Int J Mol Epidemiol Genet 2012;3(3):195-204
www.ijmeg.org /ISSN1948-1756/IJMEG1206002

Original Article

Gender difference in genetic association between *IL1A* variant and early lumbar disc degeneration: a three-year follow-up

Pasi J Eskola[1], Per Kjaer[2,3,4], Joan S Sorensen[2], Annaleena Okuloff[5], Niels Wedderkopp[3], Iita Daavittila[1], Leena Ala-Kokko[1,6], Minna Männikkö[1], Jaro Karppinen[5,7]

[1]Oulu Center for Cell – Matrix Research, Biocenter and Department of Medical Biochemistry and Molecular Biology, University of Oulu, Oulu, Finland; [2]Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark, Clinical Locomotion Network, Odense, Denmark; [3]Research Department, Spine Centre of Southern Denmark, Hospital Lillebaelt, Institute of Regional Health Services Research, Clinical Locomotion Network, University of Southern Denmark, Middelfart, Denmark; [4]Wilhelm Johannsen Centre For Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark; [5]Finnish Institute of Occupational Health, Health and Work Ability, and Disability Prevention Centre, Oulu, Finland; [6]Connective Tissue Gene Tests, Allentown, USA; [7]Institute of Clinical Medicine, Department of Physical and Rehabilitation Medicine, University of Oulu, PL 5000, 90014 Oulu, Finland

Received June 26, 2012; accepted August 5, 2012; Epub August 31, 2012; Published September 15, 2012

Abstract: Objective: The purpose of the present study was to analyze the associations between specific genetic markers and early disc degeneration (DD) or early disc degeneration progression (DDP) defined by magnetic resonance imaging (MRI). Methods: We selected eleven of the most promising single nucleotide polymorphisms (SNP) and compared the distributions of these genetic markers between groups defined by MRI in a Danish adolescent population (N=166) over a three-year follow-up period. Results: We observed a ten-fold higher annual incidence of endplate changes than previously reported in adults. The gender difference in *IL1A* rs1800587 association with DD remained significant and another association with DDP emerged in follow-up assessment. Among girls, the rs1800587 T-allele was associated both with DD (OR 2.82 [95% CI 1.29–6.16]) and with DDP (OR 2.45 [95% CI 1.03–5.82]). Among boys, the *IL6* rs1800795 genotype G/C was protective in both DD (OR 0.26 [95% CI 0.09–0.72]) and DDP (OR 0.32 [95% CI 0.12–0.88]) with the *IL6* rs1800797 genotype G/A was associated with a decreased likelihood of DD (OR 0.27 [95% CI 0.10–0.77]). Gender-genotype interactions were significant for polymorphisms in both *IL1A* and *IL6.* Correction for multiple testing weakened the associations for *IL6* polymorphisms. Conclusion: We conclude that gender specific effects in lumbar disc degeneration and its progression are possible. However, further evaluations in larger populations are needed. Our results provide some support to the hypothesis that early disc degeneration is an especially important phase in the cascade of degenerative disc disease.

Keywords: Disc degeneration, disc degeneration progression, adolescents, genetics, interleukins

Introduction

For a long time, adolescent low back pain (LBP) was considered a rare illness, normally indicating a severe disease. Epidemiological studies have now shown that LBP is a common condition already during childhood, not to speak of adolescence [1, 2], and that it has a negative impact on the quality of life when untreated [3]. While the underlying reason for the pain is often blurred, lumbar disc degeneration (DD) has been shown to be one of the reasons for LBP [4], especially among adolescents [5-7]. Furthermore, it has been suggested that in some adults, LBP may be preceded by asymptomatic lumbar changes, which are visible already in youth [8].

Several twin studies have implicated heritability in DD [9-11]. This has prompted a justified search for specific causative genetic mutations and associations [12], of which replications have often failed. The problems experienced when attempting to unravel the mystery of DD

may be related to phenotyping [13] or the cross-sectional nature of previous genetic studies. A longitudinal setting for studies in this field has been lacking for a long time. Interestingly, a recent ten-year follow-up study suggests that disc signal changes specifically in individuals aged under 50 years is an important area for the genetic research to focus on [14]. This fits exactly into the scope of the present study.

The aim of this study was to analyze the associations between specific genetic markers and DD or DD progression in adolescents. Our hypothesis was that gender specific associations with DD seen earlier [15] would be refuted during the growth spurt of puberty. We selected eleven of the most promising single nucleotide polymorphisms (SNP) and compared the distributions of these genetic markers twice in a Danish adolescent population over a three-year follow-up period.

Materials and methods

Sampling and flow of study participants

The subjects for this study were a subgroup of a Danish cohort of 771 children sampled for the European Youth Heart Study (EYHS) in 1997 [16]. In 2001, the children who had previously participated (N=589) and who were still living in the same area (N=552) were invited to take part in a magnetic resonance imaging (MRI) study at the age of 12 to 14 [7]. In all, 439 (80% of the invited 552) children were scanned at the baseline. The whole cohort (N=771) was re-invited in 2003 and interviewed on average 2.7 (range 2.2 - 3.1) years after the MRI study baseline [17]. As many as 443 individuals participated in the follow-up interview about back pain and underwent new MRI scans (N=439) within the same day. A total of 318 subjects participated in both baseline and follow-up MRI. Children of non-Caucasian ethnicity (n=29) were excluded to increase the genetic homogeneity of the study population at baseline. Written consent was obtained from both the subjects and their parents, participation was voluntary, and the ethics committee of the Vejle and Funen counties, Denmark, approved the study protocol.

