Letters to the Editor

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Conflicting Reports of Imprinting Status of Human *GRB10* in Developing Brain: How Reliable Are Somatic Cell Hybrids for Predicting Allelic Origin of Expression?

To the Editor:

We read with interest a report by Yoshihashi et al. (2000), in the August issue of the *Journal,* in which the authors detailed their results on imprinting studies and mutation screening in the human *GRB10* gene in patients with Silver-Russell syndrome (SRS). They demonstrated that *GRB10* is monoallelically expressed in human fetal brain tissues and maternally expressed in somatic cell hybrids containing a single maternally or paternally derived human chromosome 7. In addition, they screened samples from 58 patients with SRS for mutations in the 16 exons of *GRB10* (described by Angrist et al. 1998). Of the samples, 30 were from patients of Japanese origin, and 28 were obtained from the European Collection of Cell Cultures. The authors identified an amino acid substitution—P95S—in exon 3, which was maternally inherited in two unrelated Japanese patients. The authors also excluded this mutation in 100 ethnically matched controls.

To establish the frequency of the P95S mutation in a larger SRS cohort, 105 patients with SRS (74 of German origin, from the Children's Hospital, University of Tübingen; and 31 patients with SRS, from the United Kingdom) were screened. We did not identify the P95S mutation in any of the patients with SRS who were screened or in 102 healthy white controls. In addition, we have screened for mutations in 50 of the German patients with SRS in all 22 translated and untranslated exons of *GRB10* (Blagitko et al. 2000) and in 31 of the U.K. patients with SRS in 16 coding exons of *GRB10* (Hitchins et al., in press). No pathogenic mutations of *GRB10* were detected in any of these patients, although several polymorphisms were identified (Blagitko et al. 2000; Hitchins et al., in press).

GRB10 has been considered a strong candidate for SRS, on the basis of its imprinting status in mice, its suppressive effect on growth, and its localization within the duplicated region of two patients with SRS (Joyce et al. 1999; Monk et al. 2000). The general lack of mutations and the fact that the P95S variant has been identified in only 2 of 163 patients with SRS suggests that *GRB10* mutations are not a major cause of SRS. Since there is a possibility that the P95S substitution could be a rare polymorphism restricted to the Japanese population, functional experiments are required to determine whether the P95S substitution has an effect on GRB10 protein function.

As a separate issue, the inferences of Yoshihashi et al. (2000) that *GRB10* is maternally expressed in human fetal brain are opposite to the direct analyses performed by our two groups (Blagitko et al. 2000; Hitchins et al., in press). Yoshihashi et al. used an indirect approach, determining the parental origin of the transcribed allele by reverse transcription–PCR studies using human lymphocyte/Chinese hamster ovary somatic cell hybrids containing a single maternal or paternal human chromosome 7. We have used a direct approach to study the allelic origin of single-nucleotide polymorphisms within the *GRB10* transcript in different fetal tissues with accompanying maternal DNA. This demonstrates that *GRB10* is expressed specifically from the paternal allele in fetal brain and is biallelic in numerous other tissues (Blagitko et al. 2000; Hitchins et al., in press). In fact, *GRB10* imprinting is complex, demonstrating a highly tissue- and isoform-specific imprinting profile. Maternal expression of a novel splice variant, *GRB10γ1,* was detected in skeletal muscle alone. In fetal brain, all isoforms except two were expressed solely from the paternal allele (Blagitko et al. 2000). In the spinal cord, expression was exclusively from the paternal allele (Hitchins et al., in press). All *GRB10* splice variants are transcribed from both parental alleles in the majority of fetal tissues. Analysis of CpG-islands in the 5′ untranslated region of the gene showed unmethylated CpGs on both alleles, which is in good agreement with the observed biallelic expression. This is the first example of opposite imprinting in the mouse and in man. These results bring into question the reliability of using the somatic cell hybrid system for predicting the imprinting status of candidate genes. Although somatic cell hybrids have been used for the detection of imprinting, conflicting data have been reported for the imprinting status of the *GABRβ3, GABRα5,* and *GABRγ3* genes in the human 15q11-13 region. One group reported paternal-specific expression

of the $GABA_A$ receptor cluster (Meguro et al. 1997); another group found these genes to be biallelically expressed (Gabriel et al. 1998). In both reports, the same imprinted and nonimprinted controls from human 15q11-13 were used.

The finding of biallelic expression in most fetal tissues and of paternal expression in the central nervous system, taken together with the absence of primary sequence mutations in a large panel of patients with SRS, argues against a major role for *GRB10* in SRS. However, with the identification of a maternally expressed isoform in skeletal muscle and the possibility that epigenetic alterations affecting *GRB10* activity still remain, *GRB10* may yet be involved in at least a subset of patients with SRS.

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Reply to Mergenthaler et al.

To the Editor:

The thoughtful letter from Mergenthaler et al. (2001) illustrates the complex nature of the imprinting status of human GRB10. Blagitko et al. (2000) and Mergenthaler et al. (2001) have presented convincing data that human GRB10 is expressed from the paternal allele in fetal brain. This represents the first example of homologous genes being reciprocally imprinted in humans and mice. In their letter, Mergenthaler et al. make a number of valid points in relation to our report on imprinting of GRB10 as it relates to Russell-Silver syndrome (RSS). However, they also raise several issues that merit clarification.

We believe that the title of their letter, "Conflicting Reports of Imprinting Status of Human GRB10 in Developing Brain," overstates the extent of controversy. We would like to point out that we specifically concluded, in our report, only that human GRB10 is monoallelically