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Genomic Imprinting of a Human Apoptosis Gene Homologue, *TSSC3*[1]

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ABSTRACT

Genomic imprinting is an epigenetic modification of the gamete or zygote leading to parental origin-specific gene expression in somatic cells of the offspring. We have previously identified a cluster of imprinted genes on human chromosome 11p15.5, a region involved in Beckwith-Wiedemann syndrome, Wilms' tumor, and ovarian, breast, and lung cancer. Here we show that *TSSC3*, which is homologous to the mouse apoptosis gene *TDAG51* and maps to this region, is imprinted and expressed from the maternal allele in normal development. This result is important for three reasons: (*a*) *TSSC3* is the first apoptosis-related gene in any species found to be imprinted; (*b*) it is located within the tumor suppressor region of 11p15; and (*c*) it lies within 15 kb of the nonimprinted gene *hNAP2*, thus defining a small boundary interval between imprinted and nonimprinted genes on 11p.

INTRODUCTION

A major focus of many laboratories has been the identification of genes within 11p15, because this region shows frequent loss of heterozygosity in a wide variety of childhood and adult malignancies, including Wilms' tumor, rhabdomyosarcoma, hepatoblastoma, and breast, ovarian, and lung cancer (reviewed in Ref. 1). In addition, this region contains genes for BWS,[3] which predisposes to a wide variety of embryonal tumors and causes prenatal overgrowth (2). One of the surprising themes emerging from this research is that 11p15 harbors multiple imprinted genes, *i.e.*, those that are expressed from a specific parental allele. In addition, we have previously described abnormal imprinting in cancer affecting multiple genes within this region (3–5), and we and others have found direct involvement of several of these genes in BWS and cancer. For example, IGF-2, an important autocrine growth factor normally expressed from the paternal allele, shows loss of imprinting in childhood and adult cancers and BWS (3, 6, 7). In addition, H19, an untranslated RNA, shows a growth-inhibitory effect on tumor cells *in vitro* (8), and it is expressed from the maternal allele (3). Another 11p15 gene, $p57^{KIP2}$, a maternally expressed cyclin-dependent kinase inhibitor gene (9), is mutated at low frequency in BWS (10–12) and shows epigenetic silencing in Wilms' tumor (5). We have recently found that K_VLQT1 is rearranged in patients with balanced germ-line chromosomal rearrangements, and it is expressed from the maternal chromosome (13).

Within 11p15.5, we have identified a minimal region harboring a tumor suppressor gene, using a genetic complementation assay with STFs (14). This tumor-suppressing STF includes many of the imprinted 11p15 genes. As part of our effort to identify new genes in this region, we recently cloned a gene homologous to the mouse *TDAG51*, which, when overexpressed, causes Fas-mediated apoptosis in murine T-cell hybridomas (15). This gene, termed *TSSC3* (*t*umor-*s*uppressing *S*TF *c*DNA *3*) shows 73% amino acid conservation with the mouse sequence ($P < 10^{-32}$; Ref. 16). In addition, *TSSC3* lies between the known imprinted gene $p57^{KIP2}$ (9) and the nonimprinted gene *hNAP2* (17). Thus, in addition to the importance of imprinting in cancer and BWS, knowledge of the imprinting status of *TSSC3* enables us to more precisely define a boundary between imprinted and nonimprinted genes on 11p15.

MATERIALS AND METHODS

Isolation of DNA and RNA from Tissues. Normal fetal tissues were obtained from the University of Washington Fetal Tissue Bank. Specimens were maintained at −135°C until use. For DNA isolation, tissues were pulverized in liquid nitrogen and digested with proteinase K (0.2 mg/ml) in the presence of 1% SDS in buffer TE9 [0.5 M Tris-HCl (pH 9.0), 20 mM EDTA, and 10 mM NaCl] at 50°C overnight, followed by phenol-chloroform extraction and sodium acetate/ethanol precipitation. RNA was isolated by homogenizing tissues in RNAzol B (TEL-TEST, Inc.), following the manufacturer's protocol. The ages of fetus 1, 2, 3, and 4 were 58, 99, 72, and 54 days, respectively.

