<table>
<thead>
<tr>
<th>Title</th>
<th>Phenotypic and population differences in the association between CILP and lumbar disc disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Virtanen, IM; Song, YQ; Cheung, KMC; AlaKokko, L; Karppinen, J; Ho, DWH; Luk, KDK; Yip, SP; Leong, JCY; Cheah, KSE; Sham, P; Chan, D</td>
</tr>
<tr>
<td>Citation</td>
<td>Journal Of Medical Genetics, 2007, v. 44 n. 4, p. 285-288</td>
</tr>
<tr>
<td>Issued Date</td>
<td>2007</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10722/57177">http://hdl.handle.net/10722/57177</a></td>
</tr>
<tr>
<td>Rights</td>
<td>This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.; Journal of Medical Genetics. Copyright © B M J Publishing Group.</td>
</tr>
</tbody>
</table>
Phenotypic and population differences in the association between CILP and lumbar disc disease

I M Virtanen, Y Q Song, K M C Cheung, L Ala-Kokko, J Karppinen, D W H Ho, K D K Luk, S P Yip, J C Y Leong, K S E Cheah, P Sham and D Chan


Updated information and services can be found at:
http://jmg.bmj.com/cgi/content/full/44/4/285

These include:

References
This article cites 14 articles, 4 of which can be accessed free at:
http://jmg.bmj.com/cgi/content/full/44/4/285#BIBL

1 online articles that cite this article can be accessed at:
http://jmg.bmj.com/cgi/content/full/44/4/285#otherarticles

Rapid responses
You can respond to this article at:
http://jmg.bmj.com/cgi/eletter-submit/44/4/285

Email alerting service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Notes

To order reprints of this article go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to Journal of Medical Genetics go to:
http://journals.bmj.com/subscriptions/
Phenotypic and population differences in the association between CILP and lumbar disc disease

I M Virtanen, Y Q Song, K M C Cheung, L Ala-Kokko, J Karppinen, D W H Ho, K D K Luk, S P Yip, J C Y Leong, K S E Cheah, P Sham, D Chan

Background: Lumbar disc disease (LDD) is one of the leading causes of disability in the working-age population. A functional single-nucleotide polymorphism (SNP), +1184T→C, in exon 8 of the cartilage intermediate layer protein gene (CILP) was recently identified as a risk factor for LDD in the Japanese population (odds ratio (OR) 1.61, 95% CI 1.31 to 1.98), with implications for impaired transforming growth factorβ1 signalling.

Aim: To validate this finding in two different ethnic cohorts with LDD.

Methods: This SNP and flanking SNPs were analysed in 243 Finnish patients with symptoms of LDD and 259 controls, and in 348 Chinese subjects with MRI-defined LDD and 343 controls.

Results and conclusion: The results showed no evidence of association in the Finnish (OR = 1.35, 95% CI 0.97 to 1.87; p = 0.14) or the Chinese (OR = 1.05, 95% CI 0.77 to 1.43; p = 0.71) samples, suggesting that cartilage intermediate layer protein gene is not a major risk factor for symptoms of LDD in Caucasians or in the general population that included individuals with or without symptoms.

Lumbar disc disease (LDD) is one of the leading causes of disability in the working-age population. Radiological changes indicative of LDD are common, but only a proportion develops complications such as disc herniation and sciatica. Although the aetiology of LDD is not well understood, there is strong evidence for the involvement of both genetic and environmental factors.

A recent study reported an association between LDD and a functional single-nucleotide polymorphism (SNP) (rs2073711), +1184T→C, in exon 8 of the cartilage intermediate layer protein gene (CILP) in a Japanese group (odds ratio (OR) 1.61, 95% CI 1.31 to 1.98). The allelic change resulted in amino acid substitution Ile395Thr. CILP is expressed widely in intervertebral discs and its expression increases as disc degeneration progresses. CILP interacts directly with transforming growth factor (TGF)β1, inhibiting the TGFβ1-mediated induction of extracellular matrix proteins such as aggrecan and collagen II. Functional studies showed that the C allele (coding for Thr395) increased binding and inhibition of TGFβ1, suggesting that regulation of TGFβ1 signalling by CILP plays a crucial role in the aetiology and pathogenesis of LDD.

Argument for a causal role would be strengthened if the same association could be replicated in a distinct population, and in clinical cases of LDD defined by MRI changes indicative of LDD in general. Therefore, we investigated the association between CILP polymorphisms and LDD in a Finnish sample with symptoms of LDD, and in a Chinese sample with only MRI-defined LDD. These samples were informative in previous studies demonstrating association of LDD with the vitamin D receptor gene and the Gln326Trp (Trp2) allele of COL9A2 in Chinese and the Arg103Trp (Trp3) allele of COL9A3 in Finns. Thus, the Chinese sample is comparable with the Finnish dataset, and a correlation can then be drawn with the Japanese dataset.

