

Associating Mitochondrial DNA Variation with Complex Traits

To the Editor:

We read with great interest the recent paper by Saxena and colleagues describing the “comprehensive association testing of common mitochondrial DNA [mtDNA] variation in metabolic disease.”¹ We strongly support the use of large cohorts of carefully phenotyped cases and controls in studies of this type, large cohorts being essential to provide adequate power to detect small or moderate associations between mtDNA sequence variants and disease.² We do, however, have concerns that the study described by Saxena et al. is neither sufficiently accurate nor sufficiently comprehensive for exploring all possible mechanisms of disease association with common genetic variants of mtDNA. We therefore urge caution when applying their approach to other disease states, because it is not well grounded in the fundamental aspects of mtDNA phylogenetics. As a result, their approach may lead to spurious associations, as well as to false negative results.

Mitochondrial DNA is inherited strictly down the maternal line and therefore undergoes negligible intermolecular recombination, and the signature of recombination has not been detected at the population level.^{3,4} Mutations acquired throughout human history have subdivided the human population into a number of discrete clades or haplogroups.⁵ The major haplogroups have been identified by a search for common genetic variants in the population, initially through the use of restriction site analysis and subsequently through direct sequencing of both coding and noncoding regions of mtDNA.⁶ In constructing the most parsimonious phylogenetic structure of human mtDNA evolution, it has become clear that many substitutions have arisen more than once on different parts of the phylogenetic tree (i.e., homoplasies; see table 2 in the work of Herrnstadt et al.⁷ for the most complete list). Homoplasia adds complexity to the population structure of mtDNA, and it is a serious confounding phenomenon in the association approach used by Saxena et al.

Saxena and colleagues used publicly available human mtDNA coding-sequence data sets to identify mtDNA polymorphisms that occur at a frequency of >1% in the European population. To derive this key information, the authors state that they used only sequences of European origin to compile their list of polymorphisms. Thus, they report that they used 536 sequences from the MitoKor data set released by Herrnstadt et al.⁷ However, this data set contained a total of 560 sequences, of which 56 were African, 69 Asian, and only 435 European. On the basis of their report, it appears that Saxena et al. included at least 101 non-European sequences in their core data set,

some ~8% of the total. The inclusion of sequences from non-European subjects could have resulted in the identification of non-European SNPs in their initial data set or in the exclusion of specific European variants, because, in their set of 536 sequences, some rare European markers fell below the 1% threshold for inclusion. As an example, site 13105 is most often associated with African lineages, and, in the MitoKor data, it is present in 23 of 56 sequences of African origin but in only 3 of 435 sequences of European origin. As a result, this site should not have been used in the analysis of Saxena et al.

Even the strict use of European sequences is not without problems. Of the GenBank mtDNA sequences used by Saxena et al., 192 were of Finnish origin. It is now recognized that some mtDNA clades are considerably overrepresented in the Finnish phylogeny, presumably because of a recent founder event.^{8,9} For example, site m.4928 is absent in the 435 MitoKor European mtDNA sequences but is reported in 13 Finns. In a similar fashion, site 5495 is seen in only 2 of 435 MitoKor sequences but was observed in 23 Finns.⁹ As a result, there is another reason to doubt that the authors were able to analyze a representative set of mtDNA SNPs. At best, it appears that their approach is reliable only for robust disease associations with common markers.

Perhaps more importantly, although many of the U.S. and U.K. samples from the MitoKor data set were from the population controls, Herrnstadt et al. explicitly noted that the data set also included mtDNA sequences from patients with various disorders. In fact, the MitoKor set of 435 sequences of European origin included patients with type 2 diabetes ($n = 42$ [MIM 125853]), with Alzheimer disease ($n = 60$ [MIM 104300]), and with Parkinson disease ($n = 37$ [MIM 168600]), thereby accounting for at least 11% of the core data set. This is not a problem if the sequences are being used simply to draw general conclusions about the pattern of mtDNA evolution in the population. However, given the reported association between these diseases and mtDNA sequence variants,^{10–13} the use of these sequences potentially alters the spectrum of polymorphic change identified in the first phase of the study by Saxena et al. and subsequently influencing the final list of “tag” SNPs.

