Title

Reply to "A promoter polymorphism in the CASP8 gene is not associated with cancer risk"

Permalink

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cancer study (743 cases and 987 controls) includes Chinese, Japanese or Filipino cases between the ages of 25 and 74 years at the time of diagnosis (1995–2001)[7]. Cases in the colorectal cancer study (356 cases and 413 controls) were diagnosed at ages 55–74 years during 1998–2002 (ref. 5). In both studies, cases were identified through the Los Angeles County and statewide cancer registries, and controls were selected from the neighborhoods where the cancer cases resided at the time of diagnosis (**Supplementary Table 1** online). All three of these studies were approved by the appropriate institutional review boards at the University of Southern California and the University of Hawaii. In these studies, the 6-bp insertion/deletion polymorphism was genotyped using the TaqMan assay (Applied Biosystems). We assessed genotype data quality by typing 2–5% blinded replicate samples; the concordance rate was 99.4% (**Supplementary Methods** online).

The deletion variant was common in all populations examined and ranged in frequency from 16% in Japanese to 54% in African Americans (**Table 1**). No deviation from Hardy-Weinberg equilibrium was observed (at $P < 0.05$) in any racial or ethnic group. We found no significant inverse association between this deletion variant in the *CASP8* gene with risk of any of these three common cancers in pooled analyses (breast, odds ratio (OR) = 0.98, 95% confidence interval (CI) = 0.91–1.06; prostate, OR = 0.97, 95% CI = 0.89–1.05; colorectal, OR = 1.03, 95% CI = 0.94–1.13; **Table 1**); nor were

significant associations noted for heterozygous or homozygous carriers of the deletion allele (**Supplementary Table 2** online). The effects in each population were consistent, with the exception of a nominally significant positive association observed with colorectal cancer in Latinos, which may possibly be due to chance (**Table 1**). For each site, we had >99.7% power to detect an OR of 0.75 per copy of the deletion allele, which had been reported previously by Sun *et al.*[2] (assuming a log additive model, a two-sided test using $\alpha = 0.05$ and a variant allele frequency of 16%, the lowest allele frequency in any one of the populations examined). With this conservative scenario, we also had reasonably good power ($\geq 80\%$) to detect effects as small as 0.85 per allele for each cancer site. In addition to its large size and multiple cancer endpoints, this study included groups of various ancestral backgrounds with very different disease risks and allele frequencies. Thus, the likelihood that the lack of any significant association is attributable to bias or population stratification is much reduced.

Our inability to replicate the findings of Sun *et al.*[2] may be due to possible genetic and/or environmental modifiers that vary in frequency between populations of various ancestries in the United States and the Chinese populations studied by Sun *et al.*[2], or it may be because the initial association was a false positive. A recent breast cancer study by Frank *et al.*[8] (7,753 cases and 7,921 controls) also found no significant evidence that this variant has an effect on breast cancer risk among Europeans. Although we cannot com-

ment on the other cancer sites studied by Sun *et al.*[2] (lung, stomach, esophagus, cervix), our results do not support the hypothesis that this specific polymorphism, which has been proposed to be biologically functional, is an independent marker of breast, colorectal or prostate cancer risk in populations of Asian, European or African origin.

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Sun *et al*. reply:
We recently identified a 6-bp insertion/deletion polymorphism (–652 6N ins/del) in the promoter of the *CASP8* gene that removes an Sp1 binding site and that was shown to be associated with reduced *CASP8* transcription in lymphocytes. We further demonstrated that T lymphocytes with the deletion variant had lower caspase-8 activity and lower cancer cell antigen–induced cell death. We also reported that this genetic variant was associated with significantly reduced risk of several human cancers in a large case-control study in homogenous Han Chinese populations[1]. Haiman *et al.* now report a case-control study seeking to replicate our findings of this *CASP8* variant in breast, prostate or colorectal cancers in multiple US populations of various ancestries and self-declared ethnic identification (African Americans, Japanese Americans, Latinos, European Americans,

Native Hawaiians and Chinese Americans), but they did not find statistical evidence for any association[2]. Although the reasons for the discrepancies between their findings and ours are currently unknown, we have some thoughts that may offer some explanation.

First, the inconsistency may be related to sample sizes of selected populations. The overall sample sizes for each cancer site in Haiman *et al.* seem large (2,841 breast cancer cases, 1,529 colorectal cancer cases and 2,825 prostate cancer cases[2]), and comparable to those in our study (which included 1,125 individuals with breast cancer and 930 individuals with colorectal cancer[1]). We are concerned, however, that the number of cases included in some of the ancestry-specific groups for a specific cancer type may still be too limited to make a conclusive direct comparison. Perhaps most relevant for comparison to our study, the Chinese

American group (from the LABC study) in Haiman *et al.* included only 263 breast cancer cases[2]. Given that any single study may generate some spurious results[3], such initial findings need to be validated or replicated in much larger studies for each ancestry or ethnic group.

