mutation when *in cis*. The proband's sister III-2 has normal red-blood-cell (RBC) GALT activity, but her GALT genome includes three mutations. She is homozygous for N314D, and the paternally derived allele also contains the E203K mutation. Since E203K alone reduces GALT activity by 50% (Elsas et al. 1995) and the N314D homozygote has 50% reduction in activity, our interpretation of her normal RBC GALT activity in the presence of these three mutations is that the oppositely charged amino acid substitutions stabilize the dimeric active enzyme and enable catalysis of uridyl transfer. I-1 and II-1 have "near-normal" activity as well, despite one normal allele and the presence of the E203K-N314D codon changes *in cis* on the other allele, which should produce >50% reduction in GALT activity. We are eager to test these hypotheses in controlled experiments using expression systems as described by Fridovich-Keil et al. (1995). We thank Dr. Reichardt for his continued interest in our work and hope that this answers his questions.

LOUIS J. ELSAS II

Division of Medical Genetics
Emory University School of Medicine
Atlanta

References

Elsas LJ, Dembure PP, Langley S, Paulk EM, Hjelm LN, Fridovich-Keil J (1994) A common mutation associated with the Duarte galactosemia allele. Am J Hum Genet 54:1030–1036

Elsas LJ, Langley S, Steele E, Evinger J, Fridovich-Keil JL, Brown A, Singh R, et al (1995) Galactosemia: a strategy to identify new biochemical phenotypes and molecular genotypes. Am J Hum Genet 56:630–639

Fridovich-Keil JL, Langley SD, Mazur LA, Lennon JC, Dembure PP, Elsas JL II (1995) Identification and functional analysis of three distinct mutations in the human galactose-1-phosphate uridyltransferase gene associated with galactosemia in a single family. Am J Hum Genet 56:640–646

Leslie ND, Immerman EB, Flach JE, Florez M, Fridovich-Keil JL, Elsas LJ (1992) The human galactose-1-phosphate uridyltransferase gene. Genomics 14:474–480

Lin H-C, Kirby LT, Ng WG, Reichardt JKV (1994) On the molecular nature of the Duarte variant of galactose-1-phosphate uridyl transferase (GALT). Hum Genet 93:167–169

Reichardt JKV, Belmont JW, Levy HL, Woo SLC (1992*a*) Characterization of two missense mutations in human galactose-1-phosphate uridyltransferase: different molecular mechanisms for galactosemia. Genomics 12:596–600

Reichardt JKV, Levy HL, Woo SLC (1992*b*) Molecular characterization of two galactosemia mutations and one polymorphism: implications for structure-function analysis of human galactose-1-phosphate uridyltransferase. Biochemistry 31:5430–5433

Reichardt JKV, Woo SLC (1991) Molecular basis of galactosemia: mutations and polymorphisms in the gene encoding human galactose-1-phosphate uridyltransferase. Proc Natl Acad Sci USA 88:2633–2637

Address for correspondence and reprints: Dr. Louis J. Elsas II, Division of Medical Genetics, Emory University School of Medicine, 2040 Ridgewood Drive Northeast, Atlanta, GA 30322.

0002-9297/95/5704-0039$02.00

Am. J. Hum. Genet. 57:980–981, 1995

Reply to Reichardt

To the Editor:

In his letter Dr. Reichardt raises a number of issues concerning two articles recently published in the *Journal* (Elsas et al. 1995; Fridovich-Keil et al. 1995). I have addressed each of the issues relating to Fridovich-Keil et al., below:

1. Dr. Reichardt suggests that we have misattributed to him the "assumption" that nonconserved residues (within the galactose-1-phosphate uridyl transferase [GALT] sequence) are likely to be "unimportant" for function. In fact, our article described this idea as a "commonly invoked assumption" (Fridovich-Keil et al. 1995, p. 645). With regard to the R148W substitution, the Reichardt et al. article (1992*a*, p. 599) states that "the mutated arginine is not conserved. . . . However, this mutation results in an unstable polypeptide and, therefore, the mutated arginine 148 is probably not enzymatically important." We certainly apologize for any misunderstandings resulting from our interpretation, or possible misinterpretation of these words.

2. Dr. Reichardt's second point is that Fridovich-Keil et al. (1995) "disregard" his mammalian expression data "when they are at odds with" our yeast system. We have at no point disregarded his data, and in fact went out of our way to cite his work and to offer logical explanations that might account for the differences observed using the two systems. We agree with Dr. Reichardt that the most important comparisons, however, are not between the two model systems (COS cells vs. yeast), but between each of these systems and patient cells. For both of the mutations described in Fridovich-Keil et al. (1995) that have also been modeled in the COS cell system, Q188R and S135L, the activity data derived from the yeast system more closely parallel what is seen in patient cells than do the COS cell data. To summarize, Q188R-GALT expressed in yeast encodes null activity (Fridovich-Keil and Jinks-Robertson 1993), and S135L-GALT encodes ~5% wild-type GALT activity (Fridovich-Keil et al. 1995). According to Dr.

