Fat Mass and Obesity-Associated (*FTO*) Gene Polymorphisms Are Associated with Risk of Intervertebral Disc Degeneration in Chinese Han Population: A Case Control Study

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Background: The present study aimed to evaluate whether the fat mass and obesity-associated (*FTO*) gene polymorphisms are associated with risk of intervertebral disc degeneration (IDD) in a largest Chinese Han population.

Material/Methods: There were 502 IDD patients and 497 healthy controls enrolled in this study. Nineteen single nucleotide polymorphisms (SNPs) in the *FTO* gene were tested using the Sequenom MassARRAY platform. The Hardy-Weinberg equilibrium test, followed by allelic, genotypic, haplotypic association, and SNP interaction analyses were used for SNP evaluation. The Genotype-Tissue Expression (GTEx) database was used to evaluate expression quantitative trait *loci* (eQTL) value of polymorphism. Spearman rank correlation and logistic regression analyses were used for assessing the internal relation between genotypic changes and the risk of IDD.

Results: Seventeen SNPs survived the Hardy-Weinberg equilibrium test. Allelic analysis showed that allele T of SNP rs1121980 was a risk allele. Haplotypic and SNP interaction analyses suggested that 2 haplotypes and 5 SNP combinations were associated with the predisposition of IDD respectively. GTEx database revealed that the SNP rs1121980 might interfere with the expression of the *FTO* gene in the muscle-skeletal system. Through clinical statistics analysis, the different genotypes of rs1121980 can present different disease severity of IDD.

Conclusions: Our study suggests that rs1121980 can become a biomarker for the screening and prognosis of IDD. The 2 haplotype blocks and 5 SNP-SNP combinations that we discovered might be indicative of the onset of IDD. Therefore, our study might serve as evidence for future IDD molecular diagnosis.

MeSH Keywords: Genetic Linkage • Intervertebral Disc Degeneration • Low Back Pain • Polymorphism, Single Nucleotide • Prognosis

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Background

Intervertebral disc degeneration (IDD) is a commonly seen disorder that can lead to degenerative disc diseases. It has been estimated that IDD can contribute to up to 40% of low back pain (LBP) [1], which affects 80% of the population during their lifetime [2] and is a leading cause of disability in the working-age population [3]. IDD is a multifactorial process of changes in cellular and biochemical components of disc tissues that results in structural failure [4]. The pathological presentations can be manifested as disc space narrowing, sciatica, disc prolapse, or even spinal stenosis.

The exact etiological and pathological causes of IDD remain convoluted. Previous studies suggested that genetic predispositions were widely involved in the pathogenesis of IDD. In 1998, Videman et al. [5] and Jones et al. [6], proposed for the first time that genetic mutations were associated with IDD. Subsequently, increasing number of genomic studies focusing on the vitamin D receptor, extracellular matrix metabolism, intervertebral disc formation, inflammatory mediators, and a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) have been conducted [5,7–11]. Several studies have used the genome-wide association study (GWAS) to discover candidate genes related to IDD [12–14]. The *CHST3* gene and corresponding variant rs4148941 from GWAS studies have been demonstrated to be associated with IDD [13]. Candidate-gene association studies have shown that the asporin D14 allele has a high association with IDD in Chinese and Japanese individuals [14]. These studies have confirmed that there are strong correlations between genetic predisposition and the occurrence of IDD.

The fat mass and obesity-associated (*FTO*) gene comprises 2 well-defined domains, an N-(residues 32–236, exons 1–5) and a C-(residues 327–498, exons 6–9) terminal domain [15]. The N-terminal domain is the catalytic domain of *FTO* that controls lipolytic activity [16], energy homeostasis, and energy expenditure [17]. The *FTO* gene is potentially associated with the biochemical metabolism of IDD. It has been reported that the degeneration of intervertebral disc, to some extent, is determined by energy metabolism and leptin metabolism. Energy metabolism is critical to the survival of disc cells. The lack of normal energy regulation might accelerate degeneration of intervertebral disc [18]. While the *FTO* gene has close relations with energy metabolic balance [19], it can be assumed that the dysfunction of the *FTO* gene might lead to imbalance of disc energy metabolism, further causing disc degeneration. In addition, *FTO* can also regulate the function of leptin and improve leptin sensitivity [20,21]. To our knowledge, leptin can participate in disc cell proliferation and plays a role in the process of intervertebral disc degeneration [22,23]. Therefore, *FTO*

might modulate intervertebral disc metabolism via regulating leptin level and energy metabolic pathways.

Given the role of the *FTO* gene in the onset of IDD, it is worthwhile to initiate association study between single nucleotide polymorphisms (SNPs) of the *FTO* gene and IDD. However, there have been very few studies investigating *FTO* polymorphisms in IDD. To our knowledge, only 2 association studies have been reported worldwide. In 2013, Lao et al. [24] carried out a preliminary association study within 80 patients and 80 controls. Their study had a small sample size and only investigated 6 SNPs of the *FTO* gene. Similarly, our research team investigated associations between 44 SNPs in the *FTO* gene and IDD in the Han Chinese population [25]. However, the study sample size consisted of only 118 cases and 113 controls. One of the most obvious disadvantages of previous studies is that the small sample size might lead to insufficient credence supports the biological link between the gene and the disease. Therefore, we enrolled 999 participants from the Chinese Han population and conducted the largest sample size case control study to date to explore the association between polymorphisms in the *FTO* gene and the risk of IDD.

