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Hand osteoarthritis : natural course and determinants of outcome

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ASSOCIATION STUDY OF CANDIDATE GENES FOR THE PROGRESSION OF HAND OSTEOARTHRITIS

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ABSTRACT

Objective. Although a few consistent osteoarthritis (OA) susceptibility genes have been identified, little is known on OA progression. Since OA progression is clinically the most relevant phenotype, we investigated the association between *ASPN*, *BMP5* and *GDF5* polymorphisms and progression of hand OA.

Methods. Single-nucleotide polymorphisms (SNPs) *ASPN* rs13301537, *BMP5* rs373444 and *GDF5* rs143383 were genotyped in 251 hand OA patients from the Genetics ARthrosis and Progression (GARP) study and 725 controls. In a case-control comparison we assessed the association between these SNPs and radiographic progression of hand OA over 6 years, which was based on change in osteophytes or joint space narrowing (JSN), above the smallest detectable change. SNPs with suggestive evidence for association were further analysed for their effect on progression over 2 years and for the mean change in osteophytes and JSN.

Results. The minor allele of *ASPN* SNP rs13301537 was associated with hand OA progression over 6 years (OR (95%CI) 1.49 (1.06 to 2.07); $p=0.020$). The mean change in osteophytes and JSN was higher in carriers of the minor allele compared to homozygous carriers of the common allele with mean difference of 0.73 (95%CI -0.07 to 1.56; $p=0.073$) and 0.82 (95%CI 0.12 to 1.52; $p=0.022$), respectively. An association with similar effect size was found between *ASPN* SNP rs13301537 and 2-year progression, and the mean change in osteophytes and JSN was significantly higher in homozygotes.

Conclusion. *ASPN* is associated with hand OA progression. This gives insight in the pathogenesis of hand OA progression and identified a potential target for therapeutic approaches.

INTRODUCTION

Osteoarthritis (OA) is a common joint disorder characterised by degradation of cartilage and changes in subchondral bone and a leading cause of disability.¹ It is therefore a burden not only for the individual but also for society. OA is a multifactorial disease involving both genetic and environmental factors. The hand is the most frequently involved joint site.²

The role of genetic factors in influencing OA susceptibility is well documented.^{3,4} However, very few studies assessed the role of OA susceptibility genes in the disease course. One of the difficulties in studying OA progression is its gradual and slow pace. Identification of genes associated with OA progression will expand our knowledge on the pathophysiological pathways involved in this process. This can contribute to the development of much desired new treatments and to the identification of patients at high risk for progression. Earlier we reported the presence of familial aggregation in OA progression in a relatively small number of sibling pairs over a period of 2 years indicating a role for genetics in OA progression.⁵

It is generally accepted that the genetic architecture of OA onset is complex and is expected to be modulated by many genes with small effects. Genetic studies have provided a few consistent signals with relevant functional follow-up. Among these genes are growth differentiation factor 5 (*GDF5*)^{6,7}, bone morphogenic protein 5 (*BMP5*)^{8,9} and asporin (*ASPN*)^{10,11}, all involved in transforming growth factor β (TGF- β) signalling. *GDF5* and *BMP5* have been shown to be essential in the maintenance and repair of synovial joints as well as chondrogenesis and chondrocyte proliferation. The T allele of SNP rs143383 in the 5' untranslated region of *GDF5* is consistently associated with various subtypes of OA and with reduced activity in chondrogenic cells. SNP rs3734444 was shown to mark allelic imbalanced expression of *BMP5*. *ASPN* belongs to a family of small leucine-rich proteoglycans (SLRPs), which compose a major non-collagen component of the extra cellular matrix. The aspartic acid repeat (D) 14 allele was associated with an increased risk of knee OA, whereas the D13 allele may be protective. Interestingly, functional studies showed that *ASPN* binds to TGF- β and thereby inhibits its function^{10,12}. Because these genes are involved in chondrogenesis and chondrocyte proliferation, we expect them not only be involved in the onset of OA but also in the further evolution of the disease.

