



## Investigating Hardy-Weinberg equilibrium in case-control or cohort studies or meta-analysis

Andreas Ziegler, Kristel van Steen, Stefan Wellek

### ► To cite this version:

Andreas Ziegler, Kristel van Steen, Stefan Wellek. Investigating Hardy-Weinberg equilibrium in case-control or cohort studies or meta-analysis. *Breast Cancer Research and Treatment*, 2010, 128 (1), pp.197-201. 10.1007/s10549-010-1295-z . hal-00615391

**HAL Id: hal-00615391**

**<https://hal.science/hal-00615391>**

Submitted on 19 Aug 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# **Investigating Hardy-Weinberg equilibrium in case-control or cohort studies or meta-analysis**

Andreas Ziegler<sup>1</sup>, Kristel Van Steen<sup>2,3</sup>, Stefan Wellek<sup>4</sup>

<sup>1</sup> Institut für Medizinische Biometrie und Statistik, Universität zu Lübeck, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Germany

<sup>2</sup>Systems and Modeling Unit, Montefiore Institute, University of Liege, Grande Traverse 10, 4000 Liège, Belgium

<sup>3</sup>Bioinformatics and Modeling, GIGA-R, University of Liege, Avenue de l'Hôpital 1, 4000 Liège, Belgium

<sup>4</sup> Abteilung Biostatistik, Zentralinstitut für Seelische Gesundheit, Mannheim, Germany

Correspondence:

Univ.-Prof. Dr. Andreas Ziegler

Institut für Medizinische Biometrie und Statistik, Universität zu Lübeck, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Maria-Goeppert-Str. 1, 23562 Lübeck, Germany

Email: [ziegler@imbs.uni-luebeck.de](mailto:ziegler@imbs.uni-luebeck.de)

## **Abstract**

*Purpose:* Yu et al. [2009 Breast Cancer Res Treat 117:675-677] recently stated that testing for deviation from Hardy-Weinberg equilibrium (HWE) is necessary to identify systematic genotyping errors in case-control studies. They criticized a meta-analytic study for the deviation from HWE in the case group of one study. The aim of this paper is two-fold. First,

we derive recommendations on how to test for deviations from HWE in different study designs. Second, we develop a meta-analytic framework for assessing compatibility with HWE or measuring deviation from HWE.

*Methods:* We sketch possible reasons behind deviation from HWE and provide guidelines for proper investigation of HWE deviations in different study designs. We argue that the standard HWE  $\chi^2$  lack of fit test is logically flawed and provide a logically unflawed approach for measuring deviation from HWE using confidence intervals. Our method is applicable to the meta-analysis of both case-control or cohort association studies. We illustrate our approach using the meta-analysis criticized by Yu et al.

*Results:* In case-control studies, deviation from HWE should only be investigated in controls. In population-based cohort studies, deviation from HWE should be investigated using the entire sample. A simple meta-analytic framework based on confidence intervals is available for investigating deviation from HWE and establishing compatibility with HWE. Heterogeneity between studies can be assessed. The critique of Yu et al. on the paper of Frank et al. [2008 Breast Cancer Res Treat 111:139-144] can be refuted. Even more, validity of HWE can be proven for the pooled control sample.

*Conclusions:* We advocate the use of a confidence interval-based approach to assess HWE. The latter should only be investigated in control populations. In multicentre studies or meta-analysis, deviation from HWE should be analyzed using a meta-analytic approach.

**Keywords:** Disequilibrium coefficient, Meta-analysis, Quality control, Relative excess heterozygosity

## Introduction

The law of Hardy-Weinberg states that a diallelic marker having allele frequencies  $p$  and  $q = 1 - p$ , is in equilibrium if and only if the proportion of subjects with genotypes AA, Aa, and aa will be  $\pi_0 = p^2$ ,  $\pi_1 = 2pq$ , and  $\pi_2 = q^2$ . Departure from Hardy-Weinberg equilibrium (HWE) can be caused by factors such as inbreeding caused by consanguinity, assortative mating, i.e., non-random mating, selection, or migration [18]. In most human populations the effect of these causes on HWE will be small [8] although selection plays an important role in infectious diseases. Other causes which are discussed in the literature to a greater extent are population stratification and copy number variation. Population stratification always leads to a deficit of heterozygotes, while copy number variation can lead to an excess of heterozygosity (homozygote deficit).

