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EDITORIAL

Hardy–Weinberg disequilibrium and association study of insertion/deletion polymorphism of ACE gene and Alzheimer’s disease in Egyptian patients



We thank Dr Saadat’s letter to the editor [1] for his critical remarks on our study. To clarify, Dr. Saadat raised concerns with respect to genotyping errors based on the observed deviation from Hardy–Weinberg equilibrium for this particular polymorphism in our trial. Genotyping error is indeed one of the many possible sources of Hardy–Weinberg disequilibrium. To exclude this possibility and because the *D* allele in heterozygous samples is preferentially amplified, as reported in our publication [2], each sample that found to have the *DD* genotype was subjected to a second, independent PCR amplification with a primer pair that recognizes an insertion-specific sequence, with identical PCR conditions except for an annealing temperature of 67 °C. The reaction yields a 335-bp amplicon only in the presence of an *I* allele and no product in samples homozygous for *DD*. This procedure correctly identified samples with the *DI* genotype that are misclassified as *DD* with the insertion-spanning primers, which we concern it as an insuring test of our results.

Although testing for Hardy–Weinberg equilibrium is used as some quality-control measure, particularly in case-control gene association studies, it cannot be used to detect genotyping error [3,4]. Genotyping errors are generally small and do not generate sufficient deviations from Hardy–Weinberg equilibrium to be detected. In the case of a reduced number of observed heterozygous patients, as may occur in the presence of poor amplification of one of the alleles, large sample sizes are necessary to detect deviations from Hardy–Weinberg equilibrium. Therefore, testing for Hardy–Weinberg equilibrium is an unreliable tool to identify genotyping errors. Conversely, the presence of Hardy–Weinberg equilibrium does not rule out that genotyping errors might have occurred. Hence, it seems unlikely that genotyping error is the source of the Hardy–Weinberg disequilibrium observed in our study. Because approximately 10% of all genotype–phenotype association studies show deviation from Hardy–Weinberg equilibrium, the results of our trial cannot be considered “abnormal” [5]. A selection bias (population stratification) may have occurred because of inclusion and exclusion criteria of this randomized trial.

Even when deviations from HWE are detected for marker loci, without additional experimentation it is not possible to unequivocally implicate genotyping error as the cause. The power and sample sizes necessary to detect deviations from HWE for single-nucleotide polymorphism (SNP) data are examined for a variety of genotyping errors and pseudo-SNP models. For the majority of genotyping models examined, the power is poor to detect deviations from HWE. For example, for 1000 controls, if an allele with a frequency of 0.1 fails to amplify for 28% of the heterozygous genotypes producing a sample error rate of 0.05, the power is 0.51 to detect a deviation from HWE at an alpha level of 0.05. On the other hand, the detection of deviations from HWE for pseudo-SNPs for the majority of models examined produces a power of > 0.8 for sample sizes as small as 50 individuals [4].

Therefore, testing for Hardy–Weinberg equilibrium is an unreliable tool to identify genotyping errors. Conversely, the presence of Hardy–Weinberg equilibrium does not rule out that genotyping errors might have occurred. Measures of departure from HWE may be useful for detecting genotyping error, but this needs to be confirmed in other real datasets [6].

Of note, Hardy–Weinberg disequilibrium that is caused by most interesting biologic phenomena typically results in excess homozygosity, as observed in our study [2].

We noticed a high diversity in different Egyptian studies of the ACE I/D polymorphism papers mentioned by Dr. Saadat and also on other Egyptian studies, for example, as regard *D* allele frequency it was 30.7% and 38.6% in references [7] and [8] respectively, but it was 48.8% and 53% in references [9] and [10] respectively, while it was 70.6%, 71.2%, 83% and 91.3% in references [11–13] and [14] respectively. From the previous findings, we conclude that there is a serious need of more studies of the Egyptian population on larger scales. However, we agree with Dr. Saadat that violation of Hardy–Weinberg equilibrium in our study population implies a selected rather than a random sample, invalidating direct comparisons with other populations. Therefore, we share his view that our results should be regarded with caution. Our findings should be confirmed in future prospective larger-scale clinical

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trials, specifically designed and adequately powered to detect genotype-specific differences in Alzheimer patients.

