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Original Article

Genetic risk factors of disc degeneration among 12-14-year-old Danish children: a population study

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Abstract: The objective of the present study was to examine the associations between eleven putative predisposing single nucleotide polymorphisms *(COL9A3, COL11A2, IL1A, IL1B, IL6* and *VDR)* and early disc degeneration (DD). The population consisted of 12 to 14-year-old Danish children (N=352). DD was evaluated from magnetic resonance images (MRI). We analysed the association between DD and single nucleotide polymorphisms or haplotypes using logistic regression analyses. Of the 352 children studied, 73 boys and 81 girls had no MRI changes, while 30 boys and 36 girls had lumbar DD. Among girls, *IL1A* rs1800587 in CT/TT compared to CC resulted in OR 2.85 [1.19-6.83]. In *IL6* promoter polymorphism rs1800796, the C-allele was more frequent among the subjects with DD, OR 6.71 [1.71-26.3]. Of the *IL6* haplotypes, GCG was associated with DD, OR 6.46 [1.61 – 26.0]. No associations were observed among boys. Our results suggest possible roles for *IL1A* and *IL6* in early DD among girls.

Keywords: Disc degeneration, children, genetics, interleukins

Introduction

Low back pain (LBP) seems to be a common health problem already in early life, as the prevalence of non-specific LBP in adolescence is almost at the level of that of adults [1]. The role of disc degeneration (DD) in relation to LBP is open to dispute, as DD also occurs in asymptomatic subjects [2]. However, a systematic review revealed that the presence of lumbar DD was associated with LBP [3]. The positive association between DD and LBP has been confirmed in population-based cohorts of adults [4], young adults (Jani Takatalo, unpublished data) and adolescents [5].

Epidemiological studies on families and twins suggest that inheritance is the major determinant of DD [6,7,8]. Several disease-associating variations have been found in a number of different genes, suggesting that intervertebral DD is a multigenetic entity [9,10]. The degenerative process starts at an early age, as in histological samples unequivocal signs of early degeneration became visible at the age of 11 to 16 [11]. We believe that DD at a young age is more likely to be hereditary, as children have not yet been exposed to environmental risk factors such as physical workload. The objective of the current study was to examine the associations between putative predisposing single nucleotide polymorphisms (SNP) in a set of candidate genes *(COL9A3, COL11A2, IL1A, IL1B, IL6* and *VDR)* and early DD among 12 to 14-year-old Danish children. Among adults, the selected polymorphisms have been suggested predisposing to DD or sciatica [9,10].

Materials and methods

The subjects for this study were a subgroup (N=589) of a Danish cohort sampled for the

Table 1. Genetic variations analysed

Gene	dbSNP [a]	Risk allele[c]	Sample call rate	Detection methods	Reference
COL9A3	rs61734651 (C52T)	T	1.000	Sequencing, SNaPshot ™	[37], [38]
COL11A2	rs1799907 (IVS6-4A>T)	T	0.964	*BsmFI,* Sequencing, SNaP-shot ™	[39]
IL1A	rs1800587 (C-889T)[b]	T	0.977	*NcoI,* Sequencing, SNaPshot ™	[25], [26]
IL1B	rs1143634 (C3954T)	T	0.995	*TaqI,* SNaPshot ™	[25], [29]
IL6	rs1800797 (G-597A)	G	0.991	Sequencing	[29], [30]
IL6	rs1800796 (G-572C)	G	0.991	Sequencing	[29], [30]
IL6	rs1800795 (G-174C)	G	0.995	Sequencing	[29], [30]
IL6	rs13306435 (T15A)	A	1.000	Sequencing	[29], [30]
IL6	rs2069849 (132 C>T)	T	0.995	Sequencing	[29]
VDR	rs2228570 (T2C)	T	0.936	*FokI,* SNaPshot ™	[40], [41]
VDR	rs731236 (T352C)	C	0.991	*TaqI,* SNaPshot ™	[40], [41]

[a] SNP numbers as they appear in NCBI database and (prior literature).
[b] *A priori* genetic model was dominant.
[c] Putative risk allele according to previous studies.

European Youth Heart Study (EYHS) in 1997 [12,13]. In 2001, 552 of the children who were still living in the same area were invited to participate in an MRI study at the age of 12 to 14 years [5]. In all, 439 (80%) children were scanned. The children with non-Caucasian ethnicity (n=29) were excluded to increase genetic homogeneity of the study population. After excluding also those without blood samples (n=58), a total of 352 (60%) children were eligible for the analyses. Participation was voluntary and the local ethics committee approved the study protocol.