MRI protocol

We performed MRI using the same low-field 0.2T MR unit that was used in the baseline study (Magnetom Open Viva, Siemens AG, Erlangen, Germany). Axial and sagittal T2-weighted turbo spin echo sequences were used to obtain images of the lumbar spine as described elsewhere [7]. At follow-up, we changed settings for the T2-weighted MRI (number of acquisitions was 1 instead of 2), and also obtained the T1-weighted sagittal sequence for other purposes. Otherwise we used the same settings.

MRI reading and definitions

The same experienced radiologist as at baseline (JSS) evaluated all the follow-up images. The radiologist was blinded to the baseline MRI images and ratings and all health data of the subjects. We utilized the international nomenclature [18] for describing disc pathology in the definitions. We graded the *signal intensity changes* of the disc in sagittal sections on T2-weighted images using a scale from 0 to 3, where 0 = homogeneous hyper-intense (white), 1 = hyper-intense with visible intranuclear cleft (white with a dark band in the equator plane of the disc), 2 = intermediate signal intensity (all colours between white and black), and 3 = hypo-intense (dark disc without visible nuclear complex) [19] C*hanges in the disc contour* were described on a nominal scale: 0 = normal, 1= focal protrusion, 2 = broad based protrusion, 3 = extrusion and 4 = sequestration [18, 20]. Bulges were rated separately. *Defects in endplates* were graded: 0 = normal endplates, 1 = defects and 2 = large defects and Schmorl's nodes [21]. We analyzed *annular tears* (AT) including *radial tears* and *high intensity zone* (HIZ) lesions according to the existing definition [22, 23]. The detailed criteria for rating MRI at baseline and follow up has previously been published and tested for reproducibility [7].

Lumbar DD was defined if there was either a signal intensity change (grade 2 or 3) or a change in disc contour (focal protrusion or a more severe change) at one or more lumbar levels. Those with normal signal intensity (grade 0 and 1), normal disc contour (grade 0 or bulge), no annular tears, normal endplates and no other pathology in MRI were classified as subjects without DD. Subjects with intermediate findings (N=13) were not considered eligible for neither group and were thus excluded. More details regarding the selection of subjects for the baseline genetic study have been published

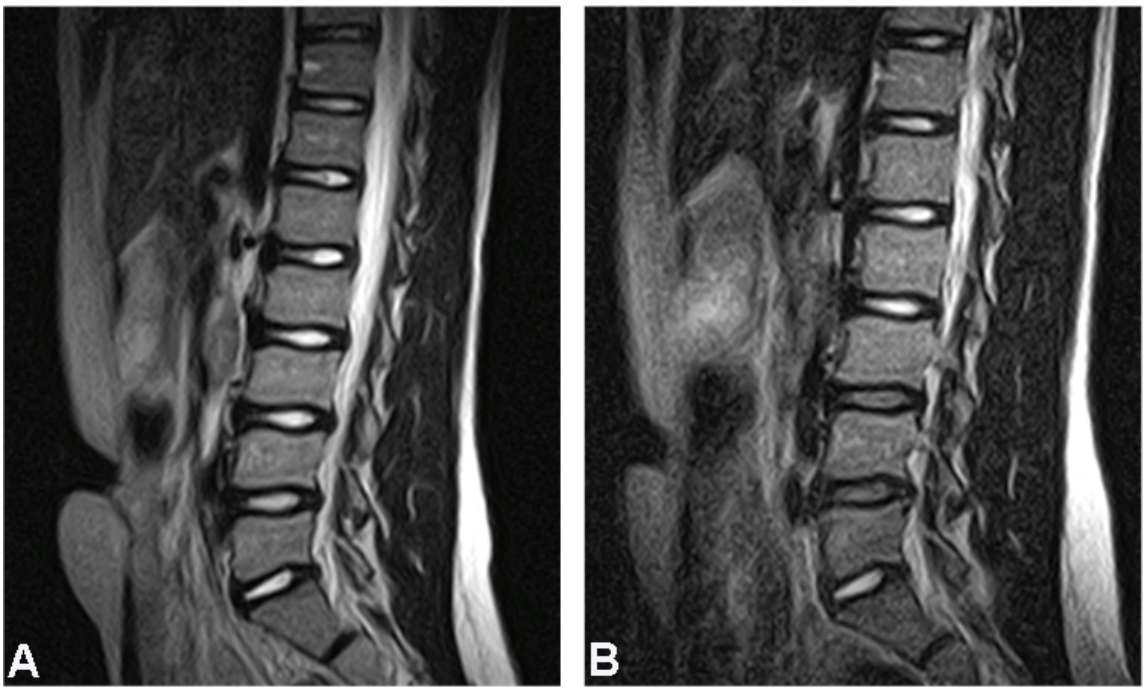

Figure 1. A 15-year-old boy with lumbar disc degeneration progression. A=baseline MRI, disc signals and contour normal, defined as subject without DD B=follow-up MRI, disc signal decrease and a new herniation at L4/L5, defined as subject with DD and DDP.

elsewhere [15].

