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Association of inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection gastric cancer risk may be due to faulty primer design

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Abstract

Rafiei *et al* recently described an association between the presence of the C150T polymorphism of the inducible nitric oxide synthase (*iNOS*) gene and *Helicobacter pylori* (*H. pylori*) induced gastric cancer. When we used primer-BLAST to find the polymerase chain reaction (PCR) product that would be generated by the primers used by these authors no products against any of the sequences present in the GenBank database were found. Further analysis of the *iNOS* sequences present in the GenBank suggest that the result from their study might come from a faulty primer design and may thus represent an artifact. Alternatively they may be correct and have identified a truly interesting explanation for the mechanism whereby *H. pylori* induces gastric cancer but some additional experiments would be in order to exclude the possibility of a PCR artifact.

Key words: *Helicobacter pylori*; Polymorphism; Inducible nitric oxide synthase

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TO THE EDITOR

Rafiei *et al*[1] recently published an interesting article in the *World Journal of Gastroenterology* entitled "Inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection affect gastric cancer risk". In this study, they investigated the possible association between inducible nitric oxide synthase (*iNOS*) genotype and gastric cancer among the 329 patients from northern Iran. They found a clear association between the presence of C150T polymorphism of the *iNOS* gene and the presence of gastric cancer in *Helicobacter pylori* (*H. pylori*) infected patients. Strikingly they found an overall frequency of the 150T allele of approximately 25% of the individuals (both controls and cases) tested[1], which came as a surprise to us for the original description by Shen *et al*[2] mentions a frequency of 13%. This discrepancy could obviously be explained by genetic differences in the ethnic populations but is not discussed by Rafiei *et al*[1] in their paper and this prompted us to carefully study the methods used to establish the C150T polymorphism. In order to establish the polymorphism the authors used the method that was described by Shen *et al*[3]. Briefly, a polymerase chain reaction (PCR) was used to produce a 255 bp amplicon of the *iNOS* gene that was subsequently digested with *Tsp* 509 I for determination of the C150T polymorphism. When we used primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast) to find the PCR product that would be generated by these primers this program was unable to find any products against

any of the sequences present in the GenBank database. Performing a manual search against the Assembled RefSeq human Genomes database (Build 37.3) did only result in two *iNOS* hit for the forward primer (database entry ref NT 010799.15 and NW 001838430.2) and no hits for the reverse primer. When we subsequently performed a basic BLAST search with the separate primers we only found hits with the forward primer in 7/9 *iNOS* sequences in the Genbank database version 25 September 2012 and no hits with the reverse primer. Further analysis of the 9 *iNOS* sequences present in the GenBank revealed that a *Tsp* 509 I site at the expected distance from the forward primer in all but one sequence (GenBank accession no DQ149843.1). This sequence (DQ149843.1) was submitted by Shen *et al*[3], the group who originally identified the C150T polymorphism. Interestingly the 3' end of this 288 bp sequence (supposedly containing the binding site for the reverse primer) differs considerably from all but one of the other *iNOS* sequences in the database; i.e., an 288 *iNOS* sequence with accession no X85772.1. This closely homologous sequence (X85772.1) differs only in the C150T position and was submitted to the database by Xu *et al*[4]. A possible explanation for the aberrant 3' end of this sequence is that the sequence from Xu *et al*[4] is assembled from two cosmid clones and as the sequence differences seem to be at the link region of these two cosmids it may represent a cloning artifact. Careful analysis of these two sequences in fact does show some similarity to the reverse primer at the expected position, but the two 3' bases clearly differ, making it almost impossible to produce a PCR product with this primer. Conclusively, it looks like the unique results of these two studies[1,2] might come from a faulty primer design and may thus represent an artifact. Alternatively they may be correct and present a truly interesting clue for the mechanism whereby *H. pylori* induces gastric cancer but we feel that some additional experiments would be in order to exclude the possible artifact as mentioned above.

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