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Susceptibility loci and polygenic architecture highlight population specific and common genetic features in inguinal hernias genetics in inguinal hernias

Keiko Hikino, M.D., Ph.D[1], Masaru Koido, Ph.D.[2,3], Kohei Tomizuka, B.S.[2], Xiaoxi Liu, Ph.D.[2], Yukihide Momozawa, D.V.M., Ph.D.[4], Takayuki Morisaki, M.D., Ph.D.[3], Yoshinori Murakami, M.D., Ph.D.[3], The Biobank Japan Project[5], Taisei Mushiroda, Ph.D.[1], Chikashi Terao, M.D., Ph.D.[2,6,7,*]

[1] *Laboratory for Pharmacogenomics, RIKEN Center for Integrative Medical Sciences, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa, 230-0045, Japan*
[2] *Laboratory for Statistical and Translational Genetics, RIKEN Center for Integrative Medical Sciences, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa, 230-0045, Japan*
[3] *Division of Molecular Pathology, Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokane-dai, Minato-ku, Tokyo 108-8639, Japan*
[4] *Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa, 230-0045, Japan*
[5] *Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokane-dai, Minato-ku, Tokyo, 108-8639, Japan*
[6] *Clinical Research Center, Shizuoka General Hospital, Shizuoka, Japan*
[7] *The Department of Applied Genetics, The School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan*

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ABSTRACT

Background: The underlying pathology of inguinal hernia is still not fully known; thus, further investigations of genetic backgrounds is needed. Here, we aimed to identify genetic factors attributing to inguinal hernias and explore the polygenic architecture of which some components are population-specific, while others are more common among populations.

Methods: We performed a genome-wide association study (GWAS) on subjects with inguinal hernias using BioBank Japan (BBJ) data with 1,983 cases and 172,507 controls, followed by a trans-ethnic meta-analysis with UK Biobank (UKBB) data. We performed downstream analyses in order to identify the mechanisms underlying inguinal hernias supported by genetic findings.

Findings: We identified a locus closest to *ELN*, which encodes elastin, at the GWAS significant level. The trans-ethnic meta-analysis revealed 23 additional significant loci, including five loci newly identified not significant in BBJ or UKBB GWAS: *TGFB2, RNA5SP214/VGLL2, LOC646588, HMCN2*, and *ATP5F1CP1/CDKN3*. Downstream analyses revealed the overlap of GWAS significant signals in extracellular components, including elastin fiber formation. We also found a highly shared polygenic architecture across different populations (trans-ethnic genetic-effect correlation = 0•77, standard error = 0•26) and population-specific lead variants in *ELN*, indicating the critical role of elastin in inguinal hernias.

Interpretation: We identified a significant locus of the *ELN* gene in the Japanese population and five additional loci across different populations. Downstream analyses revealed highly shared genetic architectures across populations and highlighted the important roles of extracellular components in the development of inguinal hernias. These findings deepen our understanding of the mechanisms underlying inguinal hernia.

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1. Introduction

An inguinal hernia occurs when there is a protrusion of the abdominal contents out of the body's surface through a weak spot in

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* Correspondence: Chikashi Terao, MD, PhD, Laboratory for Statistical and Translational Genetics, RIKEN Center for Integrative Medical Sciences, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa, 230-0045, Japan.
E-mail address: chikashi.terao@riken.jp (C. Terao).

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Research in context

Evidence before this study

Genetic features related to inguinal hernia are still not well investigated across complete sets of DNA (genome-wide) especially in non-European populations.

Added value of this study

We conducted a genome-wide association study (GWAS) for inguinal hernia in the Japanese population, which identified a previously unreported susceptibility locus at the GWAS significant level. A trans-ethnic meta-analysis (using biobank data from the UK) revealed 23 additional significant loci. Additionally, downstream analyses revealed the overlap of GWAS significant signals in enhancers of extracellular components. Lastly, we found a highly shared polygenic architecture of inguinal hernias across different populations and the important role of elastin in both populations.

Implications of all the available evidence

This is the first study showing both population-specific and common genetic features across different populations with inguinal hernias. Our findings pave a path to future research and the potential improvement of treatment for patients with inguinal hernias.

the low abdominal wall [1]. A global epidemiology study estimated prevalence is 4.88% in Southeast Asia and 4.06% in Europe [2]. Severe complications of inguinal hernia are incarceration and strangulation, and currently, surgery is the only treatment option [3]. However, the reoperation rate is not negligible, a study reported to be 8•9%, [4] and chronic groin pain developed in more than 10% of the patients after surgeries [5,6]. Multiple risk factors have been reported for inguinal hernias, such as older age, [7] male sex, [7,8] smoking, [9,10] chronic obstructive pulmonary disease (COPD) [11,12] and systemic connective tissue disorders with genetic abnormalities. [13-15] Family history is also a risk factor, [11] and studies investigating genetic risk have been conducted mainly by candidate gene analyses [16-22]. The first genome-wide association study (GWAS) was conducted by Jorgenson et al. and included European populations, with 5,295 cases as a discovery cohort and 9,701 cases as replication cohort. They identified four significant loci and their functional roles were investigated [23]. However, no additional GWAS have been published, although the single nucleotide polymorphism (SNP)-heritability has not yet been fully explained by the associated SNPs in previous studies, and the genetic architecture in non-European populations remains unknown.