Deviation from HWE (Hardy-Weinberg disequilibrium; HWD) in genetic association studies occurs in two different ways. First, population genetic causes leading to a deviation from HWE generally play a minor role in genetic association studies, with the exception of population stratification. However, the latter can be controlled by using appropriate methods, such as genomic control (for a detailed overview, see, e.g, Ref. [18]). The first standard source for deviation from HWE therefore is genotyping error.

Second, if the entire population is in perfect HWE, the presence of a genetic association, i.e., a difference in genotype frequencies between cases and controls implies that neither cases nor controls can be in HWE [16]. Because the proportion of affected subjects in a population is small, the degree of deviation from HWE is expected to be stronger in cases than in controls. Even more, Lee [7] proposed to scan the genome for disease susceptibility genes by testing for HWD in affected individuals, and several colleagues proposed to incorporate a HWD measure in the genetic association test [4, 13].

Therefore, as an indicator of genotyping quality, compatibility with HWE should be investigated in control groups only. In cohort studies no deviation from HWE is expected, and therefore the entire sample should be genotyped for assessing HWD.

In a recent publication in this journal, Yu et al. [17] criticized the work of Frank et al. [1]. Specifically, Yu et al. argued that a discussion of the potential influence of HWD in cases in a meta-analysis involving four groups was lacking. As outlined above, in the case of genetic association, HWD can be expected in cases, while it should not be strong in controls. In fact, Frank et al. clearly stated [1, p. 141, column 2] that they investigated deviations of the genotype frequencies in the controls from those expected under Hardy-Weinberg equilibrium (HWE) using Pearson's goodness-of-fit  $\chi^2$  test with 1 d.f. Thus, Frank et al. correctly used the available biological reasoning for restricting the analysis to controls. However, two comments are required on the approach taken by the authors.

First, they applied the standard procedure to assess HWE, i.e., a  $\chi^2$  test of goodness-of-fit. The null hypothesis of this test is that the locus genotype distribution is in HWE. Thus, a significant result indicates incompatibility of the observed data with HWE. However, the aim generally is to statistically show the validity of HWE. In current practice, investigators try to avoid this logical difficulty by increasing the significance bound to the p-value (e.g., from 0.05 to 0.10). Compatibility of the data with HWE is then inferred from a non significant result. Unfortunately, such direct inversion of a statistical testing procedure is not valid for establishing the alternative hypothesis that the data are in sufficiently good agreement with HWE.

Second, Frank et al. tested genetic association by pooling the results of four case-control studies. However, they investigated HWD separately for each control group. These data were not pooled, and the authors did not analyze possible heterogeneity in HWD between study groups.

In this paper we present solutions for these problems. Specifically, we use a logically unflawed solution to the problem of establishing compatibility with HWD which we have developed recently [15]. We extend this approach to the meta-analytic situation and illustrate it by re-analyzing the data of Frank et al. We demonstrate that the critique of Yu et al. can be refuted. Validity of HWE in the pooled control sample can be demonstrated.

## Methods

### *Measuring the degree of deviation from Hardy-Weinberg equilibrium*

To assess HWD by using measures of degree of HWD instead of the p-value approach from classical  $\chi^2$  goodness-of-fit test statistics has been proposed recently [15]. An overview on measures of the degree of HWD is provided, e.g., in Ref. [18]. We have argued that the relative excess heterozygosity (REH) estimated by  $\hat{\omega} = \hat{\pi}_1 / 2\sqrt{\hat{\pi}_0\hat{\pi}_2}$  is the most reasonable statistical measure of HWD, and we therefore prefer it over the disequilibrium coefficient  $\mathcal{D} = \pi_0\pi_2 - \pi_1^2/4$ . The REH has a simple genetic interpretation because it reflects the degree of HWD by the extent to which the actual proportion  $\pi_1$  of heterozygotes differs from the proportion  $2\sqrt{\pi_0\pi_2}$  of heterozygotes expected in a population which exactly conforms to HWE. The asymptotic confidence interval of the REH was derived using  $\theta = \ln \omega$  and its variance  $v = \frac{1}{n} \left( \frac{1-\pi_1}{4\pi_0\pi_2} + \frac{1}{\pi_1} \right)$ . In detail, an asymptotic confidence interval for  $\omega$  is given by  $(\hat{\omega} / \exp \{z\sqrt{\frac{1}{n} \left( \frac{1-\hat{\pi}_1}{4\hat{\pi}_0\hat{\pi}_2} + \frac{1}{\hat{\pi}_1} \right)}\}, \hat{\omega} \exp \{z\sqrt{\frac{1}{n} \left( \frac{1-\hat{\pi}_1}{4\hat{\pi}_0\hat{\pi}_2} + \frac{1}{\hat{\pi}_1} \right)}\})$ , where  $z$  denotes an upper quantile of the standard normal distribution. To test for deviation from HWE, the 5% test-level and a two-sided confidence interval are commonly used in candidate gene studies, while a pair of one-sided 95% confidence bounds is calculated for establishing compatibility with HWE. We [15] have argued that the data are sufficiently good agreement with HWE when the corresponding equal-tails confidence interval of two-sided level 90 % is within the interval  $5/7 \approx 0.7143$  to  $7/5 \approx 1.400$ .

