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► **To cite this version:**

Elias Zintzaras. Impact of Hardy-Weinberg equilibrium deviation on allele-based risk effect of genetic association studies and meta-analysis. *European Journal of Epidemiology*, 2010, 25 (8), pp.553-560. 10.1007/s10654-010-9467-z . hal-00598199

**HAL Id: hal-00598199**

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Submitted on 5 Jun 2011

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**Impact of Hardy-Weinberg equilibrium deviation on allele-based  
risk effect of genetic association studies and meta-analyses**

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## ABSTRACT

Deviations from Hardy-Weinberg equilibrium (HWE) in control subjects may bias the estimates of genetic effects in genetic association studies (GAS) and meta-analyses. A large empirical evaluation was carried out to evaluate the impact of HWE deviation and explore the effect of variance adjustment for the allele-based odds ratio in 833 individual GAS and 72 meta-analyses. In individual GAS, the variance adjustment for any deviation from HWE resulted in stronger associations, and 10 GAS (1%) became significant ( $P < 0.05$ ). One hundred sixteen GAS (14%) showed significant deviation from HWE ( $P_{\text{HWE}} < 0.05$ ); however, only 37 GAS (4%) had more than 90% power to detect significant deviation from HWE at the 5% level. In meta-analyses, adjustment for any deviation from HWE improved the significance in 53 meta-analyses (74%). Then, a formal statistical significance ( $P < 0.05$ ) was revealed for one previously negative meta-analysis whereas one meta-analysis lost its significance. Between-study heterogeneity was enhanced in 50 meta-analyses (69%). None of the meta-analyses lost the significance of heterogeneity ( $P_Q < 0.10$ ) whereas in one meta-analysis, the non significant heterogeneity became significant. Sensitivity analysis for studies not conforming to HWE ( $P_{\text{HWE}} < 0.05$ ) was applied to 45 meta-analyses (69%). Then, the significance of association was increased in 26 the meta-analyses (58%) and one meta-analysis became significant ( $P < 0.05$ ) whereas seven meta-analyses were no longer significant. In five meta-analyses, the heterogeneity lost its significance after sensitivity analysis whereas in two meta-analyses it became significant ( $P_Q < 0.10$ ). Adjustment for HWE deviation could be an effective strategy for dealing with HWE violations in GAS and meta-analyses.

**Keywords:** Hardy-Weinberg equilibrium; HWE; adjustment; bias; odds ratio, power; variance; genetic association studies; meta-analyses

## **INTRODUCTION**

Genetic association studies (GAS) assess the association between phenotypic traits and genetic variants (gene polymorphisms) in a population. GAS investigate the association without requiring information on inheritance, and thus, are conducted on a sample of unrelated cases and controls. Variants for GAS can be selected on the basis of pathophysiological hypotheses, or alternatively, genome-wide screening without any predilection of genes and variants to be tested can be performed (genome-wide association studies) [1].

The genetic association studies usually assess various contrasts of genotypes such as the allele contrast, the additive, recessive and dominant models [1]. However, the most attractive contrast for researchers is the allele-based because the sample size become twice as large (one genotype provides two alleles) providing more power to detect significant associations. In the case of a polymorphism with two alleles (mutant type-Mt and wild type-Wt), where Mt is thought to be associated with a disease, the allele contrast compares the number of alleles Mt with the number of alleles Wt in cases and controls. The magnitude and significance of a genetic association is usually estimated with the odds ratio (OR) and its 95% confidence interval (CI).

Results produced from GAS may be spurious when the genotype distribution of controls (disease-free subjects) deviates from Hardy-Weinberg equilibrium (HWE) [1]. Inspection of whether genotype frequencies of controls conform to HWE provides a surrogate of GAS quality in terms of design and conduct. Deviation from HWE can be due to laboratory/genotyping errors, population stratification, selection bias in the choice of controls and confounding factors unaccounted for [1].

When a sample is not from a single population (i.e. it is composed of sub-samples, each from a population with different frequencies of  $Mt$ ) then it is expected to have an excess of homozygotes and deficiency of heterozygotes than would be predicted by the Hardy-Weinberg law (Wahlund effect) [2]. This effect is a common cause of the departure from HWE. In population terms, lack of HWE implies existence of migration, selection, mutation and absence of random mating. Often, this population stratification includes differences between groups of ethnic origin or differences between groups of similar ethnic origin but with limited admixture [3].

A significant deviation from HWE may indicate genotyping error, because the conditions of HWE are generally applicable to the control subjects in a well designed study population [4]. Although exceptions to the conditions of HWE may explain deviation, a test of HWE is needed, and then, an evaluation of the reasons for any observed deviation is required. For example, genotyping error may exist when the variant is significantly associated with the disease, the cases are in HWE and in the controls exists an excess of homozygotes [4]. It is possible that a departure from HWE can be due to some unknown factors other than genotyping errors, especially when multiple variants that are in strong linkage disequilibrium deviate from HWE. The potential sources for genotyping error may be DNA contamination from plates and systematic errors that affect the entire research process differently in cases and controls [3]. In addition, lack of blindness may lead to bias during genotyping and scoring of the alleles, particularly for ambiguous allele calls, and this is pronounced when the inherent failure rates of genotyping are different for homozygous or heterozygous genotypes, leading to skewed gene frequencies [4].

In a sample of unrelated subjects, HWE implies that alleles are independent. Then for a genetic marker with two alleles ( $Wt$  and  $Mt$ ) the proportions of the

different genotypes Mt/Mt, Mt/Wt and Wt/Wt are  $p^2$ ,  $2p(1-p)$  and  $(1-p)^2$ , respectively. An empirical evaluation suggested that testing and reporting for HWE is often neglected in GAS and deviations are rarely admitted in published reports [4, 5]. Power calculations have shown that most studies conforming to HWE are underpowered to detect HWE deviation [5].