Identification of a Polymorphism in *TSSC3* and DNA Genotyping. The following primers were used: primer 1F, TATAAAGGCCGCGCCGGGGCCACG; primer 1R, CCGCCGTCTGGGGTTGAAGTGGTT; primer 2F, CCCGCGCTCGGCACGACATGAAATCCC; primer 2R, GGGAACAGGCTCAGGCGGTCGGAGGTG; primer 3F, ACATGAAATCCCCCGACGAGGTGCTAC; primer 3R, GATATTAGATAGTCCAATAACTTAAGG; and primer 4R, ATTTATTTGCAATGGGCACAGTGATGC. The positions of the primers are shown in Fig. 1*b*. Primer set 1F/1R was used for initial screening of genomic DNA to identify a polymorphism. PCR reactions contained 0.5 μM primers, 0.2 mM deoxynucleotide triphosphate, 50 ng of DNA, 1× PCR buffer (Life Technologies, Inc.), 2 M betaine, and 0.5 unit of Taq DNA polymerase (Life Technologies, Inc.) in 25 μl and were performed with a Robocycler (Stratagene) as follows: 30 cycles of 95°C for 45 s; 60°C for 30 s; and 72°C for 90 s; followed by extension at 72°C for 10 min. Primer set 2F/2R was then designed to type genomic DNA samples by SSCP, using the same PCR conditions, but including 1 μCi of [α-^{32}P]dATP in the reaction. The PCR products were denatured at 94°C for 3 min, chilled on ice immediately, and electrophoresed on 5% polyacrylamide gels at 4 W at room temperature for 4 h.

Analysis of Allele-specific Expression. We used RT-PCR SSCP and sequencing to analyze allele-specific gene expression. RNA was treated with RNase-free DNase (Boehringer Mannheim) before the reverse transcription reaction. Reverse transcription was carried out with avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) using ~1 μg of RNA. The first round of PCR used primer set 2F/4R in the reaction. A second round of PCR was often carried out using 0.5 μl of DNA from the first-round PCR reaction and primer set 2F/2R and including 1 μCi of [α-^{32}P]dATP in the reaction. Similar results were also achieved using primer set 2F/2R in a single round of PCR after reverse transcription of RNA, although less efficiently than with two rounds of PCR. For direct sequencing of PCR products, primer set 3F/3R was used in the second round of PCR, PCR products were separated by 2% agarose gel electrophoresis, and cDNA was purified using Qiaex II (Qiagen).

Northern Blot Hybridization. Northern blot hybridization was performed as described previously (4) on human multiple tissue Northern blots (fetal MTN II; Clontech). DNA probes were synthesized by the random priming method (18), using TSSC3 (16) or Fas cDNA as a template. The Fas cDNA template was synthesized by RT-PCR using oligo$(dT)_{15}$ for reverse transcription and nested PCR with the following primer sets: first round, GTTAATGCCCAAGTGACTGACATCAAC and TTCTGTACTTCCTTTCTCTTCACCCAA; second round, ACTGACATCAACTCCAAGGGATTGGAA and CTCTTCACCCAAACAATTAGTGGAATT. The ages of fetal tissues were

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[3] The abbreviations used are: BWS, Beckwith-Wiedemann syndrome; IGF, insulin-like growth factor; STF, subchromosomal transferable fragment; SSCP, single-strand conformational polymorphism; RT-PCR, reverse transcription-PCR.

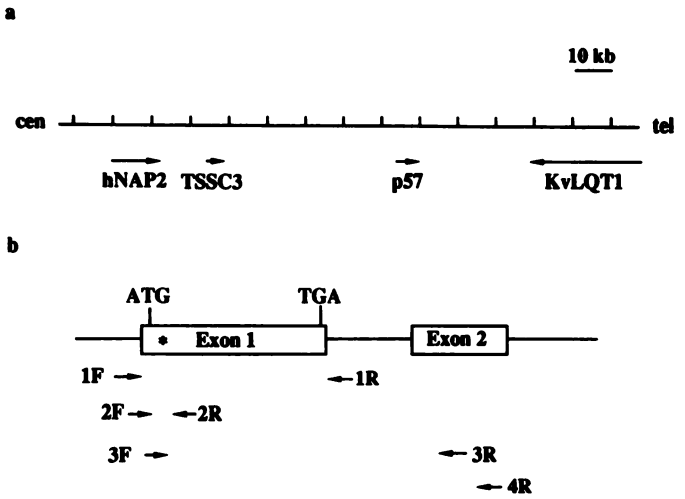

Fig. 1. Physical map and structure of *TSSC3*. *a*, map indicating the relative positions and transcriptional orientations of *hNAP2*, *TSSC3*, $p57^{KIP2}$, and K_vLQT1. Note that the distance between *hNAP2* and *TSSC3* is about 15 kb. *b*, the exon-intron structure of *TSSC3*. *, the polymorphic site at nucleotide 54. The nucleotide sequence flanking the polymorphism is AGGTGCTACGCGAGGGCGAG(T/C)TGGAGAAGCGCAGCGACAGC, with (T/C) indicating the polymorphic site. 1F, 1R, 2F, 2R, 3F, 3R, and 4R are the primers in this study (see "Materials and Methods"). *ATG* and *TGA* denote the initiation and stop codons.