Therefore we investigated the association between single-nucleotide polymorphisms (SNPs) within *GDF5*, *BMP5* and *ASPN* and radiographic hand OA progression in patients from the Genetics ARthrosis and Progression (GARP) study that were followed for 6 years. SNPs with suggestive evidence for association were further investigated for association with hand OA progression over 2 years, reflecting fast progression. We assessed hand OA since it has by far the highest prevalence in our study population.

PATIENTS AND METHODS

Study design and selection of patients

The GARP study is a cohort study aimed at identifying determinants of OA susceptibility and progression, comprising 192 Caucasian sibling pairs with symptomatic OA at

multiple sites in the hands or in at least two of the following joint sites: hand, knee, hip or spine. Patients were assessed at baseline and after 6 years. Additionally, sibling pairs with at least one subject with symptomatic hip or knee OA were evaluated after 2 years. This group only partly comprises the same patients evaluated after 6 years. Details on the recruitment and selection and on both follow-up periods have been published elsewhere.¹³⁻¹⁵ The GARP study was approved by the medical ethics committee.

Patients were eligible for the present study if they had radiographic hand OA at baseline defined as the presence of a Kellgren-Lawrence score¹⁶ of ≥ 2 in at least 2 interphalangeal joints or first carpometacarpal (CMC-1) joints. To allow for case-control comparison we included partners of the offspring in the Leiden longevity study as random control population (n=739).¹⁷

Radiographic outcome

Standardised radiographs of the hands (dorsal-volar) were obtained at baseline and after both follow-up periods by a single radiographer. Radiographs were scored in pairs (baseline-2 year, baseline-6 year) blinded for patient characteristics by consensus opinion of 2 experienced readers using the Osteoarthritis Research Society International (OARSI) atlas.¹⁸ Osteophytes and joint space narrowing (JSN) were graded 0-3 in the distal interphalangeal (DIP), proximal interphalangeal (PIP), first interphalangeal (IP-1), CMC-1, metacarpophalangeal (MCP) and scaphotrapezotrapezoidal (STT) joints, total scores ranging from 0 to 96. Intrareader reproducibility based on repeated reading of a 10% random selection of radiographs was high with intraclass correlation coefficients (ICC) for the 2-year and 6-year period, respectively, of 0.98 and 0.92 for osteophytes and 0.94 and 0.87 for JSN. Radiographic progression was defined as a change in osteophytes or JSN above the smallest detectable change (SDC), reflecting change above measurement error.¹⁹ Over 2 years the SDC was 0.9 and 0.8 for osteophytes and JSN, respectively. The SDC over the 6-year period was 1.3 for osteophytes and 1.5 for JSN.

SNP selection and genotyping

Because of our relatively small sample of hand OA patients we could only investigate a limited number of SNPs. The selection was based on SNPs that have shown consistent association with OA susceptibility within one pathway, the TGF- β superfamily, as explained in the introduction. The three SNPs are: *GDF5* rs143383, *BMP5* rs3734444 and *ASPN* rs13301537.

These genetic variants were genotyped using genomic DNA extracted from peripheral venous blood samples according to standard procedures. In total, 380 subjects from the GARP study and 725 controls were genotyped by mass spectrometry (homogeneous MassARRAY system; Sequenom, San Diego, CA) using standard conditions. PCR reactions were carried out in a final volume of 5 μ l and contained 2.5 ng of genomic DNA. Genotypes were assigned using Genotyper version 3.0 software (Sequenom, San Diego, CA). All SNPs were in Hardy-Weinberg equilibrium.

Analysis strategy

Because hand OA is by far the most prevalent OA phenotype in the GARP study, we choose to study hand OA. Radiographic progression of hand OA was assessed after 2 and after 6 years, with only partly overlap. These groups may reflect subjects with different disease progression phenotypes, fast and more gradual progression. Since the 6-year cohort has the largest number of patients and due to the longer follow-up period also the largest number of progressors, we used this group for the initial analysis. This implicates that if there is any association the chance of finding it in this group is higher compared to the 2-year cohort. In this analysis we compared subjects with OA progression over 6 years (cases) to the controls in a cross-sectional approach to establish the association between the SNPs with OA progression. To further explore SNPs showing evidence for association in this initial analysis, we repeated the case-control analysis in the 2-year cohort for these SNPs. In addition, we used a quantitative approach within the hand OA patients in both cohorts comparing mean change in osteophytes and JSN scores across the SNP genotypes showing evidence for association in the initial analysis.

