Biallelic genotype distributions in papers published in Gut between 1998 and 2003: altered conclusions after recalculating the Hardy-Weinberg equilibrium

The Hardy-Weinberg law presents a mathematical statement that describes the relationship between gene frequencies and genotype frequencies; gene frequencies at a locus in a randomly interbreeding diploid population and population genotype frequencies remain constant from generation to generation if mating is random and mutation, selection, and migration do not occur. The law states a fundamental principle of population genetics that is approximately true for small populations, and holds with increasing exactness for large populations. Should the frequencies be perturbed for any reason, they will come to the expected equilibrium frequencies after one generation of random mating.

The Hardy-Weinberg law can be used for analytical purposes. It is suitable to test the hypothesis of panmixia and evolutionary stasis. Moreover, it represents a null hypothesis to test in genetic studies. However, according to our personal experience, data for Hardy-Weinberg equilibrium (HWE) calculations in studied populations are not always presented in articles with data on the genotype distributions of biallelic polymorphisms of Mendelian inheritance. In this retrospective survey, we tested in papers published in Gut, if this important and qualifying law was checked in studies investigating genetic polymorphisms between 1998 and April 2003.

We collected genotype distributions published in papers in Gut from 1998 (volume 42) to 2003 (volume 52). Of 2389 total publications, we found 69 where genetic polymorphisms were part of the study. Of these, those articles that fulfilled the following criteria were selected: investigation of biallelic genetic polymorphism with Mendelian inheritance; use of healthy reference population in the study; and availability of genotype distribution data.

We recalculated HWE in each paper and in each study group. For this purpose, we used Arlequin software (http://anthropologie.unige.ch/arlequin/). The level of statistical significance was set at p<0.05. Deviations from HWE were further confirmed by manual recalculation.

Twenty publications in Gut fulfilled the enrolment criteria; these publications presented data on 166 genotypes. However, only four papers (20%) reported that HWE calculations were performed and genotype distribution fulfilled HW criteria. Genotype distributions in healthy reference populations did not fulfill HWE in two publications (10%). In two reports, HWE was fulfilled in controls but failed in the diseased population and this fact was not reported. In one publication, HWE was not fulfilled either in the healthy reference or in the diseased populations. In summary, we found 11 genotype distributions in five publications of the studied 166 genotypes and 20 papers where calculation of the HW law (in control or in the investigated populations) would provide additional information. We present the results of HW calculations of these 11 polymorphisms in table 1.

There are several explanations why the observed genotype frequencies may deviate significantly from those expected by HW law and why genotypes have different likelihoods of being included, even when determination of genotype was methodologically correct. One or more of the assumptions of the HW law might be incorrect, non-random mating (inbreeding or an allele effect on the mating) or gene flow may have occurred, or selection operated. There could also be an error at sampling: the studied population was not well defined or the sample size may be too small. (Interestingly, the occurrence of HWE error was independent of the number of patients enrolled; the size of study populations in the affected papers ranged between 24 and 1207.)

If the genotype distribution in the control population misses the HWE, the results should be treated cautiously because the observed genotype distribution does not represent the genotype distribution in healthy (non-diseased) people and, therefore, conclusions cannot be drawn for the significance of the investigated polymorphism.

If the genotype distribution in the investigated (diseased) population does not fulfill the HW law (while the healthy reference population fulfills it), it might be supporting evidence for the correlation between genotype and disease. Unreported or weak associations can be detected by calculating the HWE, even when statistically significant differences between genotype distributions is not present.