Lack of HWE in GAS may introduce bias and loss of precision in the estimation of genetic effects and potentially question the validity of a study [6]. This problem may be overcome when the significance testing is based on a variance adjusted for deviation from HWE. Estimates of adjusted variance have been proposed for estimating relative risks of the additive, recessive and dominant models, and differences in allele frequency [6, 7]. Sato et al. [8] provided an estimate of variance of OR for the allele contrast for GAS in the absence of HWE in cases and controls. This estimate was based on fixation indices of cases and controls [8]. Recently, Zintzaras proposed an adjustment for the same metric based on the delta method in combination with the Woolf's logit interval and the disequilibrium coefficient (D) [9].

In meta-analysis, the most commonly used approach to deal with studies not conforming to HWE is sensitivity analysis, i.e. exclusion of these studies and re-evaluation of the effect size [1]. Adjustment for HWE deviation is rarely performed for the genotype-based contrasts [10]. However, a meticulous and large empirical study to evaluate the impact of HWE deviation in GAS and the effect of adjustment for HWE deviation in meta-analysis based on the allele contrast has not been performed to date.

The present empirical study examines the impact on significance of GAS when the variance of OR for the allele contrast is adjusted for departures from HWE. The power of GAS to assess HWE is also examined. Finally, the effect of variance

adjustment in pooled OR and heterogeneity in meta-analysis is demonstrated and compared with sensitivity analysis.

## **METHODS**

### ***Selection of studies and database***

HuGE PubLit Database was searched for published meta-analyses of GAS in the year 2007. In addition, a database of meta-analyses of GAS archived in our department was utilized (<http://biomath.med.uth.gr/>).

All retrieved meta-analyses were assessed for their appropriateness for inclusion in the present empirical study. A meta-analysis was considered eligible when i) it examined biallelic polymorphisms (Wt/Mt), ii) provided the complete genotype distribution (MtMt, WtMt, WtWt) for diseased subjects and controls of individual studies included in the meta-analysis, iii) controls were non-diseased, iv) written in English, v) considered binary outcomes, and vi) contained at least three studies. In the case of overlapping meta-analyses of the same polymorphism/phenotype, all distinct individual studies were considered.

Thereafter, all published GAS included in the meta-analyses were catalogued and retrieved. Finally, each GAS was further scrutinized for eligibility according to the above i) to v) criteria.

### **Testing for association in GAS and meta-analysis**

For each GAS the association between the gene polymorphism and the disease was examined based on the allele contrast (Mt vs. Wt). Then, for each study the OR and the corresponding 95% CI were calculated.



A meta-analysis for each variant by phenotype was then performed and the pooled OR for the allele contrast was estimated using random effects (DerSimonian and Laird) model [11]. Random effects modelling assumes a genuine diversity in the results of various studies, and it incorporates to the calculations a between study variance. The heterogeneity between studies was tested using the Q-statistic [1]. If  $P_Q < 0.10$  then heterogeneity was considered statistically significant. Heterogeneity was quantified with the  $I^2$  metric, which is independent of the number of studies in the meta-analysis.  $I^2$  takes values between 0% and 100% with higher values denoting greater degree of heterogeneity [1]. The significance level (P-value) of each association (in GAS and meta-analysis) was estimated using the z-test.

### **HWE testing and power**

The distribution of the genotypes of controls of each GAS was statistically tested for HWE at  $P_{HWE} = 0.05$ . A diseased population is expected not to conform with the HWE when a true association with the examined polymorphism exists; however an independence of alleles is expected in the control group. Thus, a test for HWE was performed only for the controls. HWE was tested by using an asymptotic chi-square test [12].

Then, for each GAS, the power for testing deviation from HWE at 5% level was estimated based on the normalization of the non-central chi-squared distribution [13] which is given by the following expression:

$$z = \frac{\sqrt[3]{3.84/(1+\lambda) - [1 - 2(1+2\lambda)/(9(1+\lambda)^2)]}}{\sqrt{[2(1+2\lambda)/(9(1+\lambda)^2)]}}$$

where  $\lambda$  the non-centrality parameter, i.e.  $\lambda = \sum [(E_{1i} - E_{0i})/E_{0i}]$  ( $i=1-6$ ) and  $E_{0i}$  and  $E_{1i}$  are the expected cell frequencies under the null and the alternative hypothesis, respectively.

### **Adjustment for HWE deviation**

The precision of the genetic risk effect of each GAS was adjusted for deviation from HWE using the method proposed by Zintzaras [9]. This method provides an estimate of variance of OR (or  $\hat{\theta}$ ) for the allele contrast in the absence of HWE in cases and controls. The method is based on the delta method in combination with the Woolf's logit interval method and the disequilibrium coefficient (D). In the subsequent analysis, it was assumed that D was equal to zero for the cases since HWE was examined only for the controls.

Thus, the variance of the allele contrast was adjusted for HWE deviation using the D approach. In this approach, the variance of  $\ln \hat{\theta}$  is given by

$$\text{var}[\ln(\hat{\theta})] = \left( \frac{1}{p} + \frac{1}{(1-p)} \right)^2 \frac{p(1-p)}{n} + \left( \frac{1}{p'} + \frac{1}{(1-p')} \right)^2 \frac{p'(1-p') + D'_{Mt/Mt}}{n'}$$

where  $p$  is the estimated frequency of mutant type allele among disease cases and  $p'$  is the respective value for the controls,  $n$  and  $n'$  is the total number of alleles in cases and controls, respectively.  $D'_{Mt/Mt} = p'_{Mt/Mt} - p'^2$ , is the disequilibrium coefficient,  $p'_{Mt/Mt}$  is the frequency of mutant type homozygotes (Mt/Mt) for controls. When there is an excess or deficiency of homozygotes, it is expected the adjusted variance to be smaller than the observed one.