Saxena et al. used mathematical methods designed to measure linkage disequilibrium to identify SNPs that tag common mtDNA haplotypes as a way to identify haplogroup clusters. In a comparison of the haplotype tags described by Saxena et al. with a phylogenetic analysis of European mtDNA sequences, a number of patterns emerge. The r^2 approach reliably detects some common haplogroup-defining polymorphisms but—and this is an important point—not others. For example, mtDNA haplogroup T is reliably tagged by two tags, m.12633 and m.11812, that capture site m.8697 shared by all haplo-

group T sequences (shown in blue in fig. 1). However, their technique also identifies common homoplasies, which can account for up to 25% of substitutions in some data sets.⁹ As one example, in the MitoKor set of 560 sequences, 497 haplogroup-associated polymorphisms were identified, but 174 (35%) of these were associated with two or more haplogroups. The majority of established mtDNA haplotypes are defined by a combination of SNPs, and, by their very definition, a single homoplasmy cannot be used to reliably “tag” a single mtDNA haplotype. The approach of Saxena et al. leads to the association analysis of polyphyletic groups (e.g., as shown in fig. 1, m.709 is found independently on haplogroups U, K, T, and W). On the basis of the phylogeny shown in the figure, at least 53% of the tag SNPs identified by Saxena et al. are associated not with a single mtDNA haplotype but with two or more. Thus, their comparison of the frequencies of a common homoplasmy in cases and controls is fraught with difficulty. If one of the distal branches of the phylogenetic tree does harbor a functionally relevant disease-associated polymorphism, that association with the tagging homoplasic marker will be “diluted” by the co-occurrence of the same mtDNA substitution on other, nonassociated clades. An example is shown in red in figure 1, site m.12795 and m.709 capture m.5046 of haplogroup W. However, because of the occurrence of m.709 at multiple places in the phylogeny, many other unrelated sequences are also captured. On the other hand, if the homoplasmy itself contributes to pathogenicity, then it might be possible to use the approach developed by Saxena et al. for the identification of a disease association. However, the reported aim of this approach was to identify tag SNPs and not the functional variants themselves.

Another problem with the approach of Saxena et al. is that, by ignoring what is known about mtDNA evolutionary history, they tested a number of sites that were providing the same information on association. For example, both m.11674 and m.12414 “tag” mtDNA haplogroup W (fig. 1). On the basis of an updated reduced median network analysis of the MitoKor data set, 12 (19%) of the 64 tag SNPs studied by Saxena et al. occur in exactly the same place on the phylogeny as other tag SNPs (green boxes in fig. 1). Thus, their approach is inefficient, because of the increased superfluous genotyping, tagging the same haplotype on more than one occasion. This is an instance where less can be more. That is, by using fewer SNPs—but ones that are specifically chosen on the basis of knowledge of mtDNA phylogeny—it is possible to cover all major clades of mtDNA. This approach would reduce the number of statistical tests being performed and thus would increase the power of the study, potentially revealing a significant and biologically relevant association.

The conventional first step in studying the association between mtDNA and complex disease involves the sequential genotyping of specific SNPs that, in European populations, define the major European haplogroups. The results are interpreted in a stepwise manner, with reference