Material and Methods

Study population

A population-based case control investigation was performed for this study. The 1969 candidate participants were sequentially enrolled from the Department of Orthopedics and from the Center of Health Check-up of the Peking Union Medical College Hospital, Beijing, China, between October 2012 and September 2017. All enrolled individuals were screened by a questionnaire about standard risk factors and disease history.

The case group mainly consisted of orthopedic surgery candidates. The main inclusion criteria for the case groups were complaints of discogenic low back pain (LBP) and newly diagnosed IDD through lumbar disc magnetic resonance imaging (MRI) scanning. Participants who were assessed for Pfirrmann grade IV/V were enrolled in the case group [26]. If the participants were exposed to known environmental risk factors, such as heavy manual labor, heavy smoking, or occupational driving, then they were not recruited to this study. Participants with spinal and joint diseases, including trauma, spinal tumor, spine deformity, backbone infection, leg length discrepancy, and osteoarthritis, were also excluded from this study. The included participants met the criteria of being from the Chinese Han population and were aged between 18 and 85 years old. For the control group, the primary inclusion criterion was a lack of medical history of LBP or sciatica. According
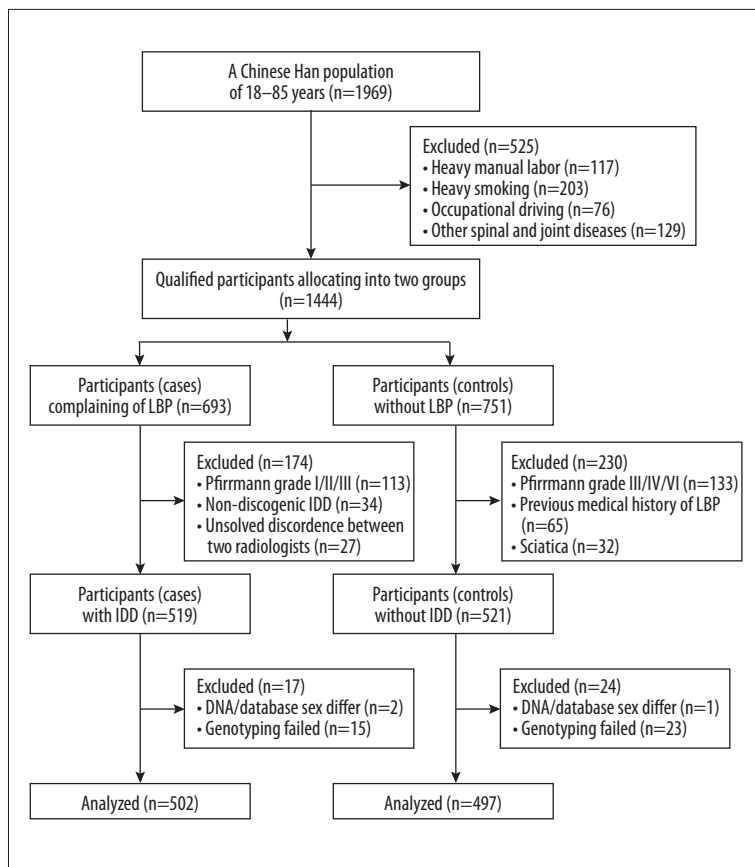
Figure 1. Flow-chart of 999 study participants in the case and control groups. The course of enrollment and grouping of participants are shown. Finally, we recruited 502 participants in the case group and 497 people in the control group.

Table 1. Demographic characteristics of participants in case (n=502) and control (n=497) groups.

Parameter	Case	Control	P values
Age (years)	50.53±12.4	49.52±11.5	0.180
Gender			
Male	276 (54.9%)	250 (50.3%)	0.145
Female	226 (45.0%)	247 (49.7%)	
BMI (kg/m^2)	25.2±3.4	25.0±3.3	0.245

Data are expressed as mean ± standard deviation (SD), or percentage. Student's t test and Chi-square test were performed to compare the difference of baseline demographic characteristics between participants with IDD and the control group. P<0.05 indicates statistical significance.

to the flow-chart (Figure 1), finally, 502 patients and 497 controls population were eligible for the study.

We asked for the detailed case history and performed thorough physical examinations. All enrolled individuals were then screened by MRI, and the imaging results were interpreted by at least 2 blinded experienced radiologists [10,11,27]. Age and gender matched in both groups. To rule out the confounding factor of body weight in our research, body mass index (BMI) was matched between the case and control groups to guarantee there was no selection bias (Table 1).

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences. All participants signed informed consent after detailed description of the project.

Genomic DNA extraction and SNP genotyping

Five milliliters of peripheral blood sample was obtained from every participant for genotyping. The blood was collected in

Table 2. Hardy-Weinberg equilibrium of the 19 SNPs of *FTO* gene.