In conclusion, we suggest that providing genotype distribution data together with detailed results of HWE calculations should be a must when results of population genetic studies are published.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Gene polymorphism</th>
<th>Subjects</th>
<th>Genotype distribution WW/WM/MM</th>
<th>HWE p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999:43:187–9</td>
<td>CITA-4 exon 1 position 49</td>
<td>Controls</td>
<td>62/47/21</td>
<td>0.034</td>
</tr>
<tr>
<td>2001:48:836–42</td>
<td>HFE H63D polymorphism haplotypes</td>
<td>Iron overload patients</td>
<td>178/85/60</td>
<td>0.001</td>
</tr>
<tr>
<td>2002:50:520–4</td>
<td>Methylenetetrahydrofolate reductase</td>
<td>Controls, 60 y&lt;sup&gt;−&lt;/sup&gt;</td>
<td>537/560/114</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td>Female colorectal cancer patients</td>
<td>204/229/34</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colorectal cancer stage: Dukes’ B</td>
<td>134/101/25</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colorectal cancer and p53 mutation</td>
<td>94/64/28</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WW/WM/MM HWE p value</td>
<td>148/116/39</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>2003:52:547–51</td>
<td>CYP1A1 exon7 polymorphism</td>
<td>Controls</td>
<td>122/22/5</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>EPHX exon 3</td>
<td>Controls</td>
<td>59/58/32</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>MOC2 gene</td>
<td>Controls</td>
<td>248/20/3</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>1007fs variant</td>
<td>Controls</td>
<td>265/12/7</td>
<td>0.129</td>
</tr>
</tbody>
</table>

WW, number of patients with homozygosity for wild allele; WM, number of patients with heterozygosity, NM, number of patients with homozygosity for mutant allele.
Author’s reply
Gyorthy et al addressed the relevant issue of deviation of biallelic genotype distributions from Hardy-Weinberg equilibrium and the interpretation of case control studies. They identified several papers that had been published in Gut in which the genotype distributions of cases, controls, or both groups deviated from Hardy-Weinberg equilibrium. They criticised the lack of reporting these observed deviations and they even suggested that the conclusions drawn from these studies should be reformulated.

We completely agree with Gyorthy et al that if the genotype distribution of the control population deviates from Hardy-Weinberg equilibrium, it should raise the suspicion of selection bias or genotyping error, and the suitability of such material for case control studies should be rechecked.

We also share the same view with these authors that if the genotype distribution in the diseased population does not fulfil the Hardy-Weinberg equilibrium, it may in fact constitute supporting evidence for the correlation between genotype and disease. Actually, in recent literature, it has been discussed that disease susceptibility loci could be searched for by testing for Hardy-Weinberg disequilibrium in a gene bank of affected individuals.1,2

In our paper, there was a deviation of the genotype distribution from Hardy-Weinberg equilibrium in the diseased subjects but not in controls. The probands were not related and the genotyping results were controlled for. In our patient group, homozygosity for the NOD2 1007fs mutation was slightly overrepresented in statistical terms, which could serve as an additional argument for the pathophysiological significance of this mutation or may represent a simple chance finding. In our opinion, the fact that the genotype frequencies of only the patients deviate from Hardy-Weinberg equilibrium does not alter the conclusions previously made.

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References

Author’s reply
Gyorthy et al calculated Hardy-Weinberg equilibrium (HWE) using data from our paper on H63D homozygotes.1 They stated that HWE was fulfilled in the control but not in the patient population. We do not think that our paper was eligible for the study of Gyorthy et al because haemochromatosis genotypes reported in our paper do not have a biallelic distribution. Data calculated in their report were based on results given in table 1 of our paper. In fact, in table 1 we listed the genotypes obtained with two different alleles of the HFE gene implicated in the pathogenesis of haemochromatosis (C282Y and H63D mutations) which have been shown to be exclusive of each other, along with the normal (wild-type) allele. Thus a total of three alleles have to be taken into account, C282Y, H63D, and wild-type. Therefore, HWE should have been calculated using a multiallelic system and not a biallelic system.

Furthermore, in table 1 of their paper, Gyorthy et al reported values used to compare the wild-type genotype (WW), homozygotes for the mutant allele (MM), and heterozygotes (WM) in each paper. For the wild-type allelic distribution of our paper, the authors took into account data relative to the C282Y mutation (homozygote (n = 178) or heterozygote (n = 85)) instead of the wild-type allele (homozygote (n = 334) or heterozygotes (n = 170)). Thus the values used to calculate the wild-type counterpart of the H63D mutation are not correct. This incorrect use of our results may obviously lead to erroneous conclusions.

Currently, it is well known that the various genotypes generated by both mutations (C282Y homozygosity, C282Y and H63D compound heterozygosity, and H63D homozygosity) and leading to iron overload have an incomplete penetrance.1 Our patient population was not a random population but a sample of patients referred for HFE analysis because of a diagnosis of iron overload. It would not have been surprising to find that, in this population, HWE was not fulfilled; abnormal genotypes were obviously overrepresented, probably according to the degree of penetrance of each.

