10. Lindley, D.V. (1988). Statistical inference concerning Hardy-Weinberg equilibrium. In Bayesian Statistics, 3, J.M. Bernardo, M.H. DeGroot, D.V. Lindley, and A.F.M. Smith, eds. (Oxford, UK: Oxford University Press), pp. 307–326.
11. Montoya-Delgado, L.E., Irony, T.Z., de B Pereira, C.A., and Whittle, M.R. (2001). An unconditional exact test for the Hardy-Weinberg equilibrium law: sample-space ordering using the Bayes factor. Genetics *158*, 875–883.
12. Wellek, S. (2004). Tests for establishing compatibility of an observed genotype distribution with Hardy-Weinberg equilibrium in the case of a biallelic locus. Biometrics *60*, 694–703.
13. Pereira, C.A.B., Nakano, F., Stern, J.M., and Whittle, M.R. (2006). Genuine Bayesian multiallelic significance test for the Hardy-Weinberg equilibrium law. Genet. Mol. Res. *5*, 619–631.
14. Wigginton, J.E., Cutler, D.J., and Abecasis, G.R. (2005). A note on exact tests of Hardy-Weinberg equilibrium. Am. J. Hum. Genet. *76*, 887–893.
15. Yates, F. (1934). Contingency tables involving small numbers and the χ^2 test. Journal of the Royal Statistical Society (Supplement) *1*, 217–235.
16. Yates, F. (1984). Tests of significance for 2 × 2 contingency tables. J. R. Stat. Soc. [Ser A] *147*, 426–463.
17. Fleiss, J.L. (1981). Statistical methods for rates and proportions, Second Edition (New York: John Wiley & Sons).
18. Frazer, K.A., Ballinger, D.G., Cox, D.R., Hinds, D.A., Stuve, L.L., Gibbs, R.A., Belmont, J.W., Boudreau, A., Hardenbol, P., Leal, S.M., et al; International HapMap Consortium. (2007). A second generation human haplotype map of over 3.1 million SNPs. Nature *449*, 851–861.
19. Haldane, J.B.S. (1954). An exact test for randomness of mating. J. Genet. *52*, 631–635.
20. Levene, H. (1949). On a matching problem arising in genetics. Ann. Math. Stat. *20*, 91–94.
21. Weir, B.S. (1996). Genetic Data Analysis II (Massachusetts: Sinauer Associates).
22. Rohlfs, R.V., and Weir, B.S. (2008). Distributions of Hardy-Weinberg equilibrium test statistics. Genetics *180*, 1609–1616.
23. Graffelman, J., and Camarena, J.M. (2008). Graphical tests for Hardy-Weinberg equilibrium based on the ternary plot. Hum. Hered. *65*, 77–84.
24. Li, M., and Li, C. (2008). Assessing departure from Hardy-Weinberg equilibrium in the presence of disease association. Genet. Epidemiol. *32*, 589–599.
25. Fardo, D.W., Becker, K.D., Bertram, L., Tanzi, R.E., and Lange, C. (2009). Recovering unused information in genome-wide association studies: the benefit of analyzing SNPs out of Hardy-Weinberg equilibrium. Eur. J. Hum. Genet. *17*, 1676–1682.
26. Minelli, C., Thompson, J.R., Abrams, K.R., Thakkinstian, A., and Attia, J. (2008). How should we use information about HWE in the meta-analyses of genetic association studies? Int. J. Epidemiol. *37*, 136–146.
27. Zou, G.Y., and Donner, A. (2006). The merits of testing Hardy-Weinberg equilibrium in the analysis of unmatched case-control data: a cautionary note. Ann. Hum. Genet. *70*, 923–933.
28. International HapMap Consortium. (2003). The international hapmap project. Nature *426*, 789–796.
29. International HapMap Consortium. (2005). A haplotype map of the human genome. Nature *437*, 1299–1320.
30. R Development Core Team. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria, 2004. URL http://www.R-project.org. ISBN 3-900051-00-3.

DOI 10.1016/j.ajhg.2009.11.019.

Response to Graffelman: Tests of Hardy-Weinberg Equilibrium

To the Editor: Testing for Hardy-Weinberg equilibrium (HWE) is perhaps the most common quality-control procedure in all of human genetics. Although there are many potential explanations for departures from HWE, the prototypical causes of departure from HWE are genotyping error and differential missing-data rates among genotypes.[1] These two are critically important because they can give rise to false positives in genetic association studies.[2] Standard practice in association studies is to test for HWE in all samples (or control samples) and to reject any marker with a p value for HWE $< \alpha$. For the HapMap project,[3,4] $\alpha = 0.001$, but other studies might elect different values.

For large samples and common alleles, a convenient means of calculating these p values is to use a simple χ^2 test. However, this χ^2 test requires two simplifying assumptions that are never true: (1) that heterozygote counts are approximately normally distributed and (b) that these counts are continuous. In a Letter to the Editor, Graffelman suggests that a continuity correction mitigates problems associated with the second assumption. In our view, the best solution to the problems associated with using a χ^2 test is the use of an exact test. A major impediment to exact tests is the associated computational burden, but that burden is greatly diminished with the use of the algorithm of Wigginton et al.[5] for calculating exact probabilities and test statistics.

Wigginton et al. note that with exact probabilities in hand, there are four possible tests of HWE. Specifically, they outline two one-tailed tests (P_{low}, P_{high}) and two two-tailed tests (P_{HWE}, $P_{2\alpha}$). They define P_{HWE} as the probability of observing a genotype configuration at least as unlikely as that actually observed and $P_{2\alpha}$ as min(1.0, $2P_{high}$, $2P_{low}$). Wigginton et al. recommend that P_{HWE} should be used in almost all circumstances and discard $P_{2\alpha}$ as too conservative (i.e., as producing incorrect probability values).