Statistical analysis

In our 6-year cohort the statistical power given a minor allele frequency (MAF) of 0.37 using a log additive model with $\alpha=0.05$ was 81% to detect an odds ratio (OR) of 1.55 or higher (Quanto software version 1.2.4 (University of Southern California, USA; <http://hydra.usc.edu/gxe>)).

Associations were analysed using SPSS, version 16.0 (SPSS, Chicago, Illinois, USA). In the initial analysis (6-year cohort) we assessed the association between the three SNPs and the presence of radiographic progression as well as hand OA susceptibility in a case-control comparison. Allelic ORs were estimated by comparing the number of alleles among cases and controls using generalised estimating equations (GEE) models with robust variance estimators to account for familial dependency among sibling pair and sex was added as covariate.

The SNPs showing evidence for association were subsequently assessed in the 2-year cohort using the same case-control comparison. For these SNPs we also used a quantitative approach within the hand OA patients comparing the mean change in osteophytes and JSN scores across SNP genotypes using linear mixed models. Adjustment was made for age, sex, BMI and a random intercept was included to adjust for family effects within sibling pairs.

RESULTS

Study population

Of the 384 patients in the GARP study, 251 fulfilled the definition of radiographic hand OA. Radiographic follow-up data over 6 years were available in 161 patients (64%). In addition, 128 patients (51%) had radiographic follow-up data over 2 years. Data over both periods was present in 86 patients.

Baseline characteristics are shown in table 1. Since patients in the 2-year follow-up study were selected based on the presence of knee or hip OA, the prevalence of

Table 1. Baseline characteristics of patients with radiographic hand osteoarthritis (OA) in the 6-year cohort and the 2-year cohort.

	6-year cohort (n=161)	2-year cohort (n=128)
Age, mean (SD), years	60.0 (7.2)	61.0 (6.7)
Women, no (%)	131 (81)	105 (82)
Postmenopausal women, no (%)	124 (95)	100 (95)
Body mass index, mean (SD), kg/m ²	27.2 (5.0)	26.2 (3.3)
Symptomatic hand OA, no (%)	131 (81)	103 (80)
Additional ROA sites, no (%)		
Knee	72 (45)	67 (52)
Hip	43 (27)	49 (38)
Facet	107 (66)	90 (70)
Spine degenerative disc	112 (69)	101 (79)

Abbreviations: ROA: radiographic osteoarthritis.

these OA phenotypes was higher in this population. In controls the mean age (SD) was 58.8 years (7.4) and 58% were female.

In the 6-year cohort 97 (60%) patients had radiographic progression of hand OA. Progression of osteophytes and JSN was present in 85 (53%) and 52 (32%) patients, respectively. Over 2 years radiographic progression was present in 50 (39%) hand OA patients. Osteophyte and JSN progression was present in 31 (24%) and 37 (29%) patients, respectively.

Genetic association with hand OA progression over 6 years

In table 2 results of the initial analysis comprising the case-control comparison in the 6 year cohort are shown. MAF and genotype distributions are provided for the *ASPN*, *BMP5* and *GDF5* variants in controls, all patients with radiographic hand OA (n=251) at baseline and patients with progression of hand OA over 6 years (n= 97). MAF were similar between controls and patients with radiographic hand OA. The minor allele of the SNP rs13301537 in *ASPN* was associated with hand OA progression with an OR (95%CI) of 1.49 (1.06 to 2.07) and a nominal p-value of 0.020. After adjustment for multiple testing (n=3) this association had suggestive evidence for association with radiographic progression (p=0.06). To further investigate this effect we stratified the analysis for progression of osteophytes and progression of JSN. This showed consistent allelic association with the *ASPN* SNP rs13301537 with an OR (95%CI) of 1.53 (1.07 to 2.11) for osteophytes (p=0.019) and an OR (95%CI) of 1.70 (1.14 to 2.55) for JSN (p=0.010).

