No evidence of an association between mitochondrial DNA variants and osteoarthritis in 7393 cases and 5122 controls. Annals of Rheumatic Diseases 2013, 72, 136-139.
CONCISE REPORT

No evidence of an association between mitochondrial DNA variants and osteoarthritis in 7393 cases and 5122 controls

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ABSTRACT

Objectives Osteoarthritis (OA) has a complex aetiology with a strong genetic component. Genome-wide association studies implicate several nuclear genes in the aetiology, but a major component of the heritability has yet to be defined at the molecular level. Initial studies implicate maternally inherited variants of mitochondrial DNA (mtDNA) in subgroups of patients with OA based on gender and specific joint involvement, but these findings have not been replicated.

Methods The authors studied 138 maternally inherited mtDNA variants genotyped in a two cohort genetic association study across a total of 7393 OA cases from the Wellcome Trust Case Control consortium 2 study. The cases were part of the arcOGEN study, and the controls were part of the W ellcome T rust Case Control consortium 2 study.

Results Following data quality control we examined 48 mtDNA variants that were common in cohort 1 and cohort 2, and found no association with OA. None of the phenotypic subgroups previously associated with mtDNA haplogroups were associated in this study.

Conclusions We were not able to replicate previously published findings in the largest mtDNA association study to date. The evidence linking OA to mtDNA is not compelling at present.

INTRODUCTION

Osteoarthritis (OA) is the most common age-related joint disease. The pathogenesis of OA is complex, with several environmental and genetic factors implicated in the aetiology.1 Genome-wide association studies implicate several nuclear genes in the risk of developing OA, but a major component of the heritability remains unexplained.2

Mitochondria are intracellular organelles involved in the synthesis of ATP, the principal source of energy within cells. Several lines of evidence implicate mitochondrial dysfunction in the pathogenesis of OA, including the disruption of respiratory chain activity in chondrocytes,3 the increased production of reactive oxygen species which compromise chondrocyte function1,5 and a central role in one apoptotic pathway.6

Mitochondria contain multiple copies of the 16.5 kb mitochondrial genome (mitochondrial DNA (mtDNA)). MtDNA codes for 13 essential respiratory chain proteins and the RNAs required for intramitochondrial protein synthesis. Point mutations of mtDNA compromise oxidative phosphorylation and are a major cause of human disease.7 This raises the possibility that more subtle polymorphic variants contribute to genetic susceptibility of complex traits, including OA. Being strictly maternally inherited, mtDNA undergoes negligible intermolecular recombination. Specific sequence variants that occurred 10–40 000 years ago define clades of the European mtDNA phylogeny called mtDNA haplogroups, each containing both distinct and shared genetic variants.8 Two studies have reported an association between different mtDNA haplogroups and OA, with one describing a reduced risk of knee OA with haplogroups J (defined by m.10398A>G) and JT (m.4216T>C), and another describing a reduced risk of hip OA with haplogroups J (defined by m.4216T>C and m.10598A>G) and JT (m.4216T>C) and another describing a reduced risk of hip OA with haplogroups J (defined by m.4216T>C and m.10598A>G) and J1c (m.14798T>C).9,10 However, each study lacked the power and resolution to reliably detect an association with all but the most common haplogroups. Moreover, despite being from the same geographic location, the studies reported different haplogroup associations with different joint involvement in OA for specific genders.9,10 While these data support the potential role of mtDNA in determining OA risk, the absence of direct replication means that the role of mtDNA in OA is thus uncertain, and the joint-specific risk is difficult to explain based on the proposed mechanism. To address this issue, we carried out a two phase study of 138 mtDNA variants in 7395 OA cases and 5122 controls as part of the arcOGEN consortium.

METHODS

We performed a two stage genetic association study. The cases were part of the arcOGEN study, and the controls were part of the Wellcome Trust Case Control Consortium 2 (WTCCC2) study.
both ascertained as described previously. In cohort 1, 3095 cases (ARC1) were compared with 2640 controls from the 1958 Birth Cohort (WTCCC-58C). In cohort 2, 4500 cases (ARC2) were compared with 2482 UK Blood Service controls (WTCCC-NBS). All cases were genotyped using the Illumina Human610 platform (Illumina, San Diego, California, USA). All controls were genotyped on the Illumina 1.2M Duo platform (Illumina). Individuals were excluded from subsequent analysis if data were absent in >10% of single nucleotide polymorphism (SNP) (cohort 1 cases=0 and WTCCC-58C=44 and cohort 2 cases=0 and WTCCC-NBS=11) and SNPs were excluded if >10% of genotypes were absent (cohort 1=5 and cohort 2=4). We excluded 50 SNPs (cohort 1=15 and cohort 2=21) with a study-wide missing data rate >5% or >1% for SNPs with a study-wide minor allele frequency (MAF) <5%. Finally, 64 SNPs with MAF<1% were removed (cohort 1=53 and cohort 2=4). We excluded 30 SNPs (cohort 1=15 and cohort 2=15) with a study-wide minor allele frequency (MAF) <5%. Statistical significance was defined as p<0.05 in both phases. Array genotypes were used to identify mtDNA haplogroup-specific sequence motifs, when compared with the mtDNA reference sequence (see online supplementary materials and table S1), allowing 98.48% of subjects to be successfully assigned to a European haplogroup.