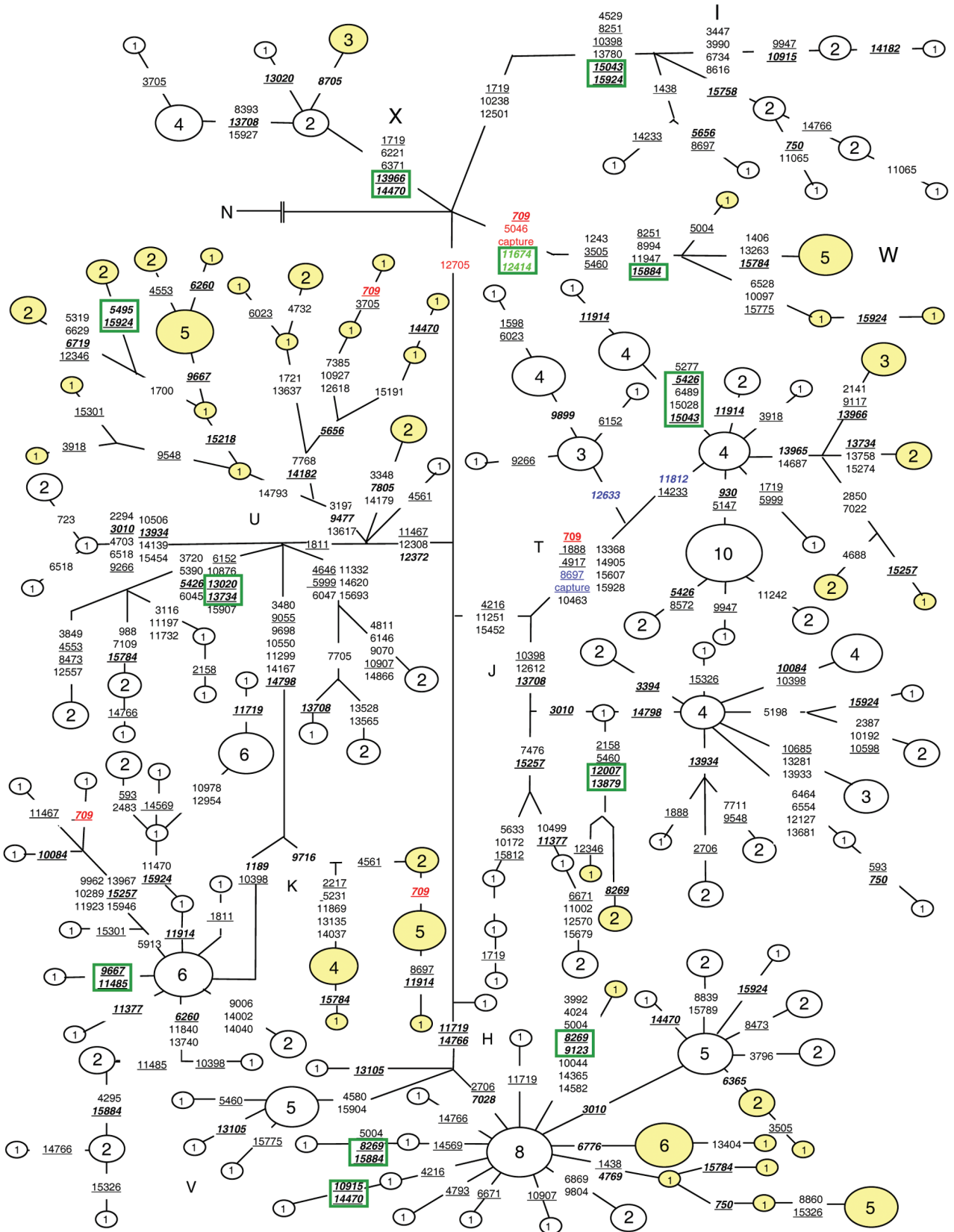
to the phylogenetic tree, to prevent misinterpretations due to the occurrence of homoplasies. For example, the presence of m.4216 defines the JT cluster, which is separated into J and T by m.13708. However, genotyping m.13708 on its own does not define J, because, among European mtDNAs, the same substitution occurs on a subgroup of haplogroup K and X.

By its very nature, the approach of Saxena et al. identifies some common haplogroup markers, but how much additional “tagging” information was acquired by their 64 SNPs? After removal of the nonunique sites ($n = 35$), which occur more than once on the phylogeny and thus do not tag a specific haplotype, followed by removal of the unique haplogroup markers ($n = 5$), 24 unique sites remain (38% of the original total). On the basis of the MitoKor data set, these 24 SNPs tag only 142 of 435 European sequences and, by extrapolation, only 33% of the European population (shown in shaded yellow in fig. 1).

