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A Letter to the Editor In Reply To “Susceptibility to Guillain-Barre syndrome is associated to polymorphisms of CD1 genes” by Caporale et al. in the J of Neuroimmunology (2006) 177:112-118.

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To the Editor. Caporale et al. (2006) described susceptibility to Guillain-Barre syndrome (GBS) as partially accounted for by the variation in CD1 genes. The claim that the genotypes of CD1 are the risk factors for GBS is supported by small p-values for tests of association (see Table 4 in Caporale et al.). However, upon examination, the p-values appear to correspond to one-sided tests, always chosen by the authors to be in the direction of the observed difference of the genotype frequencies between the GBS cases and the controls. If the observed odds ratio (OR) is greater than one, Caporale et al. consider the alternative hypothesis concerning the population OR to be $H_A: OR > 1$; otherwise they simply flip the direction of their hypothesis.

There are sometimes good theoretical reasons to prefer one-sided hypotheses if the hypothesized direction can be chosen beforehand (e.g., specified in the study protocol). For example, if safety of a drug is a concern, an increased frequency of side effects can be tested, based on a one-sided hypothesis. Caporale et al. adopted one-sided tests but did not provide any reasons for the anticipated direction of the disease risk associated with a particular genotype. Choice of the direction of the one-sided hypothesis after having looked at the data leads to greatly inflated chances to reject the null hypothesis of no association, when the null hypothesis is true. Various issues about one-sided vs. two-sided hypothesis testing have been discussed in a number of previous publications (Casella and Berger 1987; Fisher 1991; Koch 1991; Bland and Altman 1994).

In Table 1 below, we attempted to recreate Table 4 from the original publication. We reached major and minor discrepancies. It appears that some p-values reported by Caporale et al. are first computed using an inappropriate one-sided test, and then reported with typos. For example, the p-value of 0.0035 is marked as “non-significant”, at the level of 0.017, and appears to correspond to the p-value of 0.035 from the one-sided Fisher exact test. The correct two-sided p-value is 0.06. One significant result in the table becomes no longer significant. We believe that two-sided tests (with the corresponding two-sided p-values) are appropriate for all entries in the table, because no directional hypothesis was formulated *a priori*. There might be a similar issue with the results presented in Table 5 of Caporale et al., however it is impossible to verify the p-values in the absence of the joint frequency data. We note another typographical error to

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be corrected in Table 5: $p < 0.00167$ should be replaced by $p < 0.0083$ (for 0.05 α -level) in the footnote, or the row of CD1A*01/02-CD1E*01/02 should not be declared significant (compared to 0.01 α -level if this was the authors' intention).

We also calculated Fisher's exact p-values for the "overall" test that includes all three genotype classes at once. The association p-values are 0.006 (for CD1A) and 0.025 (for CD1E). Although these overall tests give support to the authors's conclusions, a potential issue of sample heterogeneity still remains. The article does not contain information on genetic homogeneity of the study sample. Heterogeneity can be a source of confounding, leading to spurious associations. To rule out sample heterogeneity, Caporale et al. conducted a HWE test. However, a single-locus HWE test is not particularly sensitive to stratification. For example, if two populations with the allele frequency difference of 0.1 are mixed together, there will be a resulting excess of the homozygote frequency of only 0.0025 (Hedrick 2005), which would be difficult to detect by a HWE test. Although the HWE may not hold for reasons other than population stratification, undetected stratification can lead to confounding, and result in false positives. Deng, Chen and Recker (2001) report that sample sizes required to detect population admixture by the HWE test using a single di-allelic marker at a time are very large. To increase power, several SNPs can be used simultaneously to detect the overall deficit of heterozygosity caused by the stratification (known as Wahlund effect). Lee (2003) suggested a simple test applicable for multiple genetic markers in linkage equilibrium.

Genetic association studies offer a great potential for uncovering mechanisms of complex diseases. Nevertheless, too few studies replicate successfully (Terwilliger and Weiss 1998; Ioannidis et al. 2001; Vieland 2001; Lohmueller et al. 2003; Lucentini 2004). There might be numerous reasons for a low replicability. These range from lack of transitivity property of linkage disequilibrium (Terwilliger and Hiekkalinna 2006) and low statistical power to publication bias and no consideration of multiple testing (Thomas and Clayton 2004). While these reasons might be to some extent beyond the investigator's control, we would like to emphasize that proper statistical techniques and careful reporting of data summary and statistical analysis should always be ensured.

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CD1A-CD1E genotypes as risk factor for Guillain-Barre syndrome (the original Table 4 in Caporale et al. with revised entries by us)

Table 1

Genotype	OR (original)	OR (revised)	Fisher's p (original)	Fisher's p (original reproduced)	Fisher's p (revised using 2-sided p)	Bonferroni Significance (original)	Bonferroni Significance (revised)
CD1A							
01/01	ND	ND	0.3902 [§]	0.1537 (U)	0.1537	NS	NS
01/02	0.28	0.28	0.0076	0.0077 (L)	0.011	*	*
02/02	2.5	2.5	0.0035 [§]	0.035 (U)	0.06	NS	NS
CD1E							
01/01	2.52	2.41	0.0036 [§]	0.0053 (U)	0.0097	*	*
01/02	0.45	0.47	0.0100	0.0143 (L)	0.025	*	NS
02/02	0.74	0.75	0.3802	0.3868 (L)	0.62	NS	NS

U and L denote upper and lower direction, respectively.

[§]We could not reproduce these values.

* p<0.016667, that is a threshold valid for 3 tests assuming 5% overall α level (i.e., family-wise error rate for false-positives) for each gene separately.