SNPs	Position	ObsHET	PredHET	HW-P value	% Geno	MAF	Alleles
rs6499640	53769677	0.2428	0.2708	0.6662	93.0	0.4804	A: G
rs1861868	53790402	0.2835	0.2790	0.6284	93.0	0.3219	A: G
rs8047395	53798523	0.4477	0.4804	0.2380	93.0	0.4143	A: G
rs62048402	53803223	0.2027	0.2038	0.6699	93.0	0.2280	A: G
rs1477196	53808258	0.3898	0.4105	0.0876	93.0	0.2690	A: G
rs1121980	53809247	0.2567	0.2854	0.5641	93.0	0.3704	C: T
rs8050136	53816275	0.2008	0.2004	0.5098	94.4	0.3225	A: C
rs9939609	53820527	0.1996	0.2040	0.6808	92.5	0.3401	A: T
rs7204609	53833605	0.4677	0.4866	0.2401	93.5	0.2181	C: T
rs17818902	53871806	0.2124	0.2400	0.1905	93.7	0.2993	G: T
rs17820875	53926790	0.1588	0.1474	0.0667	93.7	0.1190	A: G
rs11076008	53927323	0.2138	0.2049	0.8768	93.3	0.3530	A: G
rs9932411	54005163	0.3665	0.3833	0.1237	94.3	0.4533	C: T
rs9921255	54009328	0.2461	0.2496	0.2697	92.8	0.1715	C: T
rs2302673	54068121	0.2629	0.2610	0.0007	93.4	0.2782	A: G
rs2689247	54097159	0.2450	0.2194	0.0559	93.0	0.1010	A: G
rs16952951	54099427	0.2409	0.2617	0.0425	93.5	0.0970	A: G
rs16952955	54099469	0.2428	0.2177	0.0891	93.0	0.0968	A: C
rs2540766	54105971	0.2339	0.2112	0.1515	93.0	0.1374	A: G

ObsHET – marker's observed heterozygosity; PredHET – predicted heterozygosity; MAF – minor allele frequency. Nineteen SNPs were selected in the *FTO* gene. The positions were shown in this Table.

2% ethylenediaminetetraacetic acid anticoagulant tubes and fully mixed to keep from clotting. DNA was extracted using the QIAGEN Whole Blood Genomic DNA Mini Kit (QIAGEN Inc., Valencia, CA, USA) according to its standard protocol. SNP genotyping was conducted on the Sequenom MassARRAY SNP genotyping platform (Sequenom Inc., San Diego, CA, USA). Five percent of the samples were randomly selected for duplicate analyses as quality controls.

Based on the information accessed from the NCBI SNP database (*http://www.ncbi.nlm.nih.gov/SNP*) and the HapMap database (*http://www.hapmap.org*), the following SNPs within the *FTO* gene were included: rs6499640, rs1861868, rs8047395, rs62048402, rs1477196, rs1121980, rs8050136, rs9939609, rs7204609, rs17818902, rs17820875, rs11076008, rs9932411, rs9921255, rs2302673, rs2689247, rs16952951, rs16952955, and rs2540766. The criteria for the selection of the SNPs were set in accordance with the published literature [28,29]. All SNPs had a MAF above 5%.

Statistical analyses

Baseline demographic characteristics of the participants in the case and control groups were compared by Student's t-test and the Chi-square test using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA). The Hardy-Weinberg equilibrium, haplotype block, and linkage disequilibrium patterns were analyzed using the Haploview program (version 4.2, Broad Institute of MIT and Harvard, Cambridge, MA, USA). The allelic, genotypic, and genotype-phenotype association analyses were carried out using UNPHASED software (v.3.1.5 Dudbridge F, MRC Biostatistics Unit, Cambridge, UK). In this study, the sliding window size for haplotype analyses were 3 and 4. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the relative risk. To test the underlying interaction among selected SNPs within the *FTO* gene, SNP-SNP interaction analyses were also performed. The databases of SNPnexus (*http://snp-nexus.org/index.html*), UCSC genome browser (*http://genome.ucsc.edu/*) and HaploReg (v4.1) (*http://archive.broadinstitute.org/mammals/haploreg/haploreg.php*) were used for SNP annotation. The multi-tissue expression quantitative trait *loci* (eQTL)

Table 3. Distribution of allelic frequency and association test results.

SNPs	Allele (1/2)	Control		Case		OR (Allele1=1)	95% CI	*Chi*-square	*P* value
		1	2	1	2				
rs6499640	A/G	139	759	161	799	0.9089	0.7094–1.164	0.5723	0.4492
rs1861868	A/G	157	739	154	806	1.112	0.8714–1.419	0.7280	0.3935
rs8047395	A/G	535	363	578	363	0.9741	0.809–1.173	0.0770	0.7814
rs62048402	A/G	103	795	111	849	0.991	0.7452–1.318	0.0039	0.9502
rs1477196	A/G	263	635	273	687	1.042	0.8526–1.274	0.1632	0.6862
rs1121980	C/T	759	137	772	182	1.306	1.024–1.666	4.6609	0.0309
rs8050136	A/C	105	841	108	832	0.9618	0.7232–1.279	0.0716	0.7890
rs9939609	A/T	101	791	112	844	0.9622	0.7229–1.281	0.0698	0.7917
rs7204609	C/T	397	531	384	556	1.083	0.9007–1.301	0.7143	0.3980
rs17818902	G/T	127	805	134	806	0.9489	0.7305–1.233	0.1542	0.6946
rs17820875	A/G	848	84	858	82	0.9648	0.7015–1.327	0.0485	0.8256
rs11076008	A/G	117	809	99	839	1.226	0.9221–1.629	1.9706	0.1604
rs9932411	C/T	235	709	252	688	0.9049	0.7362–1.112	0.9007	0.3426
rs9921255	C/T	130	764	141	819	0.9884	0.7637–1.279	0.0079	0.9291
rs2689247	A/G	124	774	109	851	1.251	0.9499–1.647	2.5470	0.1105
rs16952955	A/C	775	123	852	108	0.7987	0.606–1.053	2.5509	0.1102
rs2540766	A/G	117	781	106	854	1.207	0.912–1.597	1.7340	0.1879