20–25 days for fetal brain (pool of 10 fetuses); 18–28 days for fetal lung (pool of 29 fetuses); and 10–32 days for fetal liver and kidney (pool of 17 fetuses).

RESULTS

Identification of a Transcribed Polymorphism in *TSSC3*. We recently reported isolation of a novel gene termed *TSSC3*, which shows homology to mouse *TDAG51* and is located between *hNAP2* and $p57^{KIP2}$ (16). Our previous study placed *TSSC3* 15 kb telomeric to *hNAP2* and 50 kb centromeric to $p57^{KIP2}$ (Fig. 1*a*; Ref. 16). *TSSC3* contains two exons, with the entire coding sequence present within the first exon (Fig. 1*b*; Ref. 16). To identify a transcribed polymorphism in *TSSC3*, we used primer set 1F/1R to amplify exon 1 from genomic DNA and sequenced the PCR products directly. In this manner, a polymorphism was identified at nucleotide 54, with the C allele present in 2 of 16 individuals, and the T allele present in 14 of 16 samples (Fig. 1*b*). We then synthesized primer set 2F/2R spanning the polymorphic site, allowing the PCR product containing alleles T_{54} and C_{54} to be resolved by SSCP analysis (data not shown).

Imprinting of *TSSC3* in Fetal Tissues. We used primer set 2F/2R for amplification of genomic DNA and SSCP to type fetal DNA samples, identifying 4 heterozygotes from 30 fetuses. The heterozygosity of *TSSC3* in these samples was also confirmed by sequencing (Fig. 2). RNA was isolated from 16 tissues derived from these fetuses. We used DNA sequencing of the RT-PCR products to investigate the allelic expression of *TSSC3*. RT-PCR was performed to amplify exons 1 and 2 spanning the polymorphic site. cDNA products were resolved on 2% agarose gels and purified for direct sequencing. All four tissues from fetus 1 showed monoallelic expression of the T allele (Fig. 2*a*, kidney, intestine, limb, and heart). Similarly, both tissues from fetus 2 showed monoallelic expression of the T allele (Fig. 2*b*, heart and placenta). In addition, two tissues from fetus 3 and three tissues from fetus 4 showed preferential expression of one allele, in this case, the C allele (Fig. 2*c*, kidney and intestine; Fig. 2*d*, heart, adrenal gland, and kidney). Although the imprinting of the T allele in heart and kidney from fetus 4 was leaky, quantification of relative allelic expression levels indicated a significant bias toward the C allele (4-fold and 3-fold, respectively; Table 1). Expression in heart and testis of fetus 3 was biallelic (Fig. 2*c*, heart and testis). Quantification of relative allelic expression after normalizing for nucleotide-specific peak height indicated that both alleles were expressed at an approximately equal level in testis and heart from fetus 3 (0.8 and 1.1, respectively; Table 1). In summary, quantification of the relative level of expression of the two alleles indicated that most tissues showed preferential expression of one allele, at least 4-fold more than the other allele, with the exception of heart and testis from fetus 3, which showed biallelic expression, and heart and kidney from fetus 4, which showed leaky but preferential allelic expression (4-fold and 3-fold, respectively; Table 1).

To determine the parental origin of the expressed allele of *TSSC3*, we were able to obtain maternal DNA samples from two of four fetuses. The T allele in both fetuses 1 and 2 was of maternal origin (Fig. 2, *a* and *b*, maternal); thus, it was the maternal allele of *TSSC3* that was expressed (Fig. 2, *a* and *b*). Finally, to confirm allele-specific expression, we reanalyzed the same specimens using RT-PCR SSCP. This analysis again showed equal representation of both alleles in DNA samples, but monoallelic expression in cDNA derived from the RNA of all tissues tested (data not shown), with the exception of heart and testis from fetus 3, confirming biallelic expression from these tissues in some individuals. Thus, *TSSC3* is imprinted in most tissues, with preferential expression of the maternal allele (Table 1).