### **Sensitivity analysis, HWE adjustment and heterogeneity**

For each GAS, and variant by phenotype meta-analysis, the effect of adjusting the variance for HWE was evaluated. For each meta-analysis the effect of sensitivity analysis was also examined and compared to HWE adjustment. Sensitivity analysis examines the effect of excluding studies with controls not in HWE and it is usually performed to establish the robustness of the estimated pooled OR.

## **RESULTS**

### ***Database and Summary statistics***

Seventeen two meta-analyses (28 from HuGE PubLit Database and 44 from our database) consisted of 833 individual GAS (347 from HUGENet and 486 from our database) met the inclusion criteria. Forty seven percent (47%) of the meta-analyses involved 10 or more GAS, 6% involved more than 30 GAS (one meta-analysis included 108 GAS) and 10% involved just 3 studies. The studies concerned 28 gene variants and 26 distinct phenotypes. In GAS, the minimum number of cases and controls was seven and six subjects, respectively, and the maximum number was 9826 and 28113 subjects, respectively.

One hundred sixteen GAS (14% of the 833 GAS) showed significant deviation from HWE ( $P_{\text{HWE}} < 0.05$ ) and 44 GAS (6%) showed marginal significance ( $0.05 \leq P_{\text{HWE}} < 0.10$ ). The disequilibrium coefficient,  $D'$ , of the studies with  $P_{\text{HWE}} < 0.05$  ranged from  $D' = -0.123$  to  $-0.002$  whereas in the studies with  $0.05 \leq P_{\text{HWE}} < 0.10$   $D'$  ranged from  $D' = -0.053$  to  $-0.003$ .

### ***The significance after adjustment for HWE deviation in GAS***

Two hundred fifteen GAS (26% of the 833 GAS) studies produced significant association ( $P < 0.05$ ). Figure 1 shows the level of significance of association for each

GAS without adjustment and with adjustment for any degree of deviation from HWE (i.e. irrespectively of the significance for HWE deviation). After adjustment for HWE, the status of significance changed for 10 studies (1%), i.e. in these studies before adjustment the P-value was  $P \geq 0.05$  and after adjustment it was  $0.02 \leq P \leq 0.048$ . The adjustment has a greater effect towards the tail of significant studies. Fifty one GAS (6%) had a reduction in P-value greater than 30% and 5 GAS (0.6%) had a reduction greater than 80%. In 324 GAS (39%) reduction was very small, less than 1%.

Among the 116 GAS with significant deviation from HWE ( $P_{HWE} < 0.05$ ), 33 GAS showed a significant association at  $P < 0.05$ . In six of the 116 GAS (5%), the adjustment for HWE changed the associations from non-significant ( $P \geq 0.05$ ) to significant ( $P < 0.05$ ).

### ***Power of HWE testing in GAS***

Fifty six GAS (7% of the 833 GAS) had a power greater than 75% to detect significant deviation from HWE at  $P_{HWE} = 0.05$  and 721 (87%) had power less than 50%. Only 37 GAS (4%) had power more than 90%. Figure 2 shows the power of studies in relation to the  $P_{HWE}$ . Power was much higher in the studies that were found to deviate significantly from HWE than those conforming to HWE.

### **The impact of HWE deviation in meta-analysis**

Forty five meta-analyses (consisting of minimum three to maximum 108 GAS) from the total of 72 meta-analyses involved studies with statistical departure from HWE ( $P_{HWE} < 0.05$ ) in controls. In 19 meta-analyses (consisting of three to 30 GAS), the proportion of individual studies within each meta-analysis not conforming to the

HWE rule ( $P_{\text{HWE}} < 0.05$ ) was more than 20%, and in five meta-analyses (consisting of four to 14 GAS), it was more than 50%.

Twenty-eight meta-analyses produced significant associations, 46 showed large heterogeneity among studies ( $I^2 \geq 25\%$ ) and 35 of them also showed statistically significant heterogeneity ( $P_Q < 0.10$ ). Six meta-analyses produced both significant pooled estimates and showed lack of heterogeneity ( $I^2 < 25\%$  and  $P_Q \geq 0.10$ ).

### *Sensitivity analysis in meta-analysis*

In the 45 meta-analyses involving GAS with statistical departure from HWE ( $P_{\text{HWE}} < 0.05$ ), a sensitivity analysis was applied to those studies. Then, the significance of association (P-value) was increased in 58% of the meta-analyses (in 26 out of 45 meta-analyses) (Figure 3). Then, one meta-analysis became significant (IL6 -174 G/C and peripheral arterial disease), where the P-value changed from  $P=0.33$  to  $P < 0.01$ , and seven meta-analyses were no longer significant: 1) MPO -463G/A and lung cancer (three out of 10 GAS were not in HWE), MTHFR C677T and diabetic retinopathy (two out of five GAS were not in HWE), MTHFR C677T and acute lymphoblastic leukemia (two out of 9 GAS were not in HWE), MTHFR C677T and Down syndrome (one out of 11 GAS were not in HWE), MTHFR C677T and diabetic nephropathy (three out of 14 GAS were not in HWE), eNOS 4b/a and hypertension (four out of 11 GAS were not in HWE), ALOX5AP SG13S89 G/A and stroke (two out of four GAS were not in HWE). In the latter case, the P-values of association of the original meta-analyses ranged from  $P=0.01$  to  $P=0.03$  and the P-values after the sensitivity analyses ranged from  $P=0.06$  to  $P=0.39$ .