### *Deviation from Hardy-Weinberg equilibrium in meta-analysis*

Detecting HWD by pooling over different studies has been discussed extensively in the literature [9-12, 14]. However, only statistical tests were considered; confidence intervals and estimates of heterogeneity which are standard in modern meta-analysis have not been provided.

An important aspect is the choice of an appropriate measure for assessing HWD in a meta-analysis. Specifically, Olson [10] noted that the disequilibrium coefficient  $\mathcal{D}$  is not constant across studies when the allele frequency varies over studies. Therefore, she and others [14] preferred the use of  $\omega^2$ , i.e., the squared REH for statistical tests in meta-analysis. We note that all these authors were not interested in interpreting the degree of deviation in the meta-analytic setting. As a result,  $\theta_i$  of study  $i$  is an appropriate measure of HWD for meta-analysis. Its estimator is  $\hat{\theta}_i$ . The weighted average in the traditional fixed effect model is calculated as  $\hat{\theta} = \sum w_i \hat{\theta}_i / \sum w_i$ , where  $w_i = 1/\hat{v}_i$ . The variance of  $\hat{\theta}$  is estimated by  $\hat{v} = 1/\sum w_i$ . The random effects model can be defined similarly [3] using weights, the pooled estimator  $\hat{\theta} = \sum w_i^* \hat{\theta}_i / \sum w_i^*$ , and its variance estimated by  $\hat{v} = 1/\sum w_i^*$ .

A test of the homogeneity of the  $\theta_i$  can be performed using Cochran's  $Q = \sum w_i (\hat{\theta}_i - \hat{\theta})^2$ , which is asymptotically  $\chi^2$  distributed with  $k - 1$  d.f.  $\hat{\tau}^2$  is obtained using the DerSimonian and Laird approach as  $\hat{\tau}^2 = \frac{Q - (k-1)}{\sum w_i - (\sum w_i^2 / \sum w_i)}$ .

Heterogeneity between studies can be quantified using  $H^2 = Q/(k - 1)$  or  $I^2 = (H^2 - 1)/H^2$ . An asymptotically valid confidence interval for  $H^2$  is given by  $\exp^2 (\ln H \pm z_{1-\alpha/2} S.E.(\ln(H)))$ , where  $S.E.(\ln(H)) = \frac{1}{2} \frac{\ln Q - \ln(k - 1)}{\sqrt{2Q} - \sqrt{2k - 3}}$  is the estimated standard error of  $\ln(H)$ . The asymptotic confidence interval for  $I^2$  are readily available from the confidence interval for  $H^2$ .

### *Data analysis*

Details on the study of Frank et al. are provided in Ref. [1]. We estimated REH and asymptotic two-sided 95% confidence intervals for every control group of the study. A fixed effects meta-analysis was performed over all four control groups. The REH and its asymptotic two-sided 95% confidence interval were calculated. Heterogeneity between studies was assessed using  $I^2$  and its two-sided asymptotic 95% confidence interval. To make the calculations for both the fixed effects model and the random effects model traceable, we have created an Excel tool (supplementary material).

### **Results**

Table 1 summarizes the results of both the fixed effects and the random effects meta-analysis for HWE in the control groups. When all control groups were analyzed separately, only a weak deviation of the REH from its expected value 1 was observed. Even more, all two-sided 95% confidence intervals included the one which represents the value of perfect agreement with HWE. This means that no deviation from HWE could be detected at the 5% test-level. Even more, all lower and upper limits of equal-tails 90% confidence intervals are within the interval  $5/7 \approx 0.7143$  to  $7/5 \approx 1.400$  which has been shown to be the appropriate equivalence margin for establishing compatibility with HWE [15]. As a result, for all four individual studies HWE holds at the 5% test-level.