SNPs – single nucleotide polymorphisms; OR – odds ratio; 95% CI – 95% confidence interval.

comparisons were evaluated and displayed by the Genotype-Tissue Expression (GTEx) database (*https://www.gtexportal.org*). Spearman rank correlation and logistic regression analyses were used to assess the internal relation between genotypic changes in SNP and the risk of IDD. The significance levels of all these tests were set to 0.05.

Results

Hardy Weinberg equilibrium in our case control study

We analyzed 19 SNPs, in a 347-kb interval spanning the *FTO* gene, in 502 cases and 497 controls. Of the 19 SNPs successfully genotyped, 2 SNPs (rs2302673 and rs16952951) with *P* values less than 0.05 deviated from the Hardy-Weinberg equilibrium and were excluded from the subsequent analyses (Table 2).

Allelic frequency analyses

Of the remaining SNPs, rs1121980 exhibited a significant allelic difference between the case and control groups (Table 3).

The risk allele was T, with an OR of 1.306 (95% CI=1.024–1.666, *P*=0.0309). Differences in the allele frequencies of other SNPs were not statistically significant.

Genotypic association analyses for the risk of IDD

The CT heterozygote of rs1121980 had significantly genotypic frequency distribution between case and control groups (*P*=0.02682). The OR (1.395) and 95% CI (1.047–1.86) value supported the odds of the event was higher in the case group. In contrast, although the AG heterozygote of rs2689247, the AC heterozygote of rs16952955 and the AG heterozygote of rs2540766 were significantly genotypic frequency distribution between case and control groups (*P*=0.004062, 0.006711, 0.01667 respectively), the value of OR and 95% CI indicated that the genotypes of these SNPs do not increase the risk of IDD in case group (Table 4).

Haplotypic analyses

We constructed the haplotype based on the genotype data of 17 SNPs in the *FTO* gene using Haploview software (version

Table 4. Genotypic frequency distribution of tested SNPs.

SNPs	Genotype	Case	Control	OR	95% CI	*Chi*-square	Individual *P*-value	Global *P*-value
rs6499640	AA	11	15	1	1–1	0.9386	0.3326	0.195998
	AG	139	109	1.739	0.7678–3.938	2.599	0.1069	
	GG	330	325	1.385	0.6265–3.06	1.473	0.2249	
rs1861868	AA	9	15	1	1–1	1.996	0.1577	0.361883
	AG	136	127	1.785	0.7544–4.222	2.527e-005	0.996	
	GG	335	306	1.825	0.7871–4.23	0.2402	0.6241	
rs8047395	AA	175	167	1	1–1	0.05394	0.8163	0.620576
	AG	228	201	1.082	0.8145–1.439	0.6976	0.4178	
	GG	77	81	0.9072	0.6221–1.323	0.6564	0.2676	
rs62048402	AA	5	6	1	1–1	0.1721	0.6782	0.885044
	AG	101	91	1.332	0.3931–4.512	0.08485	0.7708	
	GG	374	352	1.275	0.3857–4.215	0.03127	0.8596	
rs1477196	AA	44	44	1	1–1	0.1084	0.742	0.922975
	AG	185	175	1.057	0.6632–1.685	0.0184	0.8921	
	GG	251	230	1.091	0.6927–1.719	0.1057	0.7451	
rs1121980	CC	309	322	1	1–1	5.364	0.02056	0.0674199
	CT	154	115	1.395	1.047–1.86	4.902	0.02682	
	TT	14	11	1.326	0.593–2.966	0.2021	0.653	
rs8050136	AA	5	5	1	1–1	0.0001024	0.9919	0.958036
	AC	98	95	1.032	0.2893–3.678	0.08509	0.7705	
	CC	367	373	0.9839	0.2825–3.427	0.08344	0.7727	
rs9939609	AA	5	6	1	1–1	0.1757	0.6751	0.809703
	AT	102	89	1.375	0.4059–4.66	0.2694	0.6037	
	TT	371	351	1.268	0.3836–4.193	0.1589	0.6902	
rs7204609	CC	82	90	1	1–1	0.5908	0.4421	0.695358
	CT	220	217	1.113	0.7817–1.584	0.0001597	0.9899	
	TT	168	157	1.174	0.8113–1.7	0.3748	0.5404	
rs17818902	GG	9	14	1	1–1	1.159	0.2818	0.28424
	GT	116	99	1.823	0.7565–4.391	1.562	0.2114	
	TT	345	353	1.52	0.6495–3.559	0.6796	0.4097	
rs17820875	AA	394	387	1	1–1	0.1037	0.7474	0.883465
	AG	70	74	0.9291	0.6511–1.326	0.1748	0.6759	
	GG	6	5	1.179	0.3568–3.894	0.08355	0.7725	

Table 4 continued. Genotypic frequency distribution of tested SNPs.

