2284 bp cDNA sequence which contains the full putative ORF(s), the entire 3′-untranslated region (3′-UTR) (445 bp) and 337 bp of the 5′-UTR. This cDNA sequence confirms the intronless structure of this gene (Fig. 1b).

Two potential ORFs, one encoding a putative protein of 529 amino acids and a shorter one of 500 amino acids (in the same frame), can be predicted from the nucleotide sequence. The NetStart software (20) predicts the second ATG (nt 337) as the initiator codon with the highest probability; moreover, only

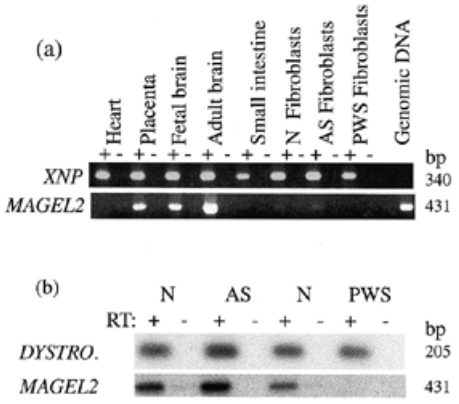

Figure 3. Expression analysis of *MAGEL2*. (**a**) RT–PCR analysis demonstrating *MAGEL2* expression compared with *XNP* expression in five different human tissues and in fibroblasts of a normal individual and PWS and AS patients. Each sample contained a roughly equivalent amount of input RNA, as indicated by control *XNP* PCR. In the latter reaction the primers amplify a 340 bp fragment from cDNA and a 700 bp fragment from genomic DNA. (**b**) Parent-of-origin expression of *MAGEL2*. RT–PCR analysis performed for *MAGEL2* and a control gene, *dystrobrevin* (*DYSTRO.*), using RNAs isolated from brain from two normal controls (N1 and N2), a typical AS deletion (AS) and a typical PWS deletion (PWS). +, −, with and without reverse transcriptase. *MAGEL2* primers amplify a 431 bp fragment.

this second ATG is conserved in mouse. A BLAST P search using the predicted 500 amino acid sequence revealed a significant homology with the MAGE protein family and NDN (discussed below; Fig. 2).

Two CpG islands were identified with the GRAIL/CpG software at positions 30310–30892 (GC score 70.59) and 70979–71280 (GC score 75.65), respectively linked to *NDN* and *MAGEL2*. One notable feature is that the CpG island associated with the *MAGEL2* gene is located 638 bp upstream of the ATG and contains 9.5 tandem copies of a 21 bp repeat (Fig. 1c).

Thus, a new intronless gene, *MAGEL2*, encoding a putative protein has been identified in proximity to the *NDN* locus.

Expression analysis and imprinting status of the human *MAGEL2* gene

By northern analysis we were unable to detect a hybridization signal on 10 µg of total RNA extracted from adult brain, placenta, small intestine, heart and fetal brain. However, by RT–PCR analysis, using the primers nMS1 and nMAS2, a 431 bp fragment was specifically amplified in adult and fetal brain and in placenta (Fig. 3a). Since *MAGEL2* is intronless, PCR amplification resulting from genomic DNA contamination was ruled out by the use of DNase I-treated RNA samples and by a control reaction without reverse transcriptase (–RT).

Uniparental expression was investigated in two different tissues. First, we tested *MAGEL2* expression by RT–PCR on RNA extracted from fibroblasts of a normal individual, of an AS patient with an abnormal methylation profile suggesting an imprinting mutation and of a PWS patient with a 15q11–q13 deletion. A low amplification of the *MAGEL2*-specific PCR product was observed in normal and AS individuals, reflecting a low level of expression in this tissue. However, no amplification could be detected in the PWS sample, whereas the non-imprinted control *XNP* gene was expressed in all samples tested (Fig. 3a). Second, RT–PCR analysis was performed with brain total RNA from two normal individuals, one patient with PWS and one with AS, both with cytogenetic deletion of 15q11–q13 (9). This analysis revealed complete absence of *MAGEL2* expression in the brain of the PWS patient whereas normal expression was detected in normal and AS individuals (Fig. 3b). These data indicate that *MAGEL2* is transcribed only from the paternal allele in adult brain and fibroblasts.