MRI was performed using an open low-field 0.2T MR unit (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 [5]. All images were evaluated by an experienced radiologist (JSS) who was blinded to the gender and all health data of the subjects. The latest international nomenclature for describing disc pathology was generally used in the definitions [14]. The *signal intensity changes* of the disc in sagittal sections on T2-weighted images was graded 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) [15,16]. C*hanges in the disc contour* were described on a nominal scale: 0 = normal, 1 = bulge, 2 = focal protrusion, 3 = broad based protrusion, 4 = extrusion and 5 = sequestration [14,17]. *Defects in endplate*s were graded: 0 = normal endplates, 1 = defects and 2 = large defects and Schmorl's nodes [18]. *Annular tear*s (AT) including radial tears and High Intensity Zone (HIZ) lesions were analysed according to the existing definition [19,20].

Lumbar DD was defined if there was either a signal intensity change (grade 2 or 3) or a change in disc contour (grade 2 or higher) at one or more lumbar levels. Those with normal signal intensity (grade 0 and 1), normal disc contour (grade 0 or 1), no annular tears, normal endplates and no other pathology in MRI were

Table 2. Prevalence of MRI findings and characteristics among subjects

MRI findings	Grading	Subjects with DD		Subjects without DD	
		N	%	N	%
Signal changes [a]	Normal (grade 0 and 1)	0	(0)	154	(100)
	Intermediate (2)	63	(95)	0	(0)
	Hypointense (3)	3	(5)	0	(0)
Disc Contour [a]	Normal (0)	40	(61)	153	(99)
	Bulging (1)	20	(30)	1	(1)
	Broadbased protrusion (2)	5	(8)	0	(0)
	Focal protrusion (3)	1	(5)	0	(0)
	Extrusion (4)	0	(0)	0	(0)
	Sequestration (5)	0	(0)	0	(0)
Endplate changes [a]	Normal	55	(83)	153	(99)
	Defects (1)	5	(8)	1	(1)
	Large Defects (2)	6	(9)	0	(0)
Annular Tears		13	(20)	0	(0)
Characteristics					
		N	%	N	%
Gender	Boys	30	(45)	73	(47)
	Girls	36	(55)	81	(53)
		Mean	SD	Mean	SD
Age		13.1	0.4	13.1	0.4
Height [b]		161.8	7.7	159.3	7.3
Weight		51	8.5	49	8.7
BMI		19.5	2.3	19.2	2.8

[a)] Prevalence rates are given by the worst grading. [b)] p=0.029

classified as subjects without DD.

Previously identified genetic risk factors for disc degeneration were analysed from genomic DNA. After polymerase chain reaction, SNaPshot ™ Multiplex Kit (Applied Biosystems) and different restriction enzyme digestions were used in genotyping. Sequencing was performed using the ABI PRISM™ 3130xl Genetic Analyzer (Applied Biosystems). The quality of DNA was not equal to all samples and therefore several genotyping methods were used for some SNPs. Concordance between different genotyping methods was verified by re-sequencing 3-7% of the samples for each SNP. The analysed sequence variations, different genotyping methods used and sample call rates are presented in **Table 1**.

Height was measured using the procedures outlined by Eurofit [21], with a SECA 221 mounted to the wall. Footwear was discarded during the measuring protocol. Weight was measured using a SECA 780 (0.2% accuracy) with the children clothed in shirts and trousers. Body mass index (BMI) was calculated by the formula weight in kilograms/height^2 in metres.

The potential deviation from the Hardy-Weinberg equilibrium (HWE) was tested using the chi-square test. Prior to polymorphism association, the possible effect of gender, BMI and height on the DD status was analysed using the SAS program (V 9.1). Allele and genotype frequencies were compared between children with and without DD using the chi-square test. Haplotypes were first estimated by SNPStats [22] and

then statistically reconstructed from population genotype data using the PHASE program with the Markov-chain method for haplotype assignments [23]. Association between DD and single SNP markers or haplotypes were analysed 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 phenotype (presence or absence of DD) 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 [24].

The effect of cluster (school) sampling was tested by the likelihood ratio test. Null hypothesis was that the variance of random effect school is zero and test statistic (LRT) is assumed to follow Chi-square distribution with one degree of freedom. The resulting p-value is conservative.

