Background: Genomic imprinting is an epigenetic chromosomal modification in the gametes or zygotesthat results in a non-random monoallelic expression of specific autosomal genes depending upon their parent of origin. Approximately 44 human genes have been reported to be imprinted. A majority of them are clustered, including some on chromosome segment 11p15.5. We report here the imprinting status of the SLC22A1 gene from the human chromosome segment 11p15.5.

Results: In order to test for allele specific expression patterns, PCR primer sets from the SLC22A1 gene were used to look for heterozygosity in DNA samples from 17 spontaneous abortuses using PCR-SSCP and DNA sequence analyses. cDNA samples from different tissues of spontaneous abortuses showing heterozygosity were subjected to PCR-SSCP analysis to determine the allele specific expression pattern. PCR-SSCP analysis revealed heterozygosity in two of the 17 abortuses examined. DNA sequence analysis showed that the heterozygosity is caused by a G>A change at nucleotide position 473 (c.473G>A) in exon 4 of the SLC22A1 gene. PCR-SSCP analysis suggested that this gene is paternally imprinted in five fetal tissues examined.

Conclusions: This study reports the imprinting status of the SLC22A1 gene for the first time. The results suggest imprinting of the paternal allele of this gene in five fetal tissues: brain, liver, placenta, kidneys and lungs.

Background
Imprinted genes are specific autosomal genes that show a non-random monoallelic expression depending upon their parent of origin [1,2]. It is estimated that the human genome contains 100–300 imprinted genes [3,4]. A catalogue of the imprinted genes maintained at the University of Otago, New Zealand [5] lists approximately 44 human genes. In addition, the catalogue lists six more genes whose imprinting status is disputable.

Studies on uniparental disomies (UPDs) have identified five imprinted segments in the human genome including the chromosome segment 11p15.5 [6,7]. The imprinted segment on chromosome 11p15.5 is involved in the pathogenesis of Beckwith-Wiedemann syndrome. It is ~1 Mb in size and harbors a total of 21 genes [8]. Of these, 11 genes are known to be imprinted, seven genes show biallelic expression and the imprinting status of the remaining three genes including the SLC22A1 gene is unknown. The
Genomic imprinting of the SLC22A1LS gene: A) diagrammatic representation of SLC22A1L and SLC22A1LS genes; positions of the primer set 22F-22R are marked by arrow heads. B) PCR-SSCP analysis of genomic DNA samples from 17 abortuses (no. 2 to no. 18) with the primer set 22F-22R; two abortuses, no. 2 and no. 9 are heterozygous for a nucleotide change. C) Direct sequence analysis of the PCR product with the primer 22F from the abortus no. 2 showing a G>A change at nucleotide position 473 (c.473G>A) marked by an arrow. D) PCR-SSCP analysis of genomic DNA samples from a control individual (N), abortus no. 2 (2), mother of the abortus no. 2 (M), and cDNA samples from lungs (L), liver (Li), brain (B), kidneys (K) and placenta (P). Note, only one allele 473G is expressed in five tissues analyzed. Since the abortus is heterozygous and the mother is homozygous, the imprinted allele 476A in the abortus should have come from its father. E) PCR-SSCP analysis of genomic DNA samples from a control individual (N), abortus no. 9 (9), mother of the abortus no. 9 (M), and cDNA samples from lungs (L), liver (Li), brain (B), and kidneys (K). Note, only one allele 473G is expressed in all four tissues analyzed, corroborating the finding from the abortus no. 2. Since both the mother and the abortus are heterozygous, it is not possible to determine the parental origin of the expressed allele.
SLC22A1LS gene contains four exons and encodes for a transcript of 1,342 bp length [9,10]. It is expressed in the liver, gastrointestinal tract, kidneys and placenta [10]. It codes for a putative protein of 253 amino acids length with unknown function. The SLC22A1LS protein does not show sequence homology to any known proteins [9,10]. The SLC22A1LS gene has no counterpart in the mouse genome [9], perhaps reflecting an example of the rapid evolution of human genomic sequences. The DNA sequence of this gene partially overlaps with that of another gene, SLC22A1L (BWR1A), which has been shown to be paternally imprinted [9,10]. These two genes are transcribed in opposite directions (Fig. 1A). We report here a study on the imprinted status of the SLC22A1LS (BWR1B) gene.

Results and discussion
PCR-SSCP analysis performed on genomic DNA samples from 17 abortuses showed heterozygosity in abortus no. 2 and no. 9 only with the primer set 22F/22R (Fig. 1B). The other two primer sets did not show heterozygosity in 17 abortuses (data not shown). DNA sequence analysis revealed that the heterozygosity in these abortuses was due to a G>A substitution at nucleotide position 473 (c.473G>A) (Fig. 1C). We have designated the two alleles as 473G and 473A, the wild type being 473G (Fig. 1C).

In order to determine if both or only one allele of this gene is expressed, PCR-SSCP analysis was performed using cDNA samples from different tissues of abortus nos. 2 and 9. The analysis showed expression of the allele 473G only in five tissues from abortus no. 2 (Fig. 1D) indicating that this gene was imprinted. Since the mother was homozygous for the expressed allele 473G and the abortus was heterozygous, the imprinted allele in this abortus must be derived from its father. This suggested imprinting of the paternal allele of the SLC22A1LS gene. The monoallelic expression of this gene was further confirmed in brain, lungs, liver and kidneys from another heterozygous abortus, no. 9 (Fig. 1E).