The BioBank Japan Project (BBJ) is a nationwide hospital-based genome cohort, which started in 2003. The BBJ has collected the data of approximately 200,000 patients with 47 target diseases including clinical data such as past medical history [24]. In this study, we aimed to identify genetics factors contributing to inguinal hernias using the data from the BBJ and explored both population-specific and common genetic architecture across populations.

2. Methods

2.1. Study Participants

The BBJ consists of DNA samples and clinical data of patients with 47 target diseases [24]. We selected cases from patients with a past medical history of inguinal hernia, documented by doctors-in-charge as previously described, [24] and controls from those with the 47 target diseases excluding COPD, which is a known risk factor of inguinal hernia [11,12].

Ethical committees at the Institute of Medical Sciences, The University of Tokyo (Tokyo, Japan) and the RIKEN Center for Integrative Medical Sciences (Yokohama, Japan) approved this study's protocol (Approval No. 17-17-16[8]). Written informed consent was provided for all patients recruited to the BBJ project. We complied with all relevant ethical regulations.

2.2. Whole-genome genotyping

We genotyped the BBJ samples by one of the following: [1] a combination of Illumina Infinium Omni Express and Human Exome, [2] Infinium Omni Express Exome v.1•0, [3] Infinium Omni Express Exome v.1•2.

2.3. Quality control (QC) of genotyping data

For QC of samples, we excluded individuals as follows: [1] sample call rates $<$0•98, [2] genetically identical to others, [3] genotypic and phenotypic sex mismatch and [4] outliers from the East Asian cluster identified by applying principal component analysis, using genotyped samples and the three major reference populations (Africans, Europeans and East Asians) from the International HapMap Project. This left 174,490 samples to be used for further analyses [25,26]. For QC of SNPs, we excluded SNPs as follows: [1] call rate $<$0•99 and [2] p-values for Hardy–Weinberg equilibrium (HWE) $<1•0 \times 10^{-6}$. We used Plink v.1•9 software for this QC process [27].

2.4. Whole-genome imputation

We generated a reference panel by using whole genome sequencing (WGS) data of 3,256 Japanese patients in the BBJ and 2,504 individuals in the 1000 Genomes Project (1KG; phase3v5) to achieve better imputation accuracy for the Japanese population (refer to Flanagan. et al. for more details) [28]. Briefly, samples were sequenced at high depth (15x, 30x) on various platforms (ex, 2 $\times$ 160-bp paired end reads on a HiSeq2500 platform Illumina with rapid run mode). The WGS data was processed, following the standardized best practice method, Genome Analysis Toolkit (GATK), [29] with the additional filters of approximate read depth and genotype quality before variant quality score recalibration (VQSR). The variants at multi-allelic sites were removed from the combined reference panel by vcftools (version 0•1•14). We estimated the haplotypes by SHAPEIT (version 2•778) and combined the data of the 1KG phase3v5 and the BBJ by using IMPUTE2 [30,31]. Quality control was then performed with bcftools (version 1•3•1) and vcftools (version 0•1•14). Variants at multi-allelic sites, monomorphic sites and singletons were excluded. We phased the genotyping data of autosomal chromosomes by SHAPEIT2 (version 2•837), and then imputed with minimac4 (version 2•0•1)(32) using the reference panel generated as described above. For X chromosome, first, we conducted phasing with SHAPEIT2 (version 2•837) and separated the variant call format files for males and females; second, we imputed with BEAGLE (version 4•1), and third, we merged males and females after excluding the variants at multi-allelic sites, monomorphic sites and singletons. The reference panel was finally composed of WGS data from 5,760 individuals, 72,406,123 autosomal variants and 3,252,444 X chromosome variants in total. Subsequently, individuals from BBJ without overlap with those in the reference panel were phased with EAGLE (version 2•3) using default parameters. In our study, we included only the variants imputed with R^2 $>$0•3 after imputation by Minimac 4 [32].

