

## Lack of association of single nucleotide polymorphism in *LRCHI* with knee osteoarthritis susceptibility

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**Abstract** A genetic association of knee osteoarthritis (OA) and a C/T transition single nucleotide polymorphism (SNP) (rs912428) located in intron 1 of the *LRCHI* gene has recently been reported in European Caucasians; however, the results are inconsistent. Our objective was to evaluate the association in different knee OA populations. Three case-control association studies were conducted in Han Chinese, Japanese, and Greek Caucasian populations. The *LRCHI* SNP was genotyped in patients who had primary symptomatic knee OA with radiographic confirmation and in matched controls, and the association was examined. We performed a meta-analysis for the studies together with results of two previous papers using the DerSimonian–Laird procedure and calculated the power of the pooled studies by the software R. A total of 1,145 OA patients and 1,266

controls were genotyped. No significant difference was detected in genotype or allele frequencies between knee OA and control groups in the three populations (all  $P > 0.05$ ). Association was not observed even after stratification by gender and Kellgren/Lawrence (K/L) scores. Meta-analysis also supported the lack of association between *LRCHI* and knee OA. The strong heterogeneity between original and replication studies was detected in Caucasian populations. However, a tendency for the increase of TT genotype was observed in the European populations (OR = 1.46,  $P = 0.06$ ). The powers for European and Asian replication studies were less than 0.8. Our results suggest that there is no association between *LRCHI* and knee OA. However, lack of association should be concluded by further replication studies.

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## Introduction

Osteoarthritis (OA, OMIM 165720) is the clinical and pathological outcome of a range of disorders that results in structural and functional failure of synovial joints (Nuki 1999). OA is the most common cause of limitation of activities of daily living after the middle aged. Knee OA, in particular, has a high prevalence in all over the world; recent studies in China show that the prevalence of radiographic knee OA increased according to age (Du et al. 2005). The situation is similar in Japan, Korea, and elsewhere (Ikegawa et al. 2003; Jin et al. 2004). OA has a strong genetic component, and several susceptibility genes for OA have been reported. Heredity will become a substantial factor in future considerations of diagnosis and treatment for OA (Holderbaum et al. 1999).

By scanning more than 25,000 single nucleotide polymorphisms (SNPs), Spector et al. (2006) detected a sound association with knee OA and a C/T transition SNP (rs912428) located in intron 1 of the *LRCHI* gene that encodes a novel protein of as-yet-unknown function. Combined with UK and Newfoundland OA cases, they show a positive association of the T allele of the SNP was a risk factor for knee OA in both genders after stratification. However, the association has not been replicated in a subsequent study in UK Caucasians (Snelling et al. 2007). The data on 2,257 individuals implied that the *LRCHI* SNP was not a risk factor for OA etiology of the knee or hip. Thus, the association of *LRCHI* and OA seems contestable. It has to be tested by independent studies preferably in different ethnic groups.

To clarify the association and its global relevance, we examined the genetic association of the *LRCHI* SNP with knee OA in Han Chinese, Japanese, and Greek Caucasian populations. To integrate the previous contradicting studies (Spector et al. 2006; Snelling et al. 2007) and evaluate the global significance of the association, we performed a meta-analysis for all the association studies. We did not detect association between *LRCHI* and knee OA.

## Materials and methods

### Subjects

Three case-control studies were conducted to replicate the association. A total of 2,411 subjects participated. The study protocol was approved by the ethnical committees of

the participating institutions (Medical School of Nanjing University, SNP Research Center of RIKEN, Medical School of University of Thessaly), and informed consent was obtained from patients and controls.

### Chinese samples

A total of 800 subjects were studied; 315 patients (205 women and 110 men) were enrolled consecutively at the Center of Diagnosis and Treatment for Joint Disease, Drum Tower Hospital, affiliated to the Medical School of Nanjing University, and 485 healthy control subjects (316 women and 169 men) were enrolled at the Center of Physical Examination. The inclusion criteria were as previously described (Jiang et al. 2006; Miyamoto et al. 2007). All subjects were Han Chinese living in and around Nanjing. For all subjects, we calculated body mass index (BMI; body weight in kilograms divided by square of height in meters) to assess obesity.