Characterization and localization of the mouse *Magel2* gene

The human *MAGEL2* sequence was used for a BLAST search against the dbEST data bank. Four mouse EST clones (GenBank accession nos AA049350, AI326859 and AI385800; IMAGE clone 479471, AA289838; IMAGE clone 601051) presented at least 80% identity with *MAGEL2*. The EST assembly machine at TigemNet was used to extend the EST contig with the EST AA198619 (IMAGE clone 657039). We sequenced two clones: 657039, which contains a poly(A) tail, and 479471, which was the largest (1121 bp). A 1121 bp contig overlapping the predicted 3′-end was first established although we could not identify any other ESTs in order to extend the sequence further 5′ (Fig. 4b).

Based on the conservation of synteny between the human 15q11–q13 region and the central part of mouse chromosome 7, we could predict that this new gene was localized close to the *Ndn* locus. A single hybridization signal was detected on genomic DNA using this new cDNA (PCR product with primers nS1 and nSas1) as probe and this signal was also detected on DNA from BAC 143C10, which contains *Ndn* (Fig. 4a). BAC 143C10 was digested with *Hin*dIII or *Bam*HI and subcloned in plasmid pBluescript. Subsequently the 1.5 kb *Bam*HI and 6 kb *Hin*dIII fragments, which both gave a positive hybridization signal, were isolated and partially sequenced. The 3664 bp of genomic sequence obtained thus far overlaps the 1121 bp cDNA contig. By comparison of this sequence with the human *MAGEL2* cDNA, a 2200 bp murine cDNA sequence was deduced which encompasses the putative ORF, the complete 3′-end (442 bp) and part of the 5′-end (269 bp) (Fig. 4b). The existence of the predicted transcription unit was confirmed by RT–PCR on brain tissue (data not shown; see below). From this sequence, two start codons could initiate translation in the same frame to encode a 552 or 490 amino acid polypeptide. By homology with the human sequence, the second ATG alone is conserved and seems the most probable. The sequence comparison between this new gene and *MAGEL2* revealed an overall 77% similarity at the protein level and 81% identity at the DNA level. The cDNA and genomic sequences are identical and confirm an intronless structure for this gene. This gene was designated *Magel2* for the mouse homologue of *MAGEL2*. The *Magel2* gene contains 11.5 tandem copies of an 18 nt partially diverged repeat, located 450 bp upstream of the ATG (Fig. 4c).

Tissue expression and imprinting status of the mouse *Magel2* gene

By northern analysis, in a large variety of adult tissues and embryonic developmental stages, we did not detect a hybridi-

