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Reply to Elson et al.

To the Editor:

We recently reported an association study of common mtDNA variation with risk of type 2 diabetes and metabolic traits.[1] We tested a simple model with few assumptions: that each common DNA sequence variant might, irrespective of its place in the mtDNA phylogeny, be associated with disease. Although Elson et al.[2] agree that our "approach will detect a robust disease association," they have three concerns: (i) that the catalogue of common variation is not representative of European mtDNAs, (ii) that our tag SNPs do not adequately capture mtDNA haplogroups, and (iii) that we failed to consider epistasis. We believe these views to be misinterpretations of our article, as well as a reflection of a difference in philosophy on limiting association studies on the basis of prior assumptions.

We developed a catalogue of common mtDNA variation on the basis of publicly available mtDNA sequences. Perhaps we were insufficiently precise in the text, as Elson et al. misinterpret which samples were used. In the European alignment, we studied 928 mtDNAs of only European origin: 429 from MitoKor, 240 from the Armed Forces Institute of Pathology, 192 from Finland, and 67 from smaller studies. (Although we used 536 MitoKor sequences for

worldwide alignment, only 429 were included in subsequent analyses.)

Elson et al. criticize our inclusion of m.13105, which they describe as "most often associated with African lineages."[2] Although it is true that m.13105 is more common in African samples, it is present at a frequency of 1.2% in the 6,000 European samples we studied and at 3% in HapMap CEU samples.[3] We are perplexed as to why Elson et al. write that this variant "should not have been used in the analysis,"[2] because it is in fact present in our samples and could, in principle, influence traits. We do agree that our catalogue is only as representative as the public database used to create it. We note that association testing performed using tags from 928 complete sequences is dramatically more complete than current standards.

Second, Elson et al. assert that our tag SNP and testing strategy captures only a small fraction of European mtDNA phylogeny.[2,4] As shown in table 4 of our article,[1] the common variants identified, as well as nine canonical European haplogroups, are well predicted by the tag SNPs and specified haplotype tests. Perhaps Elson et al. do not consider the specified haplotype tests that we performed, as, in their example (shown in red in their fig. 1), although no single SNP captures m.5046 of haplogroup W, a specified haplotype test (that we did perform) does. We do not understand why Elson et al. argue that our strategy might lead to "spurious associations,"[2] nor do Elson et al. provide an explanation of this claim.

Third, we agree that interaction among variants may alter risk, which is why we performed pairwise tests involving all SNPs with nominal $P < .1$ in the initial screen.[1(p57)] Marchini et al. showed this approach to be well powered.[5,6]

Finally, Elson et al. argue that limiting association tests to the classical phylogeny and variants with "direct functional consequences" will "increase the power of the study."[2] We agree—under the model in which their assumptions are correct. Of course, under the model in which some causal variants are not restricted to a classical haplogroup or might not yet be suspected as contributing to disease, power will decrease under the advocated approach.

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Genetic Association Analysis of *RHOB* and *TXNDC3* in Osteoarthritis

To the Editor:

In the May 2006 issue of *The American Journal of Human Genetics,* Mahr et al.[1] reported an association with osteoarthritis (OA [MIM 165720]) for a SNP (*rs49846015*) located immediately 5′ of the coding region of *RHOB* (on chromosome 2p24.1 [MIM 165370]) and for a SNP (*rs4720262*) located immediately 5′ of the coding region of *TXNDC3* (on chromosome 7p14.1 [MIM 607421]). *RHOB* codes for a GTP-binding protein whereas *TXNDC3* codes for a thioredoxin protein. The association study by Mahr et al. was performed with 171 patients with OA (74% females) who had undergone joint-replacement surgery (68% knee and 32% hip) and with 182 healthy control subjects (66% females), all of European white ethnicity. Possession of a copy of the G allele of *rs49846015* was an OA risk factor ($P = .0007$), as was possession of the T allele of *rs4720262* ($P = .0007$).

To assess the robustness of these associations, we have genotyped the SNPs in our collection of >1,500 case patients with OA (mean age 65 years; age range 56–85 years) and >700 age-matched control subjects (mean age 69 years; age range 55–89 years). As in the study by Mahr et al.,[1] our case patients were ascertained by joint-replacement surgery (hip, knee, or hip and knee) due to severe end-stage OA. Our control subjects had no signs or symptoms of arthritis or joint disease (pain, swelling, tenderness, or restriction of movement). All case patients and control subjects were individuals from the United Kingdom who are of white European ethnicity. Further details about the ascertainment of our case patients and control subjects have been published elsewhere.[2] Ethical approval for our study was obtained from the appropriate ethics