### Japanese samples

Seven hundred and twenty-three patients (600 women and 123 men) and 620 healthy control subjects (283 women and 337 men) who were living in and around Tokyo were studied. Knee OA patients who had not only definite signs and symptoms of OA but also radiographic evidence of OA were included, as previously described (Kizawa et al. 2005; Miyamoto et al. 2007).

### Greek samples

One hundred and seven OA patients (85 women and 22 men) and 161 controls (112 women and 49) who had undergone treatment for injuries and fractures were recruited for the study. All individuals were of Greek origin living in the district of Thessaly of Central Greece. All patients had pain with rest and/or night pain over 5-months duration.

In any of the studies, other etiologies causing knee diseases, such as inflammatory arthritis (rheumatoid, polyarthritic, or autoimmune disease), posttraumatic or postseptic arthritis, skeletal dysplasia, or developmental dysplasia were excluded. Radiographic OA was assessed using the Kellgren/Lawrence (K/L) grading system (Kellgren et al. 1963). Only patients with K/L grades of 2 or higher were included. None of the controls had ever had signs or symptoms of arthritis or joint diseases (pain, swelling, tenderness, or restriction of movement).

## Genotyping

The *LRCHI* SNP rs912428 was genotyped by the 5-nuclease assay (Taqman) using the M $\times$ 3000P real time polymerase chain reaction (PCR) system (M $\times$ 3000P Real Time PCR System, Stratagene) or ABI 7700 (Applied Biosystems). The primers, probes, fluorescence, and reaction conditions are available upon request. Genotyping was done by laboratory personnel blinded to case status, and a random 5% of the samples were repeated to validate genotyping procedures. Two authors independently reviewed the genotyping results, data entry, and statistical analyses.

## Statistical analysis

Standard chi-square analysis-of-contingency tables were used to compare the *LRCHI* genotype and allele distributions in the case-control study. The differences of the clinical information, age, gender, and K/L scores between the genotypes were tested using the Mann–Whitney test, the Kruskal–Wallis test, and the chi square test. These tests were performed using SPSS 12.0 system software. Hardy–Weinberg equilibrium was performed by chi-square test. Software R was used for meta-analysis. DerSimonian–Laird (DSL) procedure (DerSimonian et al. 1986) based on the random-effect model was used for analysis. Power estimates were calculated using software R.

## Results

Genotype and allele counts for the three populations were as in Table 1.

### Chinese study

The ages of patients and controls [mean  $\pm$  standard deviation (SD)] were  $58.8 \pm 14.3$  (range, 32–93) and  $56.8 \pm 12.3$  (range, 40–97) years, respectively. BMI of patients and controls (mean  $\pm$  SD) were  $24.8 \pm 3.67$  and  $23.6 \pm 6.64$  kg/m<sup>2</sup>, respectively. There was no statistical difference between the two groups. More than 70% of patients had a K/L score of 3 or 4. Distributions of genotypes in the OA and control groups were conformed to Hardy–Weinberg equilibrium ( $P = 0.886$  and  $0.967$ , respectively). Significant differences in distribution of genotype and allele frequencies were detected between Han Chinese and UK Caucasians (both  $P < 0.000001$ ) (Snelling et al. 2007). As in the European populations previously reported (Spector et al. 2006; Snelling et al. 2007), the most common genotype was CC in both patients

and controls. The genotype frequency of CC in Han Chinese was similar to that in Japanese and much higher than that in European Caucasians. No significant difference in the genotype frequency was observed in a comparison of TT vs. other genotypes combined, nor in a comparison of CC vs. other genotypes combined (Table 2). Significant association was not observed after stratification by gender (Table 2). No significant association was detected for CC vs. other genotypes combined in any comparisons. No significant differences were detected among the genotypes and between alleles after stratification by K/L scores in the Chinese samples (both  $P > 0.05$ ).

### Japanese study

The ages of patients and controls (mean  $\pm$  SD) were  $72.0 \pm 7.6$  and  $54.3 \pm 15.4$  years, respectively. Distributions of genotypes in knee OA and control groups were also conformed to Hardy–Weinberg equilibrium ( $P = 0.63$  and  $0.61$ , respectively). None of the genotype or allele frequencies differed significantly (all  $P > 0.05$ ), including stratification analysis by gender (Table 2).