We have also analyzed the allelic expression of *TSSC3* in postnatal kidney. Among five normal kidney tissues, one showed exclusively monoallelic expression, two showed biallelic expression, and two showed partial imprinting (data not shown). Thus, *TSSC3* is not as tightly imprinted in postnatal kidney as in prenatal kidney, similar to our observations regarding another imprinted 11p15 gene, K_vLQT1 (13).[4]

Northern Blot Analysis of *TSSC3* and *Fas* Expression. If *TSSC3* plays a role in Fas-mediated apoptosis like mouse *TDAG51*, one would expect similar patterns of tissue-specific expression of *TSSC3* and *Fas*. To test this hypothesis, we performed Northern blot hybridization using human multiple tissue Northern blots (fetal MTN II; Clontech), hybridizing sequentially with *TSSC3* and *Fas* probes. We detected a 0.8-kb transcript of the *TSSC3* gene in fetal lung, liver, and kidney (Fig. 3*a*), although the amount of expression was markedly reduced in fetal liver (Fig. 3*a*). Similarly, 2.5- and 2.0-kb *Fas* transcripts were predominantly observed in fetal lung and kidney, like *TSSC3*, as well as a low level of expression in fetal liver (data not shown; Fig. 3*b*). The coexpression of *TSSC3* and *Fas* in the same tissues is consistent with the hypothesis that *TSSC3* may play a role in Fas-mediated apoptosis.

We also analyzed expression of the *TSSC3* gene in adult tissues. Northern blot hybridization using human multiple tissue Northern blots (adult MTN blot and MTN blot II; Clontech) showed the presence of a 0.8-kb transcript in the placenta (Fig. 4*a*) and in the prostate and colon (Fig. 4*b*). A 6.0-kb transcript was also detected in the placenta (Fig. 4*a*). We are currently investigating whether the 6.0-kb transcript results from alternative splicing or is due to cross-hybridization to a related gene.

DISCUSSION

In summary, we have shown that *TSSC3*, which is homologous to the mouse apoptosis-related gene *TDAG51*, exhibits monoallelic expression in normal development, with preferential expression of the maternal allele. This is not due to a technical bias in the amplification of the two alleles, because two cases showed expression of the T_{54} allele, and two other fetuses showed expression of the C_{54} allele. After the submission of this paper, Qian *et al.* (19) reported imprinting of a

[4] S. Jiang, M. Hemann, M. P. Lee, and A. P. Feinberg. KvLQT1 gene imprinted in mouse but differs from human, submitted for publication.