The 64 SNPs described by Saxena et al. can be used to test two hypotheses *in part*: (1) association of a disease with deep-rooted, ancient mtDNA polymorphisms (an association occurs *either* because the specific variant has direct functional consequences *or* because it tags a clade containing functionally relevant variants); and (2) association of the disease with ancestrally young genetic variants that are present on multiple different haplogroup backgrounds. Of the 24 unique sites that do not tag major haplogroups, only 6 could have direct functional consequences (affecting tRNA genes, rRNA genes, or the protein sequence), with the majority ($n = 15$) being synonymous changes within structural genes. Interpreting the results of the analysis is therefore ambiguous—whether the outcome is positive or negative. The approach of Saxena et al. seems beautifully simple, with both statistical significance and power calculated by simulation and random permutation of the data. However, the examples presented here demonstrate the complexity and limitations of the data structure and show that it becomes very difficult to test a disease association without accounting for phylogenetic history. That is, their statistical tests are sound, but they are applied to flawed data in this instance.

Although we acknowledge that their approach will detect a robust disease association, little comfort can be taken because such associations are detected by other approaches. More importantly, we are learning that the reverse seems to be case for disease associations. That is, there is accumulating evidence that such associations will be weak and/or genetically complex.

In terms of what is emerging from other analyses, it must be noted that the approach of Saxena et al. does not test the equally plausible hypothesis that *multiple* mtDNA variants interact and subtly compromise respiratory chain function. This type of association is not without precedent, because suppressor mutations have been described in patients with mitochondrial disorders and possibly have occurred throughout mtDNA evolution.¹⁴ In terms of a specific example, an association has been found be-



tween longevity and a combination of three common polymorphisms m.150T, m.489C, and m.10398G.¹⁶ Such a disease process fits with the basic biology of the mitochondrial electron-transfer chain, in which all of the genes encoded by the mitochondrial genome contribute to the same basic biochemical process of oxidative phosphorylation.¹⁵ Mitochondrial structure and function are guided by the interaction of hundreds of nuclear- and mitochondrially-encoded subunits. It will be a major challenge to test the hypothesis that multiple mtDNA variants interact to alter disease risk—but without testing this hypothesis, no approach can be said to be “comprehensive.”

Finally, mitochondrial disorders are metabolic diseases, affecting the final common pathway of energy metabolism. Leber hereditary optic neuropathy (LHON [MIM 535000]) is the most common mtDNA disease, and >95% of cases are caused by one of three point mutations of mtDNA affecting genes that code for complex I subunits of the respiratory chain: m.3460G→A, m.11778G→A, and m.14484T→C.¹⁷ These mutations have arisen many times over human history, and they are associated with a metabolic defect of respiratory chain complex I activity. Intriguingly, only m.11778G→A and m.14484T→C preferentially occur on a specific mtDNA haplogroup background (J).^{18,19} The most likely explanation for these results is that the penetrance (or expressivity) of these pathogenic mutations is modified by the mitochondrial genetic “background.” Recent work suggests that specific subhaplogroups (J1c and J2b) are responsible for this association, possibly through polymorphic variation in the mtDNA cytochrome *b* gene.²⁰ Iranian pedigrees affected by LHON do not show the same the association with haplogroup J.²² However, the conspicuous absence of both J1c and J2b from the Iranian population probably explains this observation, illustrating the important point that a clear knowledge of the local population genetic structure, derived from phylogenetics, informs our understanding of relevant disease associations (or the lack thereof).²⁰ Although there is evidence that genetic variation of mtDNA is associated with some mitochondrial disorders, this is not always the case.²¹

An alternative approach to that of Saxena et al., as mentioned above, is to use our knowledge of mtDNA population structure to reduce the number of redundant sta-

tistical tests and thus to increase the power of the study. For example, one such hypothesis-driven approach would involve testing for an association between nonsynonymous mtDNA homoplasies and disease on different mtDNA haplogroup backgrounds. This hierarchical strategy would provide a means of independently testing a disease association multiple times within the same data set. Careful selection of specific SNPs on the basis of prior knowledge of their evolutionary conservation and likely biochemical effect might also reduce the number of statistical tests being performed. We believe that such hypothesis-driven approaches are less likely to identify a false-positive association. They will also maximize the potential of a given sample size, revealing associations that are biologically plausible and that may not be apparent after less-discriminate genotyping followed by a correction for multiple significance testing or by a simulation-based significance test not reflecting the true structure of genome evolution. In the end, we should expect that a “one size fits all” approach is simply not optimal and that analysis of large data sets with multiple hypothesis-based tests will be necessary.

