SHORT COMMUNICATION

The *MAS* Proto-Oncogene Is Not Imprinted in Humans

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Recently it was shown that the murine *Mas* gene, which is located less than 300 kb from the imprinted *Igf2r* gene, is also imprinted in Day 11.5 embryos with expression exclusively from the paternal allele. We have assigned the human *MAS* gene to chromosomal bands 6q25.3–q26 in close proximity to the *IGF2R* gene. In contrast to its murine homologue, the human *IGF2R* gene is not imprinted. By making use of a novel intragenic polymorphism, we have studied the expression of the *MAS* gene in three heterozygous human fetuses. In all tissues examined, including tongue, biallelic expression of the *MAS* gene was observed. Hence both *MAS* and the neighboring *IGF2R* gene are not imprinted in humans.

The *MAS* proto-oncogene belongs to the class of GTP-binding protein coupled receptors. Overexpression of *MAS* can induce tumor growth (18, 20). The murine *Mas* gene has been mapped to the proximal part of chromosome 17 (4) in the vicinity of the *Igf2r* gene, from which it is separated by less than 300 kb. Like *Igf2r,* which is active only on maternally derived chromosomes (2), the *Mas* gene was recently shown to be imprinted in the mouse, in line with the observation that imprinted genes tend to occur in clusters. The expression of the *Mas* gene is confined to the paternal allele in Day 11 to 12.5 embryos. After Embryonic Day 13.5 and in neonates, this paternal-specific expression persisted in the tongue, visceral yolk sac, heart, and skeletal muscle, whereas all other tissues tested showed relaxation of the maternal imprint resulting in biallelic expression (19).

In general, genes that are subject to genomic imprinting in mice seem to be imprinted in humans as well (1). The *IGF2R* gene is the only known exception to this rule; as shown by us and confirmed by others, this gene is biallelically expressed in first and second trimester fetuses (8, 10). Previous studies have mapped the human *IGF2R* and *MAS* genes to approximately the same region on chromosome 6q (8, 11, 12). This has prompted us to study the allele-specific expression pattern of the human *MAS* gene and to determine its physical distance from the *IGF2R* locus.

To this end we screened the ICI human YAC library with PCR primer pairs generated from the 5′ and 3′ ends of the published human *MAS* cDNA sequence (20) (5′UTR I6-77 GGATCCAGAAGGGTCATTC, I6-78 CTCAGGTTGGAACTCTTCAG; 3′ I6-87 GCAGTAAGAAGAAGAGATTC, I6-88 TCATCATTAATTAGTATCTCATG) and isolated a YAC clone containing the *MAS* gene. FISH mapping enabled us to assign this YAC to 6q25.3–q26, i.e., very close to the *IGF2R* gene, which has been mapped to 6q26. Moreover, FISH signals obtained with YACs carrying the *IGF2R* gene and the *MAS* gene were partially overlapping on metaphase chromosomes and in 91 of 100 interphase nuclei. Since for cosmid probes the resolution of interphase FISH is approximately 50 kb (17), these findings suggest that in humans, too, the physical distance of the *MAS* gene and the *IGF2R* gene is at most several hundred kb.

To investigate the transcriptional activity of the two parental copies of the human *MAS* gene separately, we searched for intragenic DNA polymorphisms using SSCP and sequence analysis. Amplification of genomic *MAS* fragments was performed with primers designed on the basis of the published cDNA sequence (20). PCR products of the correct size were isolated from agarose gels using the Qiaquick gel extraction kit (Qiagen) and employed as template in the sequencing reaction. Sequencing was performed using the *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems).

A silent basepair substitution (C900T) was found with primers I6-91 CATGGATATGTCATGTGC and I6-92 CCATGATGACTATGTAAAGC. Of 14 first and second trimester fetuses, 3 were found to be heterozygous for this polymorphism. RNAs from different tissues of these fetuses were tested for parental allele-specific expression. Complementary DNA was synthesized as previously described (8). To detect possible DNA contamination, in each experiment half of the RNA sample was subjected to RT-PCR without the addition of reverse transcriptase. These controls were negative in all cases. Subsequently, RT-PCR products were purified

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TABLE 1

Biallelic Expression of the *MAS* Gene in Three Heterozygous Fetuses

	F1	F4	F5
Brain	Biallelic	ND	ND
Intestine	ND	Biallelic	Biallelic
Kidney	Biallelic	Biallelic	ND
Muscle	Biallelic	Biallelic	Biallelic
Placenta	Biallelic	ND	ND
Skin	ND	ND	Biallelic
Tongue	ND	Biallelic	Biallelic

Note. F1, 19th week of gestation; F4, 12th week of gestation; F5, 12th week of gestation ND, not determined.

and sequenced. In all fetuses and tissues tested, biallelic expression was found (see Table 1). In two of the three fetuses, *MAS* expression could also be tested in the tongue (see Fig. 1), where in mice imprinting of the *MAS* gene has been reported to persist until after birth. Our results show that the *MAS* gene is biallelically expressed in three informative human fetuses and that it is apparently not imprinted in humans.

Genes that are imprinted in mice tend to be subject to imprinting in humans too. It is striking that the two known exceptions of this rule, *IGF2R* and *MAS,* map to the same chromosome region. A possible explanation for this finding is the assumption that the imprinting behavior of these genes depends on regional *cis*-acting factors that are functional in mice but defunct or lost in humans.

