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

expressed in the fetal brain, because we were unable to determine the parental origin of the expressed allele without having parental genomic DNA (Yoshihashi et al. 2000). As an alternative, we evaluated the somatic cell hybrid system and concluded that the maternal allele is expressed in that experimental system.

On the basis of paternal allele–specific expression of GRB10 in the fetal brain, Mergenthaler et al. (2001) concluded that GRB10 does not play a significant role in the pathogenesis of RSS, with rather little attention to their own finding that one isoform (gamma 1) is expressed from the maternal allele in skeletal muscle (Blagitko et al. 2000). Because northern blot analyses have indicated that GRB10 mRNA is expressed much more abundantly in muscle than in brain (Liu and Roth 1995), the importance of the maternal allele–specific expression in the skeletal muscle should not be overlooked. The tissue-specific reciprocal imprinting pattern of GRB10 in muscle and brain indeed could account for the characteristic disproportionate growth retardation of the head and the body sizes observed among patients with RSS. This peculiar growth pattern is referred to as relative macrocephaly and is characterized by greater growth of the head in comparison with severely retarded growth of the body. Tissue-specific reciprocal imprinting could exert differential effects on the growth of muscle, brain, and possibly other tissues in patients with RSS who have maternal UPD7 or duplication of 7p11.2-p13. Maternally expressed muscle transcripts would be overexpressed, whereas paternally expressed brain transcripts would not be expressed in excess. If GRB10 indeed functions as a growth suppressor (O'Neill et al. 1996), the resultant phenotype would be sparing of brain size—that is, relative macrocephaly.

We screened samples from 58 patients with RSS and identified two Japanese patients with a P95S change, which was not present among 100 normal Japanese controls (Yoshihashi et al. 2000). Subsequent screening of >300 normal Japanese individuals by Yamasaki et al. (2000) identified two individuals with the P95S sequence, suggesting the possibility that this may represent a rare polymorphism in the Japanese population. Unfortunately, the parental origin of the P95S allele in the two phenotypically normal individuals could not be determined, and thus it is not possible to make inferences on the likely expression or silence of this altered allele. It will be important to directly compare the functional properties of GRB10 containing either a P or an S residue at position 95, and these studies currently are in progress. Together with the lack of GRB10 mutations in 50 German patients (Blagitko et al. 2000) and in 31 English patients (Mergenthaler et al. 2001), we agree with the conclusion that mutations in the coding sequence of GRB10 are rare, if they occur at all, as determinants of RSS. Moreover, we hope that analysis of the complex imprinting mechanism of GRB10 and its flanking region at 7p12 will provide further insight into the pathogenesis of RSS.

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Founder Mutations of BRCA1 and BRCA2 in North American Families of Polish Origin That Are Affected with Breast Cancer

To the Editor:

We have recently identified five founder mutations in a panel of 66 families with breast/ovarian cancer who were ascertained in western Poland (Górski et al. 2000). Of the 35 families with mutations, 33 had one of the five recurrent BRCA1 mutations, including 18 families with the 5382 insC mutation. Only one BRCA2 mutation was found in the 66 families.

A significant proportion of North American families claim Polish ancestry. We were interested to see whether the same mutations were present in families residing in North America who claimed Polish ancestry. This information might be useful for genetic counseling and to facilitate mutation detection.

We attempt to record ethnicity for all families affected by breast cancer listed in our database. From a source of 1,010 families who underwent genetic evaluation, we identified 23 families of Polish ancestry. All families were tested for the five Polish founder mutations. We also completed the protein truncation test (PTT) of exon 11 of BRCA1 and exons 10 and 11 of BRCA2. A few families had mutations identified through an earlier study (Serova et al. 1997).

No BRCA2 mutation was seen in the 23 families, but 10 BRCA1 mutations were found (43%), including 6 5382insC mutations. The other mutations included one instance each of 964del4, IVS16-581del1014, C1806T, and 4184del4. None of these were present in the original panel of Polish families (Górski et al. 2000). We identified four other families in our database who did have one of the putative Polish founder mutations (other than 5382insC and 185delAG)—but none of these families were Polish. There were two unrelated Czech families with the 3819del5 mutation, one Lithuanian family with the 4153delA mutation, and one family of mixed European ancestry with the T300G mutation. These three mutations have been reported to the Breast Cancer Information Core database 13 times, 7 times, and 46 times, respectively. Only two of the families reported to BIC were said to be Polish (both had the 3819del5 mutation). The 4154delA mutation is common in Russia (Gayther et al. 1997). In summary, none of the mutations that are common in Poland appear to be specific to the population.

Our results support the claim that the majority of mutation-carrying families with Polish ancestry carry the BRCA1 5382insC mutation, in Poland and in North America. We believe that, when resources are limited, it may be efficient to offer testing for this mutation prior to undertaking a complete evaluation for Polish families seeking genetic testing. The low frequency of BRCA2 mutations in Polish families, in Poland and in North America, remains to be explained.

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Vacuoliting Megalencephalic Leukoencephalopathy

To the Editor:

In a report in the February 2000 issue of the *Journal,* Topçu et al. (2000) reported on the mapping of vacuoliting megalencephalic leukoencephalopathy with subcortical cysts to chromosome 22q, in a 3-cM interval between the markers D22S1161 and n66c4. The centromeric boundary was defined by a recombination event with the marker D22S1161. However, how n66c4 was determined as the telomeric boundary is not clear. n66c4 reaches its maximal LOD score at $\theta = .00$. The

highest multipoint LOD score was also reached with that marker, and the haplotypes shown in figure 2 of the report do not reveal any recombination events with this marker, either in the patients or in the unaffected sibs. The interval containing the gene should therefore be defined as being between D22S1161 and the telomere.

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