2.5. GWAS

We performed GWAS by applying a generalized linear mixed model using Scalable and Accurate Implementation of GEneralized mixed model (SAIGE) (version 0•29•4•2) [33]. SAIGE is composed of two steps; in step 1, a null logistic mixed model is fit by using genotype data and added covariates, incorporating both sex and the top ten principal components (PC). In step 2, single-variant association tests were performed by using imputed variant dosages. We applied the leave-one chromosome-out approach in which the chromosome with the tested candidate SNPs is excluded from calculation of the genomic relationship. In each GWAS, we excluded the variants with minor allele frequencies of <0•01 and those imputed with R^2 <0•3. We drew Manhattan plots by R (version 4•0•2). We regarded significant associations if loci showed association P values of $<5•0 \times 10^{-8}$. Significantly associated loci were defined as a genomic region within ±1 megabase (Mb) from lead variants. Novel locus was defined as those sites that did not include any known significant causal variants in inguinal hernias ($p < 5•0 \times 10^{-8}$). We generated regional association plots by LocusZoom (version 1•2) [34]. The estimated inflation factor λ_{GC} <1•05 after adjusting for sex and the top ten PCs meant little evidence of substantial inflation. For GWAS, we performed for the cases and control as described above the Study Participants sections. Additionally, we also performed GWAS, using the controls by excluding all cancer patients (with risk of muscle wasting status), fibroid patients (with risk of different sex steroid hormone levels), [35] and patients with genetic defects predisposing them to hernia (12 patients with Marfan syndrome, and one with Ehlers-Danlos syndrome, as there were no patients with cutis laxa in BBJ) so as to make sure that we excluded all possible patients with risks from controls.

2.6. Conditional analyses

Conditional analyses were performed using GCTA-COJO [36]. Additionally, stepwise conditional analyses were performed within ±1 Mb from the lead variants, and we repeated association tests by adding the dosages of the lead variants as covariates in SAIGE until no significant associations were identified.

2.7. Functional annotation of the lead variants in GWAS

Exonic variants in strong linkage disequilibrium (LD) with lead SNPs (r2 > 0•8) were annotated by ANNOVAR [37]. Similarly, we explored potential expression quantitative trait loci (eQTL) variants based on data reported by Ishigaki et al., including subgroups of immune cells, or the one from the Genotype-Tissue Expression project (GTEx) (version 8) [38,39].

2.8. Heritability enrichment analyses and genetic correlations

We estimated heritability in our GWAS results with linkage disequilibrium score regression (LDSC, version 1•0•0). We excluded variants in the human leukocyte antigen (*HLA*) region (chromosome 6: 26−34Mb). We also calculated heritability z scores and standard errors (SE) so as to assess the reliability of heritability estimation [40]. Inguinal hernia prevalence used for heritability estimation was 7•5%, 20% and 3% for all cases, only males and only females, respectively [7,41-43]. We additionally estimated genetic correlations with the BBJ's 42 target diseases [44]. We also evaluated enrichment of heritability of histone marks in 220 different cell types and 10 different tissue types and reported enrichment p-values to see enrichment correlation as described by Finucane et al. [40].

2.9. Pathway analysis

We conducted pathway analysis by Pathway scoring algorithm (Pascal), applying the corresponding LD structure [45]. Bonferroni corrections were applied. We set the statistically significant threshold as p < 0•05/1077, Bonferroni -corrected for the number of pathways tested by Pascal (REACTOME, KEGG, and BIOCARTA from Molecular Signature Database (MSigDB) version 4•0) [46,47].

2.10. Transcriptome-wide association study (TWAS)

We performed TWAS with the Multi-Tissue model in FUSION software consisting of 48 tissue types from the GTEx project (version 7) [48,49]. TWAS analysis used pre-computed gene expression weights and computed expression prediction models. Genes with nominally significant cis−SNP-heritability were used to train TWAS prediction models. FUSION fits predictive linear models for every gene. We used the summary statistics of our GWAS to estimate the associations of gene expression levels. Bonferroni corrections were applied as a stastistically significant threshold, based upon all tested genes (N=25,224).

2.11. Gene-based study

We implemented gene-based GWAS by MAGMA v1•07 software [50]. MAGMA transforms the p-values of genes in gene-sets to z-values by using an inverse normal transformation. Multiple linear principal components regression model was employed in order to account for LD between variants and to detect multi-marker associations. We used a significance threshold of $p <5•0 \times 10^{-8}$ in order to detect significance specifically at the single-variant level and to conservatively detect the gene-based specific loci across the genome.