Based on their opposite orientation and knowing that SLC22A1L is paternally imprinted (maternally expressed), it was hypothesized that the SLC22A1LS gene, which overlaps with SLC22A1L, should be maternally imprinted [9]. However, our results suggested that on the contrary, SLC22A1LS is paternally imprinted, just as is the case with its sense partner SLC22A1L. This pattern is similar to that observed in case of IGF2 and its antisense transcript IGF2-AS, both of which express the same (paternal) allele [11]. These two examples suggest that expression of sense and antisense transcripts of a gene pair may be under the same imprinting or genetic control. It is also possible that the transcript of one gene could be regulating the transcription of its partner. The ‘sense partner’ of the SLC22A1LS gene, SLC22A1L, has been found to be mutated in a breast and rhabdomyosarcoma cell lines [10]. Since patients with Beckwith-Wiedemann syndrome are known to be prone to a variety of tumors, it is possible that these genes have a role in the pathogenesis of this syndrome and in the etiology of other tumors including Wilms tumor, although this possibility remains to be investigated.

Conclusions
We report for the first time the imprinting status of the SLC22A1LS gene located in human chromosome segment 11p15.5. The results suggest imprinting of the paternal allele of this gene in different fetal tissues.

Methods
Sample collection
A total of 17 spontaneous abortuses were ascertained over a period of three years in the Department of Gynecology and Obstetrics, Kempegowda Institute of Medical Sciences, Bangalore. Following abortion, different fetal tissues were quickly dissected out and immediately frozen in liquid nitrogen. For permanent storage, tissue samples were stored at -80°C until further use. Peripheral blood samples from mothers of the abortuses were also collected in EDTA-Vacutainer® blood collection tubes (Beckton-Dickinson, USA). The study was approved by the tenets of the Declaration of Helsinki. The informed consent was obtained from human subjects included in this study.

Table 1: PCR primers used in PCR-SSCP analysis to identify heterozygosity in genomic DNA samples from abortuses.

| Sl. No. | Primer set | Sequence | Amplicon size |
|--------|------------|----------|---------------|
| 1.     | BWR3F      | 5'-AAGGCAGACAGTCTGCCGATGA-3' | 212 bp |
| 2.     | BWR3R      | 5'-CAAGCTGGCTCCACCCTCTAG-3'  | 259 bp |
| 2.     | BWR4F      | 5'-GCCAAGCTGATGGACACAGCTA-3' | 259 bp |
| 3.     | 22F        | 5'-GTACAGCGGTCTCGACAGCTC-3'  | 267 bp |
| 3.     | 22R        | 5'-GTGCCTAGTGCCTAAATCTCGA-3' | 267 bp |
Identification of heterozygosity in abortuses

Genomic DNA samples were extracted both from 17 abortuses and their mothers’ peripheral blood samples using a DNA isolation kit from Roche Diagnostics™ (Germany) following manufacturer's instructions. Three sets of primers were designed from exon 4 of the SLC22A1LS gene. Primer sequences and PCR fragment sizes are given in Table 1. These primer sequences were derived from GenBank accession number NM_007105. Genomic DNAs from 17 abortuses were amplified separately using these primer sets. PCR amplification was carried out in a 25 µl volume containing 50 ng of genomic DNA, 60 ng of each primer, 200 µmol of each dNTP, 0.25 µl αP32 dCTP (3,000 Ci/m mole; NEN, USA) and 1 unit of Taq DNA polymerase (Banglore Genei®, India) in a standard 1x buffer supplied by the manufacturer. Amplification was performed in a PTC100™ Programmable Thermal Controller (MJ Research® Inc, USA) under the following conditions: an initial denaturation at 95°C for 2 min was followed by 35 cycles at 95°C for 30 sec, 60°C for 30 sec and 72°C for 1 min with a final extension at 72°C for 5 min. PCR products were subjected to SSCP (Single-Stranded Conformation Polymorphism) analysis as described in Kumar et al. [12] on a 6% non-denaturing polyacrylamide gel containing 5% glycerol. The gel was run for 16 hrs at 22°C at 4 W with a final extension at 72°C for 5 min. PCR products were subjected to SSCP (Single-Stranded Conformation Polymorphism) analysis as described in Kumar et al. [12] on a 6% non-denaturing polyacrylamide gel containing 5% glycerol. The gel was run for 16 hrs at 22°C at 4 W using the Hoefer™ SQ3 Sequencer system (Amersham Pharmacia Biotech, USA). PCR fragment showing heterozygosity in SSCP analysis was sequenced using an fmol™ DNA Cycle Sequencing kit (Promega Inc., USA) according to manufacturer’s instructions.

Allele specific expression

In order to test for the imprinted status of this gene, total RNA samples were isolated from different tissues of abortuses showing heterozygosity in SSCP analysis using a RNase® Protect mini kit (Qiagen Inc., USA). First-strand cDNAs were synthesized using a RevertAid™H First-Strand cDNA Synthesis Kit (MBI Fermentas Inc., Canada). In order to test for allele specific expression, cDNA samples were subjected to SSCP analysis as described above.

Authors’ contributions

VB and AK carried out the molecular genetic analysis and drafted the manuscript. LK and MM were involved in sample collections.

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