METHODS

The Finnish cohort

The Finnish patient group consisted of 243 unrelated individuals (146 males, 97 females) with discogenic sciatica, representing an extended set of one published previously. All had unilateral pain radiating from the back to below the knee (sciatica) from 3 weeks to 6 months (mean (SD) duration = 2.5 (1.5) months), which did not respond to non-steroidal anti-inflammatory analgesics. The patients were examined clinically and by MRI of the spine on enrolment and 3 years later. Clinical presentation had to be concordant with MRI findings. Approximately 5% of the cohort did not have a herniated disc on MRI. In all, 29% of the subjects had been operated on for herniated discs by the time of follow-up assessment. The control group consisted of 259 unrelated individuals from the same region of Finland (128 males, 131 females).

The Chinese cohort

In the Chinese sample set, the presence and severity of LDD were assessed using Schneiderman’s classification for 691 individuals recruited from the general population. A score was given for each lumbar level, with 0 indicating no degeneration and 3 indicating a grossly degenerated disc with associated loss of disc height. The MRIs were rated by two spine specialists (KMCC and JK) independently, with good reliability. The sum of the ratings for the five disc levels provides an overall raw LDD score that is positively skewed and tends to increase in mean and variance with age. To obtain standardised, age-adjusted LDD scores, the raw LDD scores were logarithmically transformed to reduce skewness and heteroscedasticity, and then standardised to a mean of 0 and a variance of 1 in each decade of age by subtracting the decade mean and dividing by the decade SD. Finally, the sample was divided into two groups: those with higher median age-adjusted scores were classified as cases (n = 348) and those with lower scores were classified as controls (n = 343).

As this new scoring system (with age adjustment) is different from that used in a previous study, we investigated its validity. In the previous analysis, an association between the Trp2 allele and LDD was significant only after age stratification, suggesting that the association is age-dependent. Using the new scoring system, association was observed using a median split (allelic p = 0.043), and became even more significant when the extreme top and bottom 25% were compared (allelic p = 0.001).

Abbreviations: CILP, cartilage intermediate layer protein; LDD, lumbar disc disease; SNP, single-nucleotide polymorphism; TGF, transforming growth factor
Genotype analysis
For the Finnish cohort, genomic DNA extracted from white blood cells was used as a template for PCR. The recently reported SNP, +1184 T→C (rs2073711) in exon 8, associating with LDD was analysed by sequencing in all the 243 patients and 259 controls. In addition, all exons, exon boundaries and promoter regions of CILP were amplified by PCR and analysed for sequence variations by sequencing. PCR amplifications were typically performed using 20 ng of genomic DNA, 0.25 μM of forward and reverse primers, 1.5 μM MgCl₂, 0.2 mM dNTPs and 1 U of Ampli Taq Gold polymerase (Applied Biosystems, Foster City, California, USA). The PCR conditions included an initial denaturation for 12 min at 95°C, 35 cycles at 95°C for 30 s, at 58–64°C for 30 s and at 72°C for 30 s, followed by 1 cycle at 72°C for 10 min. PCR products were sequenced using an ABI PRISM 3100 sequencer and BigDye Terminator Sequencing Kit (Applied Biosystems) to define the underlying sequence variations.

For the Chinese cohort, the +1184 T→C SNP (rs2073711) and three flanking SNPs (rs1561888, rs3784447 and rs4776680) were genotyped using the Sequenom platform (Sequenom, San Diego, California, USA). The Mass ARRAY AssayDesign software (Sequenom) was used to design amplification and allele-specific extension primers for uniplex or multiplexed assays. The extension primer was designed to hybridise to the amplicon near the SNP site for the extension of a single base or a few bases depending on the genotype of the allele. PCR treatments of PCR products with alkaline phosphatase and mass extension reactions were all performed according to the manufacturer’s (Sequenom) protocol. The final base extension products were desalted using SpectroClean resin (Sequenom), mixed with 3-hydroxypicolinic acid, and analysed using a modified Brucker Autoflex MALDI-TOF mass spectrometer (Brucker, Billerica, Massachusetts, USA).

