

## Response to Abadi and Kusters, *World J Gastroenterol* 19: 429-430

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### Abstract

In a recent study, Rafiei *et al*, reported a link between a C150T polymorphism in the human inducible nitric oxide gene and *Helicobacter pylori* infection as a risk factor for gastric cancer among an Iranian population. Subsequently, Abadi and Kusters published a letter to the editor questioning the validity of the study because of a supposed flaw in primer design. Here we respond to the claims of Abadi and Kusters and show that the results reported in the original article are valid.

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**Key words:** Inducible nitric oxide synthetase; *Helicobacter pylori*; Gastric cancer

**Core tip:** In a recent Letter to the Editor, Abadi and Kusters brought into question the validity of a study published by Rafiei *et al*. Herein we respond to the claims made by Abadi and Kusters, and show that the results reported in the article originally published by Rafiei *et al*, are valid.

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### TO THE EDITOR

In a recent letter to the editor, entitled “Association of inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection gastric cancer risk may be due to faulty primer design”<sup>[1]</sup>, Amin Talebi Bezmin Abadi and Johannes Kusters questioned the validity of the results published in our manuscript<sup>[2]</sup>, the design of which was based on a previous study<sup>[3]</sup>. In their letter<sup>[1]</sup>, the authors appraised our article<sup>[2]</sup> by two main points; variation in T allele frequency and primer design. In response to their first comment regarding the T allele frequency seen in our population, we stand by the conclusion that genetic polymorphisms depend on ethnic differences; the frequency of mutant genotypes varies in different human populations. Therefore, it is likely no surprise that the frequency of the T allele seen in our Caucasian population<sup>[2]</sup> differed from that of the East Asian population<sup>[3]</sup>.

In regards to the second point made by Abadi *et al*<sup>[1]</sup>, concerning the primer specificity, we note that there is a misprint in one primer sequence in our article<sup>[2]</sup>. The primer sequence published in our paper (5'-GTCTCTG-CGGGTCTGAAG-3') differs from that of Shen *et al*<sup>[3]</sup> in that it is missing two base pairs in the 3' end of the sequence. An erratum has been submitted to the journal to note the misprinted primer sequence [On page 4919<sup>[2]</sup>, the primer sequence given for iNOS-R (5'-174 GTCTCTGCGGGTCTGAAG-3') is missing two nucleotides and should appear as iNOS-R (5'-GTCTCTGC-GGGTCTGAGAAG-3')]. However, this 2 base misprint appears not to be the core issue since the correct primer sequence (5'-GTCTCTGCGGGTCTGAGAAG-3') was

GCCCATATGTAACCAACTTCGGTGGGCTGTGAGCCTTCTCCTGCAAGCTGTGGCCAGGTTTCC  
 AG AAGAAAGGAAAAACAGTGTTTTATCATCCTGGCTTGAGAACTCTGTATTCCCTCTCTTTCTAGAAAC  
 TGAAGAAATCGCTCTTCATGCTGAAAGAGCTCAACAACAAATTCAGGTAAGCTTTCTCCGGTGTCTTAC  
 TCCTAGCCCTGCCCTGGGGCCCCAGTGTCTGGTACAGGGGATAGCTCTGGGTGTACACAGGGGGTCTTC  
**TCAGACCCGCAGAGAC**ACAGAGC

**Figure 1 Location of inducible nitric oxide synthetase-specific primer annealing sites.** The sequence of exon 16 of the inducible nitric oxide synthetase (*iNOS*) gene (<http://www.ncbi.nlm.nih.gov/nuccore/x85772>) is shown. The sequence of forward primer used in our study and by Shen *et al*<sup>[3]</sup> is in bold italics at the 5' end of the sequence above: 5'-TGTAACCAACTTCGGTGGT-3'. The sequence of the reverse primer used in our study and by Shen *et al*<sup>[3]</sup> (5'-GTCTCTGCGGGTCTGAGAAG-3') is the reverse compliment of the bold italicized bases in the 3' end of the sequence above. The C150T polymorphism is indicated by the bold underlined "C".

previously published by Shen *et al*<sup>[3]</sup> and this publication was also called into question by Abadi and Kusters. We note that as shown in Figure 1, both of the primers used in the study are specific to exon 16 of the *iNOS* gene (Figure 1), which has been mapped to chromosome 17q11.2. Direct sequence analysis of exon 16 as submitted to GenBank by Shen *et al*<sup>[3]</sup> (<http://www.ncbi.nlm.nih.gov/nuccore/x85772>) shows that the primers used in our studies are completely conserved in this sequence. Furthermore, blast analysis of the inducible nitric oxidase synthase against the human database returns 3 hits to Homo sapiens chromosome 17. Analysis of the resulting alignments shows complete conservation of the forward primer in all 3 samples. Conservation of the reverse primer is not as high; the 5' end of the primer shows several mismatches. However, the last 9 nucleotides found in the 3' end of the primer are completely conserved across all 3 samples. Thus, there should be no overt obstacles to

amplification of the gene in question. In conclusion, we stand by the results reported in our original study<sup>[2]</sup> and posit that the supposition by Abadi and Kusters that our study design is flawed is incorrect.

## REFERENCES

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