### Greek study

The ages of patients and controls (mean  $\pm$  SD) were  $70.0 \pm 8.4$  and  $71.1 \pm 8.4$  years, respectively. Distributions of genotypes in the knee OA and control groups were also conformed to Hardy–Weinberg equilibrium. None of the genotype or allele frequencies differed significantly (all  $P > 0.05$ ), including stratification analysis by gender (Table 2).

### Meta-analysis

We analyzed the seven case-control association studies between knee OA and *LRCHI* genotypes and alleles by the DSL procedure.  $P$  values for the summary odds ratio (OR) of all studies indicated that there was no positive association between *LRCHI* and knee OA. To exclude the possible publication bias of the first study (the UK discovery study), we excluded this study and analyzed the remaining six case-control studies (the “replication” studies). The  $P$  value for the summary OR for the replication studies was insignificant (Table 3).

Significant heterogeneity was detected in all studies and replication studies with CC vs. others and allele frequency mode. Therefore, we stratified the studies by ethnicity into Asian and European groups. We did not detect any association between knee OA and *LRCHI* with nonsignificant

**Table 1** Genotype and allele frequencies of C/T transition single nucleotide polymorphism (SNP) (rs912428) of the *LRCH1* gene in the three populations

Group	Number of subject	Genotype			Allele	
		TT	CT	CC	T	C
Chinese						
OA						
All	315	3 (0.010)	48 (0.152)	264 (0.838)	54 (0.086)	576 (0.914)
Female	205	2 (0.010)	36 (0.176)	167 (0.814)	40 (0.098)	370 (0.902)
Male	110	1 (0.009)	12 (0.109)	97 (0.882)	14 (0.064)	206 (0.936)
Control						
All	485	4 (0.008)	75 (0.155)	406 (0.837)	83 (0.086)	887 (0.914)
Female	316	2 (0.006)	51 (0.162)	263 (0.832)	55 (0.087)	577 (0.913)
Male	169	2 (0.012)	24 (0.142)	143 (0.846)	28 (0.083)	310 (0.917)
Japanese						
OA						
All	723	3 (0.004)	114 (0.158)	606 (0.838)	120 (0.083)	1326 (0.917)
Female	600	3 (0.005)	93 (0.155)	504 (0.84)	99 (0.083)	1101 (0.917)
Male	123	0	21 (0.171)	102 (0.829)	21 (0.085)	225 (0.915)
Control						
All	620	2 (0.003)	93 (0.15)	525 (0.847)	97 (0.078)	1143 (0.922)
Female	283	0	45 (0.159)	238 (0.841)	45 (0.080)	521 (0.920)
Male	337	2 (0.006)	48 (0.142)	287 (0.852)	52 (0.077)	622 (0.923)
Greek						
OA						
All	107	4 (0.037)	29 (0.271)	74(0.692)	37 (0.173)	177 (0.827)
Female	85	3 (0.035)	27 (0.318)	55 (0.647)	33 (0.194)	137 (0.806)
Male	22	1 (0.045)	2 (0.091)	19 (0.864)	4 (0.091)	40 (0.809)
Control						
All	161	4 (0.025)	54 (0.335)	103 (0.64)	62 (0.193)	260 (0.807)
Female	112	1 (0.009)	41 (0.366)	70 (0.625)	43 (0.192)	181 (0.808)
Male	49	3 (0.061)	13 (0.265)	33 (0.674)	19 (0.194)	79 (0.806)

Frequency in parenthesis  
OA osteoarthritis

heterogeneity (all  $P > 0.05$ ) in the Asian group. *LRCH1* was not associated in the European group with significant heterogeneity in the CC vs. others and allele frequency mode, either (all  $P < 0.05$ ). However, a tendency for the increase of TT genotype was observed in the European populations (OR = 1.46,  $P = 0.06$ ) (Table 3). The powers for European and Asian replication studies were 0.57 and 0.65, respectively.

**Discussion**

We could not detect any association of *LRCH1* with knee OA susceptibility in another European population and two East Asian populations. No significant differences were found, even when the cases were stratified by gender and severity of OA. Distribution of genotype and allele frequencies of *LRCH1* rs912428 was significantly different between East Asian and UK populations. Our meta-analysis also suggested there was no association between

*LRCH1* and knee OA in Asian and European Caucasian populations. The strong heterogeneity between original studies and replication studies were detected in the Caucasian populations. Thus, our results support the negative results of the UK replication study (Snelling et al. 2007).