Of the baseline genetic study subjects (N=220, 66 with DD and 154 without), a total of 166 participated in the follow-up. Children both with and without DD at follow-up were defined, and the follow-up status was compared to the baseline DD status. We indentified different patterns (eg. no lumbar DD – lumbar DD, no lumbar DD – no lumbar DD, no lumbar DD – not eligible, lumbar DD – no lumbar DD and lumbar DD – lumbar DD). Because some of the patterns seemed strange (lumbar DD to no lumbar DD) and because there was 2.7 years between the radiologist ratings, we compared baseline and follow-up images in 2011 to ensure correct classification of children with and without DD. All other patterns expect for no lumbar DD – no lumbar DD (N=78) were re-evaluated in joint consensus sessions by two of the authors (PK and PE) using the software OsiriX v.3.9.1 32-bit (Pixmeo SARL, Geneva, Switzerland). We evaluated sagittal MRI images from both time points simultaneously side-by-side while axial images were used to confirm herniations, bulges, HIZ lesions, annular tears or unclear signal changes. Previous MRI ratings (JSS) were available during the sessions. No more than 25 sets of images were analyzed per day to minimize the fatigue effect.

Progression was defined separately among the same individuals (N=166). Progression in DD status was defined if a subject had either a worsened or new decrease in disc signal intensity, a new bulge or a herniation, new endplate change, or a new Modic change at one or multiple lumbar levels, compared to baseline (**Figure 1**). We also rated significant new annular tears (AT) and significant high intensity zone lesions (HIZ) as progression (DDP). Individuals without these findings or the same degenerative findings as at baseline were considered non-progressive (no-DDP).

Image sets without unequivocal ratings in lumbar DD or progression after joint sessions (PK and PE) were re-evaluated and confirmed by JSS in 2011.

Genetic analyses

Previously identified genetic risk factors for disc degeneration were analyzed from genomic DNA

Table 1. Analyzed polymorphisms

Gene	dbSNP[a]	Risk allele[b]	Sample call rate	Minor allele frequency
COL9A3	rs61734651 (C52T)	T	1.000	0.09
COL11A2	rs1799907 (IVS6-4A>T)	T	0.958	0.36
IL1A	rs1800587 (C-889T)	T	0.982	0.34
IL1B	rs1143634 (C3954T)	T	0.994	0.29
IL6	rs1800797 (G-597A)	G	0.988	0.49
IL6	rs1800796 (G-572C)	G	0.988	0.05
IL6	rs1800795 (G-174C)	G	0.994	0.50
IL6	rs13306435 (T15A)	A	1.000	0.01
IL6	rs2069849 (132 C>T)	T	0.994	0.04
VDR	rs2228570 (T2C)	T	0.946	0.37
VDR	rs731236 (T352C)	C	0.988	0.40

[a]Polymorphisms as they appear in NCBI SNP data and (prior literature). [b]*A priori* genetic model was dominant.

extracted from stored whole blood. The genotyping details have been published previously [15]. We included eleven previously identified candidate SNPs in the analysis (**Table 1**).

Statistical analysis

We performed statistical analyses of the follow-up data were parallel to baseline [15], with the exception of dependent variables that were DD yes/no, DDP yes/no. We used a later version of SAS (V9.2). The potential deviation from the Hardy-Weinberg equilibrium (HWE) was tested using the chi-square test. The Mann-Whitney U-test was used in the analysis of the association between weight, height and DD status. Allele and genotype frequencies were compared between children with and without DD using the chi-square test. Haplotypes were first estimated by SNPStats [24] and then statistically reconstructed from population genotype data using the PHASE program with the Markov-chain method for haplotype assignments [25]. Association between DD/DDP and single SNP markers or haplotypes were analyzed using logistic regression analyses. Crude and adjusted ORs and their 95% confidence intervals (CIs) were calculated using the SNPStats and SAS. The dependent variable was DD/DDP phenotype (presence or absence of DD/DDP) and independent variables were genotype, allele carriage or haplotypes. In candidate gene association studies, the questions are more specific than in whole genome association studies. Therefore, in the setting of existing a priori hypotheses, multiple testing was accounted only for genes with multiple SNPs. Bonferroni-Holm method was used for multiple testing corrections [26].

Results

Clinical results at follow-up

Out of 220 individuals included at baseline a total of 166 (75%) were available for the analyses at the follow-up. Of these 166 subjects, 57.8% (N=96) were defined as subjects without DD while 34.3% (N=57) had DD. Subjects with intermediate findings (N=13) were not eligible for either group. Unexpectedly, follow-up improvement in MRI findings was seen in 12 individuals classified as subjects with DD at baseline. After consensus session and re-evaluation by the radiologist (JSS), eight individuals were rated as subjects without DD, and three subjects with intermediate findings were not eligible for either group (**Figure 2**). Subjects with DD