2.12. Meta-analysis

We subsequently conducted a trans-ethnic meta-analysis with GWAS summary statistics from the UK Biobank (UKBB, ftp://share.sph.umich.edu/UKBB_SAIGE_HRC/ downloaded on July 25th, 2019) [33]. The UKBB GWAS was composed of 15,995 cases and 361,617 controls with 21 original significant loci. The QC for samples included removing individuals without white British genetic ancestry, closely related individuals and those with sex chromosome aneuploidies. Haplotype Reference Consortium (HRC) and UK10K haplotype resources were used for imputation. The SNPs were restricted for those with an INFO score of >0.8 and the minor allele frequency of 0.1%. GWAS was performed by UKBB using SAIGE. The summary statistics for males and females separately were not publicly available in the UKBB summary data (by SAIGE). Meta-ANalysis of Transethnic Association studies (MANTRA) software (version 2•0) was used to apply a random effect meta-analysis, taking into account the heterogeneity in allelic effect across populations [51]. We regarded the log10 Bayes factor (BF) > 6 as a significant threshold [52]. While the UKBB results are unpublished, we refrain from arguing novelty of a gene if the UKBB results contain variants in the gene exceeding a GWAS significant level.

2.13. Bayesian statistical fine-mapping analysis

Statistical fine-mapping analyses were performed by FINEMAP (version 1•4) so as to prioritise causal variants in inguinal hernia susceptibile loci [53]. The FINEMAP computes a posterior probability (PP) of causality for each SNP. Candidate putative causal variants were ranked for each association signal in a descending order of their PPs, which was followed by building 95% credible set of causal variants, including the variants ranked until their cumulative PP reached 95%. We used the default priors and parameters in FINEMAP.

2.14. Machine learning-based prediction of GWAS variants regulating ncRNA transcription

We used mutation effect prediction on ncRNA transcription (MENTR) which was developed by Koido et al., a machine-learning program trained with cell-type-specific long-ncRNA and enhancer transcription data obtained by cap analysis of gene expression (CAGE) in order to predict the effect of variants on promoter/enhancer expression [54]. All significant variants in our GWAS and meta-analysis were analyzed by MENTR.

2.15. Definition and annotation of the significant SNPs in our meta-analysis

Histone modifications (H3K4me1 mark often found near regulatory elements, H3K4me3 mark often found near promoters, and H3K27ac mark often found near active regulatory elements on 7 types of cell lines from ENCODE: GM12878, H1-hESC, HSMM, HUVEC, K562, NHEK, and NHLF cells) and DNase hypersensitive sites (DNaseI hypersensitivity clusters in 125 ENCODE cell types: the names of 125 cell lines are listed as follows, "DNaseI Hypersensitivity Uniform Peaks from ENCODE/Analysis", http://genome.ucsc.edu/cgi-bin/hgTrackUi?db=hg19&g=wgEncodeAwgDnaseUniform) defined by ENCODE (version 3), [55] and, if SNPs are intergenic, enhancers (hg19) mapped by FANTOM5 (phase2•5) [56] were extracted from the University of California Santa Cruz (UCSC) database [57].

2.16. Functional mapping and annotation (FUMA) of the genes with inguinal hernia-associated SNPs in our meta-analysis

FUMA (version 1•3•6[a]: https://fuma.ctglab.nl/) performs hypergeometric tests of enrichment of the list of mapped genes in MSigDB gene sets. We used the GENE2FUNC procedure in FUMA to perform tissue specificity and pathway enrichment analyses of genes with the lead SNPs in our meta-analysis [58]. FUMA addditionally carries out gene mapping, tissue-expression analysis, and gene set enrichment analysis (GSEA).

2.17. Evidence of related gene expression of inguinal hernia risk genes newly identified in this study

We used RNA microarray data deposited by Zhao et al [35]. in the National Center for Biotechnology Information Gene Expression Omnibus database accessible under the accession no. GSE92748 (https://www.ncbi.nlm.nih.gov/geo/). With this data, we assessed the gene expression of each newly identified risk gene in this study. Total RNA was obtained from the lower abdominal muscle tissue of humanized aromatase transgenic mice, and was compared with those of wild type mice. The number of cases and controls was 6 for each. The online tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/gep2r/) was used for analysis. GEO2R performs comparisons by using the GEOquery and limma (Linear Models for Microarray Analysis) R package from the Bioconductor project [59]. Additionally, we used the Human Protein Atlas (http://www.proteinatlas.org)(60) in order to see which tissues are expressed for each gene. We considered positive RNA expression based on summary data of consensus Normalized eXpression (NX) levels, created by combining data from three transcriptomics datasets (HPA, GTEx and FANTOM5). We considered positive protein expression based on a best estimate from a knowledge-based annotation as per the Human Protein Atlas website.

2.18. Trans-ethnic genetic correlation

The python package Popcorn (ver.0•9•9) was used to estimate the genetic correlation of causal variants effect sizes across populations [61]. We used the data of 1KG for East Asians and Europeans so as to compute cross-population scores, taking into account each structure of LD [62].

2.19. Comparison of effects sizes between BBJ and UKBB data

We compared the beta coefficients and SEs of the inguinal hernia-associated SNPs in the data from UKBB with those SNPs in the data from BBJ. If the beta coefficients and SEs were plotted either in the first or third quadrant, effect size for both traits were in the same directions as both x and y take positive or negative values, which meant that inguinal hernia-associated SNPs were shared between European and Japanese populations.