Results

Of the 352 children studied, 73 boys and 81 girls had no MRI changes, while 30 boys and 36 girls were classified as having lumbar DD (**Table 2**). Subjects with other specific findings (N=132, 37.5%) were excluded from the genetic analyses. Among the subjects with DD, no other MRI findings were seen in 14 (21.2%) individuals. Lumbar disc height reduction was seen in 44 subjects (66.7%), lumbar disc bulging in 20 (30.3%), HIZ in 13 (19.7%), AT in 13 (19.7%), co -existence of AT and HIZ in 8 (12.1%), endplate changes in 11 (16.7%), and focal or broad-based disc protrusion in 6 (9.1%) individuals. Two individuals had type 1 Modic changes together with grade 3 disc signals. One female control had minor endplate changes in the lower thoracic spine, but no other abnormal MRI findings and was therefore regarded eligible as a control. Subjects with DD were on average 2.5 cm taller that those without DD (p=0.029; **Table 2**). The effect size of height was the same, but not significant, when analysed among boys and girls separately. BMI did not associate with degenerative phenotype. The extra variation caused by school-level sampling was not significantly different from zero (LRT=0.1, p=0.75) and therefore random variable school was not included in the analyses. Genotype frequencies for all SNPs were in Hardy-Weinberg equilibrium.

Among girls, a positive association between the degenerative phenotype and *IL1A* rs1800587 was observed in a dominant genotype model, where CT/TT genotypes were compared to CC genotype, OR 2.85 [1.19–6.83] (**Table 3**). In *IL6* promoter polymorphism rs1800796, the C-allele was more frequent among the subjects with DD than the subjects without DD, OR from log-additive model for C allele was 6.71 [1.71–26.3] (**Table 3**). No association between studied polymorphisms and DD was observed among boys.

Subsequent haplotype analysis of *IL6* resulted in a haplotype GCG of promoter polymorphisms rs1800797, rs1800796 and rs1800795 which associated positively with the degenerative phenotype in girls. The GCG haplotype was over-represented among subjects with DD (p=0.009; OR=6.46 [1.61–26.0]). Including the exonic SNP rs13306435 did not increase the OR (GCGT OR=6.47 [1.62–25.9]). The two most common haplotypes observed were AGCT (OR=0.58 [0.33–1.02]) and GGGT (OR=1.20 [0.69–2.10]).

Discussion

To the authors' knowledge, this is the first study to examine the genetic determinants of DD in childhood. Our results suggest possible roles for *IL1A* and *IL6* in early DD among girls and support in part the earlier findings in adult populations. Previously the T-allele in *IL1A* rs1800587 has been associated with disc bulges [25] and Modic changes (MC) [26] among men. Notably, the added risk for previously studied degenerative imaging findings has been similar as reported here; OR 2.40 [1.20–4.80] for bulges, OR 2.50 [1.09–5.71] for MC and present OR 2.85 [1.19–6.38] for early DD [25, 26]. The results emphasise the importance of *IL1A* in DD [27,28]. In the present study, however, the association was seen only among girls; T-allele frequency was similar among boys with and without DD.

A three SNP *IL6* GCG haplotype was found to be associated with early DD among Danish children. *IL6* genotypes or haplotypes have not

