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Sir,

Lumican and muscarinic acetylcholine receptor 1 gene polymorphisms associated with high myopia

Lin *et al*[1] report that polymorphisms in the lumican gene are associated with high myopia. They studied four SNPs in 182 highly myopic cases and 78 emmetropic controls, recruited from a sample of 3000 Taiwanese-born medical students aged 16–25 years old (Table 1a). They also published a second study addressing the same question,[2] testing five SNPs—including four of those above—in 201 high myopes and 86 emmetropic controls, also recruited from 3000 Taiwanese-born volunteers aged 16–25 years old. For the four SNPs common to both studies, the results were similar.

In neither article is the other study referred to, which suggests that the two investigations are unlikely to be an original study followed by a replication study. Thus, it seems possible that the same SNPs were genotyped twice (albeit using different methods) in a common set of subjects and reported as being independent results. If true, this would contravene established scientific practice (because it produces a false impression of the weight of evidence supporting a gene–disease association).

In another recent publication, Lin *et al*[3] genotyped four SNPs in the *CHRM1* gene in their Taiwanese case–control subjects. Genotype frequencies were hugely different ($P<10^{-7}$) between cases and controls for two SNPs (rs544978 and rs544269) suggesting strong association.[3]

Lin *et al* tested for departure from Hardy–Weinberg equilibrium (HWE), as this can be indicative of genotyping error[4] (Table 1a). We repeated these tests (R genetics HWE.test function) using the data in Table 2 of their article.[3] Our results (Table 1b) showed that both myopia-associated SNPs were not in HWE in controls and that Lin *et al*'s HWE test results were erroneous. Although departure from HWE can occur in case–control samples if the disease–polymorphism association is strong,[4] such strong association was not detected at the *CHRM1* locus in a high-myopia GWA in Japanese subjects.[5] The possibility of genotyping error weakens the evidence of an association between *CHRM1* polymorphisms and high myopia. We also noticed inconsistencies between the data reported by Lin[3] and the HapMap database, for example in the alleles of rs544978 and the allele frequencies of rs2186410 (Table 2a and b).

Table 1a HWE test *P*-values originally reported by Lin *et al*[3]

SNP	*HWE test* P-*value* (χ^2 *test)*	
	Cases	*Controls*
rs11823728	0.023	0.048
rs544978	NSD	0.012
rs2186410	NSD	NSD
rs542269	NSD	NSD

Table 1b HWE test *P*-values re-calculated from the data of Lin *et al*[3]

SNP	*HWE test* P-*value (*R *genetics HWE test)*	
	Cases	*Controls*
rs11823728	NSD	NSD
rs544978	NSD	1.867e−10
rs2186410	1.741e−11	1.450e−15
rs542269	NSD	8.231e−16

NSD, not statistically different from the null hypothesis of HWE.

Table 2a Characteristics of *CHRM1* polymorphisms reported by Lin *et al*[3]

SNP	*Alleles (frequencies)*	
	Cases	*Controls*
rs11823728	G/A (0.98 : 0.02)	G/A (0.99 : 0.01)
rs544978	G/A (0.87 : 0.13)	G/A (0.76 : 0.24)
rs2186410	G/A (0.17 : 0.84)	G/A (0.17 : 0.84)
rs542269	G/A (0.12 : 0.88)	G/A (0.15 : 0.85)

Table 2b Characteristics of *CHRM1* polymorphisms in the HapMap database

SNP	*Alleles (frequencies) HapMap HCB subjects Controls*	*HWE test* P-*value HapMap HCB subjects Controls*
rs11823728	C/T (1.00 : 0.00)	NA
rs544978	A/C (0.87 : 0.13)	1.00
rs2186410	G/A (0.87 : 0.13)	1.00
rs542269	C/T (0.13 : 0.87)	1.00

Conflict of interest

The authors declare no conflict of interest.

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Sir,

Reply to Guggenheim *et al*

We thank Guggenheim *et al*[1] for their critical comments on our recently published papers.[2,3] We would like to reply to their comments as follows. Myopia is a multigenetic condition involving several overlapping signalling pathways. Therefore, the effects of lumican (*LUM*) and muscarinic acetylcholine receptors 1 (*CHRM1*) in myopia are likely to be different between Taiwanese patients and those from other ethnic origin because of differences in environment and race.[4] LUM was initially described as a proteoglycan responsible for the control of collagen fibrillogenesis and interaction. The *LUM* gene is located at 12q21-23 (*MYP3*), which is a locus associated with high-grade myopia. However, the association between *LUM* gene and myopia is controversial. We performed two independent studies to identify the association between myopia and *LUM* gene. The one published in *Eye* is actually a study that was completed more than 2 years ago. The paper submission and publication process was long and complicated. After completion of the previous study, we tried to identify the possible functional roles of *LUM* in the pathogenesis of myopia, for which we performed whole gene sequencing analysis on *LUM* gene and identified one novel polymorphism. The influence of this newly identified polymorphism on the expression levels of *LUM* was also studied. Taken together, the single-nucleotide polymorphisms in *LUM* genes may have multiple effects on the expression level of *LUM*. We have thoroughly studied and listed the association between *LUM* and myopia in a manner that is not biased on account of using few patients or only one SNP.

We must admit that we made errors in the statement of Hardy–Weinberg equilibrium (HWE). The text in one of our reports reads:[5] 'In the test of HWE, there were departures from HWE for S1 in both the control and high myopia groups ($P = 0.048$ and 0.023, respectively) and for S2 in the control group ($P = 0.012$).' These values were not P values but were the results of HWEχ^2. Consequently, the exact statement of the sentence must be changed to 'In the test of HWE, there were no departures from HWE for S1 in both the control and high myopia groups (HWE$\chi^2 = 0.048$ and 0.023, respectively) and for S2 in the control group (HWE$\chi^2 = 0.012$). The S3 and S4 polymorphisms were not in HWE.' We had explained the possible problems about departures from HWE in the fourth paragraph in the text,[5] and hoped this could decrease the doubt on genotyping or population stratification. Definitely, we must make painstaking efforts in our future studies.

Conflict of interest

The authors declare no conflict of interest.

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