We also showed the distribution of risk allele frequencies (RAF) of the inguinal hernia-associated SNPs specifically in data from UKBB and inguinal hernia-associated SNPs specifically in the data from our meta-analysis.

2.20. Role of funding source

Funders' roles: Study design and data collection.

3. Results

In this study, we included 1,983 cases and 172,507 controls. Baseline demographics of the subjects are shown in Table 1, and male gender was higher in the cases as shown in previous studies [7,8]. After filtering an imputed dataset, we tested 8,443,696 autosomal variants and 181,087 chromosome X variants for GWAS by SAIGE [33] (see methods). The Manhattan and Q-Q plots are shown in Fig. 1 and Supplemental Fig.1 where no evidence for inflation was observed. A significant locus, rs118109209 in the intergenic region of *ELN*/*TMEM270* genes in chromosome 7 (the closest gene was *ELN*), was significantly associated with inguinal hernias ($p=4•7 \times 10^{-9}$, Table 2, Fig. 1 and Fig. 2a). This locus has not been reported to reach GWAS significance level in previous literature. GWAS excluding all cancer, fibroid and genetic defects predisposed to inguinal hernia from controls groups showed the same *ELN* locus reached GWAS significance level ($p=1•6 \times 10^{-8}$), confirming that the difference between controls caused no significant biases. The *ELN* gene encodes elastin, a protein that is one of the two components of elastic fibers, [63] and mutations of the *ELN* gene are observed in patients with cutis laxa, a rare connective tissue disorder, who are at a higher risk

Table 1
Baseline characteristics of patients included for this study (n = 174,490)

	Cases	Controls
Characteristic	n = 1,983	n = 172,507
Age, mean (SD)	64.8 (14.4)	62.7 (14.6)
Men (%)	n = 1,622 (81.8)	n = 90,335 (52.4)
Genotyping array	(1) A combination of Illumina Infinium Omni Express and Human Exome (2) Infinium Omni Express Exome v.1.0 (3) Infinium Omni Express Exome v.1.2	

n, number; SD, standard deviation.

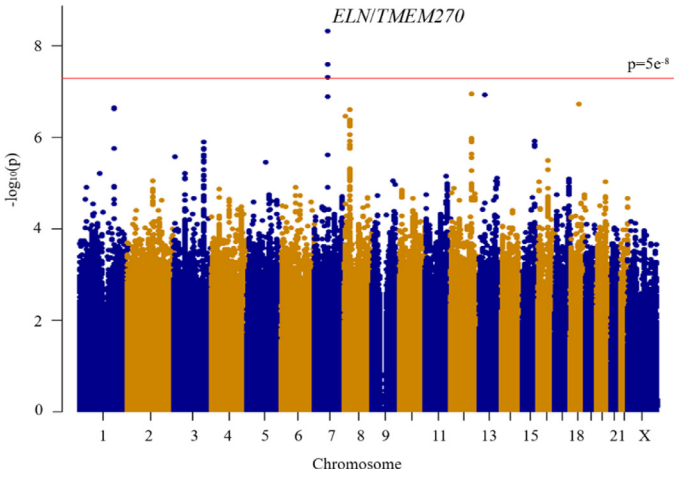

Fig. 1. Manhattan plot of genome-wide markers for inguinal hernia (1,983 cases and 172,507 controls). We performed genetic association tests, adjusted for sex and genetic ancestry (PCs 1 through 10). Results are plotted as −log10 p values on the y-axis by position in chromosome (x-axis) (NCBI build 37). The red line represents the genome-wide significance level. Gene name is shown next to the top locus.

of developing inguinal hernias [64]. No exonic SNPs were in strong LD with the lead variant and no eQTL studies report the lead variant as an eQTL [39].

Conditional analyses for the significant locus by the lead SNP detected no additional independent signals. We conducted a gene-based test in order to identify the association signals of novel susceptibility genes, but no additional genes were identified (Supplemental Fig. 2). SNP heritability estimate for inguinal hernia was 25•3% (SE of 5•5%) by LD score regression, proving its firmly inherited feature. We conducted a genetic correlation analysis by bivariate LD score regression to evaluate shared polygenic architecture between inguinal hernia and BBJ target diseases, [44] showing that no traits reached Bonferroni-corrected significance (Supplemental Table 1). Additionally, we separately conducted GWAS for males and females, and estimated heritability (Table 3, Supplemental Fig. 3 and 4). We found higher heritability for males, which may be consistent with previous studies showing a higher risk of developing inguinal hernias in males [7,8].