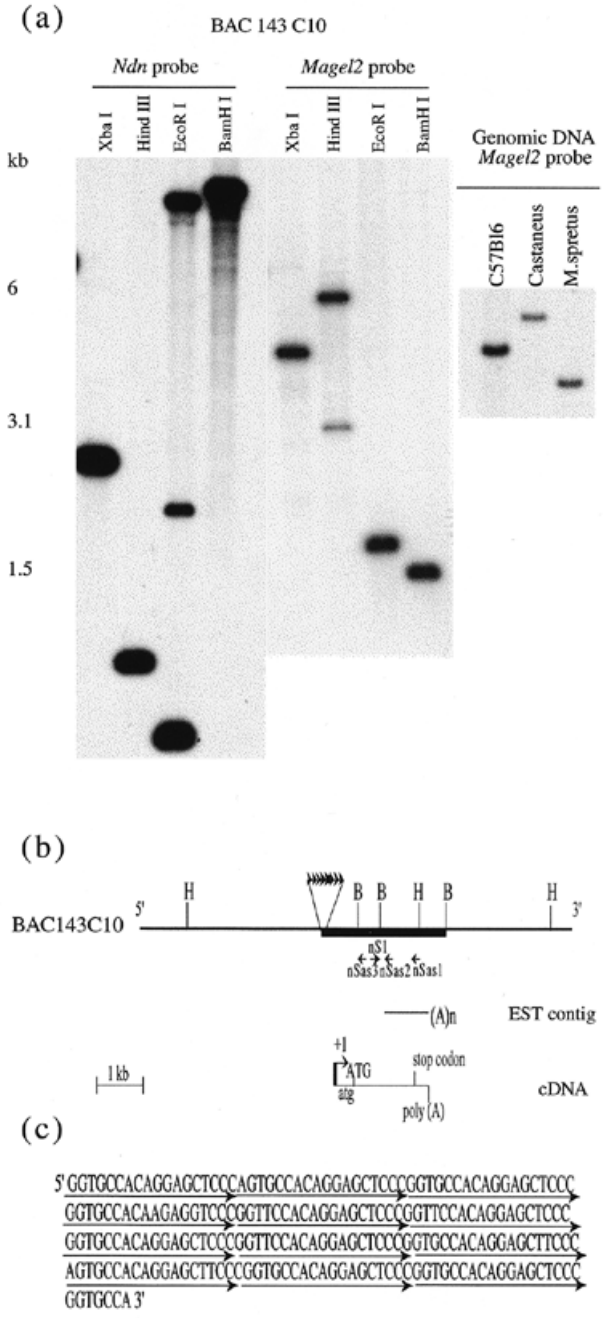

Figure 4. Mapping, genomic structure and transcript of *Magel2*. (**a**) Mapping of *Magel2* by hybridization to BAC 143C10. Hybridization of an *Ndn* cDNA probe to a Southern blot of BAC 143C10 genomic DNA (left); a *Magel2* cDNA probe was hybridized to the same blot (centre) and to a blot of mouse genomic DNA extracted from different strains (right). BAC 143 C10 DNA was digested with different restriction enzymes (as indicated); mouse genomic DNA was digested with *Xba*I. (**b**) Physical map of the *Magel2* locus on BAC 143C10. Primers used for genomic and cDNA sequencing and for RT–PCR as well as the EST contig and cDNA features corresponding to the *Magel2* transcript are indicated. (**c**) Sequence of the CpG island with 11.5 tandem copies of an 18 bp repeat, located 450 bp upstream to the ATG.

zation signal using a *Magel2* probe. RT–PCR analysis was performed using the primers nS1 and nSas1 and a specific 1037 bp amplification product encompassing the 3′-UTR was

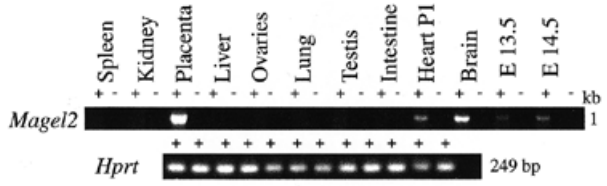

Figure 5. Tissue expression analysis of *Magel2*. RT–PCR (+, –, with and without reverse transcriptase, respectively) analysis demonstrating *Magel2* expression compared with control *HPRT* expression (+RT only) in different mouse tissues. Primers used are nS1 and nSas1, which amplify a 1049 bp fragment including the 3′-non-coding region. Each sample contained a roughly equivalent amount of input RNA, as indicated by control *HPRT* PCR with Hprt1 and Hprt2 PCR primers that amplify a 249 bp fragment from cDNA only. E13.5, E14.5 and P1 indicate embryonic days 13.5 and 14.5 and post-natal day 1, respectively.

detected in brain and placenta and at embryonic days 13.5 and 14.5 (Fig. 5).

In order to examine the imprinting status of *Magel2*, we sequenced the *Magel2* PCR product with primers nS1 and nSas1 from genomic DNA from two different strains (C57Bl6 and *Mus spretus*). Four polymorphisms were detected between the two strains, with one of these resulting in the loss of an *Mbo*II restriction site in *M.spretus* (Fig. 6a). This feature was used to test functional imprinting of *Magel2* in F_1 mice derived from matings between *M.spretus* males and C57Bl6 females and in N_2 mice derived from matings between F_1 females and C57Bl6 males. Three N_2 mice were genotyped to determine the parent-of-origin allele at the *Ndn* locus (14). The physical distance between the *Ndn* locus and the *Magel2* locus is ~40 kb and a recombination event between these loci is improbable; therefore, two mice that inherited the *M.spretus* allele from their F_1 mother and a paternal C57Bl6 allele were used to examine the imprinting status of *Magel2*.