SNPs	Genotype	Case	Control	OR	95% CI	*Chi*-square	Individual *P*-value	Global *P*-value
rs11076008	AA	4	9	1	1–1	2.016	0.1556	0.250686
	AG	91	99	2.068	0.6157–6.948	0.5624	0.4533	
	GG	374	355	2.37	0.7235–7.766	1.289	0.2562	
rs9932411	CC	41	31	1	1–1	1.55	0.2131	0.456327
	CT	170	173	0.743	0.4451–1.24	0.02366	0.8777	
	TT	259	268	0.7307	0.4446–1.201	0.2675	0.605	
rs9921255	CC	14	10	1	1–1	0.4238	0.5151	0.767397
	CT	113	110	0.7338	0.3127–1.722	0.1442	0.7041	
	TT	353	327	0.7711	0.3378–1.76	0.01776	0.894	
rs2689247	AA	14	7	1	1–1	1.935	0.1642	0.0078855
	AG	81	110	0.3682	0.1422–0.9535	8.256	0.004062	
	GG	385	332	0.5798	0.2313–1.454	5.172	0.02295	
rs16952955	AA	385	333	1	1–1	4.827	0.02802	0.0150537
	AC	82	109	0.6507	0.4716–0.8978	7.349	0.006711	
	CC	13	7	1.606	0.6335–4.073	1.455	0.2278	
rs2540766	AA	12	6	1	1–1	1.653	0.1985	0.0298244
	AG	82	105	0.3905	0.1406–1.085	5.731	0.01667	
	GG	386	338	0.571	0.212–1.538	3.562	0.05913	

SNPs – single nucleotide polymorphisms; OR – odds ratio; 95% CI – 95% confidence interval.

4.2). The pairwise linkage disequilibrium D' values between SNPs and the linkage disequilibrium plots were presented in Figure 2. We identified 2 haplotype blocks with significant associations with predisposition to IDD. The first block contained rs62048402, rs1477196, rs1121980, rs8050136, rs9939609, and rs7204609, while the second block contained rs2689247, rs16952955, and rs2540766.

SNP-SNP interactions on the risk of IDD

By using UNPHASED software, the results of SNP-SNP interaction analyses showed that there were 5 SNP combinations significantly associated with IDD (1st: rs62048402G-rs1477196G-rs1121980T, Global *P* value=0.015; 2nd: rs1477196G-rs1121980T-rs8050136C, Global *P* value=0.039; 3rd: rs8047395A-rs62048402G-rs1477196G-rs1121980T, Global *P* value=0.035; 4th: rs62048402G-rs1477196G-rs1121980T-rs8050136C, Global *P* value=0.028; 5th: rs1121980T-rs8050136C-rs9939609T-rs7204609C, Global *P* value=0.007) (Table 5).

SNPs annotation

Through SNPnexus databases, which is a database used for SNP annotation and UCSC genome browser, we can know the basic information of certain SNP. In genotypic association analyses, the 4 SNPs (rs1121980, rs2689247, rs16952955, and rs2540766) with *P*-value less than 0.05 were located in the intronic region. By means of HaploReg annotation, we found the 4 intronic SNPs were associated with regulation of promoter and/or enhancer histone (data not shown). They might play biology function in this way in patients.