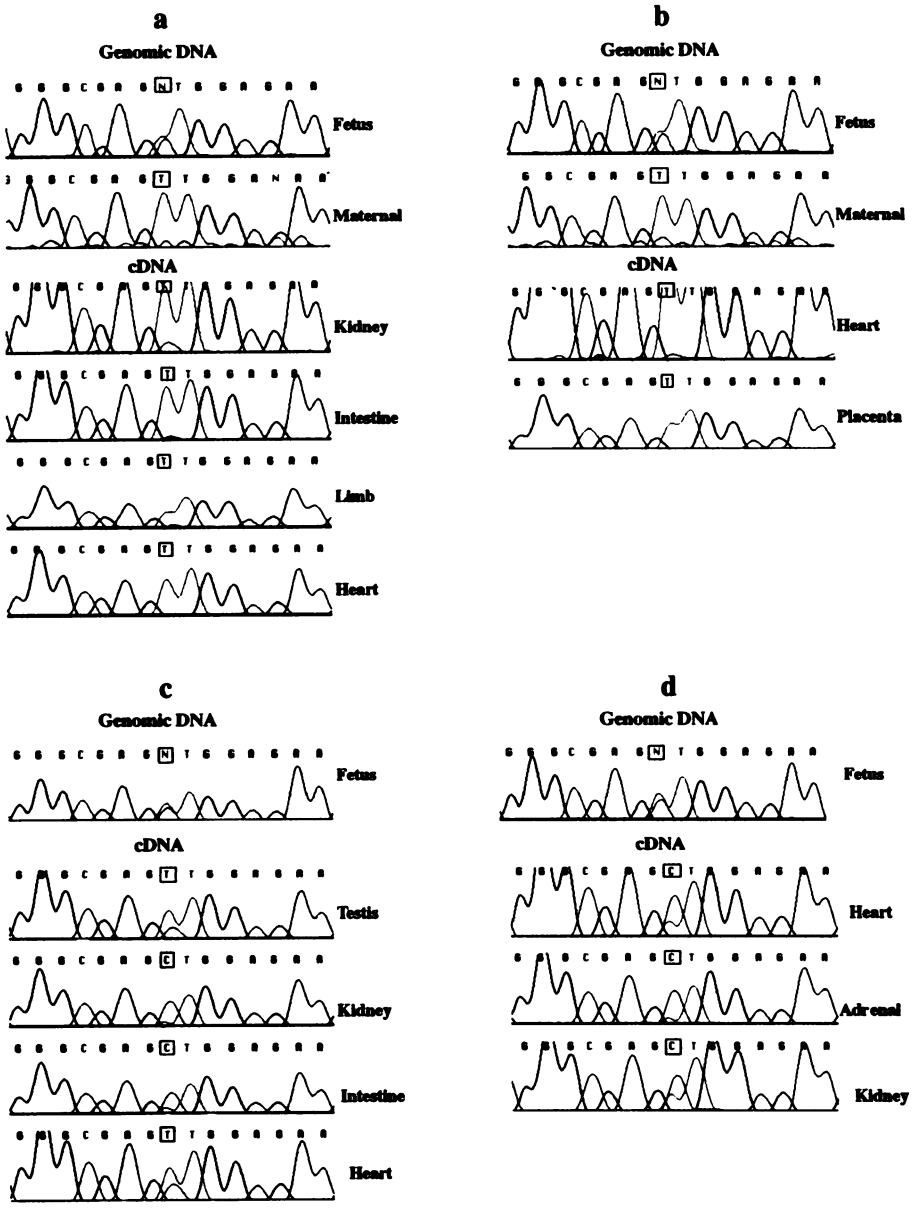

Fig. 2. Sequencing analysis of allele-specific gene expression of *TSSC3* in fetal tissues. *a, b, c,* and *d* correspond to fetuses 1, 2, 3, and 4, respectively. Genomic DNA tracings demonstrate heterozygosity of *TSSC3* in each fetus, and maternal tracings are from DNA of maternal decidua. cDNA tracings are from sequencing of RT-PCR products amplified from RNA isolated from the indicated tissues. RT-PCR reactions were always performed across intron-exon boundaries and spanning the polymorphic site to eliminate any possible genomic DNA contamination. The polymorphic C and T nucleotides are *boxed*.

Table 1 *Genomic imprinting of TSSC3*

C and T indicate the polymorphism at nucleotide 54. T_{mat} indicates that the T allele is derived from the maternal chromosome. C/T in the RNA column indicates biallelic expression. The imprinting status for adrenal gland and kidney from fetus 2 and lung from fetus 4 is derived from RT-PCR SSCP, and the imprinting status for the rest of samples is derived from both RT-PCR sequencing and SSCP. Ratio is calculated to two significant digits, based on the peak heights of tracing after normalizing for nucleotide-specific peak height. It is expressed as the ratio of expressed allele to silenced allele. NA, not available.

Fetus	Tissue	DNA	RNA	Ratio
1	Kidney	C/T_{mat}	T_{mat}	4.6
1	Intestine	C/T_{mat}	T_{mat}	9.0
1	Limb	C/T_{mat}	T_{mat}	13
1	Heart	C/T_{mat}	T_{mat}	13
2	Heart	C/T_{mat}	T_{mat}	9.0
2	Placenta	C/T_{mat}	T_{mat}	13
2	Adrenal gland	C/T_{mat}	T_{mat}	NA
2	Kidney	C/T_{mat}	T_{mat}	NA
3	Testis	C/T	C/T	0.8
3	Kidney	C/T	C	10
3	Intestine	C/T	C	6.7
3	Heart	C/T	C/T	1.1
4	Heart	C/T	C	4.0
4	Adrenal gland	C/T	C	99
4	Kidney	C/T	C	3.0
4	Lung	C/T	C	NA