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Web Resource

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for Alzheimer disease, LHON, Parkinson disease, and type 2 diabetes)

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Figure 1. Revised and updated reduced median network analysis of European mtDNA sequences from the MitoKor data set, adapted from the work of Herrnstadt et al.⁷ A reticulation at the haplogroups W, I, and X has been resolved. An example of haplogroup H4 has been added. Tag SNPs described in table 4 of Saxena et al.¹ are shown in bold italics (not all are present on the network). Underlined sites are homoplasies in the 435 European sequences of the MitoKor data set. In a larger data set, it is likely that the number of homoplasies would be greater; thus, the data set used by Saxena et al. will have fewer unique sites. *Blue*, An example of good tagging of a cluster of related mtDNA sequences. *Red*, An example of a tag SNP that is a common homoplasy, present at many different sites on the phylogeny. *Green*, An example of two SNPs that uniquely occur at exactly the same point on the phylogeny and thus provide the same information regarding that particular clade. *Green boxes*, Other examples of SNPs that occur at the same point on the phylogeny. *Yellow bubbles*, The areas of the phylogeny captured by the unique sites of Saxena et al. The total number of sequences captured (142) is 66 greater than shown on the network, which does not show all the H sequences in the data set of 435 European mtDNA sequences (because of space limitations).

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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

committees, and informed consent was obtained from each individual studied.

When planning our investigation, we noted that *rs49846015* was absent from dbSNP. A correspondence with Sandra Mahr (personal communication) revealed that *rs585017* was the correct accession number for this SNP. *rs585017* and *rs4720262* were genotyped by mass spectrometry (homogeneous MassARRAY system [Sequenom]), and the genotype and allele distributions in case and control groups were compared using standard χ^2 analysis-of-contingency tables. Tables 1 and 2 list the results for *rs585017* and *rs4720262*, respectively. Both SNPs were in Hardy-Weinberg equilibrium in the case and control groups. There were no significant differences (all *P* values >.05) in genotype or allele frequencies for either SNP between the case and control groups. This was also the case when the data were stratified by sex, with male case patients compared with male control subjects and female case patients compared with female control subjects.

The frequency of the G allele of *rs585017* in our study is comparable to that in the study by Mahr et al.,¹ with a frequency of 27.4% in our control group and 23.9% in Mahr et al.'s control group (*P* = .20). However, the frequency of the T allele of *rs4720262* shows a highly significant difference between the two studies, with a frequency of 28.8% in our control group and 13.4% in Mahr et al.'s control group (*P* < .0005). In dbSNP, the T allele of *rs4720262* is listed as having a frequency of 31.2% in the AFD-EUR panel (23 unrelated American individuals of European descent and one sample from a human variation panel of 50 whites) and a frequency of 21.7% in a HapMap-CEU panel (30 mother-father-child trios from the CEPH collection of Utah residents with northern and western European ancestry). Our T-allele frequency of 28.8% is between these two dbSNP-reported frequencies, whereas the T-allele frequency of 13.4% reported by Mahr et al. is substantially lower than both database frequencies. This implies that the control frequencies reported for this

Table 1. Association of *RHOB* SNP *rs585017* between Our Case Patients with OA and Control Subjects