After exclusion of the studies with statistical departure from HWE ( $P_{\text{HWE}} < 0.05$ ), the heterogeneity ( $P_Q < 0.10$ ) among studies lost its significance in five

meta-analyses: IL6 -174 G/C and peripheral arterial disease ( $P_Q=0.26$ , two out of four GAS were not in HWE, ApaI A/a and osteoporosis ( $P_Q=0.91$ , four out of seven GAS were not in HWE), MTHFR C677T and congenital heart defects ( $P_Q=0.22$ , one out of 10 GAS were not in HWE), BDNF G196A and Parkinson 's disease ( $P_Q=0.11$ , two out of six GAS were not in HWE), and MTHFR C677T and diabetic retinopathy ( $P_Q=0.20$ , two out of five GAS were not in HWE). On the contrary, the heterogeneity in two meta-analyses became significant after sensitivity analysis: MTHFR C677T and stomach cancer (from  $P_Q=0.12$  changed to  $P_Q=0.04$ , three out of eight GAS were not in HWE) and MTHFR C677T and acute lymphoblastic leukemia (from  $P_Q=0.22$  changed to  $P_Q<0.01$ , two out of nine GAS were not in HWE).

The sensitivity analysis decreased the between study heterogeneity ( $I^2$ ) in 22 meta-analyses (almost 50% of the meta-analyses). In 11 and five meta-analyses,  $I^2$  increased more than 10% and 50%, respectively. However, in 16 and eight meta-analyses, the decrease in  $I^2$  was more than 10% and 50%, respectively.

### ***Adjustment for HWE deviation in meta-analysis***

In considering all 72 meta-analyses, the adjustment for any degree of deviation from HWE improved the significance (i.e. in terms of P-value) in the majority of meta-analyses (74%, i.e. 53 out of 72). The adjustment reduced the P-value by more than 20% in 15 meta-analyses (21% of meta-analyses). In six meta-analyses (8% of meta-analyses), the change in P-value was greater than 50% (Figure 4).

In one meta-analysis a non significant association ( $P=0.09$ ) became strong ( $P=0.02$ ) after adjustment for HWE (the association between MTHFR C677T and multiple myeloma). There is also one significant association ( $P=0.02$ ) in the unadjusted analysis that became non significant after HWE adjustment ( $P=0.08$ )

(MTHFR C677T and Down syndrome). The later meta-analysis, included only one individual study with statistical departure for HWE ( $P_{\text{HWE}} < 0.05$ ).

When the 45 meta-analyses with at least one study not statistically conforming with the HWE ( $P_{\text{HWE}} < 0.05$ ) were considered, an improvement in significance was shown in 31 studies (69% of the meta-analyses) after adjustment for HWE. In 12 meta-analyses (27% of the meta-analyses), the improvement in significance was greater than 20% (Figure 3).

Regarding the effect of HWE adjustment in the between study heterogeneity of the 72 meta-analyses, the adjustment increased the between study heterogeneity ( $I^2$ ) in 50 meta-analyses and decreased it in five (Figure 5). In 12 cases, the increase in  $I^2$  was more than 10% after adjustment, whereas, in six cases,  $I^2$  decreased by more than 50%. Heterogeneity became formally statistically significant ( $P_Q = 0.07$ ) in one meta-analysis where it was previously not formally significant ( $P_Q = 0.23$ ) (MTHFR C677T and acute lymphoblastic leukemia). In none of the meta-analyses, was the significance of testing ( $P_Q < 0.10$ ) for heterogeneity lost after adjustment.

## **DISCUSSION**

The present empirical study evaluated the impact of HWE deviation and the effect of adjustment for this deviation across a wide range of GAS and meta-analyses in the field of genetic epidemiology. In this large dataset consisting of 833 studies, the proportion of studies with HWE violations was not high (14%) and of similar magnitude to previously reported results (10%) [5]. However, these estimates can be misleading since the proportion of adequately powered studies was small.

In testing the impact of adjustment of HWE deviation on allele-based ORs for individual GAS, new significant associations were revealed for a small fraction GAS.

Although the adjusted variance was lower than the corresponding unadjusted resulting in reductions in the P-values [9], the magnitude of these reductions was small for the majority of GAS. Adjustment for HWE had more profound effects in studies not conforming to HWE.

When testing the impact of adjustment for HWE deviation on the meta-analyses results versus the commonly used sensitivity analysis, changes in P-values were observed at similar directions: the adjustment approach resulted in reductions in P-values in the majority of the meta-analyses (74%) and the sensitivity analysis approach, where applicable, increased the P-values in 58% of the meta-analyses. These changes were translated in revealing one formally significant result with the adjustment for HWE deviation, whereas the sensitivity analysis approach identified another one significant association but masked seven previously significant results. The HWE deviation adjustment approach was more influential in meta-analyses with a small number of included studies. Regarding heterogeneity statistics, the adjustment approach increased the  $I^2$  metric in the majority of meta-analyses, although shifting in the status of formal statistical significance occurred only in one meta-analysis. This improved capacity for heterogeneity detection and quantification was not observed with the sensitivity analysis approach.

The power of detecting a significant association based on variance adjusted for HWE deviation depends on the magnitude of  $D'$  and is described by a sigmoid function; the power is getting greater when the  $D'$  increases [9]. In the current study, testing for deviation from HWE was performed by using an asymptotic chi-square test [12], since it is the most commonly used method in the literature of GAS and provides sufficient estimates [5, 17]. When dealing with low allelic frequencies, the performance may not be optimal and then an exact test could be a reasonable



alternative [5]. However, our dataset included GAS and meta-analyses investigating the role of common variants.

In GAS, an alternative test of association for the allele contrast is the Armitage's trend test, which is asymptotically chi-squared when the studied populations are in HWE and in general provides higher P-values. The significance of a genetic association when a chi-squared test is used may not be valid when the alleles are not independent in the studied population [14]. Therefore, the size of the Armitage's trend test will not be correct if the HWE does not hold and then the type-I error rate would also increase [6].