In our paper, we did not verify if the HWE was applicable but we compared statistically the patient and control populations, which was a random well representative sample.1 We showed that there was a significant difference between the presence of H63D homozygotes among patients and controls. The statistical difference was much higher for C282Y homozygotes and compound heterozygotes, but this result was not reported as it was beyond the scope of the paper.

In conclusion, we do not think that HWE would have contributed to the analysis of the patient population in our paper except to check if the control population had been correctly chosen.

References

Table 1 HFE genotype of the whole population referred for a personal or family history of iron overload (patients) compared with a control group (controls) from our area (1276 unselected newborns)1

<table>
<thead>
<tr>
<th>HFE genotype</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>m1m1</td>
<td>178</td>
<td>18.4</td>
</tr>
<tr>
<td>m1N</td>
<td>141</td>
<td>14.6</td>
</tr>
<tr>
<td>m1m2</td>
<td>85</td>
<td>8.8</td>
</tr>
<tr>
<td>m2m2</td>
<td>60</td>
<td>6.2</td>
</tr>
<tr>
<td>m2N</td>
<td>170</td>
<td>17.6</td>
</tr>
<tr>
<td>NN</td>
<td>334</td>
<td>34.5</td>
</tr>
<tr>
<td>Total</td>
<td>968</td>
<td>100</td>
</tr>
</tbody>
</table>

m1, C282Y; m2, H63D; N, wild-type.

NOTICES

British Society of Gastroenterology
Paul Brown Travel Fellowships
The Paul Brown Travel Fellowships are awarded by the Endoscopy Committee of the BSG. They are intended to assist trainee gastroenterologists and established consultants in visits to units outside the United Kingdom for specialist experience and training in endoscopy.

Specialist registrars who have not achieved their CCST are expected to have the approval of their Postgraduate Dean and their Regional Training Director when they apply for a Travel Fellowship. Applicants are expected to provide confirmation that they have been accepted for training in the unit that they wish to visit.

Successful applicants will be expected to provide a brief written report to the Endoscopy Committee of the outcome of their visit.

Application forms are available from the British Society of Gastroenterology Office, 3 St Andrew’s Place, London NW1 4LB. Email: bsg@mailbox.ulcc.ac.uk

39th Annual Meeting of the European Association for the Study of the Liver
This meeting will be held on 15–19 April 2004 in Berlin, Germany. Further details:

European Postgraduate Gastro-surgical School (EPGS) Courses 2004
The EPGS at the Academic Medical Center of the University of Amsterdam will be holding the following courses during the year: ‘Benign Hepato-Biliary Disorders’ will be held on 22 & 23 April 2004, ‘Endosonography live in Amsterdam’ will be held on 2, 3 & 4 June 2004, and ‘Update in Coloproctology’ will be held on 28 & 29 October 2004. For further information, please contact: J Goedkoop (tel: (31) 616 3926; fax: (33) 267 5594; e-mail: j.goedkoop@amc.uva.nl; website: www.epgs.nl).

8th Southeast European Symposium of Paediatric Surgery
The 8th Southeast European Symposium of Paediatric Surgery will focus upon ‘Infectious Problems in Paediatric Surgery.’ The event will be held between 24–25 September 2004, at the University of Graz, Austria. For further information, please contact: Professor M E Hollwarth, Department of Paediatric Surgery, Medical University of Graz, Austria, Auenbruggerplatz 34, 8036 Graz (tel: + 43 316 385 3762; fax: tel: + 43 316 385 3775; e-mail: kinderchirurgie@uni-graz.at).

Biennial Conference of the Asian Pacific Association for Study of the Liver
A Biennial Conference of the Asian Pacific Association for the Study of Liver (APASL) will take place in New Delhi, India, on 11–15 December 2004. The deadline for submission of abstracts is 30 June 2004. For further information, please contact Dr S K Sarin, President APASL, Room No. 201, Academic Block, Department of Gastroenterology, G B Pant Hospital, New Delhi-110002, India (tel: 91-11-23232013; fax: 91-11-23219710; e-mail: welcome@apaslindia2004.com; website: www.apaslindia2004.com).
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