The difference in ascertainment criteria is unlikely to account for the discrepancy (Jiang et al. 2006; Ikegawa et al. 2006). Radiographic criteria were similar: all referred to K/L grading system, and all patients were of K/L grade 2 or more. The degree of severity of OA was different: the European cases contained more terminal OA (K/L grade > 2) than East Asian cases. However, the difference in OA severity was also an unlikely explanation according to the negative correlation between allele frequencies of the susceptibility T allele and radiographic severity in the Chinese population. Sample size was not the explanation for the discrepancy, either. The sample size of the Chinese population was comparable with previous studies (Spector et al. 2006), and that of Japanese population was much larger. Therefore, our results were much more powerful to

**Table 2** Association of the *LRCHI* C/T polymorphism with knee osteoarthritis in the three populations

Study	Comparison									
	CC vs. others			TT vs. others			All genotypes <sup>a</sup>	C allele vs. T allele		
	OR	<i>P</i> value	95%CI	OR	<i>P</i> value	95%CI		<i>P</i> value	OR	<i>P</i> value
Chinese										
All	0.99	0.97	0.69–1.48	1.16	0.85	0.25–5.20	0.98	1	1	0.70–1.43
Female	1.13	0.66	0.71–1.79	1.55	0.60	0.22–11.7	0.82	1.13	0.56	0.74–1.74
Male	0.74	0.83	0.36–1.51	0.77	0.48	0.07–8.55	0.7	0.75	0.40	0.39–1.46
Japanese										
All	1.07	0.67	0.79–1.43	1.29	0.78	0.22–7.70	0.89	1.07	0.65	0.81–1.41
Female	1.01	0.97	0.68–1.48	–	0.23	–	0.49	1.04	0.83	0.72–1.50
Male	1.18	0.56	0.68–2.06	–	0.39	–	0.53	1.12	0.68	0.66–1.90
Greek										
All	0.79	0.38	0.47–1.33	1.52	0.55	0.37–6.23	0.48	0.88	0.57	0.56–1.37
Female	0.91	0.75	0.51–1.63	4.06	0.19	0.41–39.8	0.36	1.01	0.96	0.61–1.68
Male	0.33	0.09	0.08–1.26	0.73	0.79	0.07–7.44	0.42	2.41	0.12	0.13–1.30

CI confidence interval, OR odds ratio

<sup>a</sup> CC, CT, TT genotypes were grouped together; and a 2 × 3 contingency-table analysis was performed

**Table 3** Meta-analysis: summary of association and heterogeneity of the *LRCHI* C/T polymorphism in knee osteoarthritis

Study	Comparison											
	CC vs. others				TT vs. others				C allele vs. T allele			
	Summary		Heterogeneity		Summary		Heterogeneity		Summary		Heterogeneity	
OR	95%CI	<i>P</i> value	<i>P</i> value	OR	95%CI	<i>P</i> value	<i>P</i> value	OR	95%CI	<i>P</i> value	<i>P</i> value	
All	1.16	0.91–1.48	0.24	0.002	1.43	0.98–2.08	0.06	0.92	1.16	0.95–1.40	0.14	0.01
Replication	1.10	0.84–1.44	0.47	0.003	1.34	0.89–2.03	0.16	0.91	1.11	0.90–1.37	0.33	0.02
European	1.21	0.85–1.71	0.28	0.0005	1.46	0.98–2.17	0.06	0.75	1.20	0.93–1.57	0.17	0.004
Asian	1.04	0.82–1.31	0.75	0.77	1.21	0.38–3.83	0.75	0.93	1.04	0.84–1.30	0.72	0.79

All all studies for the association of *LRCHI* and OA, *Replication* all studies except UK discovery sample. *European* all European samples, *Asian* Chinese and Japanese replication samples, OR odds ratio, CI confidence interval

detect an effect on OA susceptibility of the SNP. The combined result of our Greek population and the UK population (Snelling et al. 2007) was negative for ethnic difference as the explanation for the discrepancy.

Our results suggest that there is no association between *LRCHI* and knee OA in Asian and European populations; however, more than 80% power at a significance level of 5% is desired for the “negative” meta-analysis (Bertram et al. 2007). Lack of association should be concluded by further replication studies.

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