Testing for HWE is included in guidelines for the critical appraisal of GAS in meta-analysis [1, 15]. However, reporting of HWE testing in the GAS literature is suboptimal [5] or the genotype distribution of controls is not always provided [16]. Additionally, lack of evidence for departure from HWE does not necessarily imply conformation with HWE, given the power considerations described above. Different strategies have been proposed for dealing with GAS with deviations from HWE in meta-analyses. Although the most popular one is to perform a sensitivity analysis for the studies violating HWE, the superiority of this commonly used approach over others (exclusion of these studies or adjustment of variance of risk effect for HWE deviation) has not been demonstrated [5, 9, 16-17]. In performing sensitivity analysis, the change in the magnitude of risk effect suggests the extent of bias due to studies with departure from HWE. In correcting for departure from HWE, the ORs and the corresponding variances of the individual GAS included in the meta-analysis are corrected to account for departures from HWE, and then, the meta-analysis is performed based on the corrected (adjusted) OR of the individual GAS. Previous empirical work was not conclusive regarding the use of sensitivity analysis versus

exclusion of HWE violating studies, since HWE violation could be considered as a red flag of biases [5, 9, 16-17].

The current analysis demonstrated that an adjustment of variance of ORs provided enhanced power to detect significant allele-based estimated of genetic effect, compared to sensitivity analysis. This adjustment for HWE deviation resulted in revealing new significant associations, especially in borderline cases. The adjustment approach also made even more prominent the diversity in the results of studies on the same postulated association. The between-study heterogeneity is important to describe, quantify, and try to explain and meta-analysis should aim at more than simply arriving to a grand mean, since dissection of heterogeneity can provide some valuable information [1].

In genome-wide association studies, genotypes violating HWE have been routinely eliminated during the quality check stages, as possible genotyping miscalls [1]. However, recent work based on simulations showed that true disease susceptibility loci can be largely out of HWE and, thus, be candidates for removal before association testing. It was also shown that these loci may maintain sufficient statistical power even under extreme error models. In addition, random miscalls of null SNPs, independent of the phenotype, may do not induce bias in GAS and then, a significant deviation from HWE may not prevent a variant from being tested when conducting genome-wide association studies. [17]. Nevertheless, significant findings for variants not conforming with the HWE should be treated with caution.

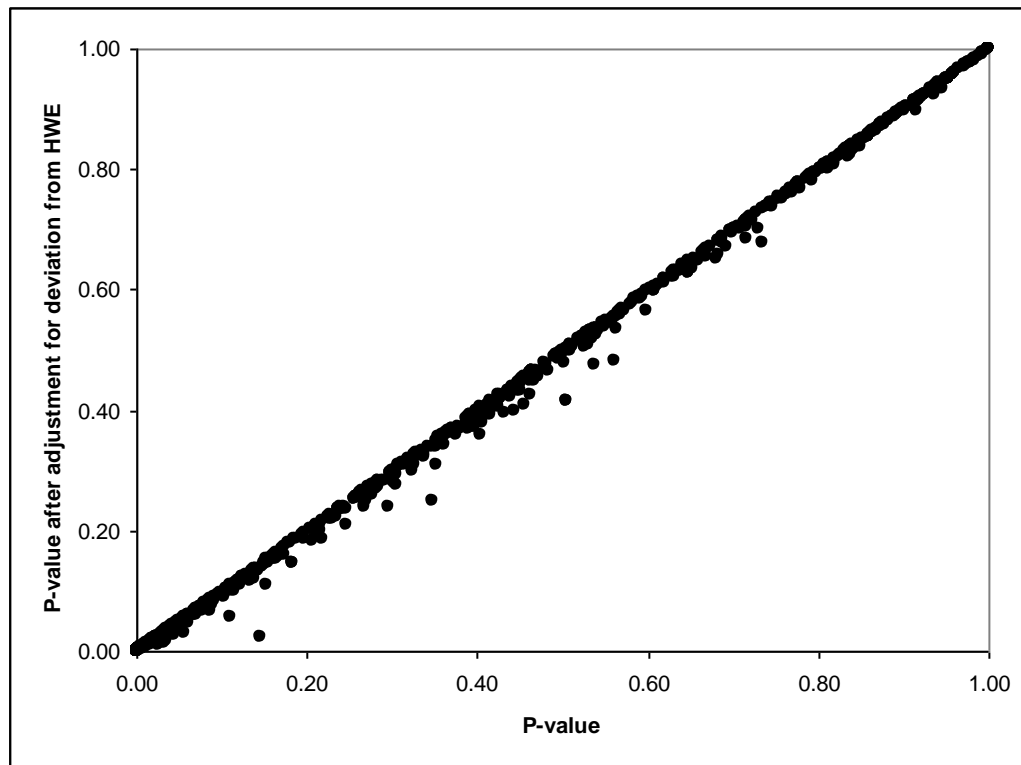
However, when there is indication that genotyping errors may include the miscalling of homozygous and heterozygotes, as well as amplification preference of alleles, then, we may consider omitting these variants (after retyping them by alternative techniques) when they show huge deviation from HWE. Otherwise, the

chance of false-positive associations is considerably inflated [6]. Since no study can really claim immunity to genotyping errors, correction for departures from HWE should be applicable even when the probability for substantial genotyping error is low [18].