We tested pathway analysis by Pascal in order to evaluate disease-associated pathways driven by polygenic components, [45] resulting in a possibly significant signal of the focal adhesion pathway (p-value of $1•7 \times 10^{-4}$, Supplemental Table 2). Given the mechanism of inguinal hernias and a previous report showing significant enrichment in the focal adhesion pathway, this finding is intriguing. [65] The partitioned heritability analysis in cell groups using LD score regression revealed enrichment in the connective/bone and gastrointestinal (GI) tissues (p-value of 0•0011 for connective/bone tissues and 0•0045 for GI tissues, see Fig. 3). Analyses of detailed cell types demonstrated significant heritability enrichment in the H3K9ac in colon smooth muscle (p-value of $1•8 \times 10^{-4}$, Supplemental Table 3). Interestingly, we observed near significant enrichments in the smooth muscles of multiple GI tracts, including H3K27ac in the duodenum and H3K9ac in the stomach (p-value of $6•5 \times 10^{-4}$ and $8•5 \times 10^{-4}$, respectively, Supplemental Table 3). TWAS by FUSION was performed in order to investigate the transcriptional landscape regulated by genetic components of inguinal hernia. [48] We observed a significant association of the *CALD1* gene in chromosome 7 with suprapubic skin (not sun exposed, p-value of $7•9 \times 10^{-5}$). This is supported by *CALD1*'s involvement in the regulation of smooth muscle contraction (including the GI tract), [66] which is reasonable to be seen, as a network analysis investigating causative proteins related to inguinal hernia showed enrichment in the regulation of actin cytoskeleton in a previous study. [65] These findings indicate the importance of gene regulation in focal adhesion, the GI tract (the contents of protrusion), and skin covering the protrusions in the pathology of inguinal hernia.

Subsequently, we conducted a trans-ethnic meta-analysis, using the summary statistics of GWAS for inguinal hernia from UKBB. We tested 4,846,078 autosomal variants available for both the data from BBJ and UKBB. In meta-analysis, 23 significant loci were identified (Table 4). Among the 21 significant loci in the UKBB GWAS, 18 loci remained statistically significance in the meta-analysis. Most importantly, 5 out of 23 significant loci, namely, *TGFB2, RNA5SP214*/*VGLL2, LOC646588, HMCN2*, and *ATP5F1CP1*/*CDKN3,* were unreported; these were unidentified in GWAS by BBJ and UKBB, indicating a shared genetic component between these two populations. Two genes in these loci seemed relevant to inguinal hernias. First, the *TGFB2* (transforming growth factor beta 2) gene in chromosome 1 encodes a secreted ligand of the transforming growth factor-beta (TGF-beta) superfamily of proteins. [67] Overexpression of one of the isoforms of TGF-beta, TGF-beta1, is reported in patients with inguinal hernias, [68] and previous GWAS also showed that another isoform, *TGFB3*, is regulated by *WT1*, the significant locus in their study. [23] Second, the *VGLL2* (vestigial like family member 2) gene in chromosome 6 encodes a protein that may act as a co-factor of transcriptional enhancer factor 1 (TEF-1) regulating gene expression during skeletal muscle development. [69] When exploring potential eQTL variants based on the data reported by GTEx (version 8), [39] we found evidence suggesting that some signals in these novel loci are associated with gene regulation. Specifically, eQTL signals were noted in the lead SNP in the *RNA5SP214*/*VGLL2* gene in the esophagus (mucosa) and in the *HMCN2* gene, especially prominent in skin (not sun exposed, suprapubic, Supplemental Table 4). As per the UCSC browsers we used to define and annotate significant SNPs, the lead SNP in the *TGFB2* gene has overlap in the H3K27ac and H3K4me3-marked region, while the lead SNP in the *RNA5SP214*/*VGLL2* gene has overlap in the H3K27ac-marked region, H3K4me1-marked region, and DNaseI hypersensitivity clusters. Finally, the lead SNP in the *HMCN2* gene has overlap in the H3K4me1-marked region (Supplemental Table 4). [57] We also performed statistical finemapping analyses for the five loci using both BBJ and UKBB sets of data. The lead variant in the meta-analysis in *ATP5F1CP1/CDKN3* and in the *TGFB2* were prioritized in both BBJ and UKBB with highly ranked PP (Supplemental Table 5), suggesting shared causal variants across populations in these loci. Lead variants in the other three loci showed top or near top PP in the UKBB, but not BBJ data. However, lead SNPs are not necessarily always the causal SNPs and variants in high LD with lead variants showing comparable PP with lead variants might be causal and functional. We discovered an additional candidate of causal variant, rs10951082 in *LOC646588* in high LD with the lead SNP (R-square of 0.95), showing the 4th highest PP in

Table 2
Significant loci associated with inguinal hernia among patients at BioBank Japan Project identified by genome-wide association study

Chr	Position	SNP id (rs)	Gene	Ref/var	Location	AF.Cases	AF.Controls	OR	95% CI	p-value
7	73389541	rs118109209	*ELN*/*TMEM270*	A/T	Intergenic	0.071	0.052	1.64	1.25, 2.15	4.7×10^{-9}

Allele frequencies in the 1000 Genomes: East Asian A=0.9742, T=0.0258; Europe A=1.0000, T=0.0000.
Chr, chromosome; Ref, reference allele; Var, variant allele; AF.Cases, variant allele frequency in cases; AF.Controls, variant allele frequency in controls; OR, odds ratio; CI, confidence interval.