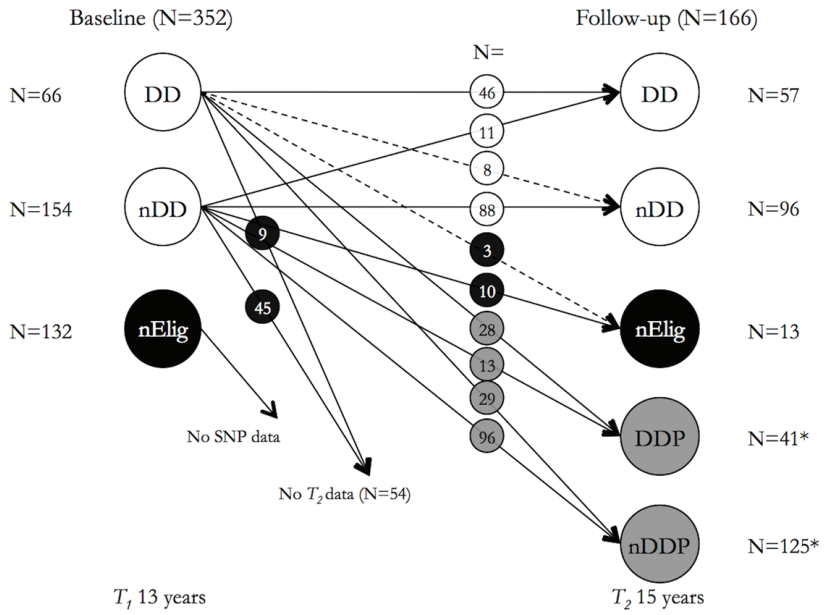

Figure 2. Transitions of study subjects between groups. Abbreviations: DD=subjects with lumbar disc degeneration, nDD=subjects without lumbar disc degeneration, nElig=subjects with other specific MRI findings, DDP=progression of lumbar disc degeneration, nDDP=no progression of lumbar disc degeneration. *Additional definition of phenotype (DDP+nDPP=DD+nDD+nElig at follow up). Dash line indicates illogical pathways, in which subjects were re-evaluated thrice.

were on average 5 cm taller that those without DD (p=0.008; **Table 2**). They also tended to weigh more than subjects without DD (**Table 2**).

DDP was seen in 33.8% of the boys (N=25/74) and in 17.4% (N=16/92) of the girls. Out of subjects with DDP (N=41), it was defined based on a decrease in disc signal intensity in 15.7% (N=26), a new or clearly worsened endplate change in 15.1% (N=25), a new disc herniation in 7.2% (N=12), a new AT in 2.4% (N=4), a new bulge in 1.8% (N=3), and a new HIZ in 1.2% (N=2). DDP definition was based on one change in 36.6% (N=15), two changes in 51.2% (N=21) and three changes in 12.2% (N=5) of the DDP subjects. Multiple level DDP was present in 56.1% (N=23) of the subjects while the two lowest levels (L4/L5 and L5/S1) were the most often affected (68.3%, N=28).

Genetic associations at follow-up

The genotyping of the selected polymorphisms produced good average sample call rates (0.985) and we were able to genotype all the selected SNPs (**Table 1**). All the genotype frequencies analyzed were in the Hardy-Weinberg equilibrium. Among girls, the T-allele of *IL1A* rs1800587 was associated in additive manner with both DD (OR 2.82 [95% CI 1.29–6.16] in the additive genotype model, p=0.0064) and with DDP (OR 2.45 [95% CI 1.03–5.82] in the additive genotype model, p=0.037). Gender-genotype interaction was significant; the p-value of test for interaction in the trend was 0.024 (**Figure 3**). Among boys, the *IL6* rs1800797 genotype G/A was associated with a decreased likelihood of DD (OR 0.27 [0.10–0.77], p=0.012, in the over-dominant genotype model). *IL6* rs1800795 genotype G/C was similarly protective in both DD (OR 0.26 [95% CI 0.09–0.72], p=0.0079, in the co-dominant genotype model) and DDP (OR 0.32 [95% CI 0.12–0.88], p=0.024, in the over-dominant genotype model) among boys. Gender-genotype interactions were also significant for *IL6* polymorphisms; the p-value for test of interaction in the trend was 0.017 (DD) in rs1800797 (**Figure 3**) and 0.023, 0.043 (DD, DDP, respectively) in rs1800795. However, after correction for multiple testing the associations with the *IL6* polymorphisms were significant only in over-dominant genotype model. We observed no statistically significant associations between the other SNPs or haplotypes and DD or DDP.

Discussion

To the authors' knowledge, this is the first report of associations between genetic markers and lumbar disc degeneration progression in adolescence.

In this study we observed DDP in 24.7% and DD in 34.3% of the subjects at the follow-up. Of those with DD at baseline, the degenerative

Table 2. Prevalence of MRI findings and characteristics among subjects with DD and without DD at baseline (BL) and follow-up (FU).

MRI findings	Grading	Subjects with DD at BL (N=66) N %	Subjects without DD at BL (N=154) N %	Subjects with DD at FU (N=57) N %	Subjects without DD at FU (N=96) N %
*Signal changes**	Normal (grade 0 and 1)	0 (0)	154 (100)	4 (7)	96 (100)
	Intermediate (2)	63 (95)	0 (0)	48 (84)	0 (0)
	Hypointense (3)	3 (5)	0 (0)	5 (9)	0 (0)
*Disc Contour**	Normal (0)	40 (61)	152 (99)	37 (65)	96 (100)
	Bulging (1)	20 (30)	1 (1)	16 (28)	0 (0)
	Broadbased protrusion (2)	5 (8)	0 (0)	3 (5)	0 (0)
	Focal protrusion (3)	1 (5)	0 (0)	1 (2)	0 (0)
	Extrusion (4)	0 (0)	0 (0)	0 (0	0 (0)
	Sequestration (5)	0 (0)	0 (0)	0 (0)	0 (0)
*Endplate changes**	Normal	55 (83)	153 (99)	37 (65)	0 (0)
	Defects (1)	5 (8)	1 (1)	15 (26)	0 (0)
	Large Defects (2)	6 (9)	0 (0)	5 (9)	0 (0)
Annular Tears		13 (20)	0 (0)	10 (18)	0 (0)
Modic changes	Normal (0)	0 (0)	0 (0)	55 (96)	0 (0)
	Grade 1 (1)	2 (3)	0 (0)	2 (4)	0 (0)
Characteristics		N %	N %	N %	N %
Gender	Boys	30 (45)	73 (47)	30 (53)	36 (38)
	Girls	36 (55)	81 (53)	27 (47)	60 (62)