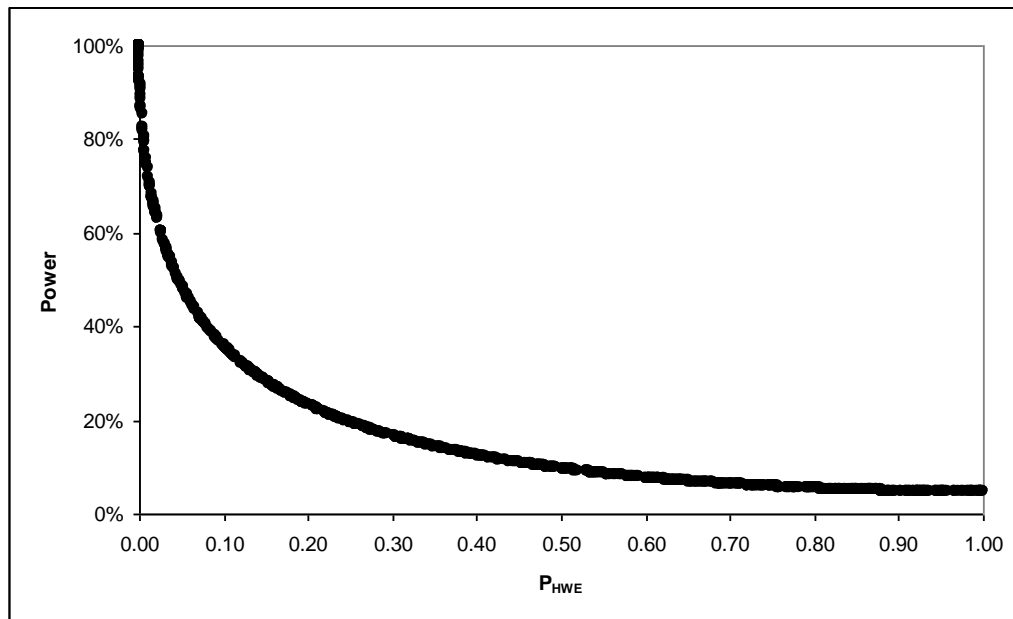
An adjustment to account for deviations from HWE based on the inbreeding coefficient has been proposed to examine difference of the estimated allele frequencies between cases and controls when there is excess of homozygotes [6]. Sato et al. estimated the variance of allele odds ratio based on an estimate of a fixation index [8]. Although, the use of inbreeding or fixation coefficients to describe departures from HWE has some merit, it has the disadvantage that the parameters involved are estimated as ratios of genotypic frequencies, and it is difficult to estimate the statistical properties of ratios [9].

In conclusion, the application of the adjusted variance of allele-based OR for HWE deviations proved to be an effective strategy in detecting associations in GAS and meta-analyses of GAS, which could have been missed by using sensitivity analysis. Additionally, significant heterogeneity in meta-analyses of GAS was more often detected by the variance adjustment method. Although the impact of the variance adjustment method was evident by small size changes, it resulted in shifting of significance status in borderline cases. In the era of genome-wide association studies, the field has shifted towards consortial efforts that will allow the detection of the diminishing, in terms of effect sizes, genetic associations [19]. Eliminating studies and datasets on the basis of HWE violations alone seems unjustified and could limit the power for new discoveries. The proposed adjustment of variance represents a useful technique that can enhance the power to detect associations in GAS and meta-analyses.

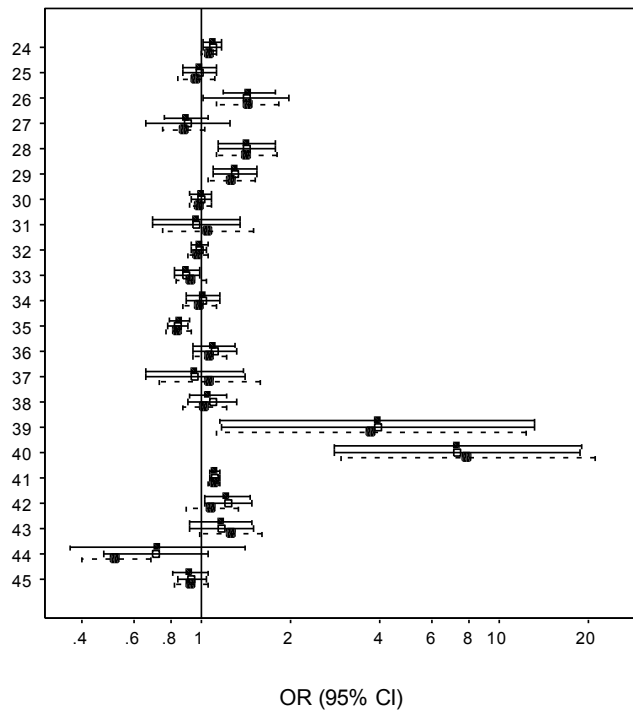
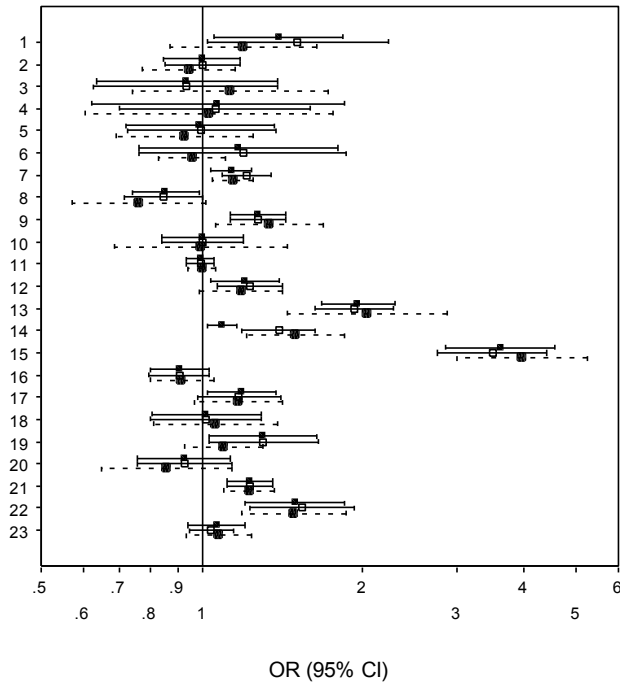
**Figure 1.** Level of significance (P-value) of association for the 833 GAS with and without adjustment for HWE deviation.