Adult brain RNA was isolated from *M.spretus*, C57Bl6, F_1 and N_2 mice. These RNAs were reverse transcribed, cDNAs were amplified with the primers nS1 and nSas1 and the PCR products were digested with *Mbo*II (Fig. 6b). The results clearly show that *Magel2* is exclusively expressed from the paternal (*M.spretus*) allele in F_1 and paternal (C57BL6) allele in N_2 mice. Moreover, the paternal *M.spretus* expressed allele in the F_1 offspring becomes the maternal silenced allele in the N_2 offspring. These data reveal the maternal imprinting of *Magel2* in mouse adult brain with this imprint being erased and reset from one generation to another.

Comparison of the mouse *Magel2* and human *MAGEL2* genes

Mouse and human MAGEL2 are homologous polypeptides of 500 and 490 amino acids sharing an overall 77% similarity (65% identity). The degree of homology is weak relative to the average value of 85% identity calculated in a comparison of 1196 mouse and human cDNAs (21), but the first 144 amino acids and the 237 C-terminal amino acids show a stronger homology with 82 and 83% similarity, respectively. The central part (amino acids 144–263) of the predicted protein sequence shows a lower homology with only 57% similarity. These three regions of differing homology could define three domains with calculated pI of 9.80, 4.50 and 6.29, respec-