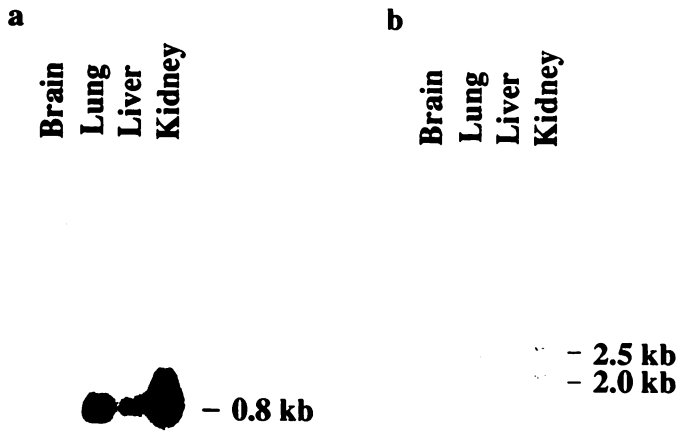

Fig. 3. Northern blot analysis of *TSSC3* and *Fas* expression in fetal tissues. *Human* multiple tissue Northern blots (fetal MTN II; Clontech) were hybridized with a *TSSC3* probe. The probe was stripped (*a*), and the blot was rehybridized with a Fas probe (*b*). Both exposures were for 24 h. Longer exposures of Fas blots also detected a lower level of expression in fetal liver, correlating with a similar lower level of expression of *TSSC3*.

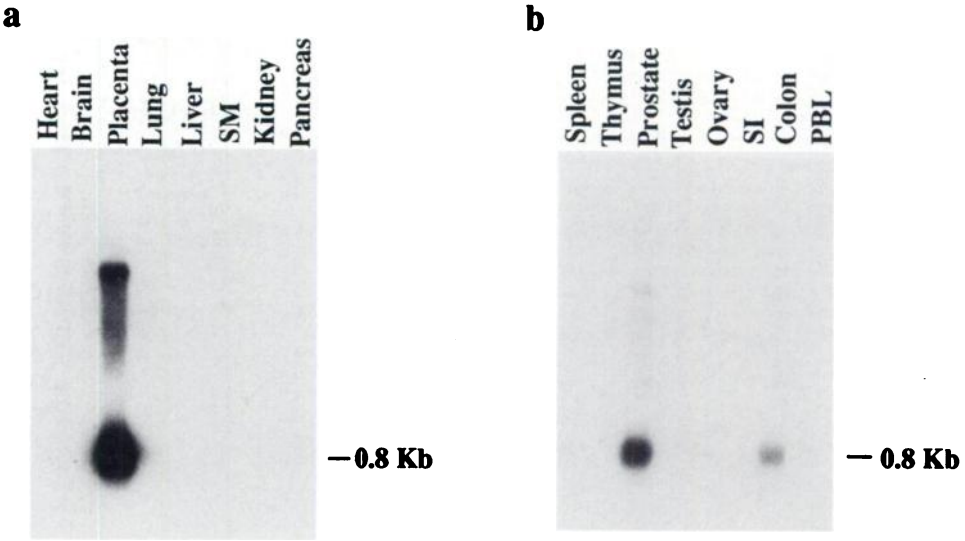

Fig. 4. Northern blot analysis of *TSSC3* expression in adult tissues. Human multiple tissue Northern blots, MTN (*a*) and MTN II (*b*), were hybridized with a *TSSC3* probe. The exposures were for 24 h. *SM*, *SI*, and *PBL* indicate skeletal muscle, small intestine, and peripheral blood leukocytes, respectively.

gene that they termed *IPL*. The DNA sequences of *IPL* are identical to the *TSSC3* sequence we have reported (Ref. 16; GenBank accession number AF019953). Qian *et al.* (19) observed imprinting in the placenta and liver only and thus referred to the gene as *IPL* (*i*mprinted in *p*lacenta and *l*iver). In contrast, we have found that *TSSC3* is imprinted in almost all fetal tissues tested, with the exception of heart and testis in one fetal specimen (Fig. 2*c*; Table 1). Thus, we believe the nomenclature *TSSC3* is more appropriate.