**Figure 2.** Power of studies for detecting significant HWE in relation to the level of significance ( $P_{HWE}$ ).

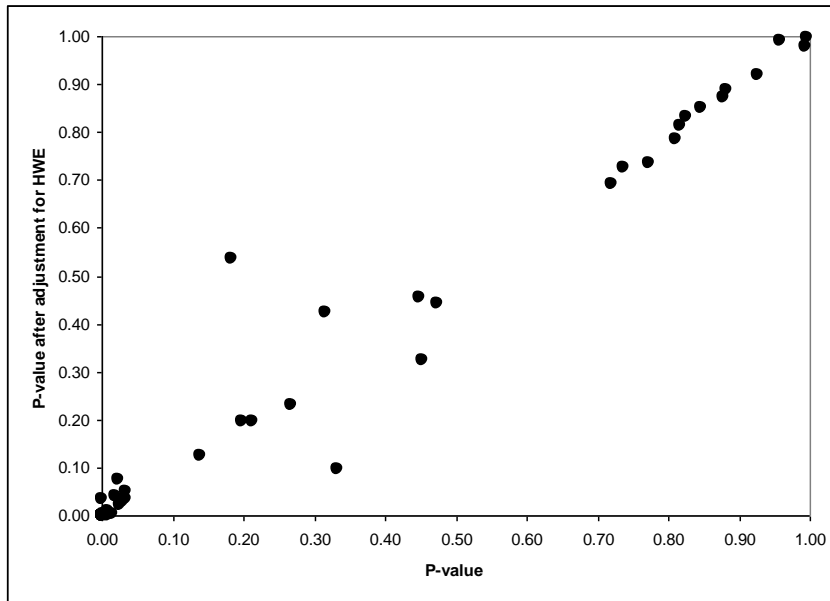


**Figure 3.** Pooled odd ratios (OR) with the corresponding 95% confidence intervals (CI) unadjusted (solid square) and adjusted (open square) for deviation from HWE, and sensitivity analysis (solid circle) for 45 meta-analysis that included studies deviated from HWE. The identification numbers on the y-axis corresponds to the meta-analyses. The first author, the publication year, the gene polymorphism, the disease and the number of studies included and the number of studies deviated from HWE for each meta-analysis are shown.



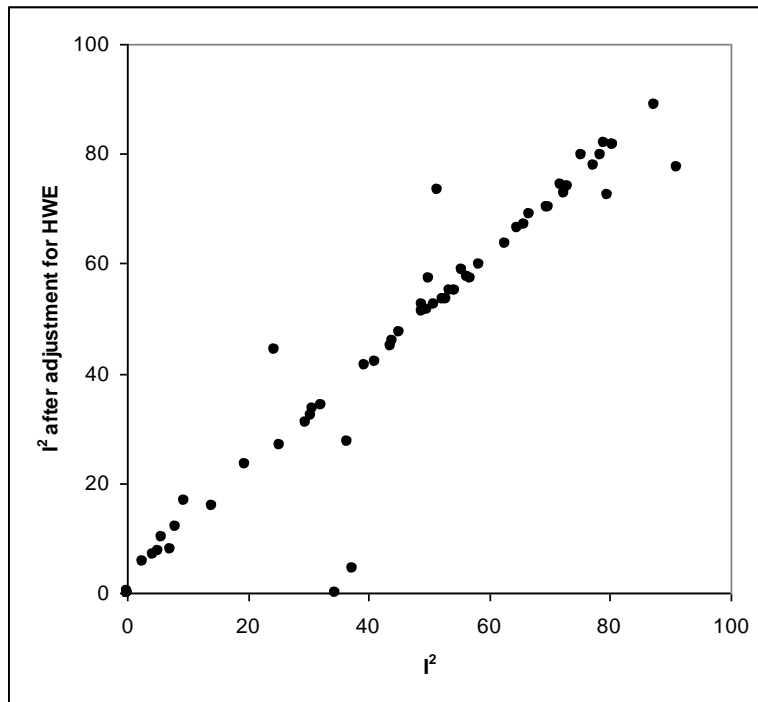
1. Zintzaras et al. 2005 (5/2), MTHFR C677T, diabetic retinopathy
2. Zintzaras et al. 2005 (6/2), BDNF G196A, Parkinson's disease
3. Zintzaras et al. 2006 (14/7), VDR BsmI B/b, osteoporosis
4. Zintzaras et al. 2006 (7/1), TaqI T/t , osteoporosis
5. Zintzaras et al. 2006 (7/4), ApaI A/a, osteoporosis
6. Zintzaras et al. 2006 (3/1), FokI F/f , osteoporosis
7. Zintzaras et al. 2006 (10/1), MTHFR C677T, schizophrenia
8. Zintzaras et al. 2006 (9/2), MTHFR C677T , acute lymphoblastic leukemia
9. Zintzaras 2006 (8/3), MTHFR C677T , Stomach cancer
10. Zintzaras 2006 (4/2), MTHFR A1298C , stomach cancer
11. Zintzaras et al. 2006 (15/2), eNOS Glu298Asp , hypertension
12. Zintzaras et al. 2006 (11/4), eNOS 4b/a , hypertension
13. Zintzaras et al. 2006 (39/3), ADH2 2/1, alcoholism
14. Zintzaras et al. 2006 (28/6), ADH3 1/2 , alcoholism
15. Zintzaras et al. 2006 (30/11), ALDH2 2/1 , alcoholism
16. Zintzaras et al. 2006 (23/2), CYP 2/1 , alcoholism
17. Zintzaras 2007 (11/1), MTHFR C677T, Down syndrome
18. Zintzaras 2007 (7/1), MTHFR A1298C, Down syndrome
19. Zintzaras et al. 2007 (14/3), MTHFR C677T , diabetic nephropathy
20. Zintzaras 2007 (5/2), BDNF G196A, schizophrenia
21. Amorim et al. 2008 (37/3), MTHFR C677T, Neural Tube Defects
22. Banerjee et al. 2008 (10/2), MTHFR C677T, stroke
23. Banerjee et al. 2008 (6/2), ACE I/D, stroke
24. Begovich et al. 2008 (13/1), FCRL3 -169T/C, rheumatoid arthritis
25. Lin et al. 2008 (13/2), 5-HTT S/L promoter, obsessive compulsive disorder
26. Loza et al. 2008 (12/2), IL4R Q551R, atopic asthma
27. Loza et al. 2008 (12/1), IL4R I50V, asthma
28. Medica et al. 2008 (14/2), AGT Met235Thr, preeclampsia
29. Medica et al. 2008 (11/1), ACE I/D, preeclampsia
30. Naoe et al. 2008 (8/1), BDNF Val66Met, schizophrenia
31. Pereira et al. 2008 (8/1), TNF G308A, ischemic stroke
32. Qian et al. 2008 (16/2), BDNF Val66Met, schizophrenia
33. Taioli et al. 2008 (10/3), MPO -463G/A, lung cancer
34. Tsantes et al. 2008 (14/1), PAI-1 4G/5G, ischemic stroke
35. Tsantes et al. 2008 (23/2), PAI-1 4G/5G, Venous Thrombosis
36. Verkleij-Hagoort et al. 2008 (10/1), MTHFR C677T , congenital heart defects
37. Zintzaras et al. 2008 (6/1), COMT G472A, panic disorder
38. Zintzaras et al. 2008 (6/1), MTHFR A1298C, multiple myeloma
39. Zdoukopoulos et al. 2008 (6/1), FV Leiden R506Q, placental abruption
40. Zdoukopoulos et al. 2008 (3/1), F II G20210A , placental abruption
41. Zintzaras et al. 2008 (108/19), ACE D/I , coronary artery disease
42. Zintzaras et al. 2009 (4/2), ALOX5AP SG13S89 G/A, stroke
43. Zintzaras et al. 2009 (6/1), MTHFR C677T , peripheral arterial disease
44. Zintzaras et al. 2009 (4/2), IL6 -174 G/C, peripheral arterial disease
45. Zintzaras et al. 2009 (6/1), ACE D/I, peripheral arterial disease