A principal components analysis (PCA) was performed on the X-Y ratios of raw intensities for all SNPs for the combined dataset of cases (n=7595) and controls (n=5122) using R. The k-means function of the R cluster package was used to cluster individuals into 5, 10, 15 and 20 groups using these PCA scores. We then studied mtDNA haplogroups using two independent approaches: (1) manual haplogroup calling based on phylogenetics (for methods see online supplementary table S1) and (2) hypothesis-free approach based on raw fluorescence intensities in a principal components analysis. Analysis of mtDNA haplogroups between cases and controls failed to replicate previous studies (table 1). Stratifying by gender, joint involvement (knee or hip) and method of case ascertainment (radiograph or arthroplasty) failed to reveal any significant associations with mtDNA haplogroups (see online supplementary table S3). PCA analysis identified the main mtDNA haplogroups previously identified through phylogenetic analysis. These appear as clusters in figure 1, where each cluster represents a major European mtDNA haplogroup. However, there was no significant difference in cluster membership for any of the disease or control cohorts, or when the cases and controls were pooled (figure 1, online supplementary table S4). The data were only pooled after confirming that the allele frequencies were not significantly different between the two case groups or between the two control groups.

**RESULTS**

Using established QC criteria we rejected 90 SNPs, leaving 48 in both cohorts. There was no association between any mtDNA variant passing QC and OA. Stratifying by gender, joint involvement (knee or hip) and method of case ascertainment (radiograph or arthroplasty) failed to reveal any significant associations (see online supplementary table S2).

We then studied mtDNA haplogroups through PCA. This approach was adopted for the following reasons: (i) manual haplogroup calling based on phylogenetics involves non-synonymous substitutions in different parts of the world. Homoplasy accounts for up to 20% of mtDNA variation, and often involves non-synonymous substitutions. If the homoplasies are having a functional effect, then this might lead to different haplogroup associations in different studies across the globe. Finally, it is possible that geographic differences in the fine detail of the sub-haplogroup structure of mtDNA could account for inconsistencies between studies, as

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Haplogroups determined manually, as described in the Methods section and online supplementary table S1. Uncorrected $p^2$ comparison of haplogroup frequencies showing: (i) cohort 1 (ARC1, n=3903 and controls WTCCC-58C, n=2640); (ii) cohort 2 (ARC2, n=4300 and controls WTCCC-NBS, n=2482); and (iii) pooled arcOGEN cases (n=7593) and controls (n=5122). Hap=mtDNA haplogroup, $F$ = frequency (percentage in brackets) and $p=uncorrected Pearson’s$ $p^2$ probability. For study and control group acronym see the Methods section.
described for Leber’s hereditary optic neuropathy, where subbranches of haplogroup J are associated with either an increased or decreased risk of visual failure in different populations, largely due to specific differences in the cytochrome B protein sequence. However, in the largest mtDNA association study to date, we found no association between OA and the major European mtDNA haplogroups in either cohort. If an association does exist between OA and mtDNA, it is likely that this will only be resolved through extensive genotyping, ideally at the whole mtDNA level, in a much larger cohort of cases and controls and should be consistent in more than one geographic region.