Reichardt's COS cell system, Q188R-GALT retains ~10% residual activity (Reichardt et al. 1991), and S135L-GALT is "a polymorphism because it encodes normal GALT specific activity" (Reichardt et al. 1992*b*, p. 5432). Hemolysates from seven Q188R homozygotes (Elsas et al. 1994) and lymphoblast extracts from three unrelated Q188R homozygotes (Fridovich-Keil and Jinks-Robertson 1993) have all demonstrated a complete absence of GALT activity, and both hemolysates and lymphoblast extracts from patients (or carriers) with the S135L mutation have demonstrated extremely low, if any, GALT activity attributable to the S135L allele (Fridovich-Keil et al. 1995).

With regard to Dr. Reichardt's concern over the current lack of published data illustrating detection of human GALT protein (not just activity) in yeast; these data were alluded to in an abstract published in the *Journal* in 1994 (Fridovich-Keil et al. 1994), and some of these data are also included in a manuscript currently under review (J. L. Fridovich-Keil, B. B. Quimby, L. Wells, L. A. Mazur, and J. P. Elsevier, unpublished data).

3. Dr. Reichardt is correct that "no reports on expression of the common N314D mutation in yeast have been published." These data are, however, included in the manuscript under review. Dr. Elsas referred to these data when he stated that "N314D in yeast encodes near normal activity" (see letter above). Similarly, Reichardt and Woo (1991, p. 2636) have reported from their COS cell work that "the aspartate-314 polymorphism actually increases the specific activity of the GALT enzyme."

I would like to stress, however, that regardless of how many times the yeast system may "give the right answer" in terms of modeling human biochemical phenotypes, it, as with any other model system, including one that uses mammalian cells, is still a model, and must always be interpreted with caution as such.

JUDITH L. FRIDOVICH-KEIL
Department of Genetics and Molecular Medicine
Emory University School of Medicine
Atlanta

References

Elsas LJ, Dembure PP, Langley SD, Paulk EM, Hjelm LN, Fridovich-Keil JL (1994) A common mutation associated with the Duarte Galactosemia allele. Am J Hum Genet 54:1030–1036

Elsas LJ, Langley S, Steele E, Evinger J, Fridovich-Keil JL, Brown A, Singh R, et al (1995) Galactosemia: a strategy to identify new biochemical phenotypes and molecular genotypes. Am J Hum Genet 56:630–639

Fridovich-Keil JL, Elsevier JP, Mazur LA, Quimby BB (1994) Studies of subunit interactions in the human enzyme gal-1-uridyltransferase. Am J Hum Genet Suppl 55:A342

Fridovich-Keil JL, Jinks-Robertson S (1993) A yeast expression system for human galactose-1-phosphate uridylyltransferase. Proc Natl Acad Sci USA 90:398–402

Fridovich-Keil JL, Langley SD, Mazur LA, Lennon JC, Dembure PP, Elsas LJ II (1995) Identification and functional analysis of three distinct mutations in the human galactose-1-phosphate uridyltransferase gene associated with galactosemia in a single family. Am J Hum Genet 56:640–646

Reichardt JKV, Belmont JW, Levy HL, Woo SLC (1992*a*) Characterization of two missense mutations in human galactose-1-phosphate uridyltransferase: different molecular mechanisms for galactosemia. Genomics 12:596–600

Reichardt JKV, Levy HL, Woo SLC (1992*b*) Molecular characterization of two galactosemia mutations and one polymorphism: implications for structure-function analysis of human galactose-1-phosphate uridyltransferase. Biochemistry 31:5430–5433

Reichardt J, Packman S, Woo SLC (1991) Molecular characterization of two galactosemia mutations: correlation of mutations with highly conserved domains in galactose-1-phosphate uridyl transferase. Am J Hum Genet 49:860–867

Reichardt JKV, Woo SLC (1991) Molecular basis of galactosemia: mutations and polymorphisms in the gene encoding human galactose-1-phosphate uridylyltransferase. Proc Natl Acad Sci USA 88:2633–2637

Address for correspondence: Dr. Judith L. Fridovich-Keil, Department of Genetics and Molecular Medicine, Emory University School of Medicine, 1462 Clifton Road N.E., Room 432, Atlanta, GA 30322.

0002-9297/95/5704-0040$02.00

Am. J. Hum. Genet. 57:981–982, 1995

Defining "Proband"

To the Editor:

Bennett et al. (1995) presented recommendations of the Pedigree Standardization Task Force of the National Society of Genetic Counselors. As the authors clearly state, the importance of standardized nomenclature is without question for reducing the chances of incorrect interpretation of patient and family information and for facillitating communication between researchers and clinicians involved in genetic family studies.

Most of the recommendations are appropriate and allow recording of the complex situations that can result from today's reproductive and diagnostic technologies. However, there is a problem with the definition presented for "proband" (Bennett et al. 1995, fig. 1, p. 746), i.e., the "first affected family member coming to medical attention".

This definition illustrates the dichotomy that seems to have developed in the use of the term "proband" by clinicians versus researchers. The Bennett et al. (1995) definition is the one that clinicians seem to have evolved