Multi-tissue eQTL comparisons by GTEx database

Figure 3 shows that genotype change of rs1121980 can significantly interfere with the *FTO* gene expression in muscle-skeletal system comparing with other tissues (single-tissue *P*-value=3.163e-6). Therefore, this multi-tissue eQTLs comparison of rs1121980 will provide important data that rs1121980 can interfere with the *FTO* gene expression. Through the GTEx
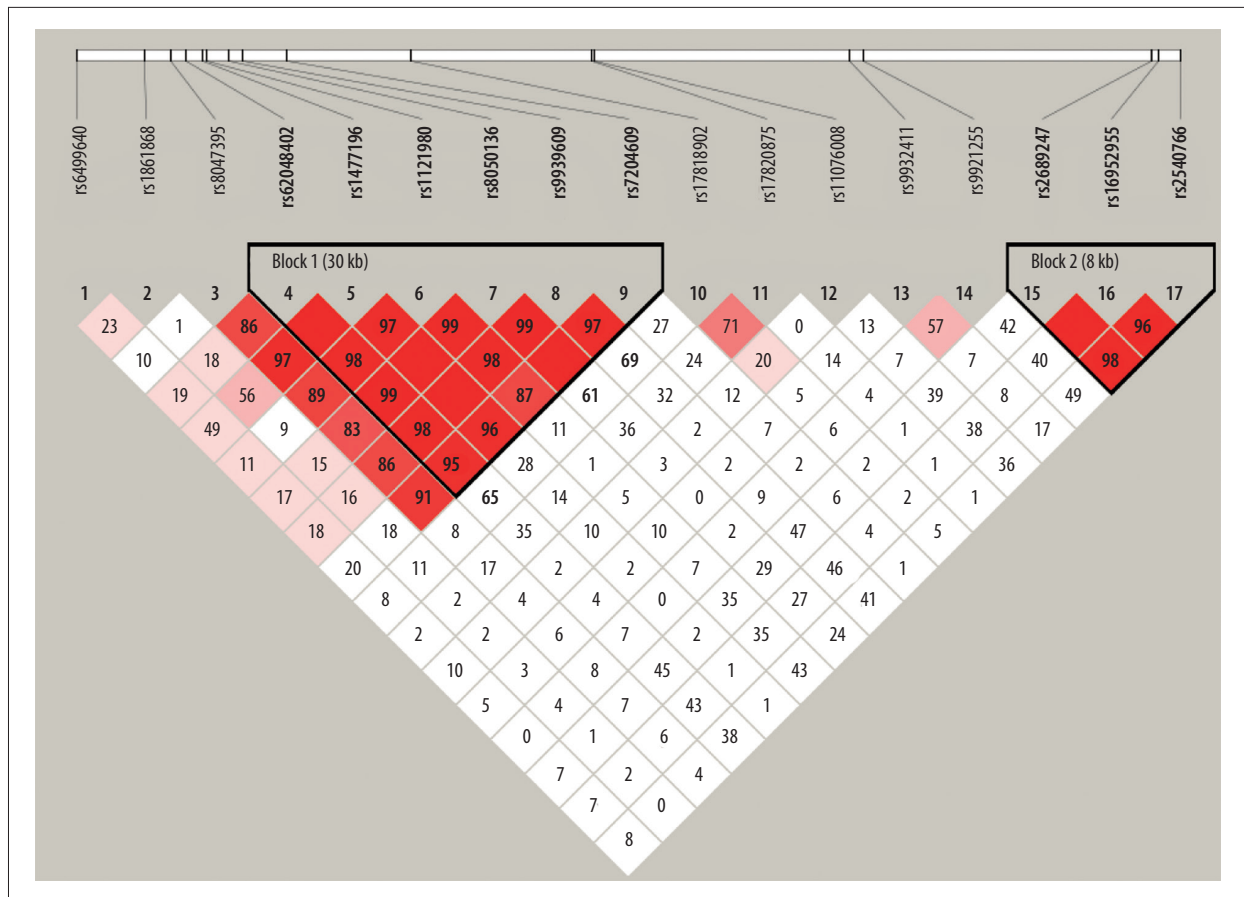
Figure 2. Linkage disequilibrium structures for the 17 SNPs genotyped in the *FTO* gene. The numbers inside the diamonds indicate the D' for pairwise analyses. The colors of the diamonds are shown according to the confidence interval model. SNPs – single nucleotide polymorphisms. *FTO* gene – fat mass and obesity-associated gene.

Table 5. Significant SNP-SNP interactions in *FTO* gene with IDD.

SNP combination	Haplotype	Frequency in case	Frequency in control	Lower 95% CI	Higher 95% CI	Individual *P* value	Global *P* value
rs62048402-rs1477196-rs1121980	G-G-T	0.07218	0.04028	6.498e-008	1.509e+005	0.002159	0.0151691
rs1477196-rs1121980-rs8050136	G-T-C	0.07559	0.04363	0	0	0.003677	0.0396061
rs8047395-rs62048402-rs1477196-rs1121980	A-G-G-T	0.07084	0.04028	0	9.573e+041	0.00267	0.0354699
rs62048402-rs1477196-rs1121980-rs8050136	G-G-T-C	0.07331	0.04021	0	4.488e+042	0.002048	0.0280349
rs1121980-rs8050136-rs9939609-rs7204609	T-C-T-C	0.01563	0	1.509e+005	1.195e+006	0.001291	0.00780653