The mean change in osteophytes and JSN scores across *ASPN* SNP rs13301537 genotypes was higher in homozygous and heterozygous carriers of the minor allele compared to homozygous carriers of the common allele (table 3), with a mean difference in change score of 0.73 (95%CI -0.07 to 1.56; p=0.073) for osteophytes and 0.82 (95%CI 0.12 to 1.52; p=0.022) for JSN. There was no dose response effect of the allele.

Table 2. Association between three single nucleotide polymorphisms (SNPs) and the presence of hand osteoarthritis as well as the progression of hand osteoarthritis over 6 years.

ASPN rs13301537							
	TT	CT	CC	MAF	n	Allelic OR (95%CI)*	p-value
Controls	366	297	62	0.29	1418		
Presence hand ROA	119	104	26	0.32	489	1.15 (0.88 to 1.49)	0.309
Radiographic progression	37	47	12	0.37	189	1.49 (1.06 to 2.07)	0.020
BMP5 rs373444							
	TT	CT	CC	MAF	n	Allelic OR (95%CI)*	p-value
Controls	271	334	104	0.38	1388		
Presence hand ROA	82	113	44	0.42	469	1.12 (0.87 to 1.43)	0.375
Radiographic progression	33	45	14	0.40	181	0.99 (0.72 to 1.37)	0.954
GDF5 rs143383							
	CC	CT	TT	MAF	n	Allelic OR (95%CI)*	p-value
Controls	105	330	290	0.37	1418		
Presence hand ROA	31	131	86	0.39	487	0.98 (0.78 to 1.23)	0.859
Radiographic progression	14	44	38	0.37	189	1.04 (0.72 to 1.51)	0.833

*Adjusted for family effects within sibling pairs and sex.

Abbreviations: MAF: minor allele frequency, ROA: radiographic osteoarthritis.

Table 3. Mean change in osteophytes and joint space narrowing (JSN) in the hand over 6 years for ASPN SNP rs13301537 genotypes.

	TT (N=67)	CT (N=73)	CC (N=19)
Change osteophytes, mean (SD)	1.8 (2.2)	2.5 (2.8)	2.7 (3.0)
Change JSN, mean (SD)	1.0 (1.7)	1.8 (2.6)	1.7 (1.8)

Association of ASPN SNP rs13301537 with hand OA progression over 2 years

Subsequently ASPN SNP rs13301537 was assessed in the 2-year cohort showing a similar effect size for the association with hand OA progression over that period compared to the 6-year cohort (table 4). However, only the association for progression of osteophytes reached nominal statistical significance.

The mean change in osteophytes and JSN score was considerably higher in homozygous carriers of the minor allele of the ASPN SNP rs13301537 compared to the other genotypes (table 5), with a mean difference in change score of 1.10 (95%CI 0.24 to 1.96; $p=0.012$) for osteophytes and 0.91 (95%CI 0.10 to 1.72; $p=0.028$) for JSN.

Because the ASPN SNP rs13301537 has shown to be in linkage disequilibrium with the frequently described D14 and D13 allele in the ASPN D-repeat polymorphism we performed haplotype analysis involving a second ASPN SNP rs331377, to assess whether the effect is attributable to these D-repeat polymorphisms. We found that the D14 and D13 allele were not associated with progression of hand OA over both periods, suggesting that the effect of the ASPN SNP rs13301537 is independent of D14 and D13.

Table 4. Association between *ASPN* SNP rs13301537 and progression of hand osteoarthritis over 2 years.

	TT	CT	CC	MAF	n	Allelic OR (95%CI)*	p-value
Controls	366	297	62	0.29	1418		
Radiographic progression	19	25	6	0.37	99	1.48 (0.95 to 2.32)	0.087
Progression osteophytes	10	16	5	0.43	61	1.85 (1.08 to 3.18)	0.025
Progression JSN	14	18	5	0.38	73	1.55 (0.92 to 2.60)	0.102

*Adjusted for family effects within sibling pairs and sex.
Abbreviations: JSN: joint space narrowing; MAF: minor allele frequency.

Table 5. Mean change in osteophytes and joint space narrowing (JSN) over 2 years for *ASPN* SNP rs13301537 genotypes.