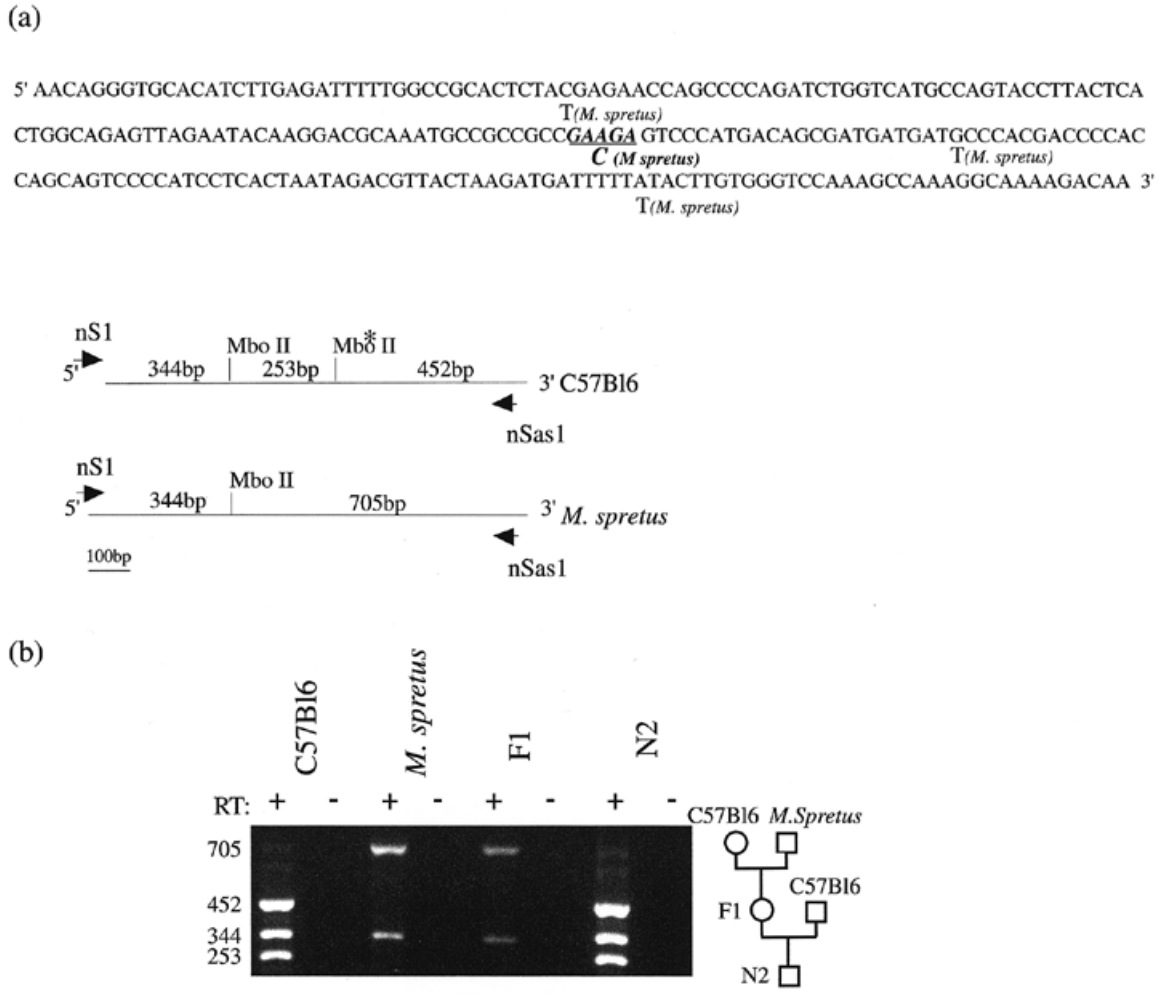

Figure 6. Parent-of-origin expression of *Magel2*. (**a**) Polymorphisms observed between C57Bl6 and *M.spretus*. Nucleotide sequence of *Magel2* genomic DNA product amplified with primers nS1 and nSas1 (the four single nucleotide differences detected in *M.spretus* are indicated below each relevant nucleotide of the C57Bl6 sequence). One of these single base pair (C in *M.spretus* instead of A in C57Bl6, in bold italic) changes in *M.spretus* abrogates the *Mbo*II (*) restriction site present in C57Bl6 (bold italic). Therefore, three *Mbo*II restriction fragments (344, 253 and 4452 bp) are generated from PCR products amplified with primers nS1 and nSas1 from genomic DNA or cDNA (*Magel2* is intronless) of C57Bl6 whereas only two restriction fragments (344 and 705 bp) are generated from the same PCR products amplified from *M.spretus*. (**b**) Paternal expression of *Magel2* in mouse adult brain. RT–PCR products with primers nS1 and nSas1 from adult brain RNAs of C57Bl6, *M.spretus*, (C57Bl6 × *M.spretus*)F_1 and [(C57Bl6 × *M.spretus*) × C57Bl6]N_2 mice digested with *Mbo*II restriction enzyme, separated by gel electrophoresis and visualized by ethidium bromide staining.

tively. No known conserved protein motif could be detected in these sequences.

At the nucleotide level, the mouse and human sequences share an overall 81% identity in the coding part. Although we did not define the transcription start, we were able to map it to a 75 bp interval upstream of the human and mouse cDNA sequences by RT–PCR using primers designed from the genomic sequence. No canonical TATA or CAAT boxes were identified in the vicinity of these putative mouse and human transcription starts (data not shown).

Both genes contain a non-transcribed CpG island at the 5′-end with similar sized tandem repeat motifs of 9.5 and 11.5 units in human and mouse, respectively. The sequences of these repeat motifs are not conserved (60% identity). Such tandem repeats are often associated with imprinted genes (22) and could define a structural regulatory element.

The human and mouse 3′-UTRs are of the same size and contain a UAUAA poly(A) signal instead of the canonical AAUAA. They share 79% identity in a 140 bp region which contains AU-rich elements (AREs). In mouse, this sequence is characterized by 51% uridine, one AUUUA, three AUUUU and one AUUG motifs. These sequence features are characteristics of AREs found in the 3′-UTR of many mRNAs that encode for proto-oncogenes or nuclear transcription factors and they target these molecules for rapid and selective degradation (23). Therefore, the *MAGEL2*/*Magel2* RNAs could be unstable and difficult to detect by northern blotting.

