# Missed Calculations and New Conclusions: Re-Calculation of Genotype Distribution Data Published in *Journal of Investigative Dermatology*, 1998–2003

To the Editor:

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 (Stern, 1943). The Hardy–Weinberg law (Hardy, 1908; Weinberg, 1908) states that should the genotype frequencies be perturbed for any reason, they will come to the expected equilibrium frequencies after one generation of random mating.

Population-genetic studies investigating genetic polymorphisms of Mendelian inheritance should always test the presence of the Hardy–Weinberg criteria. According to our experience however, data about HWE calculations are not always presented in articles. In this retrospective survey, we tested whether this law was checked in papers published in the *Journal of Investigative Dermatology* since 1998.

The number of publications about genetic polymorphisms between January 1998 and May 2003 in the *Journal of Investigative Dermatology* was 76. Of these, those articles ($n = 22$) that fulfilled the following criteria were selected: investigation of biallelic genetic polymorphism with Mendelian inheritance; use of healthy reference population at the study; and availability of genotype distribution data. We recalculated HWE in each available genotype using the Arlequin software (http://anthropologie.unige.ch/arlequin/). The level of statistical significance was set at $p < 0.05$.

Only eight of the selected 22 (36%) publications performed HWE calculations. HWE error occurred in nine papers and remained unreported in each case. In each case, we analyzed how the lack of HW criteria affects the interpretation of results (see Table I).

Gonzalez *et al* (2000) checked the presence of OTF3 gene polymorphisms and mutations of the MHC and other HLA genes in psoriasis. HW criteria were not fulfilled in the case of MHC S393 polymorphism in healthy reference subjects; therefore, the significance of this genetic polymorphism in psoriasis cannot be evaluated in this study. On the other hand, the MHC S394 polymorphism did not fulfill HW criteria in the diseased population, and this may suggest a possible association between the MHC S394 allele and psoriasis.

Mee and Cork (1998) found no correlation between VDR genotype and clinical responsiveness to calcipotriol; however, patients' genotype altogether missed HWE, suggesting a possible correlation between VDR BsmI and psoriasis. This theory is in line with the observed correlation between VDR BsmI and psoriasis in Japanese patients (Saeki *et al*, 2002). The calculated HW disequilibrium in the study would further strengthen the presence of the association between this genotype and psoriasis.

Pyo *et al* (2003) found that the TAP and HLA-DM alleles could lead to genetic susceptibility toward psoriasis in Koreans. They calculated HWE, but not for each gene. The exception was TAP2, as, according to their report, they could not discriminate heterozygotes by their typing system. Indeed, the TAP2-665 polymorphism misses the HWE in their population and this questions the conclusions drawn from their TAP2-665 results.

According to the finding of Richter-Hintz *et al* (2003) "variant alleles coding for phase II enzymes only GST M1, but not GST T1 or NQOR, correlated with a risk to contract psoriasis. Interestingly, heterozygosity for CYP2C19 alleles n1A and n2A was associated with increased risk for "late onset" psoriasis, whereas this genotype was protective for psoriatic arthritis." Although according to their report genotype distribution fulfilled HWE, NQOR and the GST M1 polymorphisms missed HWE in their reference population, and this questions their conclusions.

McGregor *et al* (2002) inferred functional differences between polymorphic forms of p53 that are likely to be relevant to skin carcinogenesis. The p53–72 genotype misses HWE in their study in healthy individuals with skin type V. Therefore, this finding should be treated cautiously for individuals with this skin type and should be further tested using another cohort of healthy reference subjects.

Another skin cancer study (Kennedy *et al*, 2002) detected no associations between MICA gene polymorphism and increased risk for skin cancer. The MICA 5.1 polymorphism missed the HWE in reference subjects, in basal cell carcinoma patients, and also in malignant melanoma patients.

Cairey-Remonnay *et al* (2002) found no correlation with human papillomavirus status in the TP53 polymorphism of exon 4 at codon 72 in cutaneous squamous cell carcinoma and immunocompetent individuals. The TP53 polymorphism of exon 4 at codon 72 misses, however, HWE in immunocompetent blood samples and, therefore, their finding (i.e., the TP53 polymorphism could present a potential risk factor for squamous cell carcinoma in renal transplant recipients) needs further support.