Table 3. Genotype and allele frequencies among boys and girls

			Boys		Girls		
	Frequency %		Frequency %		Frequency %		
Gene	Subjects without DD	Subjects with DD	Subjects without DD	Subjects with DD	Subjects without DD	Subjects with DD	
COL9A3 rs61734651							
CC	79.9	86.4	80.8	93.3	79.0	80.6	
CT	19.5	13.6	19.2	6.7	19.8	19.4	
TT	0.6	0	0	0	1.2	0	
T allele	10.3	6.8	9.6	3.3	11.1	9.7	
COL11A2 rs1799907							
TT	44.9	36.9	42.9	37.9	46.8	36.1	
TA	40.1	43.1	38.6	37.9	41.6	47.2	
AA	15.0	20.0	18.6	24.1	11.7	16.7	
A allele	35.0	41.5	37.9	43.1	32.5	40.3	
IL1A rs1800587							
CC	46.7	34.9	44.4	48.1	48.8	25.0	
CT	42.1	52.4	40.3	37.0	43.8	63.9	
TT	11.2	12.7	15.3	14.8	7.5	11.1	a)
T allele	32.2	38.9	35.4	33.3	29.4	43.1	
IL1B rs1143634							
CC	52.9	47.0	56.9	50.0	49.4	44.4	
CT	34.6	43.9	27.8	46.7	40.7	41.7	
TT	12.4	9.1	15.3	3.3	9.9	13.9	
T allele	29.7	31.1	29.2	26.7	30.2	34.7	
IL6 rs1800797							
AA	24.8	23.1	23.3	13.8	30.0	16.7	
GA	51.0	50.8	57.5	55.2	45.0	47.2	
GG	24.2	26.1	19.2	31.0	25.0	36.1	
G allele	49.7	51.5	47.9	58.6	47.5	59.7	
IL6 rs1800796							
GG	91.5	83.1	86.3	89.7	96.2	77.8	
GC	7.9	15.4	12.3	10.3	3.8	19.4	
CC	0.7	1.5	1.4	0	0	2.8	b)
C allele	4.6	9.2	7.5	5.2	1.9	12.5	
IL6 rs1800795							
CC	24.8	21.2	19.2	26.7	30.0	16.7	
GC	51.0	50.0	57.5	53.3	45.0	47.2	
GG	24.2	28.8	23.3	20.0	25.0	36.1	
G allele	49.7	53.8	52.1	46.7	47.5	59.7	
IL6 rs13306435							
TT	97.4	98.5	98.6	100	96.3	97.2	
TA	2.6	1.5	1.4	0.0	3.7	2.8	
A allele	1.3	0.8	0.7	0	1.8	1.4	
IL6 rs2069849							
CC	92.8	95.5	94.4	96.7	91.4	94.4	
CT	7.2	4.5	5.6	3.3	8.6	5.6	
T allele	3.6	2.3	2.8	1.7	4.3	2.8	
VDR rs2228570							
CC	29.1	44.6	25.4	41.4	32.4	47.2	
CT	58.9	40.0	58.2	48.3	59.5	33.3	
TT	12.1	15.4	16.4	10.3	8.1	19.4	
T allele	41.5	35.4	45.5	34.5	37.8	36.1	
VDR rs731236							
TT	37.0	43.8	39.7	37.9	34.6	48.6	
TC	48.0	42.2	49.3	48.3	46.9	37.1	
CC	14.9	14.1	11.0	13.8	18.5	14.3	
C allele	39.0	35.2	35.6	37.9	42.0	32.9	

a) dominant model CT/TT vs CC: OR = 2.85 [1.19 – 6.83], p_{corr}=0.028
b) log-additive model: OR = 6.71 [1.71 - 26.3], p_{corr}=0.0105

been linked to DD in either childhood/adolescence or adulthood, although *IL6* haplotype GGGA, including the SNPs studied here, has previously been associated with sciatica [29] and indicated as putative prognostic factor for future low back and sciatic pain [30]. Here the GGGA haplotype frequency was less than 0.02 and it was not associated with early DD. The discrepancy may be related to sample origin, sample size or different phenotypes; early disc degeneration vs. sciatica. However, the two most common haplotypes in the present study were parallel with the earlier findings, AGCT (OR 0.58 [0.33–1.02] vs. OR 0.74 [0.54–1.00]) and GGGT (OR 1.20 [0.69–2.10] vs. OR 1.28 [0.94–1.75]) (29). The effects of the *IL6* promoter polymorphisms are not completely elucidated and it appears that the effect on transcription is more complex than was initially expected [31].

Significant associations were observed only in girls. The underlying reason besides true gender differences may be that the number of individuals studied was too low. In the present study the girls had at least partially entered puberty, which was recently proposed to increase reporting of back pain among Danish girls [32], while late puberty decreased the risk of low back pain hospitalizations during early adulthood among Finnish males [33].

Other previously identified predisposing polymorphisms in *COL9A3*, *COL11A2, VDR* and *IL1B* [9,10] were not associated with early DD in the present study. However, a potential association can not be excluded and further studies using larger samples are required.

In the present study, height was associated with DD. Height has been found to be a risk factor of disc herniations [34], but its role is still controversial [35]. Height may be associated with disc degeneration via impaired nutrition [36] or the present association might be affected by puberty [32].

The study design used here is likely to offer a better chance of identifying predisposing polymorphisms because the subjects have not been exposed to confounding environmental factors such as smoking or heavy physical workload. In addition analyses were made in a relatively genetically homogenious population. Furthermore, genotype distributions in the study population were similar compared to prior Caucasian distributions (NCBI dbSNP; Build130), thus supporting appropriate sample selection. A limitation of the study is the very low number of subjects, even though the size of the EYHS cohort, from which the subjects were originally selected, was quite adequate. In the present study, children with relatively mild MRI changes were defined as subjects with DD. There are no studies on MRI-defined DD progression from childhood to middle-age, where the incidence of symptomatic lumbar disc disease peaks, and the importance of different MRI findings in early DD are still open to debate. However, this is the first report of a positive genetic association with MRI -defined DD among young adolescents.

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