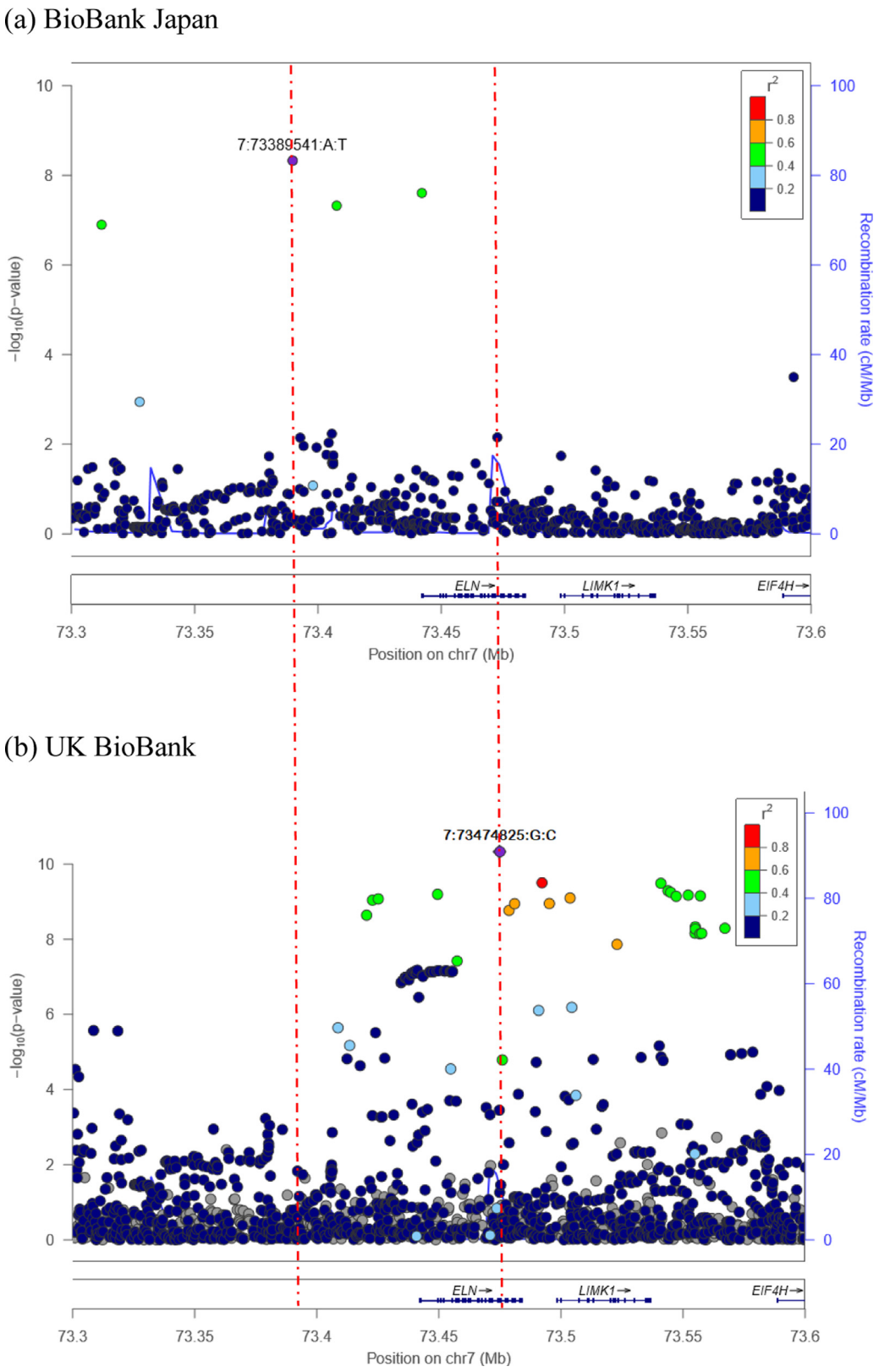

Fig. 2. Locus zoom plot of results of genetic association tests at chromosome 7 regions. Locus zoom plots at significant loci in chromosome 7 are shown in the BioBank Japan (a) and UK Biobank data (b). Coloring is based on LD (genome build hg19/1KG for both populations) with the top hits in the regions for inguinal hernia. The left red dashed line represents the position of the top locus in BBJ, and the right one is for the top locus in UKBB for this region.