	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (Years)	13.1	0.4	13.1	0.4	15.7	0.3	15.7	0.3
Height[a] (cm)	162	7.7	159[a]	7.3	173	9.3	168[b]	7.9
Weight (kg)	51	8.5	49[c]	8.7	63	10.4	59	9.1
BMI (kg/m²)	19.5	2.3	19.2	2.8	20.9	2.7	20.8	2.6

*Prevalence rates are given by the worst grade at one or more of the five lumbar levels. [a] p=0.029, [b] p=0.008, [c] p=0.042 (Mann-Whitney U-test).

changes progressed in every second subject (51%) during the follow-up period (**Figure 2**). Decrease in disc signal intensity was the prominent marker of progression (seen in 15.7% of the subjects) while deterioration in endplates was nearly as common (in 15.1%). New disc herniations were less common (in 7.2%) and annular tears, bulges and HIZ lesions were relatively rare (in 1-2%). Previous longitudinal studies among adults have reported annual progression of degenerative changes in ca. 5-10% of subjects [27, 28]. Annual DDP in the present study was similar, as it was seen in 9.1% of the subjects. Progression in disc signal changes has been reported in 9-24% of individuals after three-to-five year follow-ups [27, 29], which is in line with our findings (15.7% in 2.7 years). A study on adult Finnish male twins [30] reported that new annular tears or HIZ lesions were relatively rare, as they were present in ca. 3-5% of subjects after a five-year follow-up (compared to our 1-2% in three years). The same study reported that new endplate defects were also quite rare, as annual incidence of reported progression in endplate irregularities was only ca. 0.4% [30]. In our study, the annual incidence of

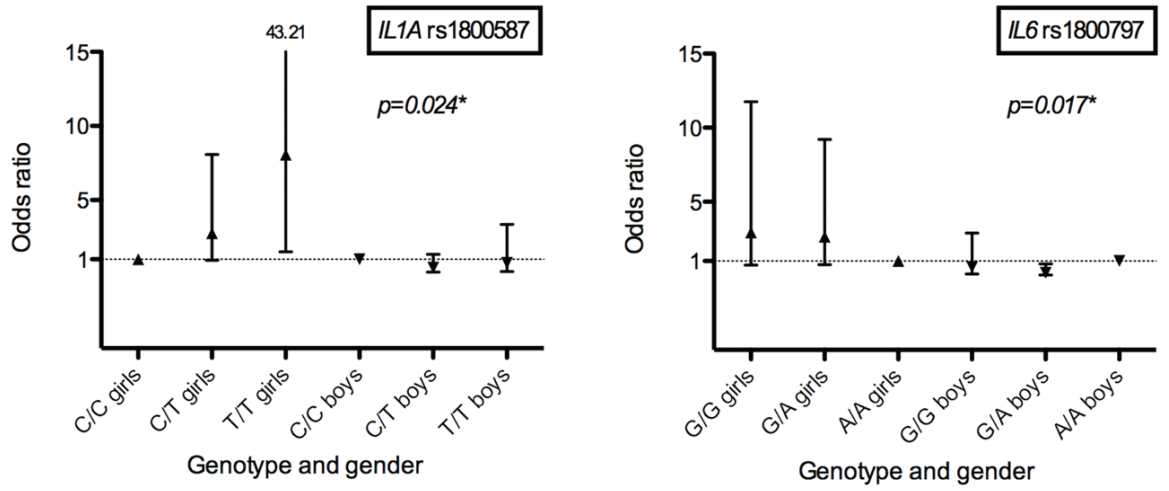

Figure 3. Interaction analyses results with covariate gender. Gender-genotype interaction; p-value of test for interaction in the trend.

new endplate findings was over ten-fold higher (5.6%) than the earlier observation in adults. This might be partly explained by differences in MRI definitions. Also the Scheuermann's disease can cause endplate irregularities during adolescence. However, based on the low annual incidence of endplate changes in later life, the authors of the previous study suggested that endplate changes might develop primarily before middle adulthood [30]. Endplate irregularities are quite common in later life, as they have been reported in 6-30% of adults [30, 31]. Interestingly, a slow "recovery" in the endplate changes has been also seen before [30]. It may be that the differences in phenotypes of endplate changes in previous studies as well as possible differences in healing capacity of endplate changes in adolescence vs. later in life lie behind the intricate interplay. However, our findings support the recently risen perception that early disc degeneration should be taken under a more careful observation [8, 32].

The *IL1A* rs1800587 T-allele has been associated previously with disc bulges and Modic changes in occupational cohort of men [33]. Based on animal studies it is also known that IL-1 is the dominant cartilage-destructive cytokine [34]. Furthermore, the IL1α has been shown to inhibit proteoglycan synthesis in the interverbral discs in a rabbit model [35] and is bioactive already as a precursor unlike that of IL1β [36]. The *IL1A* rs1800587 T-allele has been previously shown to act as a marker for another polymorphism (+4845 G > T) resulting to capability of higher level of cellular release of IL-1α [37]. Our results are in line with the earlier findings with the exception of gender difference, which is a novel finding.