These results have several important implications. This is the first apoptosis-related gene that has been shown to be imprinted. Therefore, apoptosis is likely to be added to the list of cellular pathways involving imprinting, which already include cell signaling, ion channel conductance, cell cycle control, and embryonic development. In addition, *TSSC3* lies within the embryonal tumor suppressor gene region of 11p15. The fact that the gene is expressed from the maternal allele is consistent with a potential growth-inhibitory effect, because imprinted genes expressed from the maternal allele, particularly in this region, tend to inhibit cell proliferation, and those expressed from the paternal allele tend to stimulate growth. These data suggest that a cluster of growth-inhibitory genes lie within a relatively small domain of 11p15.5, including *TSSC3*, $p57^{KIP2}$, and *H19*. A recent study suggests that regulation of Fas expression may play a role in breast cancer (20). *TSSC3* was also expressed in breast tissue (data not shown). Thus, it is conceivable that inactivation of *TSSC3*, due to the combination of imprinting of one allele and loss of heterozygosity of the other, could block *Fas*-mediated apoptosis in breast cancer, although such an effect need not be limited to breast cancer, particularly given the strong expression of *TSSC3* and *Fas* in the lung and kidney. In this regard, it is particularly interesting that Hueber *et al.* (21) and Harrington *et al.* (22) have recently reported involvement of *c-myc* and *IGF-1* in the same pathway downstream of *Fas*-mediated apoptosis. Our demonstration that *TSSC3* (which is upstream of *Fas*) is in the same linked group of imprinted genes with *IGF-2* suggests that *TSSC3* and *IGF-2* could be genetically linked antagonists, consistent with the Haig hypothesis of linked antagonists in imprinted gene domains (23).

This study defines a centromeric boundary to the multigene imprinted domain of 11p15.5, *i.e.*, between *hNAP2*, which is not imprinted, and *TSSC3*, which is imprinted. A similar 40-kb boundary region is defined at the telomeric end between *H19* and *L23MRP* (24). The centromeric imprinted domain boundary defined by the present study is much smaller (only 15 kb). Thus, this work should greatly facilitate efforts to identify functional boundary elements within this domain.

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REFERENCES

1. Feinberg, A. P. A developmental context for multiple genetic alterations in Wilms' tumor. J. Cell Sci., *18:* 7–12, 1994.
2. Mannens, M., Hoovers, J. M. N., Redeker, E., Verjaal, M., Feinberg, A. P., Little, P., Boavida, M., Coad, N., Steenman, M., Bliek, J., Niikawa, N., Tonoki, H., Nakamura, Y., de boer, E. G., Slater, R. M., John, R., Cowell, J. K., Junien, C., Henry, I., Tommerup, N., Weksberg, R., Pueschel, S. M., and Westerveld, A. Parental imprinting of human chromosome region 11p15.3-pter involved in the Beckwith-Wiedemann Syndrome and various human neoplasia. Eur. J. Hum. Genet., *2:* 3–23, 1994.
3. Rainier, S., Johnson, L. A., Dobry, C., Ping, A. J., Grundy, P. E., and Feinberg, A. P. Relaxation of imprinted genes in human cancer. Nature (Lond.), *362:* 747–749, 1993.
4. Steenman, M. J. C., Rainier, S., Dobry, C., Grundy, P., Horon, I., and Feinberg, A. P. Loss of imprinting of IGF2 is linked to reduced expression and abnormal methylation of H19 in Wilms' tumour. Nat. Genet., *7:* 433–439, 1994.
5. Thompson, J. S., Reese, K. J., DeBaun, M. R., Perlman, E. J., and Feinberg, A. P. Reduced expression of the cyclin-dependent kinase inhibitor gene $p57^{KIP2}$ in Wilms' tumor. Cancer Res., *56:* 5723–5727, 1996.
6. Ogawa, O., Eccles, M., Szeto, M. R., McNoe, L. A., Yun, K., Maw, M. A., Smith, P. J., and Reeve, A. E. Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms' tumour. Nature (Lond.), *362:* 749–751, 1993.
7. Weksberg, R., Shen, D. R., Fei, Y. L., Song, Q. L., and Squire, J. Disruption of insulin-like growth factor 2 imprinting in Beckwith-Wiedemann syndrome. Nat. Genet., *5:* 143–150, 1993.
8. Hao, Y., Crenshaw, T., Moulton, T., Newcomb, E., and Tycko, B. Tumour-suppressor activity of H19 RNA. Nature (Lond.), *365:* 764–767, 1993.
9. Matsuoka, S., Thompson, J. S., Edwards, M. C., Barletta, J. M., Grundy, P., Kalikin, L. M., Wade Harper, J., Elledge, S. J., and Feinberg, A. P. Imprinting of the gene encoding a human cyclin-dependent kinase inhibitor $p57^{KIP2}$, on chromosome 11p15. Proc. Natl. Acad. Sci. USA, *93:* 3026–3030, 1996.
10. Hatada, I., Ohashi, H., Fukushima, Y., Kaneko, Y., Inoue, M., Komoto, Y., Okada, A., Ohishi, S., Nabetani, A., Morisaki, H., Nakayama, M., Niikawa, N., and Mukai, T. An imprinted gene $p57^{KIP2}$ is mutated in Beckwith-Wiedemann syndrome. Nat. Genet., *14:* 171–173, 1996.
11. Lee, M. P., DeBaun, M., Randhawa, G., Reichard, B. A., Elledge, S. J., and Feinberg, A. P. Low frequency of $p57^{KIP2}$ mutation in Beckwith Wiedemann syndrome. Am. J. Hum. Genet., *61:* 304–309, 1997.
12. O'Keefe, D., Dao, D., Zhao, L., Sanderson, R., Warburton, D., Weiss, L., Anyane-Yeboa, K., and Tycko, B. Coding mutations in $p57^{KIP2}$ are present in some cases of Beckwith-Wiedemann syndrome but are rare or absent in Wilms' tumors. Am. J. Hum. Genet., *61:* 295–303, 1997.
13. Lee, M. P., Hu, R-J., Johnson, L. A., and Feinberg, A. P. Human K_VLQT1 shows tissue-specific imprinting and is physically disrupted by Beckwith Wiedemann syndrome chromosomal rearrangements. Nat. Genet., *15:* 181–185, 1997.