Werth *et al* (2002a) extensively investigated the genomics of dermatomyositis. In their first work, they concluded that

Abbreviation: HWE, Hardy–Weinberg equilibrium

**Table I. Genotype distribution of gene polymorphisms missing the Hardy–Weinberg equilibrium in publications of *Journal of Investigative Dermatology* published between 1998;110 and 2003;120**

| Publication | Gene polymorphism | Subjects | Genotype distribution AA/AB/BB | HWE p value |
|---|---|---|---|---|
| Mee and Cork (1998) | VDR BsmI | Psoriatic patients | 36/35/21 | 0.032 |
| Gonzalez *et al* (2000) | MHC S393 (Gly/Val) | Control | 102/0/2 | 0.001 |
| | MHC S394 (Ser/Leu) | Psoriatic patients | 41/51/3 | 0.006 |
| Pyo *et al* (2003) | TAP2-665 (Ala/Thr) | Controls | 20/103/61 | 0.016 |
| Richter-Hintz *et al* (2003) | GST M1 | Patients | 131/136/0 | 0.001 |
| | | Controls | 89/132/0 | 0.001 |
| | NQOR | Controls | 169/52/13 | 0.004 |
| McGregor *et al* (2002) | p53–72 genotype | Healthy individuals with skin type V | 16/16/16 | 0.021 |
| Kennedy *et al* (2002) | MICA 5.1 (major histocompatibility complex class I chain-related proteins) | Controls | 84/88/75 | 0.001 |
| | | Basal cell carcinoma patients | 84/98/79 | 0.002 |
| | | Malignant melanoma patients | 38/44/29 | 0.034 |
| Cairey-Remonnay *et al* (2002) | TP53, codon 72 of exon 4 | Immunocompetent blood samples (control) | 17/7/5 | 0.031 |
| Werth *et al* (2002a) | association of TNF −308A and HLA DR3 | Controls | 33/42/135 | 0.001 |
| | | Dermatomyositis | 12/9/18 | 0.001 |
| Werth *et al* (2002b) | MBL Asp54 | Controls | 119/37/11 | 0.005 |
| | | Caucasian DLE | 16/1/2 | 0.011 |
| | Association of TNF GG and MBL Asp54 | Controls, TNF GG group | 80/30/10 | 0.01 |

the tumor necrosis factor −308A polymorphism is associated with dermatomyositis. The lack of HWE, however, occurred in the combination of TNF-308A and HLA DR3. Therefore, the association of TNF-308A and HLA DR3 is further supported by this finding.

In the second publication (Werth *et al*, 2002b), the polymorphism of MBL Asp54 fulfilled HWE criteria neither in the control group nor in Caucasian DLE cases. As the control group missed HWE, the overrepresentation of this polymorphism in patients needs further investigations.

If the control population misses the HWE, the results should be treated cautiously because the observed genotype distribution in the reference population does not represent the genotype distribution in healthy people and, therefore, conclusions are of limited value. If HWE criteria are fulfilled in the healthy reference group, but not in the diseased population, this may be a further evidence for the existence of the association between disease and polymorphism (for statistical details, see Deng *et al*, 2001; Hoh *et al*, 2001).

There are several explanations as to why the observed genotype frequency deviates significantly from the expected one. One or more of the assumptions of the model may be incorrect, non-random mating or gene flow occurred between the sampled and another population, or selection has operated. If the HW law is not fulfilled, there might also be a sampling error (Hedrick, 1983).

In conclusion, in line with the recommendation of Tiret and Cambien (1995), we suggest that providing genotype distribution data along with the results of performed HWE calculations should be a must when the results of population-based genetic studies are published.

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DOI: 10.1111/j.0022-202X.2004.22334.x

Manuscript received July 31, 2003; revised September 24, 2003; accepted for publication September 29, 2003

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