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TECHNICAL NOTE

Examination of Hardy-Weinberg equilibrium in papers of *Kidney International*: An underused tool

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Examination of Hardy-Weinberg equilibrium in papers of *Kidney International*: An underused tool.

Background. Population-genetic studies investigating genetic polymorphisms of Mendelian inheritance should always test whether the measured genotype frequencies deviate from the expected one. For this purpose Hardy-Weinberg (HW) criteria are generally used. If genotype distribution of control population misses HW equilibrium, the results should be treated cautiously because the observed genotype distribution in control population does not represent genotype distribution in the overall population. If HW criteria are not fulfilled in the investigated population, this may be further evidence for the correlation between genotype and investigated condition.

Methods. Between September, 1998, and September, 2003, we tested papers published in *Kidney International* if HW criteria were ordinarily and correctly checked in studies investigating genetic polymorphisms. Seventy-five genotype distributions of the selected 39 articles were reanalyzed.

Results. HW calculation was reportedly performed in 25 papers (64%). The observed genotype distribution deviated significantly from the expected one in three control, and in 16 patient populations and in three populations of association studies of 15 papers overall; however, this fact was not mentioned in 12 papers.

Conclusion. Although the deviation of genotype distribution from the expected one is important information, HW calculations are not performed routinely for each investigated subject groups in these papers investigating genetic polymorphisms.

During the past decade the association of genetic polymorphisms with the risk and development of renal diseases has been extensively studied. According to the PubMed database, more than 1300 papers containing the key words "human kidney" and "genetic polymorphism" had been published by November 2003.

Key words: Hardy-Weinberg equilibrium, gene polymorphism, population genetics.

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A significant part of these studies investigates biallelic genetic polymorphisms of Mendelian inheritance. While the accuracy of laboratory methods is excellent, population genetic studies may be still biased by other points of methodology, especially by the selection of subjects. If genotype distribution of enrolled groups is skewed and this fact remains undetected, the drawn conclusions are possibly biased. Population-genetic studies should therefore always test whether measured genotype frequencies deviate significantly from the expected one. For this purpose the Hardy-Weinberg (HW) criteria are generally used [1–6]. However, according to our personal experience, data about HW calculations in the studied populations are not always presented, or HW values are often miscalculated. Between 1998 and 2003, in this retrospective survey we tested papers published in *Kidney International* if HW criteria were ordinarily and correctly checked in studies investigating genetic polymorphisms.

METHODS

Subjects and methods

Using the Medline Database we selected 103 papers from past issues of *Kidney International*, which reports laboratory data about population genetics. Detailed genotype distribution was presented in 39 papers.

Data analysis

We systematically reviewed these articles, and two independent observers recalculated Hardy-Weinberg equilibrium in each paper in each study group. For this purpose the Arlequin software (http://anthro.unige.ch/arlequin/) was used [7]. The level of statistical significance was set at $P < 0.05$. Deviations from HW equilibrium were confirmed by manual recalculation.

We also tested whether authors calculated HW criteria and presented any data about the results of their calculations.

Table 1. Summary of papers with unreported Hardy-Weinberg (HW) values under $P = 0.05$

Reference	Genotype distribution			Total no. of subjects	Affected population	Investigated polymorphism	HW, *P* value
	AA	AB	BB				
17	3	87	169	259	Association study	AGT-235	0.028
8	8	22	0	30	Patient	TGF-β1 codon10	0.009
	10	31	132	173	Control	TNF-α -308	0.001
13	85	37	11	133	Patient	COLIA 1	0.041
	10	2	5	17	Patient	COLIA 1	0.003
11	24	24	17	65	Patient	TGF-β1 codon10	0.045
18	66	125	109	300	Association study	IL-10–1082	0.009
12	16	27	0	43	Patient	TGF-β1–509	0.016
	13	27	3	43	Patient	TGF-β1 codon10	0.052
15	10	29	3	42	Patient	GLUT-1 (XbaI-)	0.011
9	29	123	68	220	Control	VDR BsmI	0.027
19	46	27	12	85	Association study	MTFR–677	0.036
16	43	78	10	131	Patient	GLUT-1 (XbaI-)	0.002
	12	48	4	64	Patient	GLUT-1 (XbaI-)	0.001
14	64	116	28	208	Patient	PAI-1	0.047
	42	88	22	152	Patient	PAI-1	0.031
	28	58	12	98	Patient	PAI-1	0.036
10	19	48	8	75	Control	ACE I/D	0.009