Group	Genotype			<i>P</i> for Genotype	Allele		<i>P</i> for Allele	OR for T Allele (95% CI)
	AA	AG	GG		A	G		
All case patients ^a (<i>n</i> = 1,501):				.32			1.0	1.00 (.87–1.16)
Count	800	577	124		2,177	825		
Frequency (%)	53.3	38.4	8.3		72.5	27.5		
All controls (<i>n</i> = 702):			
Count	365	289	48		1,019	385		
Frequency (%)	52.0	41.2	6.8		72.6	27.4		
Female case patients (<i>n</i> = 856):				.28			.84	1.03 (.84–1.25)
Count	448	338	70		1,234	478		
Frequency (%)	52.3	39.5	8.2		72.1	27.9		
Female controls (<i>n</i> = 356):			
Count	182	153	21		517	195		
Frequency (%)	51.1	43.0	5.9		72.6	27.4		
Male case patients (<i>n</i> = 645):				.78			.84	.97 (.79–1.20)
Count	352	239	54		943	347		
Frequency (%)	54.6	37.1	8.4		73.1	26.9		
Male controls (<i>n</i> = 346):			
Count	183	136	27		502	190		
Frequency (%)	52.9	39.3	7.8		72.5	27.5		
All knees ^a (<i>n</i> = 352):				.08			.94	1.01 (.83–1.24)
Count	192	125	35		509	195		
Frequency (%)	54.5	35.5	9.9		72.3	27.7		
Female knees (<i>n</i> = 195):				.07			.71	.94 (.71–1.24)
Count	111	66	18		288	102		
Frequency (%)	56.9	33.8	9.2		73.8	26.2		
Male knees (<i>n</i> = 157):				.54			.53	1.11 (.83–1.49)
Count	81	59	17		221	93		
Frequency (%)	51.6	37.6	10.8		70.4	29.6		
All hips ^a (<i>n</i> = 1,067):				.60			.87	1.02 (.87–1.18)
Count	560	422	85		1,542	592		
Frequency (%)	52.5	39.6	8.0		72.3	27.7		
Female hips (<i>n</i> = 615):				.43			.52	1.08 (.88–1.32)
Count	310	255	50		875	355		
Frequency (%)	50.4	41.5	8.1		71.1	28.9		
Male hips (<i>n</i> = 452):				.78			.62	.94 (.75–1.17)
Count	250	167	35		667	237		
Frequency (%)	55.3	36.9	7.7		73.8	26.2		

^a In this analysis, 82 case patients who had undergone both hip replacement and knee replacement surgery were not included and were studied as a separate stratum in the stratification analysis because of the small sample size.

Table 2. Association of *TXNDC3* SNP *rs4720262* between Our Case Patients with OA and Control Subjects

Group	Genotype			<i>P</i> for Genotype	Allele		<i>P</i> for Allele	OR for T Allele (95% CI)
	CC	CT	TT		C	T		
All case patients ^a (<i>n</i> = 1,515):				.92			.76	1.03 (.89–1.18)
Count	749	643	123		2,141	889		
Frequency (%)	49.4	42.4	8.1		70.7	29.3		
All control subjects (<i>n</i> = 706):			
Count	353	299	54		1,005	407		
Frequency (%)	50.0	42.4	7.6		71.2	28.8		
Female case patients (<i>n</i> = 868):				.35			.17	1.15 (.95–1.39)
Count	406	384	78		1,196	540		
Frequency (%)	46.8	44.2	9.0		68.9	31.1		
Female control subjects (<i>n</i> = 360):			
Count	183	151	26		517	203		
Frequency (%)	50.8	41.9	7.2		71.8	28.2		
Male case patients (<i>n</i> = 647):				.48			.26	.88 (.72–1.08)
Count	343	259	45		945	349		
Frequency (%)	53.0	40.0	7.0		73.0	27.0		
Male control subjects (<i>n</i> = 346):			
Count	170	148	28		488	204		
Frequency (%)	49.1	42.8	8.1		70.5	29.5		
All knees ^a (<i>n</i> = 354):				.82			.68	1.05 (.86–1.28)
Count	170	157	27		497	211		
Frequency (%)	48.0	44.4	7.6		70.2	29.8		
Female knees (<i>n</i> = 196):				.21			.098	1.26 (.97–1.65)
Count	86	90	20		262	130		
Frequency (%)	43.9	45.9	10.2		66.8	33.2		
Male knees (<i>n</i> = 158):				.29			.24	.82 (.61–1.11)
Count	84	67	7		235	81		
Frequency (%)	53.2	42.4	4.4		74.4	25.6		
All hips ^a (<i>n</i> = 1,080):				.89			.90	1.01 (.87–1.17)
Count	541	450	89		1,532	628		
Frequency (%)	50.1	41.7	8.2		70.9	29.1		
Female hips (<i>n</i> = 626):				.54			.30	1.12 (.91–1.37)
Count	297	276	53		870	382		
Frequency (%)	47.4	44.1	8.5		69.5	30.5		
Male hips (<i>n</i> = 454):				.41			.32	.89 (.71–1.11)
Count	244	174	36		662	246		
Frequency (%)	53.7	38.3	7.9		72.9	27.1		