When the studies were analyzed jointly using the meta-analytic approach proposed above, the first finding was that results from fixed effects and random effects meta-analysis were similar. The REH of the fixed effects model was 1.0144, and the equal-tails 90% confidence interval ranged from 0.9665 to 1.0629. We calculated the heterogeneity between studies to be  $I^2 = 6.25\%$  (95% confidence interval 0.00%–60.39%). Thus, the heterogeneity between studies was very low according to Ref. [6].



## Discussion

In genome-wide association (GWA) studies, testing for deviation from HWE is a standard quality control filter [19]. While it should be used for the entire sample in a cohort study, in case-control studies it should only be applied to controls because a deviation from HWE in cases may indicate a genetic association. However, to detect genotyping errors, repeated genotyping of the same probands is preferable over HWE testing. Unfortunately, it is costly, and it only reveals specific genotyping errors that are caused by technical artifacts. Specifically, it will not identify other errors such as sample swap.

Although testing for HWD in cases is not meaningful for quality control, Yu et al. [17] criticized Frank et al. [1] who explicitly stated in the Methods section that they checked for HWD in controls. These authors performed the  $\chi^2$  goodness-of-fit test with 1 d.f. separately for each study. Although this approach is the commonly applied one to show that the data are in sufficiently good agreement with HWD, it is logically flawed. Many tests have been devised to determine whether a finite population follows Hardy-Weinberg proportions. However, the most classical way to check HWD is to compare observed to expected genotype frequencies using a formal  $\chi^2$  testing procedure. It has been shown by Guo and Thompson [2] that asymptotic tests can fail. And even for large samples, as in the presence of multi-allelic markers [2], exact testing procedures are advocated. Exact testing is a tedious job and may be computationally intensive, despite several efforts to speed up the exact testing procedure using improved Monte-Carlo algorithms [5]. The one-sided confidence interval approach [15] offers a statistically sound alternative to investigate HWE.

For estimation, we have used the relative excess heterozygosity (REH). Other measures include the inbreeding coefficient which is also termed fixation coefficient, and the disequilibrium coefficient [18]. However, both measures depend on the allele frequency of the SNP, and we therefore suggest using the REH as measure of HWD.

In this paper, we have extended the confidence interval method for HWE to the meta-analytic situation of Frank et al. [1]. We were able to show that all their control groups and the overall sample were in sufficiently good agreement with HWE. Furthermore, the  $I^2$  measure of heterogeneity indicated that the relative excess heterozygosity in the control groups was very homogeneous across the different studies.

In conclusion, we have refuted the critique of Yu et al. [17] on the study of Frank et al. [1] by using population genetic arguments. Furthermore, we have derived an approach for investigating deviation from HWE in meta-analyses of small candidate gene studies or even huge consortia of GWA studies.

### **Acknowledgments**

This work was funded by the German Research Foundation (ZI 591/17-1) and the German Ministry of Education and Research (NGFN-Plus, 01GS0831). This work was completed during the sabbatical of AZ at the University of Liège, Belgium.

### **Conflicts of interest**

The authors declare that they have no conflicts of interest.

### **References**

1. Frank B, Rigas SH, Bermejo JL, Wiestler M, Wagner K, Hemminki K, Reed MW, Sutter C, Wappenschmidt B, Balasubramanian SP, Meindl A, Kiechle M, Bugert P, Schmutzler RK, Bartram CR, Justenhoven C, Ko YD, Bruning T, Brauch H, Hamann U, Pharoah PP, Dunning AM, Pooley KA, Easton DF, Cox A, Burwinkel B (2008) The CASP8 -652 6N del promoter polymorphism and breast cancer risk: a multicenter study. *Breast Cancer Res Treat* 111:139-144. doi: 10.1007/s10549-007-9752-z
2. Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361-372.
3. Higgins JP, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. *Stat Med* 21:1539-1558. doi: 10.1002/sim.1186
4. Hoh J, Wille A, Ott J (2001) Trimming, weighting, and grouping SNPs in human case-control association studies. *Genome Res* 11:2115-2119. doi: 10.1101/gr.204001