The *MAGEL2*/*Magel2* genes belong to the 'MAGE gene superfamily'

A BLASTP search using the mouse or human MAGEL2 protein sequence reveals significant homology to the

Table 1. Similarity (%) of the 240 MAGEL2/Magel2 C-terminal amino acids with human homologous proteins

	Magel2	MAGE D	APR-1	NDN
MAGEL2	83	67	64	60
Magel2		61	57	53
MAGE D			63	63
APR-1				69

melanoma antigen gene expression (MAGE) proteins with the most significant alignment to a 606 amino acid MAGE-like protein recently identified as MAGE D (24), a gene mapped to Xp11.1–11.4. There is also significant homology with necdin (NDN) and with apoptosis-related protein 1 (APR-1) (Fig. 2). However, the homologous region corresponds to the third region of MAGEL2, i.e. the 240 C-terminal amino acids, and the presence of a functional domain with some consensus sequences is suggested in this region (Fig. 2 and Table 1).

DISCUSSION

In this manuscript we report the identification and characterization of a new human gene *MAGEL2* and its mouse homologue *Magel2*. These are located in the human 15q11–q13 region and in the mouse 7C region, close to the *NDN*/*Ndn* locus, respectively. Both encode putative proteins belonging to the MAGE–NDN family of proteins. Moreover, the genes are imprinted, being expressed only from the paternal allele in brain.

The genomic organization of the mouse and human genes is similar. The genes are intronless with tandem direct repeat sequences contained within a CpG island in the 5′-UTR. These two characteristics are often associated with imprinted genes and have been proposed to play a role in imprinting regulation (22). Another property of imprinted genes, monoallelic methylation, has not yet been tested for *MAGEL2*/*Magel2*. The imprinting mechanism in the 15q11–q13 region has been mainly studied at the regional level through the involvement of the IC (4). Other types of important regulatory element found associated with other imprinted genes, such as tandem repeat sequences, CpG islands, differential methylation and the so-called imprinting box, are not well described for genes in this region, except for *SNRPN*/*Snrpn* (25,26). *Magel2* may prove to be a model locus for studying whether and how such elements interact with the IC.

Expression of the *MAGEL2* gene has been detected in fetal and adult brain and in placenta. Among the tissues tested, the same expression pattern is observed in human and mouse, as is the case for most imprinted genes. *MAGEL2*/*Magel2* transcripts are detectable only by RT–PCR. This low level of expression of steady-state mRNA could reflect a cell-specific expression in brain or the instability of the mRNAs. In this regard, a conserved sequence containing AU-rich elements associated with rapid mRNA degradation is contained in the 3′-UTR of *MAGEL2*/*Magel2* (23). Such elements have been found in the adjacent *ZNF127*/*Zfp127* genes (8,15).

The *MAGEL2* and *Magel2* genes encode putative proteins of 500 and 490 amino acids, respectively. The 240 C-terminal amino acids show a strong homology with the MAGE, NDN and APR-1 proteins and could define a functional domain. This C-terminal region in NDN has been shown to interact with E2F1 (27) and P53 (28); these data suggest a role as a post-mitotic growth suppressor for NDN. *MAGE* genes such as *MAGE A*, *MAGE B* and *MAGE C* (29), which encode 300 amino acid polypeptides, are not expressed in normal tissues with the exception of testis. The *MAGE D* gene, which encodes a 606 amino acid polypeptide, presents the highest level of homology with the *MAGEL2* and *APR1* genes. Its ubiquitous expression pattern and its genomic structure, with 10 exons for the coding part, are different from the other *MAGE* genes (24). Localization of the *MAGEL2*/*Magel2* gene close to *NDN*/*Ndn* and the intronless genomic structure of both genes could be consistent with evolution through a duplication event.