The pleiotropic cytokine IL6 is one of the most important mediators of inflammatory reactions [38] and it has also been reported that IL-6 is produced at the site of lumbar disc herniation [39]. The *IL6* promoter polymorphisms have been previously associated with sciatica [40]. Reports of protective effects of heterozygotes of either *IL6* rs1800797 or 1800795 in DD or DDP are not available. However, based on recent research, the effects of *IL6* promoter polymorphisms on gene expression are likely to be more complex than what has been initially expected [38]. Interestingly, a recent *in vitro* study using blood cells from prepubescent children reported gender differences in IL6 production [41]. However, it is to be noted that correction for multiple testing weakened the associations for the *IL6* polymorphisms in this study.

Could the negative replications in genetic studies with DD [12] be partly explained by gender? In osteoporosis and osteoarthritis the gender-specificity of the effects of genetic variants is well recognized [42, 43]. In sciatica, the prevalence in men has long been known to be 1.5–3 times higher compared to women [44]. This has been conventionally explained by the fact that men tend to work in more physically demanding occupations. However, in childhood and adolescence the situation might even be vice versa, at

least when it comes to LBP, as girls have been reported to have higher prevalence of pain [45]. Similarly, gender differences in LBP reporting were seen in a study with partly same population as in the present study [17]. These dissimilarities on the other hand have been suggested to be due to hormonal differences and the onset of menstruation-related pains in girls [46]. The effects of body height and weight on LBP have been reviewed and discussed previously [47, 48], however, their association with adolescent DD observed in this study is yet another interesting finding.

Strengths of the present study include the longitudinal setting, which has not been used previously in a candidate gene study neither on adolescent DD nor on DDP. The early disc degeneration is likely to be important for the later status of the disc. Early changes are associated with spinal deformities and endplate changes that may predispose the intervertebral disc to further degeneration via impaired spinal biomechanical function [6, 49-51]. The young spine is also highly vulnerable to trauma especially during the growth spurt [52]. The disc signal decrease as well as disc herniations have been recently found to be valid phenotypes to investigate the genetic component of DD progression [14]. Furthermore, the endplate changes, that were relatively prominent in the present study, have been often suspected to be the initiating event of DD [53]. Schmorl's nodes, that were included in our definition of endplate changes, have been also earlier found to be strongly genetically determined and shown to associate with disc degeneration [31]. Sample size is limited in our study. However, it is to be noted that we have included more subjects than majority of the previous studies focusing on disc degeneration progression [27-30]. Follow-up improvement in MRI findings was seen in eight individuals classified as subjects with DD at baseline. This phenomenon has been seen before in adults [14, 30] and is likely not a source of significant bias. The present study has a unique approach to the topic as, in addition to DD, we analyzed associations between genetic markers and DDP. After all, a large amount of individuals develop some degenerative changes with age but all do not develop symptoms. We assume that individuals with DDP at the follow-up assessment might be the ones that develop symptomatic degenerative disease in the future [5, 6]. Many individuals in this study had multiple markers of DDP, which is often the case when evaluating patients in the clinical world. The present results emphasize the importance of further investigations on early disc degeneration.

We observed unexpected gender-specific genetic associations in adolescent lumbar disc degeneration and progression of degenerative lumbar changes as well as high incidence of new endplate changes. While the present study has limitations, we conclude that possible gender effects should be better acknowledged in genetic studies of lumbar DD.

Acknowledgments

We thank Ms. Irma Vuoti and Ms. Helena Satulehto for their expert technical assistance. This work was supported by the Academy of Finland (121620, 200868 to JK and 115471 to LAK) and by the Päivikki and Sakari Sohlberg Foundation (PJE). PK was supported by a grant from the Danish Agency for Science, Technology and Innovation (271-08-0374). We also received additional funding for a short-term research visit to Denmark from the Research Council of the University of Oulu (PJE).

Address correspondence to: Dr. Jaro Karppinen, Finnish Institute of Occupational Health, Health and Work Ability, and Disability Prevention Centre, Oulu, Finland; Institute of Clinical Medicine, Department of Physical and Rehabilitation Medicine, University of Oulu, PL 5000, 90014 Oulu, Finland Tel: +358414462859; Fax: +35883153501; E-mail: jaro.karppinen@ttl.fi

References

[1] Auvinen J, Tammelin T, Taimela S, Zitting P, Karppinen J. Associations of physical activity and inactivity with low back pain in adolescents. Scand J Med Sci Sports 2008; 18: 188-194.

[2] Leboeuf-Yde C, Kyvik KO. At what age does low back pain become a common problem? A study of 29,424 individuals aged 12-41 years. Spine (Phila Pa 1976) 1998; 23: 228-234.

[3] Fontecha CG, Balague F, Pellise F, Rajmil L, Aguirre M, Pasarin M, Cedraschi C, Ferrer M. Low Back Pain in Adolescents: Is Quality of Life Poorer in Those Seeking Medical Attention? Spine (Phila Pa 1976) 2011; 36: 1154.

[4] Endean A, Palmer KT, Coggon D. Potential of magnetic resonance imaging findings to refine case definition for mechanical low back pain in epidemiological studies: a systematic review.