P_{DOST} = min($2P_{high}$, $2P_{low}$), the statistic proposed by Graffelman, is just an imperfect approximation of $P_{2\alpha}$. P_{DOST} often takes values $>$ 1.0 and still produces

incorrect p values whenever allele frequencies are unequal. If we denote $P_{DOST}(\xi)$ as the value of P_{DOST} associated with sample-configuration ξ, we can guarantee that, under the null hypothesis of HWE, $P(P_{DOST} \leq P_{DOST}(\xi)) < P_{DOST}(\xi)$ whenever allele frequencies are unequal. In contrast, using P_{HWE}, P_{low}, P_{high} guarantees a properly calibrated test statistic so that, for example, $P(P_{HWE} \leq P_{HWE}(\xi)) = P_{HWE}(\xi)$, regardless of allele frequency.

A simple example is illustrative. Consider a sample of 100 individuals in whom two copies of the rare allele are present. Two configurations are possible, one with two heterozygotes and another with a single rare allele homozygote. The first configuration has probability of 198/199, and the second has a probability of 1/199. Suppose a single homozygote is observed. This gives a χ^2 test p value of $< 10^{-22}$ (without continuity correction) or $< 10^{-6}$ (with continuity correction). Both are clearly wrong. Using P_{DOST} is "better," giving p = 2/199, but still incorrect. In contrast, P_{HWE} correctly specifies that a configuration such as this occurs with p = 1/199. For rare alleles and unlikely configurations, P_{DOST} and $P_{2\alpha}$ are effectively equal to $2P_{HWE}$. For common alleles and large samples, the three statistics converge to more similar values, but, in those settings, χ^2 test approximations can be conveniently used.

The fact that P_{DOST} and P_{2a} detect fewer departures from HWE is not a virtue. It simply reflects that they are poorly calibrated statistics. If an investigator wishes to discard fewer SNPs due to HWE departures, it is more appropriate to lower α, the threshold for rejection. Just as we found no reason to recommend $P_{2\alpha}$ originally, we find no reason to recommend P_{DOST} now.

David J. Cutler[1,*] and Gonçalo R. Abecasis[2,*]
[1]Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322, USA; [2]Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI 48109, USA
*Correspondence: djcutle@emory.edu (D.J.C), goncalo@umich.edu (G.R.A)

References

1. McCarthy, M.I., Abecasis, G.R., Cardon, L.R., Goldstein, D.B., Little, J., Ioannidis, J.P., and Hirschhorn, J.N. (2008). Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat. Rev. Genet. *9*, 356–369.
2. Mitchell, A.A., Cutler, D.J., and Chakravarti, A. (2003). Undetected genotyping errors cause apparent overtransmission of common alleles in the transmission/disequilibrium test. Am. J. Hum. Genet. *72*, 598–610.
3. International HapMap Consortium. (2003). The International HapMap Project. Nature *426*, 789–796.
4. Frazer, K.A., Ballinger, D.G., Cox, D.R., Hinds, D.A., Stuve, L.L., Gibbs, R.A., Belmont, J.W., Boudreau, A., Hardenbol, P., Leal, S.M., et al; International HapMap Consortium. (2007). A second generation human haplotype map of over 3.1 million SNPs. Nature *449*, 851–861.
5. Wigginton, J.E., Cutler, D.J., and Abecasis, G.R. (2005). A note on exact tests of Hardy-Weinberg equilibrium. Am. J. Hum. Genet. *76*, 887–893.

DOI 10.1016/j.ajhg.2010.03.012.

Tuba8 Is Expressed at Low Levels in the Developing Mouse and Human Brain

To the Editor: Sheridan and colleagues recently reported that mutations in the tubulin gene *TUBA8* result in polymicrogyria with optic nerve hypoplasia (PMGOH [MIM 613180]).[1] This conclusion is based on the mapping of two consanguineous families of Pakistani origin to a 7.42 Mb region on chromosome 22q11.2 that contains ~230 genes including *TUBA8*. Drawing on our previous finding that mutations in *TUBA1A* cause lissencephaly[2] and that mutations in *TUBB2B* cause asymmetric polymicrogyria,[3] Sheridan and colleagues sequenced *TUBA8* and found a 14 bp deletion in intron 1 that affects splicing. They provide further evidence that *TUBA8* is involved in the disease state by analyzing its expression in the developing mouse brain by in situ hybridization. They report that *Tuba8* is widely expressed in developing neural structures, with strongest expression in the cortical plate at E15.5 and E18.5 and in the cortical plate, subplate, and hippocampus at P0.

A meaningful analysis of individual tubulin gene expression by in situ hybridization requires the use of probes that avoid cross-hybridization among the highly conserved coding regions, relying exclusively on either the variant 5′ or 3′ untranslated regions. The probe employed by Sheridan and colleagues was 443 nucleotides in length, of which 415 correspond to sequences contained within the conserved coding region. Consequently, this probe shares a very high sequence homology with other α-tubulins.[4] An *Ensembl* BLAST search with the Sheridan probe against total mouse cDNA results in six other hits, each being at least 300 nucleotides in length with at least 80% sequence identity. Each of these hits corresponds to one of the six other members of the α-tubulin family and includes a 374 nucleotide stretch that shares 84.2% identity with the coding sequence of *Tuba1a*, a gene that is highly expressed in the developing CNS.[5]

To establish whether the results reported by Sheridan and colleagues are a consequence of cross-hybridization, we conducted in situ hybridization on the developing (E14.5, E16.5, and P0) and adult mouse brain employing their probe and two others that we designed. We first confirmed the sequence of *Tuba8* mRNA by amplifying