For the issue concerning the plasma ACE activity, for the first insight into the results, we also thought there will be a significant difference of the activity between AD patient groups, but statistics revealed no significant difference which could be referred to the number of AD patients having the II genotype.

Conflicts of interest

The author declares no conflict of interest. There is no financial and personal relationship with other people or organizations that could inappropriately influence this work.

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References

- [1] Saadat M. Hardy-Weinberg equilibrium and association study of insertion/deletion polymorphism of ACE gene and Alzheimer's disease in Egyptian patients. *Egypt J Med Hum Genet* 2014;15(4):405–6.
- [2] Hassanin OM, Moustafa M, El Masry TM. Association of insertion–deletion polymorphism of ACE gene and Alzheimer's disease in Egyptian patients. *Egypt J Med Hum Genet* 2014;15(4):355–60.
- [3] Zou GY, Donner A. The merits of testing Hardy–Weinberg equilibrium in the analysis of unmatched case-control data: a cautionary note. *Ann Hum Genet* 2006;70:923–33.
- [4] Leal SM. Detection of genotyping errors and pseudo-SNPs via deviations from Hardy–Weinberg equilibrium. *Genet Epidemiol* 2005;29:204–14.
- [5] Trikalinos TA, Salanti G, Khoury MJ, Ioannidis JP. Impact of violations and deviations in Hardy–Weinberg equilibrium on postulated gene-disease associations. *Am J Epidemiol* 2006;163:300–9.
- [6] Attia John, Thakkinian Ammarin, McElduff Patrick, Milne Elizabeth, Dawson Somer, Scott Rodney J, et al. Detecting genotyping error using measures of degree of Hardy–Weinberg disequilibrium. *Stat Appl Genet Mol Biol* 2010; 9(1) [Art. 5].
- [7] Elshamaa MF, Sabry SM, Bazaraa HM, Koura HM, Elghoroury EA, Kantoush NA, et al. Genetic polymorphism of ACE and the angiotensin II type1 receptor genes in children with chronic kidney disease. *J Inflamm (Lond)* 2011;8:20.
- [8] Morsy MM, Abdelaziz NA, Boghdady AM, Ahmed H, Abu Elfadl EM, Ismail MA. Angiotensin converting enzyme DD genotype is associated with development of rheumatic heart disease in Egyptian children. *Rheumatol Int* 2011;31: 17–21.
- [9] Badr Soheir, Raboh Naglaa Raafat Abd, Ali Samia. Angiotensinogen M235T and angiotensin-converting enzyme I/D gene polymorphism and their association with type 2 diabetes in Egypt. *Life Sci J* 2012;9(2), <http://www.lifesciencesite.com>.
- [10] El-Shafei MS, Farres MN, Shahin RY. Evaluation of angiotensin converting enzyme gene polymorphism and susceptibility to bronchial asthma among Egyptians. *Allergol Immunopathol (Madr)* 2012;40:275–80.
- [11] Raslan HM, Amr KS, Elhosary YA, Ezzat WM, Abdullah NA, El-Batae HE. Possible role of angiotensin-converting enzyme polymorphism on progression of hepatic fibrosis in chronic hepatitis C virus infection. *Trans R Soc Trop Med Hyg* 2011; 105:396–400.
- [12] Settin Ahmad, ElBaz Rizk, Abbas Amr, Abd-Al-Samad Ayman, Noaman Ahmed. Angiotensin-converting enzyme gene insertion/deletion polymorphism in Egyptian patients with myocardial infarction. *J Renin Angiotensin Aldosterone Syst* 2009;10:96, <http://jra.sagepub.com/content/10/2/96>.
- [13] Abde-Aziz AF, Afaf EL-Said, Khaled EL-Dahshan, Basma AL-Sayed Ebead. Association of angiotensin-i-converting enzyme (ace) insertion/deletion gene polymorphism with end stage renal disease in Egyptian patients. *SDI Paper Template Version 1 1.6* Date 11.10.2012.
- [14] Abdel-Aziz AF, Elsaid A, Elmongy R, Abd-Al-Samad A, Elwaseef AM. Angiotensin converting enzyme (ACE) insertion/deletion polymorphism in Egyptian patients with coronary artery disease. *Int J Biochem Res* 2012;2:106–19.

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