Spine (Phila Pa 1976) 2011; 36: 160-169.
[5] Takatalo J, Karppinen J, Niinimaki J, Taimela S, Nayha S, Mutanen P, Sequeiros RB, Kyllonen E, Tervonen O. Does lumbar disc degeneration on MRI associate with low back symptom severity in young Finnish adults? Spine (Phila Pa 1976) 2011; 2180-2189.
[6] Samartzis D, Karppinen J, Mok F, Fong DYT, Luk KDK, Cheung KMC. A population-based study of juvenile disc degeneration and its association with overweight and obesity, low back pain, and diminished functional status. J Bone Joint Surg Am 2011; 93: 662-670.
[7] Kjaer P, Leboeuf-Yde C, Sorensen JS, Bendix T. An epidemiologic study of MRI and low back pain in 13-year-old children. Spine (Phila Pa 1976) 2005; 30: 798-806.
[8] Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. Spine (Phila Pa 1976) 2002; 27: 2631-2644.
[9] Battie MC, Videman T, Gibbons LE, Fisher LD, Manninen H, Gill K. 1995 Volvo Award in clinical sciences. Determinants of lumbar disc degeneration. A study relating lifetime exposures and magnetic resonance imaging findings in identical twins. Spine (Phila Pa 1976) 1995; 20: 2601-2612.
[10] MacGregor AJ, Andrew T, Sambrook PN, Spector TD. Structural, psychological, and genetic influences on low back and neck pain: a study of adult female twins. Arthritis Rheum 2004; 51: 160-167.
[11] Sambrook PN, MacGregor AJ, Spector TD. Genetic influences on cervical and lumbar disc degeneration: a magnetic resonance imaging study in twins. Arthritis Rheum 1999; 42: 366-372.
[12] Battie MC, Videman T, Kaprio J, Gibbons LE, Gill K, Manninen H, Saarela J, Peltonen L. The Twin Spine Study: contributions to a changing view of disc degeneration. Spine J 2009; 9: 47-59.
[13] Modic MT. Degenerative disc disease: genotyping, MR imaging and phenotyping. Skeletal Radiol 2007; 36: 91-93.
[14] Williams FM, Popham M, Sambrook PN, Jones AF, Spector TD, Macgregor AJ. Progression of lumbar disc degeneration over a decade: a heritability study. Ann Rheum Dis 2011; 70: 1203-1207.
[15] Eskola PJ, Kjaer P, Daavittila IM, Solovieva S, Okuloff A, Sorensen JS, Wedderkopp N, Ala-Kokko L, Mannikko M, Karppinen JI. Genetic risk factors of disc degeneration among 12-14-year-old Danish children: a population study. IJMEG 2010; 1: 158-165.
[16] Wedderkopp N, Leboeuf-Yde C, Andersen LB, Froberg K, Hansen HS. Back pain reporting pattern in a Danish population-based sample of children and adolescents. Spine (Phila Pa 1976) 2001; 26: 1879-1883.
[17] Kjaer P, Wedderkopp N, Korsholm L, Leboeuf-Yde C. Prevalence and tracking of back pain from childhood to adolescence. BMC Musculoskelet Disord 2011; 12: 98.
[18] Fardon DF, Milette PC. Nomenclature and classification of lumbar disc pathology. Recommendations of the Combined task Forces of the North American Spine Society, American Society of Spine Radiology, and American Society of Neuroradiology. Spine (Phila Pa 1976) 2001; 26: 93.
[19] Weishaupt D, Zanetti M, Hodler J, Boos N. MR imaging of the lumbar spine: prevalence of intervertebral disk extrusion and sequestration, nerve root compression, end plate abnormalities, and osteoarthritis of the facet joints in asymptomatic volunteers. Radiology 1998; 209: 661-666.
[20] Milette PC. The proper terminology for reporting lumbar intervertebral disk disorders. AJNR Am J Neuroradiol 1997; 18: 1859-1866.
[21] Videman T, Battie MC, Gill K, Manninen H, Gibbons LE, Fisher LD. Magnetic resonance imaging findings and their relationships in the thoracic and lumbar spine. Insights into the etiopathogenesis of spinal degeneration. Spine (Phila Pa 1976) 1995; 20: 928-935.
[22] Yu SW, Sether LA, Ho PS, Wagner M, Haughton VM. Tears of the anulus fibrosus: correlation between MR and pathologic findings in cadavers. AJNR Am J Neuroradiol 1988; 9: 367-370.
[23] Aprill C, Bogduk N. High-intensity zone: a diagnostic sign of painful lumbar disc on magnetic resonance imaging. Br J Radiol 1992; 65: 361-369.
[24] Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics 2006; 22: 1928-1929.
[25] Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001; 68: 978-989.
[26] Holm S. A simple sequentially rejective multiple test procedure. Scand J Statist 1979; 6: 65-70.
[27] Elfering A, Semmer N, Birkhofer D, Zanetti M, Hodler J, Boos N. Risk factors for lumbar disc degeneration: a 5-year prospective MRI study in asymptomatic individuals. Spine (Phila Pa 1976) 2002; 27: 125-134.
[28] Borenstein DG, O'Mara JW, Boden SD, Lauerman WC, Jacobson A, Platenberg C, Schellinger D, Wiesel SW. The value of magnetic resonance imaging of the lumbar spine to predict low-back pain in asymptomatic subjects : a seven-year follow-up study. J Bone Joint Surg Am 2001; 83 -A: 1306-1311.
[29] Jarvik JG, Hollingworth W, Heagerty PJ, Haynor DR, Boyko EJ, Deyo RA. Three-year incidence of low back pain in an initially asymptomatic cohort: clinical and imaging risk factors. Spine (Phila Pa 1976) 2005; 30: 1541-1518.