Support for the existence of such regional control elements has come from the imprinted gene clusters on human chromosomes 11 and 15, where pairs of genes that are differentially imprinted are located. Functional loss, through deletion or uniparental disomy of the paternally or maternally derived 15q11–q13 segment, leads to Prader–Willi (PWS) or Angelman (AS) syndrome, respectively (for review see (9)). For both syndromes, however, patients have been identified with a normal biparental inheritance but with abnormal methylation and expression patterns, i.e., only maternal for PWS and only paternal for AS. In Beckwith–Wiedemann syndrome (BWS), too, patients with biparental inheritance of chromosome 11p15 but paternal-specific methylation and expression patterns

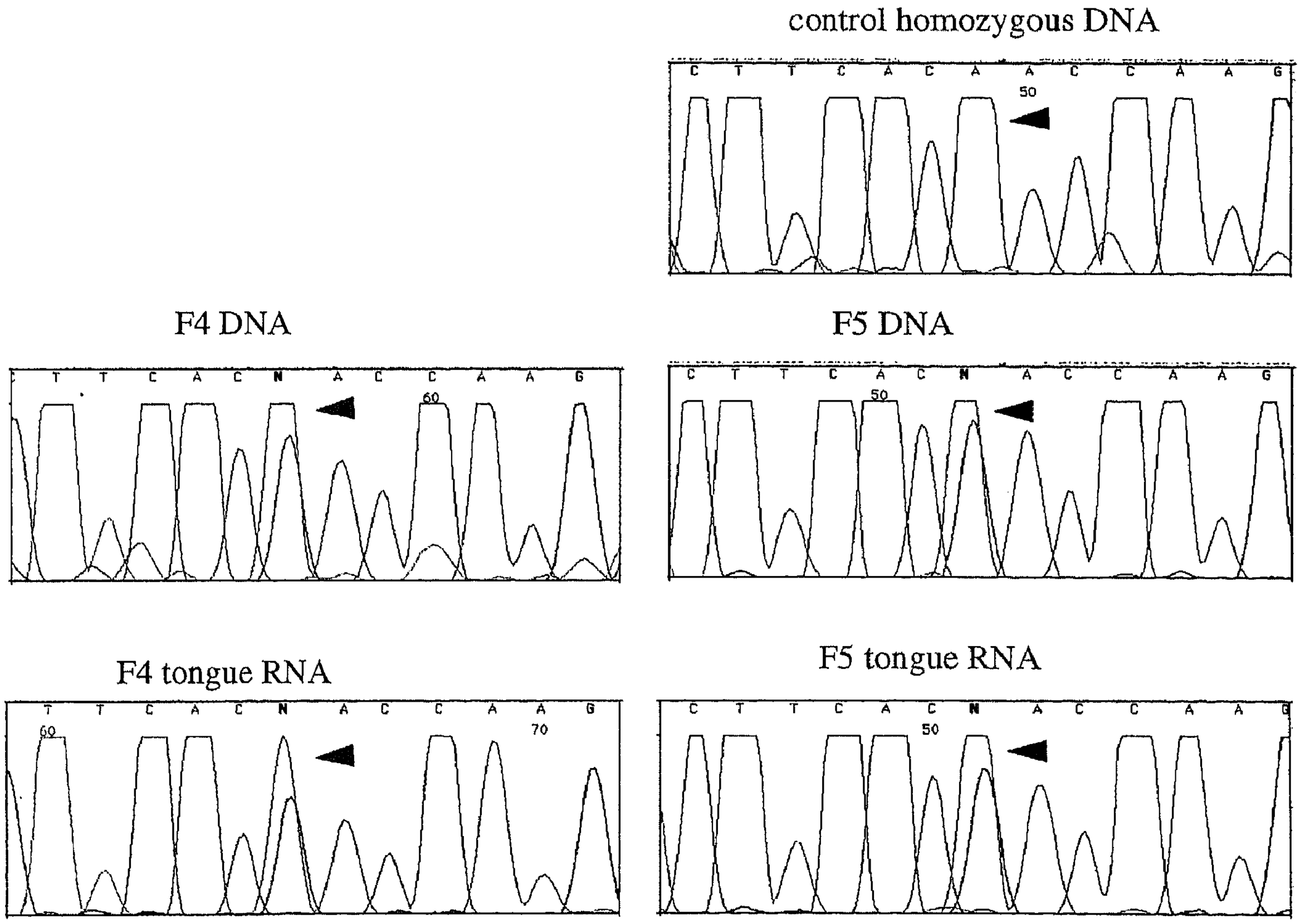

FIG. 1. Sequence of RT-PCR products of tongue RNA of two heterozygous fetuses. Sequencing was performed with primer I6-92. Both alleles are present in F4 and F5 DNA and RNA as shown by overlapping signals, whereas in control homozygous DNA only one allele is present (arrowheads).

have been identified. These cases have been explained as mutations affecting imprinting control elements (ICEs) in the vicinity of the PWS, AS, and BWS genes (3, 13, 14). Nothing is known so far about the molecular nature of these postulated ICEs, nor about their mode and range of action.

In mice, *Igf2r* and *Mas* map to the *T* locus region on chromosome 17 not far from the major histocompatibility cluster (6), whereas in humans, genes from this region map to opposite ends of chromosome 6 (7). At least formally it is possible, therefore, that the evolutionary rearrangement that separated these genes also dislocated the *IGF2R* and *MAS* genes from their ICE or vice versa. This possibility can be tested, e.g., by physical fine mapping of the respective evolutionary breakpoint. On the other hand the functional need to imprint these two genes is clearly absent in humans, and biallelic expression may even be advantageous, as it has been shown that *IGF2R* can function as a tumor suppressor gene in liver carcinogenesis (5). If so the parent-specific methylation and asynchronous replication of *IGF2R* (15, 16) may just be an evolutionary remnant.

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