	TT (N=59)	CT (N=58)	CC (N=10)
Change osteophytes, mean (SD)	0.4 (1.0)	0.6 (1.1)	1.5 (3.0)
Change JSN, mean (SD)	0.4 (1.2)	0.4 (1.1)	1.3 (2.0)

DISCUSSION

In this study we investigated whether SNPs within *ASPN*, *BMP5* and *GDF5* are related to the progression of hand OA over 6 years in participants of the GARP study. Subsequently, SNPs with suggestive evidence for association were investigated for association with hand OA progression over 2 years, reflecting fast progression. We found that the SNP rs13301537 in *ASPN* was associated with radiographic progression of hand OA over 6 years ($p=0.020$). The minor allele of this variant was more common in patients with progression of hand OA compared to healthy controls. In addition, the mean change in osteophytes and JSN was higher in C-allele carriers compared to the TT genotype. In the 2-year cohort similar effect sizes were found, with the mean change in osteophytes and JSN being significantly higher in homozygous C-allele carriers. In patients with progression over both time periods ($n=25$), effect sizes for *ASPN* were similar to the risk in the whole 6-year and 2-year cohort implying that the effects over the long term and short term are independent.

This study is the first to assess specific genes associated with hand OA progression. To our knowledge there is only one other study investigating the association between specific SNPs and OA progression, which concerned knee OA.²⁰ One of the reasons for the lack of genetic association studies on OA progression is the lack of follow-up data in combination with genotype data, especially when the hand is concerned. It is of interest to assess OA progression because this phenotype is clinically most relevant with respect to development of new interventions and patient management.

The *ASPN* SNP we found to be associated with hand OA progression was originally identified by Kizawa et al. as susceptibility gene for both knee and hip OA in two independent Asian populations.¹⁰ Apart from the genetic association they demonstrated that *ASPN* is abundantly expressed in articular cartilage and inhibits expression of genes encoding aggrecan and type II collagen. Our association for *ASPN*

SNP rs13301537 with OA progression was independent from the D14 and D13 alleles in the D-repeat polymorphisms, although these SNPs are in linkage disequilibrium. In the studies of Kizawa et al., D14 allele in the *ASPN* D-repeat polymorphism was found to increase OA risk, the D13 allele was protective. In a Greek population the association between the D-repeat polymorphisms was confirmed for knee OA.²¹ In Spanish and British populations no relationship with knee or hip OA was observed.^{22,23} A study on the relationship between various *ASPN* polymorphisms and hand OA susceptibility showed no association.²⁴

ASPN inhibits both early- and late-stage chondrogenesis through suppression of TGF- β , a central player among growth factors in articular cartilage.^{10,12} Excessive *ASPN* activity reduces TGF- β function to less optimal levels, leading to cartilage degeneration. Our findings suggest that this imbalance between *ASPN* and TGF- β is an ongoing process leading not only to the development, as shown in earlier research, but also to progression of OA. This was evident for long-term progression and probably also for progression on the short-term. This interaction between *ASPN* and TGF- β , leading to suboptimal TGF- β levels is a potential target for therapeutic approaches.

We did not find an association between hand OA presence at baseline and the *ASPN* SNP. An explanation is that the SNP is associated with a subset of more progressive hand OA. The whole group comprises a wide variety of hand OA types, both slow and faster progressive types. Therefore analysis in the whole group does not show a relationship with the SNP.

There are a number of potential limitations to be addressed. Although it is generally known that small studies are subject to spurious results and need replication to assess robust effects, we did not replicate our results. This is mainly due to a lack of populations with both radiographic follow-up data on hand OA and genotype data, implying that replication of our data was not possible at this time. Therefore, this study should be appreciated as an initial result for further research on the role of specific polymorphisms in OA progression. Despite this limitation we found an association between hand OA progression and *ASPN* over both follow-up periods. As discussed above, these effects seem independent, increasing the credibility of the association.

In conclusion, we found that *ASPN* SNP rs13301537 was associated with progression of hand OA over both 6 years and 2 years. This finding gives insight in the pathogenesis of hand OA progression and identified a potential target for the development of therapeutic approaches.

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