Statistical analysis
Genotype data of each SNP were checked for Hardy–Weinberg disequilibrium using standard $\chi^2$ goodness-of-fit tests. Genotype data were then converted to allele counts in cases (a, b) and allele counts in controls (c, d). These allele counts were used to calculate OR (ad/bc), and 95% CI (using the formula $1/a + 1/b + 1/c + 1/d$) for calculating the sampling variance of the natural logarithm of the OR. The allele counts were also subjected to Pearson’s $\chi^2$ tests for association. These analyses were done by implementing the formulae on an EXCEL spreadsheet. In addition, analysis of variance testing for differences in mean LDD scores between groups was done using the SPSS software. Power calculations of the cohorts were determined using a Genetic Power Calculator, assuming an OR of 1.6 as found in the Japanese cohort.

RESULTS

The Finnish cohort
Sequence analysis of the CILP SNP (rs2073771), +1184T→C in exon 8, found no significant association, with an OR of 1.35 (95% CI 0.97 to 1.87) and a p value of 0.14 (table 1). Furthermore, the frequencies of the corresponding genotypes did not differ between the two groups (table 1). Sequence analysis of the CILP promoter region, all exons and exon boundaries detected five additional SNPs that are in Hardy–Weinberg equilibrium, but all were present in both patients and controls with similar frequency (data not shown). Four of the variations were intronic at −45C→T (intron 4), −12T→C (intron 6), −19T→C (intron 6) and +19G→A (intron 6), and one was a synonymous change, +3496G→A, in exon 9.

The Chinese cohort
Two SNPs in CILP (rs2073711 and rs1561888) and two in genes flanking CILP, rs3784447 in RASL12 and rs4776680 in PARP16, were genotyped in this sample set. All four SNPs were in Hardy–Weinberg equilibrium in both cases and controls. The functional CILP SNP (rs2073771, +1184T→C in exon 8) had an OR of only 1.05 (95% CI 0.77 to 1.43) and a p value of 0.71 (table 1). The frequencies of the corresponding genotypes did not differ between the two groups (table 1). Furthermore, similar results were obtained when more extreme cut-offs were used for defining cases and controls (table 1), and when the age-adjusted scores for SNP rs2073711 were treated as a continuous variable in a regression analysis using analysis of variance (F = 0.112, with p = 0.74). We also observed no significant allelic association of another SNP (rs1561888) within CILP or of SNPs flanking CILP (rs3784447 and rs4776680) with LDD (data not shown).

Power calculation
Assuming a prevalence of 0.1–0.35 and an OR of 1.6 (per allele, multiplicative risk model), we consider the power to be reasonable with an estimate of over 80% for both the Chinese and Finnish cohorts, for a significance level of 0.05.

DISCUSSION
Many of the candidate genes identified to be associated with LDD are extracellular matrix components (recently reviewed by Chan et al10). CILP is expressed in all structures of the intervertebral discs, and its expression increases with progression in disc degeneration.1 Thus, the finding by Seki et al12 further highlights the importance of extracellular matrix components in the aetiology of LDD, and the role of extracellular matrix in the structural integrity of the tissue and also in regulating signalling molecules in tissue repair and maintenance. This finding opens new ideas for the search for candidate genes for LDD as well as novel therapeutic treatments that target specific pathways such as TGFβ1 signalling. Its significance however needs validation in other populations.

The lack of association in the current study does not imply that the finding in the Japanese cohort is a false-positive result. This is also unlikely, given the strong statistical power of the study with a p value of 0.00002 following correction for multiple testing.1 On the contrary, our findings add important information to the Japanese study. In the Finnish set, the recruitment criteria for disease and control groups were very similar to those in the Japanese study. Thus, the disparity in association with the CILP polymorphism may reflect ethnic differences, suggesting that genetic risk factors for LDD are likely to differ between the Japanese and Northern European populations. This conclusion is supported by previous findings of predisposing collagen IX alleles, Gh326Trp (Trp2) in the α2 chain13 14 and Arg103Trp (Trp3) in the α3 chain.15 Trp2 and Trp3 were found in about 5% and 24% of Finnish patients with LDD, respectively. While Trp2 was found in about 20% of Southern Chinese1 and Japanese14 individuals, Trp3 was absent in Southern Chinese.16 Ethnic variations are further highlighted in the different allele frequency of the rs20073711 SNP in CILP between Japanese and Finnish populations.

