LETTERS TO THE EDITOR

Strong evidence that the N21I substitution in the cationic trypsinogen gene causes disease in hereditary pancreatitis

EDITOR,—We read with great interest the paper by Nishimori *et al* (*Gut* 1999;44:259–263) that reported the presence of two cationic trypsinogen gene mutations, the N21I in exon 2 and the R117H in exon 3, in two Japanese families with hereditary pancreatitis. Furthermore, this report showed that these mutations are worldwide and that the defect in cationic trypsinogen is important in the pathogenesis of hereditary pancreatitis. However, these authors questioned whether the N21I mutation could alter the molecular nature of the enzyme dramatically to produce pancreatitis, based on the observation that the first 38 N-terminal amino acids of the secreted cationic trypsinogen containing this mutation are identical to that of the native anionic trypsinogen isoform. This comparison may be important, but any possible functional effect of the mutation should be evaluated in the context of the tertiary structure of the native cationic trypsinogen itself.

The N21I mutation was first identified in two American families with hereditary pancreatitis by Gorry and colleagues¹ in 1997; it has now been shown to be present in three German² and two French³ families. The proposal that N21I also causes disease is strongly supported by the huge body of available research data, the linkage analysis, its perfect segregation with the dominant disease phenotype in several unrelated families with hereditary pancreatitis worldwide, its absence in the control chromosomes, and the previously identified R117H mutation in the same gene.^{4,5}

At issue is how to interpret the mutation and, in this regard, it is important to note that the physicochemical characteristics of isoleucine are quite different from those of asparagine. These differences could significantly affect the local conformational structure. Through computer analysis, Nishimori *et al* predicted that the N21I substitution could change the native turn structure of the flanking region of wild type cationic trypsinogen to sheet structure, on mutant type enzyme. Moreover, the N21 is located on the surface of the molecule and near the R117 site, revealed by the crystallographic structure of trypsin.⁶ Thus, taking into account of the self destructive model of R117H,⁴ it is reasonable to predict that the N21I mutation may impair trypsin inactivation through altering the accessibility of trypsin-like enzymes to R117, and/or pro-

tecting the adjacent C22-C157 disulphide bond to prolong survival of trypsin after limited hydrolysis.¹

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- 1 Gorry MC, Ghabaizadeh D, Furey W, *et al*. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. *Gastroenterology* 1997;113:1063–8.
- 2 Teich N, Mossner J, Keim V. Screening for mutations of the cationic trypsinogen gene: are they of relevance in chronic alcoholic pancreatitis? *Gut* 1999;44:13–16.
- 3 Ferec C, Raguene O, Salomon R, *et al*. Mutations in the cationic trypsinogen gene and evidence for genetic heterogeneity in hereditary pancreatitis. *J Med Genet* 1999;36:228–32.
- 4 Whitcomb DC, Gorry MC, Preston RA, *et al*. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996;14:141–5.
- 5 Cotton RGH, Scriver CR. Proof of "disease causing" mutation. *Hum Mutat* 1998;12:1–3.
- 6 Whitcomb DC. The first international symposium on hereditary pancreatitis. *Pancreas* 1999;18:1–12.

Speed of gastric emptying and metabolism of ethanol

EDITOR,—There seem to be several possible problems with the recent paper by Oneta and colleagues (*Gut* 1998;43:612–619). Firstly, blood ethanol concentrations said to result from an ethanol dosage of 0.225 g/kg were in the range of 0.2–0.3 mg/100 ml (figs 3–6 of Oneta *et al*'s paper). These values are about 1% of the expected blood concentration for this dosage; presumably there was a 100-fold error in the labelling of the vertical axes of these four figures. Secondly, differences in the area under the curve were used to assess first pass metabolism when it has been repeatedly pointed out that this technique cannot be used when there is saturation of metabolism, as is the case with the ethanol dosage given. The authors irrationally justify their use of this inaccurate methodology by indicating that there may be problems with other methods. Furthermore, an appreciable fraction of the difference in the area under the curve seen with intravenous versus oral administration of ethanol seems to be attributable to incomplete distribution of the more rapidly delivered intravenous ethanol rather than a difference in metabolism. Finally, the authors found a positive correlation between first pass metabolism and gastric alcohol dehydrogenase (ADH) activity, which just missed reaching statistical significance ($p=0.057$ in antral biopsy samples). This positive correlation seems to be almost totally accounted for by the values of two subjects who had very high negative first pass metabolisms of –25% and –50%, respectively. Because, of course, there cannot be negative first pass metabolism, the values of these two subjects are suspect.

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Reply

EDITOR,—We appreciate Furne and Levitt's comments on our paper and the error they noticed in figures 3–6. The concentrations on the vertical axes should indeed be 100-fold higher than published; they should read 20 mg/100 ml instead of 0.2 mg/100 ml. We regret this mislabelling; however, neither first pass metabolism of ethanol (given in percentages) nor its modulation by changes in gastric emptying are affected by this.

Differences in the area under the ethanol concentration time curves have been used previously to describe first pass metabolism of ethanol and give reliable approximations, although we are aware that this measurement has some disadvantages. Others have also used these differences,^{1,2} as mentioned in the discussion of our paper and which need not be repeated here. Ethanol was given intravenously over 30 minutes which allows some time for distribution. However, it is true that the input rate of ethanol affects its bioavailability and the area under the ethanol concentration time curve. If the input rate of ethanol through the different administration rates is similar—for example, comparable time profiles, the area under the curve method should still be valid.³

We did not find a significant positive correlation between first pass metabolism and gastric ADH activity as discussed in the results section of our paper, and it was emphasised in the discussion that such a correlation could not be confirmed. Thus, it did not seem necessary to exclude the two (statistically questionable) subjects with negative first pass metabolism in order to show that there was no correlation. Furthermore, it was not the purpose of this paper to give evidence for such a correlation. In contrast, changes in gastric emptying time may explain changes in first pass metabolism.

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- 1 Frezza M, DiPadova C, Pozzato G, *et al*. High blood alcohol levels in women: the role of decreased gastric alcohol dehydrogenase activity and first pass metabolism. *N Engl J Med* 1990;322:95–9.
- 2 Brown AS, Fiatarone JR, Wood P, *et al*. The effect of gastritis on human gastric alcohol dehydrogenase activity and ethanol metabolism. *Aliment Pharmacol Ther* 1995;9:57–61.
- 3 Ammon E, Schaefer C, Hoffmann U, *et al*. Disposition and first pass metabolism of ethanol in humans: is it gastric or hepatic and does it depend on gender? *Clin Pharmacol Ther* 1996;59:503–13.