Letter

# Replication of association of the D-repeat polymorphism in asporin with osteoarthristis

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Published: 29 June 2006
This article is online at http://arthritis-research.com/content/8/4/403


*Arthritis Research & Therapy* 2006, **8:**403 (doi:10.1186/ar1992)

See related research by Rodriguez-Lopez *et al*., http://arthritis-research.com/content/8/3/R55

Rodriguez-Lopez and colleagues [1] describe a replication study of our previous association between osteoarthritis (OA) and asporin [2]. The authors were unable to find this association in the Spanish population and question the association we found in the Japanese population. Their report also contains an interpretation of two previous papers on the same topic [3,4] that differs from those of the original study authors. We have concerns about their interpretation of data and about the conclusions drawn.

It is not surprising that an association of a gene with a disease is found in some populations but not in others. Such diversity has been established for many common complex diseases with several explanations [5]. In this particular case, one explanation is the difference in the inclusion criteria used to recruit study participants. Whereas we recruited symptomatic OA patients with supporting radiographic evidence, Rodriguez-Lopez and colleagues used joint replacement surgery as inclusion criteria (Table 1).

Another explanation is ethnic diversity, which is apparent in the very different allelic frequencies between the Spanish and Japanese populations. We question the authors' generalization of the three European populations (Spanish, Greek and UK) as 'European Caucasian', given the diverse frequencies of asporin alleles in the three populations [1,3,4] (Table 2), as well as their history and geography. The Spanish population in particular is distinct from the others; for example, the frequency of the common allele, Asp13 (D13), in the Spanish control groups shows statistically significant differences ($p = 0.00088$ versus UK; $p = 0.021$ versus Greek). The allelic frequency in hip OA also is very different.

However, it is notable that in studies of knee OA for all three European populations, the allelic frequency of D13 is decreased and that of D14 is increased in the case group – the same trend observed in our Japanese study (Table 2). In all four populations, the odds ratios exceed 1. Given that the deviation of the odds ratio is random, the probability for its

**Table 1**

**Association studies of asporin and osteoarthritis**

| | Case | | | | | |
|---|---|---|---|---|---|---|
| Study | Knee | Hip | Total | Control | Association | Inclusion criteria |
| Japan | 530 | 593 | 1123 | 608 | Knee, hip OA | Symptomatic OA with radiograph |
| UK | 278 | 904 | 1182 | 748 | Male hip OA | TKR/THR |
| Greece | 155 | (–) | 155 | 190 | Decrease of D13 | TKR |
| Spain | 188 | 303 | 491 | 294 | None | TKR/THR |

OA, osteoarthritis; THR, total hip replacement; TKR, total knee replacement; –, not studied.

CI = confidence interval; D13 = Asp13; D14 = Asp14; OA = osteoarthritis.

**Table 2**

**Allelic frequencies of D13 and D14 repeats of asporin in osteoarthristis**

| | Allelic frequency (%) | | | |
|---|---|---|---|---|
| | D13 repeat | | D14 repeat | |
| Study | Case | Control | Case | Control |
| Knee | | | | |
| Japan | 58.7 | 65.2 | 8.6 | 4.8 |
| UK | 46.4 | 50.3 | 13.7 | 12.7 |
| Greece | 38.1 | 49.7 | 15.2 | 13.9 |
| Spain | 41.5 | 42.2 | 14.9 | 12.6 |
| Hip | | | | |
| Japan | 61.6 | 64.0 | 7.9 | 4.8 |
| UK | 47.5 | 50.3 | 14.3 | 12.7 |
| Spain | 43.3 | 42.2 | 9.7 | 12.6 |

occurrence by chance is $(1/2)^4 = 1/16$, which is substantially low. If we combine data for all three European populations, the association becomes significant ($p = 0.030$; odds ratio 1.26, 95% confidence interval 1.02 to 1.56). We believe that this estimation is valid because the inclusion criteria are the same, provided that the ethnicity is consistent as the Spanish group itself proposed. If so, the association of asporin has been replicated in the European Caucasian population. The low odds ratio given above suggests that the Spanish study might be under-powered to detect the low-risk gene. It remains under-powered even when we postulate the moderate risk (power = 0.56 to 0.71 at a relative risk of 1.4 to 1.5 [6]).

OA is a serious disease with global impact, and it has proven refractory to genetic (etiologic) study. The questions raised by Rodriguez-Lopez and colleagues [1] provide further incentive to build common platforms for phenotype definition, inclusion criteria, genotyping and analytical methods, and to unite the ethnically diverse resources available for study. Such efforts would increase the accuracy and power of the research for our 'common' enemy.

## Authors' response

Julio Rodriguez-Lopez, Manuel Pombo-Suarez, Myriam Liz, Juan J Gomez-Reino and Antonio Gonzalez

The letter from Ikegawa and colleagues highlights some difficulties in defining what constitutes replication of previous genetic association in the context of studies with different patient selections, ethnicities, environmental and cultural influences and a multiplicity of tests. Our article [1] did not question the results described in the Japanese population [2]. We merely concluded that, among European Caucasians, there was no evidence for an important effect of the asporin D repeat polymorphism; this was similar to the conclusion of the authors of the UK study [3].

Our conclusion was based in the analysis of the three available studies in Europeans [1,3,4]. We were well aware of differences in allele frequencies between the European populations and, consequently, we used the appropriate techniques to combine data. All the comparisons done were not significant or were, at best, inconclusive. For example, in the comparison between D14 and D13 allele frequencies in relation to knee OA that is mentioned by Ikegawa and colleagues, the crude combination of data shows a significant effect, but it is not significant if the variability between studies is adequately accounted for (Mantel–Haenszel odds ratio 1.23; 95% confidence interval 0.99 to 1.56; $p = 0.07$).

Ikegawa and colleagues also call our attention to the coincidence in direction of the odds ratio from the different studies in relation to knee OA, giving its probability as $1/16 = 0.0625$, and that this is unlikely to have occurred by chance alone. However, this analysis includes the result used as reference, the Japanese study, in the subject of the comparison. The correct probability is $1/8 = 0.125$.

Regarding the comment on the power of our study, we have already shown that it is enough to detect effects of the size observed in the Japanese study (with the exceptions mentioned in our article). In addition, the larger power of the combined European studies did not result in significant differences, as made explicit in this reply.

In essence, our conclusion is fully supported by the available evidence. In our article we were careful not to rule out a role of the asporin D repeat polymorphism in OA susceptibility among Caucasians. Only an important effect, similar to that found in the Japanese study, was excluded.

## Competing interests

The authors declare that they have no competing interests.

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