PWS results from the absence of paternal expression of a gene(s) localized in the 15q11–q13 region. Because there is no evidence of PWS cases resulting from point mutations, the phenotype is likely to be a contiguous gene syndrome involving imprinted genes located in a critical interval of 4 Mb (Fig. 1a) (30) and regulated by a genomic imprinting mechanism. Four mouse models of PWS have been reported. The first results from a partial maternal uniparental disomy (UPD) for a large interval including the region of conserved synteny to the human PWS/AS region (31). The second model results from a 40 kb deletion of the putative mouse IC including the first exon of *Snrpn* (17). The phenotype observed in both cases is perinatal lethality associated with poor feeding, this phenotype being consistent with the feeding difficulties and failure to thrive that characterize PWS infants (2). The third model is characterized by a complete inheritable deletion of the mouse PWS/AS-homologous region that results from a transgene-induced mutation. These PWS-like mice live several days longer than those with UDP and an imprinting mutation (32). The fourth model, in which the paternal region extending from *Snrpn* to *Ube3a* has been deleted, results in hypotonia, growth retardation and 80% lethality; these findings suggest that an important PWS candidate gene(s) is contained between *Snrpn* and *Ipw* (18). However, this last model does not exclude that other loci outside the deleted region contribute to neonatal lethality or that gene deficiency in this region may cause neonatal lethality in relation to the strain background (18). Furthermore, the features of PWS are complex and many of them, such as behavioural anomalies or hyperphagia, have not yet been reported in any mouse model.

Targeted mutagenesis of mouse homologues of the human candidate PWS genes *Snurf-Snrpn*, *Ndn*, *Zfp127* and *Ipw* does not result in any of the main reported features of mouse Prader–Willi-like syndrome such as lethality or hypotony and mutant mice are not sterile or obese. Behavioural studies have not been reported for any of these mouse models. Moreover, discrepancies between the phenotype may be observed in null mutants generated by different laboratories. In our case, for example, of 241 genotyped adult mice born from a mating between a heterozygous (+/–) male, with a null *Ndn* allele, and a wild-type (+/+) C57Bl6 female, we observed that only 38% had inherited the null allele, instead of the 50% expected. Therefore, we deduced 12% lethality in mice deficient for *Ndn* (F. Muscatelli, unpublished data), these data being inconsistent with the previously published data of Tsai *et al*. (19).

Therefore, characterization of human *MAGEL2*, in the 15q11–q12 region, and of its mouse homologue, *Magel2*, both

being new imprinted genes, remains of great interest. These genes encode putative proteins and are paternally expressed in brain; *MAGEL2* is not expressed in the brain of a PWS patient and is thus a good candidate for involvement in the aetiology of PWS.

MATERIALS AND METHODS

Accession numbers

cDNA sequences have been submitted to the EMBL Nucleotide Sequence Database. The accession number for the human *MAGEL2* cDNA is AJ243531 and the ID no. is HSA243531. The accession number for the mouse *Magel2* cDNA is AJ243608 and the ID no. is MMU243608.

ESTs and mouse BAC analysis

Human and mouse IMAGE clones corresponding to the ESTs described in the manuscript were obtained from the UK-HGMP resource. These clones were amplified as indicated by the provider. Sequencing was performed using T3 and T7 or M13F and M13R dye-labelled primers in a Gene Rider 4200 (LI-COR system) DNA analyser.

Internal sequencing of these EST and genomic clones was performed by Genome Express (Grenoble, France) with the following primers.

Human primers:
nMs1j, 5′-CTAAGAAGCTCATCACCGAAG-3′;
nMs2j, 5′-CCACAGCCCAATATGAATGCC-3′;
nMs3j, 5′-AGCAGGCCCAAGGCATCGGGTCCG-3′;
nMas1j, 5′-TACTGATGAGTCATTTGAACGTTC-3′;
nMas2j, 5′-GGCAGATACGAAACCAAGTTG-3′;
nMas3j, 5′-CAGGACAAGCATCTTGCTGG-3′.

Mouse primers:
nS1, 5′-GACCAAGCCAAGGTGCCTGTCCAG-3′;
nS2, 5′-AACTCAGCTGCCGACCCCACC-3′;
nSas1, 5′-CAGACAGTATTTTACCGATGAGTC-3′;
nSas2, 5′-AAGGTGCACTCCAGCTTAGTG-3′;
nSas3, 5′-GGCAGATGGACCTCTAAAGG-3′.

