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.

Richa Saxena, Paul I. W. de Bakker, Leif C. Groop, Mark J. Daly, and David Altshuler

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From the Center for Human Genetic Research, Massachusetts General Hospital (R.S.; P.I.W.d.B.; M.J.D.; D.A.), and Program in Medical and Population Genetics, Broad Institute of Harvard and MIT (R.S.; P.I.W.d.B.; M.J.D.; D.A.), Boston; and Department of Clinical Sciences–Diabetes and Endocrinology at University Hospital MAS, Lund University, Lund, Sweden (L.C.G.)

Address for correspondence and reprints: Dr. Richa Saxena, Department of Molecular Biology, Massachusetts General Hospital, Simches Research Building, 175 Cambridge Street, CPZN-6818, Boston, MA 02114. E-mail: saxena@molbio.mgh.harvard.edu

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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			*P* for Genotype	Allele		*P* for Allele	OR for T Allele (95% CI)
	AA	AG	GG		A	G		
All case patients[a] ($n = 1{,}501$):				.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 ($n = 702$):				…			…	…
Count	365	289	48		1,019	385		
Frequency (%)	52.0	41.2	6.8		72.6	27.4		
Female case patients ($n = 856$):				.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 ($n = 356$):				…			…	…
Count	182	153	21		517	195		
Frequency (%)	51.1	43.0	5.9		72.6	27.4		
Male case patients ($n = 645$):				.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 ($n = 346$):				…			…	…
Count	183	136	27		502	190		
Frequency (%)	52.9	39.3	7.8		72.5	27.5		
All knees[a] ($n = 352$):				.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 ($n = 195$):				.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 ($n = 157$):				.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] ($n = 1{,}067$):				.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 ($n = 615$):				.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 ($n = 452$):				.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			*P* for Genotype	Allele		*P* for Allele	OR for T Allele (95% CI)
	CC	CT	TT		C	T		
All case patients[a] (n = 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 (n = 706):				…			…	…
Count	353	299	54		1,005	407		
Frequency (%)	50.0	42.4	7.6		71.2	28.8		
Female case patients (n = 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 (n = 360):				…			…	…
Count	183	151	26		517	203		
Frequency (%)	50.8	41.9	7.2		71.8	28.2		
Male case patients (n = 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 (n = 346):				…			…	…
Count	170	148	28		488	204		
Frequency (%)	49.1	42.8	8.1		70.5	29.5		
All knees[a] (n = 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 (n = 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 (n = 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] (n = 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 (n = 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 (n = 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 ⩾99.7% for all comparisons, and the power to detect an OR of 2.26 for the association with *rs4720262* was ⩾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 ⩾80% and a Significance Level of 5%

Comparison	Minimum Detectable OR	
	RHOB SNP *rs585017*	*TXNDC3* SNP *rs4720262*
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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From the Institute of Musculoskeletal Sciences, Botnar Research Centre, University of Oxford, Oxford, United Kingdom (J.L.; Z.M.; A.C.); Section of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands (I.M; J.M.; P.E.S.); and Departments of Human Genetics and Biomathematics, David Geffen School of Medicine at University of California–Los Angeles, Los Angeles (J.S.S)

Address for correspondence and reprints: Dr. John Loughlin, University of Oxford, Institute of Musculoskeletal Sciences, Botnar Research Centre, Nuffield Orthopaedic Centre, Oxford, OX3 7LD, United Kingdom. E-mail: john.loughlin@ndos.ox.ac.uk

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Reply to Loughlin et al.

To the Editor:

We very much appreciate the effort Loughlin and colleagues[1] 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*.

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