[30] Videman T, Battie MC, Ripatti S, Gill K, Manninen H, Kaprio J. Determinants of the progression in lumbar degeneration: a 5-year follow-up study of adult male monozygotic twins. Spine (Phila Pa 1976) 2006; 31: 671-678.
[31] Williams FMK, Manek NJ, Sambrook PN, Spector TD, Macgregor AJ. Schmorl's nodes: common, highly heritable, and related to lumbar disc disease. Arthritis Rheum 2007; 57: 855-860.
[32] Andersson GB, An HS, Oegema TR, Setton LA. Intervertebral disc degeneration. Summary of an AAOS/NIH/ORS workshop, September 2005. J Bone Joint Surg Am 2006; 88: 895-899.
[33] Karppinen J, Solovieva S, Luoma K, Raininko R, Leino-Arjas P, Riihimaki H. Modic changes and interleukin 1 gene locus polymorphisms in occupational cohort of middle-aged men. Eur Spine J 2009; 18: 1963-1970.
[34] van dB, Bresnihan B. Pathogenesis of joint damage in rheumatoid arthritis: evidence of a dominant role for interleukin-I. Baillieres Best Pract Res Clin Rheumatol 1999; 13: 577-597.
[35] Maeda S, Kokubun S. Changes with age in proteoglycan synthesis in cells cultured in vitro from the inner and outer rabbit annulus fibrosus. Responses to interleukin-1 and interleukin-1 receptor antagonist protein. Spine (Phila Pa 1976) 2000; 25: 166-169.
[36] Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood 2011; 117: 3720-3732.
[37] Kawaguchi Y, Tochimoto A, Hara M, Kawamoto M, Sugiura T, Saito S, Kamatani N. Contribution of single nucleotide polymorphisms of the IL1A gene to the cleavage of precursor IL-1alpha and its transcription activity. Immunogenetics 2007; 59: 441-448.
[38] Smith AJP, Humphries SE. Cytokine and cytokine receptor gene polymorphisms and their functionality. Cytokine Growth Factor Rev 2009; 20: 43-59.
[39] Burke JG, Watson RW, McCormack D, Dowling FE, Walsh MG, Fitzpatrick JM. Intervertebral discs which cause low back pain secrete high levels of proinflammatory mediators. J Bone Joint Surg Br 2002; 84: 196-201.
[40] Noponen-Hietala N, Virtanen I, Karttunen R, Schwenke S, Jakkula E, Li H, Merikivi R, Barral S, Ott J, Karppinen J, Ala-Kokko L. Genetic variations in IL6 associate with intervertebral disc disease characterized by sciatica. Pain 2005; 114: 186-194.
[41] Casimir GJ, Heldenbergh F, Hanssens L, Mulier S, Heinrichs C, Lefevre N, Desir J, Corazza F, Duchateau J. Gender differences and inflammation: an in vitro model of blood cells stimulation in prepubescent children. J Inflamm (Lond) 2010; 7: 28.
[42] Karasik D, Ferrari SL. Contribution of gender-specific genetic factors to osteoporosis risk. Ann Hum Genet 2008; 72: 696-714.
[43] Valdes AM, Loughlin J, Oene MV, Chapman K, Surdulescu GL, Doherty M, Spector TD. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. Arthritis Rheum 2007; 56: 137-146.
[44] Frymoyer JW. Lumbar disk disease: epidemiology. Instr Course Lect 1992; 41: 217-223.
[45] Diepenmaat ACM, van dW, de Vet HCW, Hirasing RA. Neck/shoulder, low back, and arm pain in relation to computer use, physical activity, stress, and depression among Dutch adolescents. Pediatrics 2006; 117: 412-416.
[46] Wedderkopp N, Andersen LB, Froberg K, Leboeuf-Yde C. Back pain reporting in young girls appears to be puberty-related. BMC Musculoskelet Disord 2005; 6: 52.
[47] Shiri R, Karppinen J, Leino-Arjas P, Solovieva S, Viikari-Juntura E. The association between obesity and low back pain: a meta-analysis. Am J Epidemiol 2010; 171: 135-154.
[48] Coeuret-Pellicer M, Descatha A, Leclerc A, Zins M. Are tall people at higher risk of low back pain surgery? A discussion on the results of a multipurpose cohort. Arthritis Care Res (Hoboken) 2010; 62: 125-127.
[49] Paajanen H, Erkintalo M, Kuusela T, Dahlstrom S, Kormano M. Magnetic resonance study of disc degeneration in young low-back pain patients. Spine (Phila Pa 1976) 1989; 14: 982-985.
[50] Erkintalo MO, Salminen JJ, Alanen AM, Paajanen HE, Kormano MJ. Development of degenerative changes in the lumbar intervertebral disk: results of a prospective MR imaging study in adolescents with and without low-back pain. Radiology 1995; 196: 529-533.
[51] Swischuk LE, John SD, Allbery S. Disk degenerative disease in childhood: Scheuermann's disease, Schmorl's nodes, and the limbus vertebra: MRI findings in 12 patients. Pediatr Radiol 1998; 28: 334-338.
[52] Baranto A, Hellstrom M, Nyman R, Lundin O, Sward L. Back pain and degenerative abnormalities in the spine of young elite divers: a 5-year follow-up magnetic resonance imaging study. Knee Surg Sports Traumatol Arthrosc 2006; 14: 907-914.
[53] Chan WCW, Sze KL, Samartzis D, Leung VYL, Chan D. Structure and biology of the intervertebral disk in health and disease. Orthop Clin North Am 2011; 42: 447-464.