5. Huber M, Chen Y, Dinwoodie I, Dobra A, Nicholas M (2006) Monte Carlo algorithms for Hardy-Weinberg proportions. *Biometrics* 62:49-53. doi: 10.1111/j.1541-0420.2005.00418.x
6. Huedo-Medina TB, Sanchez-Meca J, Marin-Martinez F, Botella J (2006) Assessing heterogeneity in meta-analysis: Q statistic or I<sup>2</sup> index? *Psychol Methods* 11:193-206. doi: 10.1037/1082-989X.11.2.193
7. Lee WC (2003) Searching for disease-susceptibility loci by testing for Hardy-Weinberg disequilibrium in a gene bank of affected individuals. *Am J Epidemiol* 158:397-400.
8. Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J (2007) How should we use information about HWE in the meta-analyses of genetic association studies? *Int J Epidemiol* 37:136-146. doi: 10.1093/ije/dym234
9. Nam JM (1997) Testing a genetic equilibrium across strata. *Ann Hum Genet* 61:163-170. doi: 10.1046/j.1469-1809.1997.6120163.x
10. Olson JM (1993) Testing the Hardy-Weinberg law across strata. *Ann Hum Genet* 57:291-295.
11. Olson JM, Foley M (1996) Testing for homogeneity of Hardy-Weinberg disequilibrium using data sampled from several populations. *Biometrics* 52:971-979.
12. Schaid DJ, Batzler AJ, Jenkins GD, Hildebrandt MA (2006) Exact tests of Hardy-Weinberg equilibrium and homogeneity of disequilibrium across strata. *Am J Hum Genet* 79:1071-1080. doi: 10.1086/510257
13. Song K, Elston RC (2006) A powerful method of combining measures of association and Hardy-Weinberg disequilibrium for fine-mapping in case-control studies. *Stat Med* 25:105-126. doi: 10.1002/sim.2350
14. Troendle JF, Yu KF (1994) A note on testing the Hardy-Weinberg law across strata. *Ann Hum Genet* 58:397-402.
15. Wellek S, Goddard KA, Ziegler A (2010) A confidence-limit-based approach to the assessment of Hardy-Weinberg equilibrium. *Biom J* 52:253-270. doi: 10.1002/bimj.200900249
16. Wittke-Thompson JK, Pluzhnikov A, Cox NJ (2005) Rational inferences about departures from Hardy-Weinberg equilibrium. *Am J Hum Genet* 76:967-986. doi: 10.1086/430507
17. Yu KD, Di GH, Fan L, Shao ZM (2009) Test of Hardy-Weinberg equilibrium in breast cancer case-control studies: an issue may influence the conclusions. *Breast Cancer Res Treat* 117:675-677. doi: 10.1007/s10549-009-0353-x
18. Ziegler A, König IR (2010) *A Statistical Approach to Genetic Epidemiology: Concepts and Applications*. With an e-learning course by Friedrich Pahlke. 2nd ed. Wiley-VCH, Weinheim
19. Ziegler A, Thompson JR, König IR (2008) Biostatistical aspects of genome-wide association studies. *Biom J* 50:8-28. doi: 10.1002/bimj.200710398

**Table 1** Relative excess heterozygosity and asymptotic confidence intervals

Study <sup>f</sup>	Genotype	Controls		REH <sup>a</sup>	1-/2-sided <sup>c</sup>	95% CI <sup>b</sup>	
		n	%				
GFBCS	ins/ins	270	25.99	0.9494	2	0.8407	1.0722
	ins/del	506	48.70		1	0.8573	1.0515
	del/del	263	25.31				
SBCS	ins/ins	245	20.87	1.0840	2	0.9662	1.2162
	ins/del	608	51.79		1	0.9843	1.1939
	del/del	321	27.34				
GENICA	ins/ins	285	28.33	0.9629	2	0.8506	1.0900
	ins/del	492	48.91		1	0.8678	1.0685
	del/del	229	22.76				
SEARCH	ins/ins	1149	25.68	1.0243	2	0.9660	1.0862
	ins/del	2263	50.58		1	0.9751	1.0760
	del/del	1062	23.74				
Pooled FE <sup>d</sup>				1.0144	2	0.9700	1.0608
					1	0.9665	1.0629
Pooled RE <sup>e</sup>				1.0135	2	0.9770	1.0532
					1	0.9739	1.0548

a Relative excess heterozygosity

b Asymptotic 95% confidence interval according to [15]

c One-sided or two-sided asymptotic confidence interval. The one-sided confidence interval is used for establishing compatibility with HWE, while the two-sided confidence interval is used for investigating lack of fit

d Fixed effects meta-analysis pooled over all four control groups

e Random effects meta-analysis pooled over all four control groups

f Heterogeneity  $I^2$  between studies was estimated as 6.25% (two-sided 95% confidence interval 0.00%-60.39%)