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Acknowledgements. The UK samples used in arcOGEN derive from eight centres: Nottingham, London, Oxford, Sheffield, Southhampton, Newcastle, Wansbeck and Edinburgh. For Nottingham we acknowledge Arthritis Research UK for funding the collection of the majority of extant cases (cohort 1) and we thank Sarah Phipps and Maggie Wheeler for organising cohort 2 patient ascertainment. For London we thank the staff from the TwinsUK unit and the Clingford Study for cohort 1 patient ascertainment, we thank Fiona O’Neill for organising cohort 2 patient ascertainment, and we acknowledge financial support from Arthritis Research UK, from the Wellcome Trust, from the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre (BRC) award to Guy’s & St Thomas’ NHS Foundation Trust in partnership with King’s College London, and from the European Commission framework 7 programme TREAT-OA (grant 200800). For Oxford we acknowledge funding support from the Coliission Foundation, the Botnar Foundation and the Jeun Sharks Foundation for cohort 1 patient ascertainment, we acknowledge the NIHR for supporting the Biomedical Research Unit (BRU) at the University of Oxford, and we thank Bridget Watkins, Anne Hanley and Kim Clipsham for cohorts 1 and 2 patient ascertainment. For Sheffield we acknowledge the NIHR for supporting the Sheffield Musculoskeletal BRU collection site, the South Yorkshire Clinical Research Network for part-funding the Sheffield research nurses and for clerical support, the Royal College of Surgeons of England and the Cavendish Hip Foundation, Andrew Gordon for cohort 1 patient ascertainment, and Diane Steventon and James Talbot for cohort 2 patient ascertainment. For Southampton we acknowledge the Wellcome Trust Clinical Research Facility at Southampton General Hospital, we thank Philippa-Kate Battley and Elizabeth Arden for organising cohort 2 patient ascertainment, and we thank Richard Keen and Anna Bara for facilitating access to the Arthritis Research UK-funded VIDE study. For Newcastle we acknowledge Sheryl Mitchell and the Freeman Hospital orthroplasty surgeons Nigel Brewster, David Deehan, Craig Gerrand, Andy Gray, Munawar Hashmi, Jim Holland and David Weir for assistance in cohort 2 patient ascertainment, we thank Karen Bettinson for her assistance, we acknowledge the support of the UK NIHR BRC for Ageing and Age-related disease award to the Newcastle upon Tyne Hospitals NHS Foundation Trust and the support of the Northumberland, Tyne and Wear CLRN. For Wansbeck we thank Kirsten Walker and Donna Gray for organising cohort 2 patient ascertainment, and we acknowledge the Northumbria NHS Foundation Trust orthroplasty consultants Mr Partington, Mr Muller, Mr Emmerson, Mr Prasad, Mr Kramer, Mr Jones, Mr Murtys, Mr Asaad, Mr Leitch, Mr Pratt, Mr Townshend and Miss Van Kampen for accrual. For Edinburgh we acknowledge the contribution of Nichola Koller, Gayle Scott and Helen Watters for recruitment and ascertainment of cohort 2 cases. We acknowledge cohorts 1 and 2 sample management undertaken by the UK DNA Banking Network funded by the Medical Research Council at CIGMR—the Centre for Integrated Genomic Medical Research, University of Manchester, with special thanks to Kate Sherburn and Kate Dixon. Genotyping was performed at the Wellcome Trust Sanger Institute and we thank Emma Gray, Sarah Edkins and Cordelia Langford for their assistance. We thank Vanessa Cox of the MRC Epidemiology Resource Centre, Southampton, for database, and computing services for the arcOGEN cohort 2 patient questionnaires.

Contributions. GH and IW carried out the primary analysis, supervised by PJC, DCS and JL. KP, LS, NWR, NA, FB, IC, AC, KC, KD, MD, AMcC, WERO, SHR, MRR, TDS, AMW, GMM, JMW, EZ and JL generated the primary data. PJC, JL and GH wrote the manuscript, who modified following comments from the other authors. The authors declare no conflict of interest.

Funding. arcOGEN (http://www.arcogen.org.uk/) was funded by a special purpose grant from Arthritis Research UK (grant 18030). This study used genotype data from population controls that were generated by the Wellcome Trust Case Control Consortium 2 (a full list of the investigators who contributed to the generation of the data is available from http://www.wtccc.org.uk, funded by The Wellcome Trust (grant 083948). The population controls were from the 1958 British Birth cohort collection funded by the Medical Research Council at CIGMR—the Centre for Integrated Genomic Medical Research, University of Manchester, with special thanks to Kate Sherburn and Kate Dixon. Genotyping was performed at the Wellcome Trust Sanger Institute and we thank Emma Gray, Sarah Edkins and Cordelia Langford for their assistance. We thank Vanessa Cox of the MRC Epidemiology Resource Centre, Southampton, for database, and computing services for the arcOGEN cohort 2 patient questionnaires.

Competing interest. None.
Ethics approval

Ethics approval was provided by Newcastle REC.

Provenance and peer review

Not commissioned; externally peer reviewed.

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Ann Rheum Dis 2013 72: 136-139 originally published online September 14, 2012
doi: 10.1136/annrheumdis-2012-201932

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