^a In this analysis, 81 case patients who had undergone both hip replacement and knee replacement surgery were not included and were studied as a separate stratum in the stratification analysis because of the small sample size.

SNP by Mahr et al. may not accurately reflect the true frequency of this SNP in Europeans of white ancestry.

To assess the power of our study, we conducted power calculations by using Quanto, version 1.5,^{3,4} with the following options: an unmatched case-control study design, a population risk of severe OA of 5%, a significance level of 0.05, a G-allele frequency of 23.9% for *rs585017*, a T-allele frequency of 13.4% for *rs4720262*, and a log-additive inheritance mode. The allele frequencies and the inheritance mode were selected to agree with the results of Mahr et al. Table 3 lists, for each comparison, the minimum odds ratio (OR) detectable with 80% power for our study. We calculated an OR of 2.05 for the association with *rs585017* and an OR of 2.26 for the association with *rs4720262* from the results reported by Mahr et al. All the ORs given in table 3 are lower than these values, indicating that the sample sizes used in our study are more than adequate to detect the ORs previously observed by Mahr et al. In fact, the power to detect an OR of 2.05 for the association with *rs585017* was $\geq 99.7\%$ for all compari-

sons, and the power to detect an OR of 2.26 for the association with *rs4720262* was $\geq 99.6\%$ for all comparisons.

Overall, our study does not replicate the previous findings of an association between OA and the *RHOB* SNP *rs585017* and the *TXNDC3* SNP *rs4720262*. Our study was adequately powered to detect an association comparable to that reported by Mahr et al., and we avoided potential confounding factors by using the same disease ascertainment (joint replacement of the hip or knee) and the same ethnic group (Europeans of white ethnicity) used in the original study. As more studies are reported, an accurate estimation of the effect of these two SNPs on OA susceptibility will become apparent.

JOHN LOUGHLIN, INGRID MEULENBELT, JOSINE MIN,
ZEHRA MUSTAFA, JANET S. SINSHEIMER,
ANDREW CARR, AND P. ELINE SLAGBOOM

Web Resources

Accession numbers and URLs for data presented herein are as follows:

Table 3. The Minimum Detectable ORs for Our Analysis under the Log-Additive Model with Power $\geq 80\%$ and a Significance Level of 5%

Comparison	Minimum Detectable OR	
	<i>RHOB</i> SNP <i>rs585017</i>	<i>TXNDC3</i> SNP <i>rs4720262</i>
All case patients vs. all control subjects	1.23	1.30
Female case patients vs. female control subjects	1.34	1.42
Male case patients vs. male control subjects	1.36	1.45
All knees vs. all control subjects	1.35	1.43
Female knees vs. female control subjects	1.49	1.62
Male knees vs. male control subjects	1.54	1.68
All hips vs. all control subjects	1.25	1.31
Female hips vs. female control subjects	1.36	1.47
Male hips vs. male control subjects	1.38	1.49

dbSNP, <http://www.ncbi.nlm.nih.gov/projects/SNP/> (for *rs585017* and *rs4720262*)

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for osteoarthritis, *RHOB*, and *TXNDC3*)
 Quanto, <http://hydra.usc.edu/gxe> (for power calculations)

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In our study,² odds ratios (ORs) of 2.1 and 2.3 describe the association between osteoarthritis and the *RHOB* and *TXNDC3* SNPs, respectively, and we had 80% power to detect ORs as small as 1.6 and 1.8, respectively. Both SNPs were in Hardy-Weinberg equilibrium in the control group, so we can confidently rule out obvious methodological flaws.