Second, the discrepant findings may be related to population-specific differences not only in genetic variation, but also in gene–environment interactions. Other variants have also recently been suggested to have population-specific differences in association with different cancers. For example, the *CASP8* D302H and *CASP10* V410I SNPs have been shown to be associated with reduced risk of breast cancer in populations of predominantly European ancestry[4]; however, both SNPs seem extremely rare (*CASP8* D302H) or nonexistent (*CASP10* V410) in some Asian populations (from a search of

the HapMap Project and the Environmental Genome Project databases). Similarly, we identified the *CASP8* −652 6N ins/del variation as a risk modifier of several cancers including breast cancer in a Chinese population[1], but this variant was not found to be associated with breast cancer risk in another recent study involving four independent European breast cancer cases-control studies[5]. Instead of signaling a false association, these studies reporting failures to replicate

in other populations may signal the population-specific nature of the association.

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Analysis of association of the TIRAP (MAL) S180L variant and tuberculosis in three populations

To the Editor:

Khor *et al.*[1] have recently reported an association of four different infectious diseases, including tuberculosis, with the amino acid–changing polymorphism rs8177374 (C→T, S180L) in the toll-interleukin-1 receptor domain–containing adaptor protein (TIRAP, also known as MyD88 adaptor-like, or MAL). This association was observed across a wide range of populations, and its estimated effect in tuberculosis (odds ratio (OR) = 0.23) was considerably larger than the confirmed effects of the causal polymorphisms in other complex diseases ($0.5 < OR < 2$)[2–5]. The tuberculosis associations in Khor *et al.* were observed in 675 patients and 605 controls from West Africa (a combined sample of individuals from Gambia, Guinea-Bissau and the Republic of Guinea; $P = 0.013$), and in families from West Africa and Algeria ($P_{one-tailed} = 0.075$ and 0.02, respectively).

We attempted to replicate the association between this TIRAP S180L variant and tuberculosis in three independent sample collec-

tions. These collections each were larger than the tuberculosis case-control collection of Khor *et al.* and represented a European, an African and an Asian population. We genotyped rs8177374 in 9,441 subjects, comprising 1,867 HIV-negative, culture-confirmed adult pulmonary tuberculosis cases and 2,076 controls from Russia[6], 1,913 HIV-negative, smear-positive pulmonary tuberculosis cases and 2,293 controls from Ghana[7], and 611 HIV-negative culture-confirmed adult pulmonary tuberculosis cases and 681 controls from Indonesia (**Supplementary Note** online). We found no evidence of association with tuberculosis in any of these collections (**Table 1**), indicating neither a multiplicative allelic effect nor a protective effect of Ser/Leu heterozygosity, as in the model proposed by Khor *et al.* Our sample collections were large and had statistical power to detect an effect with OR = 0.23 estimated by Khor *et al.* (**Table 1**). Given that the Leu180 allele is much more common in the Russian population than in West African populations (11.3% and <1%, respectively),

the Russian sample was also powered to detect smaller effects (for example, >90% power to detect OR = 0.75 for Ser/Leu heterozygotes at α = 0.05). Importantly, our Ghanaian collection originates from West Africa, thereby resembling the population studied by Khor *et al.* Therefore, it seems very unlikely that our study missed a true association, and we conclude that rs8177374 does not have a measurable effect on tuberculosis in these populations. Of note, a recent study from Vietnam reported association of a neighboring polymorphism in TIRAP with meningeal tuberculosis in a sample of 358 tuberculosis cases and 392 controls, but it was underpowered to evaluate the effect of rs8177374 (ref. 8).

In complex diseases, the prior probability of a true association is low even for a biologically plausible candidate gene. Given the marginal level of statistical support obtained for each of the individual collections studied by Khor *et al.* ($P = 0.08$–0.003), the probability of false association for each of the diseases remained high[9]. Khor *et al.* considered the association

Table 1 Association analysis of the rs8177374 (C>T, S180L) polymorphism in the TIRAP gene with tuberculosis

Samples	Leu/Leu (%)	Ser/Leu (%)	Ser/Ser (%)	MAF (%)	OR (95% CI)		P value	Power[e] (%)
Russian								
Controls	34 (1.6)	400 (19.3)	1,642 (79.1)	11.3	0.98[a]	(0.85–1.13)	0.79[c]	100/100
TB cases	23 (1.2)	369 (19.8)	1,475 (79.0)		1.03[b]	(0.88–1.21)	0.69[c]	
Ghanaian								
Controls	0 (0)	6 (0.3)	2,287 (99.7)	0.1	0.97[b]	(0.29–3.25)	0.96[d]	51.8/ 81.3
TB cases	0 (0)	5 (0.3)	1,908 (99.7)					
Indonesian								
Controls	1 (0.1)	17 (2.5)	663 (97.4)	1.4	1.06[a]	(0.55–2.02)	0.90[c]	86.3/ 97.5
TB cases	1 (0.2)	16 (2.6)	594 (97.2)		1.05[b]	(0.53–2.10)	0.91[c]	

95% CI, 95% confidence interval; OR, odds ratio; MAF, minor allele frequency.

[a]OR for the Leu allele. [b]OR for the Ser/Leu heterozygotes versus Ser/Ser and Leu/Leu homozygotes. [c]Calculated by the Mantel-Haenszel test controlling for the origin of the sample (**Supplementary Methods** and **Supplementary Table 1** online). [d]Calculated by the Mantel-Haenszel test adjusted for the ethnic group. [e]Statistical power to detect OR = 0.23 for Ser/Leu heterozygotes at one-tailed P value = 0.05/one-tailed P value = 0.2. We did not detect one-tailed $P < 0.2$ for the protective effect of Ser/Leu in any of our sample collections, although all three of them, including the Ghanaian collection, had > 80% power to detect it.