Mouse BAC 143C10 was obtained from Research Genetics (Huntsville, AL) (33). DNA from the BAC was isolated following the mini-preparation protocol recommended by Research Genetics. *Bam*HI and *Hin*dIII subcloning were done in the plasmid pBluescript-SK. Bacterial colonies were lifted on PALL Biodyne A membranes and filters were screened by radioactive hybridization using an *Ndn* cDNA probe (9) and a *Magel2* cDNA probe (PCR product with primers nS1 and nSas1). Hybridization and washing conditions were identical to those described for Southern analysis.

Southern blot analysis

Aliquots of 10 µg of mouse genomic DNA and 500 ng of BAC DNA were digested with restriction enzymes and separated by gel electrophoresis through a 1% gel in 1× TBE. Alkaline transfer was made on PALL Biodyne B membranes as recommended by the manufacturer. Hybridizations were performed in Church solution with ^{32}P-random-labelled DNA probes at 65°C for 16 h. Filters were washed three times at a stringency of 0.5× SSC, 0.1% SDS at 65°C for 20 min and exposed to X-ray film at –70°C. The probes used are described above.

Mice

C57BL/6J mice were purchased from IFFA CREDO. Outbred *M.spretus* males were kindly provided by Dr J.L. Guenet (Pasteur Institute, Paris, France). Outcrosses and backcrosses were performed at the university facility.

Genotyping of backcross progeny was performed as previously described (14).

Polymorphism analysis

PCR products amplified with primers nS1 and nSas1 (1037 bp) from mouse genomic DNA from C57Bl6 and *M.spretus* were purified using a High Pure PCR Product Purification kit (Roche Diagnosis, Meylan, France) and sequenced using nS1 and nSas1 dye-labelled primers on a Gene Rider 4200 DNA analyser. PCR was performed in a volume of 50 µl under standard conditions indicated by the manufacturer [*Taq* DNA polymerase (Sigma Aldrich, St Quentin Fallavier, France), 2.5 mM $MgCl_2$]. PCR conditions were 94°C for 90 s followed by 35 cycles of 94°C for 20 s, 57°C for 30 s and 72°C for 60 s.

Mouse RNA isolation and RT–PCR analysis

RNAs and cDNAs were prepared as previously described (14). Primers nS1 and nSas1 and PCR conditions were as described above. Thirty-five cycles were performed. To test the imprinting status, PCR products were purified and digested with *Mbo*II for 45 min. DNA fragments were separated in a 2% agarose gel and visualized by ethidium bromide staining.

A control PCR was performed with *HPRT* (GenBank MUSHPRT) primers Hprt1 (5′-GCTGGTGAAAAGGACCTCT-3′) and Hprt2 (5′-CACAGGACTAGAACACCTGC-3′). Primers have been choosen in two different exons and overlap two exons. A specific 249 bp PCR product could be amplified from cDNA. PCR conditions were 94°C for 120 s followed by 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 30 s under standard conditions (see above).

Human RNA isolation and RT–PCR analysis

RNAs and cDNAs were prepared as previously described (9). *MAGEL2* PCR was performed using primers nMS1 and nMAS2 and a 431 bp fragment was amplified. PCR conditions were 94°C for 120 s followed by 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 30 s. A control PCR was performed with *XNP* primers Xnp1 (5′-AAGCAACAAACAGTGTGACAGC-3′) and Xnp2 (5′-GGCTGGCTTGTCTACTTAATGC-3′). A specific 340 bp PCR product could be amplified from cDNA whereas a 740 bp product was specific for genomic DNA. PCR conditions were 94°C for 120 s followed by 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 30 s under standard conditions. DNA fragments were separated in a 1.5% agarose gel and visualized by ethidium bromide staining.

PCR primers and conditions for *dystrobrevin* and for RNAs extracted from brain of PWS, AS and control individuals have been previously reported (9).

Patients

Patients in this study have been previously reported (9).

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