**Figure 4.** P-values of pooled estimates unadjusted and adjusted for deviation from HWE for the 72 meta-analyses.





**Figure 5.** Heterogeneity values ( $I^2$ ) without adjustment and after adjustment for deviation from HWE for the 72 meta-analyses.



## REFERENCES

1. Zintzaras E, Lau J. Synthesis of genetic association studies for pertinent gene-disease associations requires appropriate methodological and statistical approaches. *J Clin Epidemiol.* 2008;61:634-45.
2. Maynard Smith J. *Evolutionary Genetics.* Oxford University Press. New York 1998.
3. Wittke-Thompson JK, Pluzhnikov A, Cox NJ. Rational inferences about departures from Hardy-Weinberg equilibrium. *Am J Hum Genet.* 2005;76:967-86.
4. Xu J, Turner A, Little J, et al. Positive results in association studies are associated with departure from Hardy-Weinberg equilibrium: hint for genotyping error? *Hum Genet.* 2002;111:573-4.
5. Salanti G, Amountza G, Ntzani EE, et al. Hardy-Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power. *Eur J Hum Genet.* 2005;13:840-8.
6. Schaid DJ, Jacobsen SJ. Biased tests of association: comparisons of allele frequencies when departing from Hardy-Weinberg proportions. *Am J Epidemiol.* 1999;149:706-11.
7. Lathrop GM. Estimating genotype relative risks. *Tissue Antigens* 1983;22:160-6
8. Sato Y, Suganami H, Hamada C, et al. The confidence interval of allelic odds ratios under the Hardy-Weinberg disequilibrium. *J Hum Genet.* 2006; 51:772-80
9. Zintzaras E. Variance estimation of allele-based odds ratio in the absence of Hardy-Weinberg equilibrium. *Eur J Epidemiol.* 2008;23:323-6.

10. Zintzaras E, Koufakis T, Ziakas PD, et al. A meta-analysis of genotypes and haplotypes of methylenetetrahydrofolate reductase gene polymorphisms in acute lymphoblastic leukemia. *Eur J Epidemiol.* 2006;21:501-10.
11. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7:177-88.
12. Emigh TH. Comparison of tests for Hardy-Weinberg Equilibrium. *Biometrics.* 1980;36:627-42.
13. Severo NC, Zelen M. Normal approximation to the chi-square and non-central F probability functions. *Biometrika.* 1960;47:411-6.
14. Sasieni PD. From genotypes to genes: doubling the sample size. *Biometrics.* 1997;53:1253-61.
15. Munafo MR, Flint J. Meta-analysis of genetic association studies. *Trends Genet.* 2004; 20:439-44.
16. Minelli C, Thompson JR, Abrams KR, et al. How should we use information about HWE in the meta-analyses of genetic association studies? *Int J Epidemiol.* 2008;37:136-46.
17. Fardo DW, Becker KD, Bertram L, et al. Recovering unused information in genome-wide association studies: the benefit of analyzing SNPs out of Hardy-Weinberg equilibrium. *Eur J Hum Genet.* 2009; 17:1676-82.
18. Trikalinos TA, Salanti G, Khoury MJ, et al. Impact of violations and deviations in Hardy-Weinberg equilibrium on postulated gene-disease associations. *Am J Epidemiol.* 2006;163:300-9.
19. Goldstein DB. Common genetic variation and human traits. *N Engl J Med.* 2009; 360:1696-8.