To the Editor:

Pyruvate kinase (PK) deficiency is the most common disorder among glycolytic enzyme defect causing hereditary hemolytic anemia. Since the first case was reported 30 years ago, over 300 cases have been reported. In 1988, we isolated the cDNA clones for human L-type PK, and last year we reported the cDNA cloning of R-type PK and the single amino acid substitution (Thr194 to Met) caused by a point mutation (1151C to T) in the R-type PK cDNA of the Japanese homozygous patients, PK Tokyo. We then expressed the L-type PK cDNA carrying the point mutation in Escherichia coli, and successfully reproduced the thermolabile, electrophoretically slow-moving character of the variant PK.

In the recent issue of this journal, the point mutations found in Turkish and Lebanese patients were published without citing our report. We would like to send our best respects to their study. However, we object to their discussion.

As they stated in the report, they did not sequence the whole coding sequences of the L-gene which transcribed to the R-type PK mRNA. Because there is no evidence that both erythrocyte (R-type) and liver (L-type) PK are affected in their cases, whole nucleotide sequence encoding R-type PK must be examined for detecting mutation(s). Although they sequenced all of the residues consisting of the active site of the enzyme, mutations that cause severe phenotype do not necessarily exist in the active site. Moreover, they did not perform any expression experiments that were essential, particularly in their cases, to distinguish mutations from polymorphisms. The discussion based on the nucleotide changes found by the partial sequence of the gene seems to be too speculative.

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REFERENCES

human R-type pyruvate kinase (PK) and identification of a single amino acid substitution (Thr to Met) affecting enzymatic stability in PK variant (PK Tokyo) associated with hereditary hemolytic anemia. Proc Natl Acad Sci USA 1991 (in press)


RESPONSE

The pyruvate kinase (PK) isozymes in liver (L-type) and erythrocyte (R-type) are transcribed from the same gene and are identical in 541 amino acids. However, in the R-type isozyme there are an additional 31 amino acids at the N-terminus, presumably due to alternative promoter usage. The N-terminal domain does not contribute to the active site of the protein, as shown by structural analysis. This is emphasized by the finding that R- and L-type isozymes, except for different molecular weights, have identical biochemical properties as shown by many approaches. In our recent publication in this journal we describe two distinct point mutations found in the L-type PK genes of two children with hemolytic anemia caused by homozygous PK deficiency.

In PK-Linz (the Turkish child), a unique change in the substrate binding pattern of the variant enzyme was found. Our conclusion that the mutation which we showed in this variant is the reason for the altered properties of the enzyme rests on the following evidence. We have sequenced 95% of the PK coding sequence of this patient, and it was the only deviation from the published sequence that we found. Importantly, the residue involved has been shown before to be part of the active site of the enzyme. The basic arginine 132 is substituted by a neutral cysteine resulting in a change of the overall charge density at a position within the active site of the enzyme. Both parents are healthy and are heterozygous at this position with one mutated and one normal allele present. Therefore, we conclude that there is merely a remote theoretical chance that a second homozygous mutation responsible for the disease exists in the remaining 5% of the sequence (that again the parents would have to be heterozygous for), and that the Arg-Cys exchange in the active site which we have documented would actually be neutral.

In PK-Beirut we found a threonine to methionine exchange at position 353. The residue affected is clearly located outside of the substrate binding site and, consistent with this location, enzyme kinetic parameters are close to normal. Precisely the same mutation has apparently also been identified in PK-Tokyo by Kanno et al (note that because the R-type enzyme is 31 residues longer, our position 353 in the L-type enzyme corresponds to position 384 in the R-type enzyme).

Finally, I feel obligated to say that we are fully aware of the pioneering work of Dr Miwa's group concerning PK deficiency, and in our recent report we believe that we have made this point clear and that we have given proper credit. Citations 1, 5, and 7 refer to work from their laboratory. However, the report referred to by Kanno et al is actually not a manuscript but merely a congress meeting abstract that was not available in international libraries when we prepared our manuscript. (The ASH meeting was held in November/December 1990 and our manuscript was received by the Blood journal on January 17, 1991.) Nevertheless, we sincerely regret not having been aware of these results, for obviously the independent finding of the same mutation in two unrelated individuals suffering from the same disease corroborates the significance of our data.

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REFERENCES


Single-nucleotide substitution in pyruvate kinase deficiency [letter; comment]

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