SNP – single nucleotide polymorphism; 95% CI – 95% confidence interval. Odds ratio and 95% confidence internals were calculated to estimate the relative risk. P<0.05 is statistically significant.
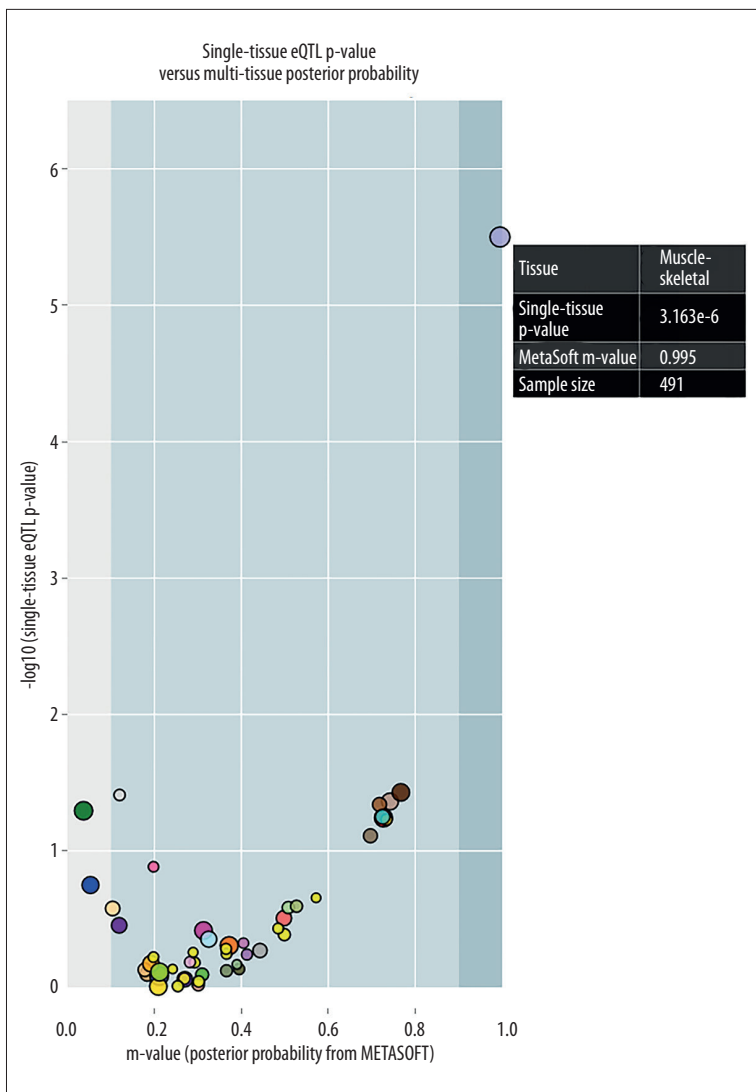
Figure 3. Multi-tissue eQTL comparison of rs1121980 in GTEx analysis database. The dispersion points of different colors represent the expression of the *FTO* gene in different tissues. The *P* value: from a *t*-test that compares observed beta from single-tissue eQTL analysis to a null beta of 0; the m-value: the posterior probability that an eQTL effect exists in each tissue tested in the cross-tissue meta-analysis. GTEx – Genotype-Tissue Expression; eQTL – expression quantitative trait *loci*; *FTO* gene – fat mass and obesity-associated gene.
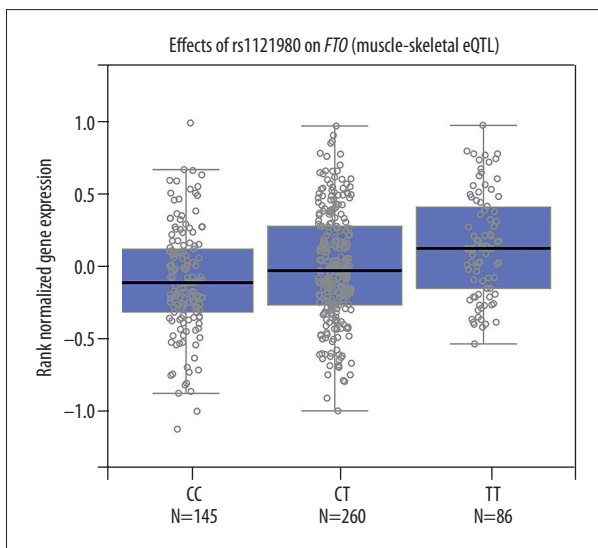
Figure 4. Different rs1121980 genotypes of the *FTO* gene expression in muscle-skeletal tissues. The GTEx database indicated that as the genotype changes (CC→CT→TT), the expression level of the *FTO* gene increases (*P* value=0.0000032). *FTO* gene – fat mass and obesity-associated gene; GTEx – Genotype-Tissue Expression.

database, we also found that rs1121980 and its different genotypes were significantly associated with increased expression of the *FTO* gene (*P* value=0.0000032) (Figure 4). The box plot in Figure 4 provided detailed evidence to demonstrate different genotypes of rs1121980 were associated with different *FTO* gene expressions. The other 3 SNPs (rs2689247, rs16952955, and rs2540766) had no significant eQTLs in the GTEx database.

Table 6. The Pfirrmann grade distribution of study participants.

Pfirrmann grade	No. of patients
Grade I	5
Grade II	88
Grade III	45
Grade IV	41
Grade V	28

The relation between different genotypes of SNPs and severity of IDD

In order to understand the clinical significance of this SNP, we needed to figure out the internal connection between different genotypes and the severity of IDD. Since the previous data showed rs1121980 could affect the expression of the *FTO* gene, from the clinical perspective, we wanted to know whether the genotype changes of rs1121980 would affect the manifestations of lumbar intervertebral disc degeneration. Therefore, in order to clarify the clinical manifestations of the SNP rs1121980, 207 participants whose MRI would qualitatively record the Pfirrmann grade were enrolled in the next analysis processes (Table 6).

We first analyzed whether genotypic changes in rs1121980 were related to the changes in clinical phenotypes. Thus, we performed Spearman rank correlation analyses using SPSS 16.0. The results showed that the genotypes of CC/CT/TT were related to the changes of Pfirrmann grade, that is, different genotypes of CC/CT/TT can interfere with the Pfirrmann grade (P value <0.05) (Table 7). This was also consistent with the results of eQLT, in which genotypic was closely linked to *FTO* gene expression.

Second, we performed logistic regression analysis to determine if participants who carried TT, CT, and CC respectively were at progressively increasing risk for IDD. Through logistic regression analyses, we found that with the change of rs1121980

genotype, the risk of TT was higher than CT (OR=2.784, 95% CI=1.052–7.368, P=0.039), CT was higher than CC (OR=1.697, 95% CI=1.282–2.245, P<0.01). Also, the risk of TT was significant higher than CC (OR=4.474, 95% CI=1.722–11.628, P=0.02). From CC to CT to TT, the risk of IDD gradually increases followed by the values of odd ratio increasing.

However, when performing the same statistical methods for rs2689247, rs16952955, and rs2540766, the results showed that these SNPs had no significant effect on the risk of IDD (data not shown).

Discussion

In this study, we investigated the association of *FTO* gene polymorphisms with risk of IDD in a case control study of 999 Chinese Han participants. To the best of our knowledge, the sample size in this research was the largest among similar association studies. Our results showed that SNP rs1121980 had an allelic association with IDD, and the risk allele of the same SNP was T. Furthermore, we also found 2 haplotype blocks and 5 SNP-SNP combinations that might be indicative of the onset of IDD.