Loughlin et al.¹ used the same disease ascertainment that we did (joint replacement of the hip and/or knee), and they too analyzed Europeans of white ethnicity. Why then did the study fail? And, by extension, why did all the previous genomewide scans—no matter how comparable the strategies to elucidate the genetics of osteoarthritis^{3,4}—not culminate in a coherent set of results?

Obviously, one can always call for an even higher statistical power or claim that reported associations are spurious. But let us refocus on the disease at hand; osteoarthritis is a complex disease, and if there was a simple genetic pattern involved, surely we would have identified it by now.⁵ Assuming that the osteoarthritis pathogenesis requires a delicate interplay between individual genetic polymorphisms and regional environmental changes, we need to question the comparability of a British study and an eastern German study for disease-associated genes. Additionally, we need to identify the strata that reflect the different etiologies. In fact, table 3 in the letter by Loughlin et al.¹ might also suggest that increased ORs cannot be excluded for the strata “osteoarthritis of the knee.” Our own data support a recessive model for *RHOB* and a dominant model for *TXNDC3*. The combination of both risk factors yields an OR >9 ; however, our sample numbers are small. To facilitate testing our hypothesis in the U.K. cohort, we emphasize the suggestion that all tested data be reported as online supplements in their original forms.⁵

Loughlin et al.¹ also raise the issue of the different frequencies for the *TXNDC3* SNP *rs4720262* in the control subjects—13.4% in our cohort and 28.8% in the U.K. cohort. Indeed, the Ensembl Genome Browser reports a high variability for this particular SNP, with 2.2% in African Americans as the lowest. Among Americans of European descent, the frequencies vary from 21.7% (in HapMap, among 60 individuals) to 31.2% (in PERLEGEN, among 25 individuals). This points to an elevated variability in

Reply to Loughlin et al.

To the Editor:

We very much appreciate the effort Loughlin and colleagues¹ took in trying to replicate our finding of an association between osteoarthritis (MIM 165720) and the *RHOB* (MIM 165370) SNP *rs585017* and the *TXNDC3* (MIM 607421) SNP *rs4720262*.

whites, since these particular PERLEGEN and HapMap cohorts are not independent of each other (Coriell Institute for Medical Research, Ensembl Genome Browser, and HapMap).

Indeed, the frequency of 13.4% in our white control subjects is striking. However, there is a precedent of different SNP frequencies in British and German populations,⁶ which calls for detailed investigations of individual European populations—down to the haplotype level. We will certainly perform further analyses to increase the understanding of our control population. It is also important, in this context, to specify the origin of all individuals genotyped; in the study of Loughlin et al.,¹ 268 of the patients are not mentioned in the reference cited. The situation for *RHOB* SNP *rs585017* is different; the recessive effect of the GG genotype is reflected by a frequency of 4.9% in our control subjects and 20.5% in our case patients. Loughlin et al. found a comparable frequency in control subjects (6.8%; $P = .4$), but the frequency in case patients was much different (8.3%; $P = 3 \times 10^{-6}$), which illustrates the points we discussed above.

We are confident that the future of osteoarthritis genetics will encompass both (i) the analysis of even larger cohorts, with sample cohorts crossing regional boundaries—either genetic or environmental—and thus the possible dilution of effects, and (ii) strategies for zooming in on regional effects and for designing smart experiments to test individual traits.

SANDRA MAHR, HOLGER KIRSTEN, AND BRIGITTE MÜLLER

Web Resources

The URLs for data presented herein are as follows:

Coriell Institute for Medical Research, <http://www.coriell.org/>
Ensembl Genome Browser, <http://www.ensembl.org/>
HapMap, <http://www.hapmap.org/>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for *OH*, *RHOB*, and *TXNDC3*)

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