For abbreviations of affected mutations see original articles. HW, *P* value: observed vs. calculated expected frequencies.

RESULTS

Finally, 75 genotype distributions of the selected 39 articles were reanalyzed. HW calculation was reportedly performed in 25 papers (64%). According to our calculation, the genotype distribution deviated significantly from the expected one in three reference populations in 16 patient populations and in 3 populations of association studies altogether in 15 papers. However, this fact was mentioned in only three papers. The authors of five articles stated that HW criteria are fulfilled, in spite of the presence of significant deviations of genotype distributions. For further details see Table1.

DISCUSSION

HW values do have profound effects on the possible conclusions that could be drawn from a population-genetic study. If genotype distribution misses HW equilibrium in controls, the results should be treated cautiously because the observed genotype distribution in the reference (non-diseased) population does not represent the genotype distribution in healthy people. This bias was present in three papers. Therefore, it needs further studies with new reference groups to investigate the association between tumor necrosis factor-α (TNF-α) −308 polymorphism and renal function after transplantation [8], VDR BsmI polymorphism, and severity of hyperparathyroidism in patients on dialysis [9], ACE I/D polymorphism, and essential hypertension or nephroangiosclerosis [10].

If HW criteria are not fulfilled in the investigated (diseased) population, this might be further evidence for the correlation between genotype and disease (as in 13 of reanalyzed papers). Even in the absence of significant difference between genotype frequencies, unreported or weak associations could be detected by calculating the HW equilibrium. However, our results suggest that this analytical tool is largely underused; 18% of the 39 reanalyzed studies did not recognize the abnormal distribution of genotype in patient population.

Melk et al [8], Wong et al [11], and Cotton et al [12] did not notice that genotype distributions of transforming growth factor-β1 (TGF-β1) polymorphisms were out of HW equilibrium in transplanted patients with stable renal function, in patients with diabetic nephropathy, and in patients with renal parenchymal scarring, respectively. The distribution of COLIA polymorphism was skewed in patients with osteoporotic men [13]. Genotype distributions of PAI and GLUT-1 polymorphisms did not fulfill HW criteria in patients with diabetic nephropathy [14–16].

Although the conclusions of these studies are probably correct, the unreported deviations of genotype distributions would have provided additional supporting evidence for the observed associations between genotype and disease.

When the impact of genotype on continuous variables and/or disease state is investigated in association studies, the fulfilment of HW criteria is not a must. However, we are convinced that the significant deviation of the genotype distribution is also worth mentioning in these reports, especially when the enrolled subjects are randomly selected from a larger population (as in three papers [17–19]).

CONCLUSION

Interestingly, the authors stated that genotype distribution data fulfilled HW criteria in five of the affected papers. Indeed, this statement was established for the

reference, but not for the patient groups. Probably in these cases HW values were not calculated for each genotype distributions in each subgroup of subjects in the study. This finding urges that the numeric results of HW calculations for each investigated subject groups be presented instead of the general statement that "genotype distribution fulfilled HW criteria." The discrepancy between published and recalculated HW values also suggests that rechecking some papers' HW calculations might be of value for the interested readers. Unfortunately, many reports publish exclusively allele frequencies, and state the fulfillment of HW criteria without detailed presentation of genotype frequencies. Therefore, it is highly recommended for the authors to publish the prevalence of each genotype together with allele frequencies.

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