Smaller ethnic differences are expected between Japanese and Chinese, and this is reflected in the similar allele frequency for the rs20073711 SNP in CILP. The major difference here is the definition of LDD, wherein the Chinese sample set comprises all individuals with LDD defined by MRI, independently of symptoms. LDD is not always with symptoms, and patients with sciatica represent only a small fraction, and individuals requiring surgical invention are a further subset.
Within the Chinese sample set, we also evaluated a small subset of individuals who are clearly symptomatic versus controls with herniation or sciatica and find no association, with p values of 0.42 and 0.83, respectively. Thus, the lack of association in the Chinese sample suggests that the Japanese sample is not representative of LDD in general but is a subset of individuals with painful disc herniations.

Furthermore, we investigated the possibility that the Japanese sample set represents an even more severe subset of the two samples (p = 0.14 for Finnish and p = 0.71 for Chinese) with painful disc herniations found in the Japanese population. Although this may suggest that the CILP association is unique in the Japanese sample, it may be explained by other factors, including differences in ethnicity and phenotype definition.

**ACKNOWLEDGEMENTS**

This work was supported by grants from the University Grants Committee of Hong Kong (AoE/M-04/04), the Research Grants Council of Hong Kong (HKU7509/03M) and the Academy of Finland.

**Authors' affiliations**

I M Virtanen*, L Ala-Kokko, Collagen Research Unit, Biocenter and Department of Medical Biochemistry and Molecular Biology, University of Oulu, Oulu, Finland

Y Q Song*, D W H Ho, K S E Chee, D Chan, Department of Biochemistry, The University of Hong Kong, Pokfulam, Hong Kong, China

K M C Cheung*, K D K Luk, Department of Orthopaedics and Traumatology, The University of Hong Kong, Pokfulam, Hong Kong, China

P Sham, The Genome Research Centre, The University of Hong Kong, Pokfulam, Hong Kong, China

J Karppinen, Finnish Institute of Occupational Health, Helsinki, Finland

S P Yip, Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong, China

J C Y Leong, The Open University of Hong Kong, Hong Kong, China

*These authors contributed equally to this work.

Competing interests: None declared.

Correspondence to: Dr D Chan, Department of Biochemistry, The University of Hong Kong, Faculty of Medicine Building, 21 Sassoon Road, Pokfulam, Hong Kong, China; chand@hkusua.hku.hk

Received 13 October 2006 Revised 11 December 2006 Accepted 18 December 2006

Published Online First 12 January 2007

**REFERENCES**


5 Jim JJ, Noponen-Hietala N, Cheung KM, Ott J, Karppinen J, Sahinarovand A, Luk KD, Yip SP, Sham PC, Song YQ, Leong JCY, Cheah KSE, Ala-Kokko L, Chan D.

---

**Table 1** Genotype and allele frequencies of the functional cartilage intermediate layer protein (CILP) +1184T→C single-nucleotide polymorphism in the Finnish and Chinese samples

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Finnish Cases</th>
<th>Finnish Controls</th>
<th>Chinese Cases</th>
<th>Chinese Controls</th>
<th>Chinese [extreme set]*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>82 (0.36)</td>
<td>73 (0.28)</td>
<td>4 (0.01)</td>
<td>4 (0.01)</td>
<td>3 (0.01)</td>
</tr>
<tr>
<td>CT</td>
<td>116 (0.50)</td>
<td>141 (0.55)</td>
<td>88 (0.26)</td>
<td>83 (0.25)</td>
<td>65 (0.25)</td>
</tr>
<tr>
<td>TT</td>
<td>33 (0.14)</td>
<td>43 (0.17)</td>
<td>249 (0.73)</td>
<td>251 (0.74)</td>
<td>151 (0.72)</td>
</tr>
<tr>
<td>C</td>
<td>280 (0.61)</td>
<td>287 (0.56)</td>
<td>96 (0.14)</td>
<td>91 (0.13)</td>
<td>61 (0.15)</td>
</tr>
<tr>
<td>T</td>
<td>182 (0.39)</td>
<td>227 (0.44)</td>
<td>586 (0.86)</td>
<td>585 (0.87)</td>
<td>357 (0.85)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>p Value (genotype)</th>
<th>0.24</th>
<th>0.93</th>
<th>0.88</th>
</tr>
</thead>
<tbody>
<tr>
<td>p Value (allele)</td>
<td>0.14</td>
<td>0.71</td>
<td>0.93</td>
</tr>
</tbody>
</table>

*Sample set established by selecting the bottom 30% of the normalised distribution representing a control group of 70 males and 138 females with a mean (SD) age of 45 (4) years, and the top 30% representing a more severe patient group of 93 males, 119 females with lumbar disc disease with a mean (SD) age of 42 (9) years.

---
The TRP2 allele of COL9A2 is an age-dependent risk factor for the development and severity of intervertebral disc degeneration. *Spine* 2005;30:2735–42.