14. Koi, M., Johnson, L. A., Kalikin, L. M., Little, P. F. R., Nakamura, Y., and Feinberg, A. P. Tumor cell growth arrest caused by subchromosomal transferable DNA fragments from human chromosome 11. Science (Washington DC), *260:* 361–364, 1993.
15. Park, C. G., Lee, S. Y., Kandala, G., Lee, S. Y., and Choi, Y. A novel gene product that couples TCR signaling to Fas(CD95) expression in activation-induced cell death. Immunity, *4:* 583–591, 1996.
16. Hu, R-J., Lee, M. P., Connors, T. D., Johnson, L. A., Burn, T. C., Su, K., Landes, G. M., and Feinberg, A. P. A 2.5-Mb transcript map of a tumor-suppressing subchromosomal transferable fragment from 11p15.5, and isolation and sequence analysis of three novel genes. Genomics, *46:* 9–17, 1997.
17. Hu, R-J., Lee, M. P., Johnson, L. A., and Feinberg, A. P. A novel human homologue of yeast nucleosome assembly protein, 65 kb centromeric to the $p57^{KIP2}$ gene, is biallelically expressed in fetal and adult tissues. Hum. Mol. Genet., *5:* 1743–1748, 1996.
18. Feinberg, A. P., and Vogelstein, B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem., *132:* 6–13, 1983.
19. Qian, N., Frank, D., O'Keefe, D., Dao, D., Zhao, L., Yuan, L., Wang, Q., Keating, M., Walsh, C., and Tycko, B. The IPL gene on chromosome 11p15.5 is imprinted in human and mice and is similar to TDAG51, implicated in Fas expression and apoptosis. Hum. Mol. Genet., *6:* 2021–2029, 1997.
20. Keane, M. M., Ettenberg, S. A., Lowrey, G. A., Russell, E. K., and Lipkowitz, S. Fas expression and function in normal and malignant breast cell lines. Cancer Res., *56:* 4791–4798, 1996.
21. Hueber, A-O., Zornig, M., Lyon, D., Suda, T., Nagata, S., and Evan, G. I. Requirement for the CD95 receptor-ligand pathway in c-myc-induced apoptosis. Science (Washington DC), *278:* 1305–1309, 1997.
22. Harrington, E. A., Bennett, A. F., and Evan, G. I. c-myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. EMBO J., *13:* 3286–3295, 1994.
23. Haig, D., and Graham, C. Genomic imprinting and the strange case of the insulin-like growth factor II receptor. Cell, *64:* 1045–1046, 1991.
24. Tsang, P., Gilles, F., Yuan, L., Kuo, Y-H., Lupu, F., Samara, G., Moosikasuwan, J., Goye, A., Zelenetz, A. D., Selleri, L., and Tycko, B. A novel L23-related gene 40 kb downstream of the imprinted H19 gene is biallelically expressed in mid-fetal and adult human tissues. Hum. Mol. Genet., *4:* 1499–1507, 1995.

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