At the allelic level, we found a significant association of the T allele of SNP rs1121980, which locates in the intron region of *FTO*. Scheid et al. created an *FTO* risk score based on 5 SNPs, including rs1121980, and found that the *FTO* risk score did not increase variance accounted for beyond individual *FTO* SNPs [30]. Adeyemo et al. investigated the variation of the *FTO* gene in West Africans and replicated the association between rs1121980 and rs7204690 among this population [31]. The selection of SNPs in their study might be attributed to the representation of tag SNPs. Some SNPs were able to represent other SNPs in the gene. In addition, the rs1121980 SNP was also associated with hip fracture in women with AA allele as the risk allele, suggesting a potential relationship between this SNP and bone metabolism [32]. Since IDD is a degenerative disease affecting the intervertebral disc and the

Table 7. Spearman rank correlation analyses between rs1121980 and Pfirrmann grade.

		rs1121980	L4 - L5 Pfirrmann grade
rs1121980	Correlation coefficient	1.000	0.334
	Sig.(2-tailed)	.	0.000*
	N	207	207
L4–L5 Pfirrmann grade	Correlation coefficient	0.334	1.000
	Sig.(2-tailed)	0.000*	.
	N	207	207

* p-value <0.05.

surrounding vertebrae, the association between rs1121980 and hip fracture might serve as evidence supporting our results from a biological point of view.

The hypothesis of our SNP analysis was that if SNPs are associated with IDD, the proportions of different alleles would distribute differently in disease and normal controls. Therefore, we conducted a 2-step verification of rs1121980 and its internal relations with the risk of IDD. First, through GTEx database, we found SNP rs1121980 might interfere with the normal expression of the *FTO* gene in the muscle-skeletal system. Meanwhile, we also found that the rs1121980 and its different genotypes were significantly associated with increased expression of the *FTO* gene. Then, by using Spearman rank correlation and logistic regression analyses, it can be considered that different genotypic changes in rs1121980 might refer to different risk of IDD. Therefore, the present study demonstrated that genotypic changes of rs1121980 in its different alleles were closely related to the risk of IDD. In this sense, the T allele of rs1121980 might be a biomarker for the screening and prognosis of IDD.

SNPs rs2689247, rs16952955, and rs2540766 were reported to be related to osteoporosis phenotypes. In a previous study investigating the association of *FTO* with osteoporosis, the 3 SNPs showed significant association with bone mineral density (BMD) in the hip and spine [33]. Two independent experiments of osteoporotic animal models revealed that osteoporosis plays a certain role in the processes of intervertebral disc degeneration and can accelerate the degeneration of intervertebral disc at specific time [34,35]. However, in the present case control association study, we found that the 3 SNPs could be protective factors. In other words, participants who carried these SNPs might have lower risk against IDD than others in our study cohort. Therefore, our association study showed that there was no direct relationship between rs2689247, rs16952955 and rs2540766 and the risk of IDD.

At the haplotypic level, we identified 2 haplotype blocks significantly associated with the risk of IDD. The haplotype is a set of SNPs that are closer together. Accordingly, those SNPs are more likely to be inherited together [36]. The aim of the linkage disequilibrium analysis was to identify those SNPs in IDD patients. The 2 haplotype blocks that we discovered might be indicative of the onset of IDD. The SNP-SNP interaction analysis showed that there were 5 SNP combinations significantly associated with IDD. These SNP combinations showed significant statistical differences in our study cohort. This suggested that those SNP combinations might provide clinical biomarkers for prognosing IDD and also further demonstrated that these genetic variants in the *FTO* gene are significantly associated with the predisposition of IDD.

Two previous studies have reported on the relationship between SNPs of the *FTO* gene and IDD [24,25]. Nevertheless, this study had the following strengths, by comparing the results of other similar studies. We collected larger sample size and had more stringent inclusion and exclusion criteria to insure the power and reliability of our study. Moreover, our results added novel evidence to support the association between the *FTO* gene and IDD, i.e., the T allele of rs1121980 was the risk allele for IDD. However, there were also some limitations to this study. First, notwithstanding the significant SNP results in our study, functional studies focus on the exact biological mechanisms are important to corroborate, confirm and interpret the role of SNPs in IDD. Nutrient diffusion distance to the nucleus pulpous [37], energy metabolism [18], leptin regulation [38], and increased risk of mechanical failure under loading [39] are the possible mechanisms accounting for the generation of IDD. Therefore, further functional studies are needed to elucidate the associations and clarify the precise mechanisms. Second, the genetic architecture and allele frequencies in Chinese people might differ from those in other ethnic populations. Therefore, more generalized studies with larger sample sizes, especially beyond the Chinese Han people, should be performed to investigate whether *FTO* gene polymorphisms are associated with risk of IDD in other ethnic groups.

Conclusions

The present study showed that SNPs of the *FTO* gene were associated with risk of IDD. The T allele of rs1121980 was the risk allele for IDD and it might become a biomarker for the screening and prognosis of IDD. The 2 haplotype blocks and 5 SNP-SNP combinations that we discovered might be indicative of the onset of IDD. Therefore, our study might serve as evidences for future IDD molecular diagnosis.

Acknowledgement statement

Conflicts of interests

None.

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