UKBB and the 2nd highest PP (higher than the lead variant) in BBJ. Additionally, the variant overlapped with the H3K27ac marks.

We further investigated variants among all significant loci in the meta-analysis with potential to modulate gene expression levels that are difficult to identify with conventional eQTL studies. We applied MENTR, a newly developed machine-learning model to predict an alternative allele's mutation effect, or ability to change the expression of transcribed promoter/enhancer which was defined by CAGE

Table 3
Heritability

Population	Heritability (SE)
All	0.25 (0.055)
Male	0.35 (0.098)
Female	0.23 (0.23)

SE, standard error

sequencing. [54] As a result, five SNPs in the *TGFB2, LOX*, and *WT1-AS*/*WT1* genes are shown to be potential functional variants with high confidence (Supplemental Table 6). In particular, rs2234580 (chr1: 32,457,138 at hg19) in the *WT1-AS*/*WT1* genes was predicted to increase expression of the promotors in relevant tissues, namely, epithelial folds, anatomical walls, epithelial cells of alimentary canal, and endo-epithelial cells. These findings again support the theory of involvement of extracellular components, GI tract, and dermatological pathologies in the development of inguinal hernias.

Subsequently, we explored the functional annotation of genes with lead SNPs in our meta-analysis by using FUMA. [58] Prioritized genes were overrepresented in extracellular matrix pathways (adjusted P-value of 0•036, GO cellular component (MsigDB c5), Supplemental Fig. 5a). GSEA conducted by FUMA using the curated gene sets showed enrichment in elastic fiber formation (Reactome) and NABA_MATRISOME (ensemble of genes encoding extracellular matrix and extracellular matrix-associated proteins) [46,70] (adjusted P-values of 0•018 and 0•046, respectively, Supplemental Fig. 5b), both of which are consistent with the mechanisms of inguinal hernia. These two pathways contained functionally relevant genes including *TGFB2, EFEMP1, LOX, ADAMTS6, HMCN2* and *CDCA2* supported by eQTL and/or histone marks described above, together with the *CRISPLD2* gene. While we did not find functional evidence of the lead variant for *CRISPLD2,* the pathway analysis suggested this gene is causal for inguinal hernia in this region. These findings suggest that the majority of lead signals in the meta-analysis are putative causal variants, pointing to the need for future investigations. The significant loci containing genes whose biological functions relevant to inguinal hernia were shown in previous studies are illustrated in Fig. 4. Statistical finemapping analyses for the known significant loci showed rs6749170, an intronic variant of the *ADCY3* gene, had the highest PP for both the BBJ and UKBB data in addition to *ATP5F1CP1*/*CDKN3* and *TGFB2* (Supplemental Table 5).

Subsequently, we assessed related gene expression for the five inguinal hernia risk loci newly identified in this study, in addition to the *ELN*. Gene expression for four of the six genes (*ELN, TGFB2, VGLL* and *CDKN3*) was available in the RNA microarray data deposited by Zhao et al. [35], which was obtained from the lower abdominal muscle tissues of humanized aromatase transgenic mice. For the *ELN* gene, expression was upregulated 2.3 times more compared to wild type mice (p-value of 0.0027) and for *TGFB2, VGLL* and *CDKN3*, gene expression was 1.19, 1.02, and 1.08 times more upregulated, respectively (p-values of 0.016, 0.81, and 0.023, respectively). Next, data from expressed tissues for five of the six genes (*ELN, TGFB2, VGLL, HMCN2* and *CDKN3*) were available from the Human Protein Atlas [60]. RNA and protein expression were seen in multiple tissues related to inguinal hernia (Supplemental Table 7). These findings highlight that most of the newly identified risk genes were also supported by gene expression and/or protein expression in relevant tissues.

Of note, we also confirmed that the results of meta-analysis did not change dramatically when excluding all forms of cancer, fibroid and genetic defects predisposed to inguinal hernia from controls groups. However, additionally, an unreported locus, rs1925281, was found to be significant, an intergenic variant in *EMX2*/*RAB11FIP2* in chromosome 10 with a 6.10 log-arithm of the Bayes Factor and posterior probability of heterogeneity of 0.10 (those were 5.93 and -0.066, respectively, in analysis with the original control samples). This locus did not reach GWAS significance level in either of the BBJ or UKBB GWAS. In GTEx data, eQTL signals were noted in this SNP in the cultured fibroblasts. This locus has overlap in the H3K27ac-marked region, according to UCSC browsers. In the finemapping analyses, this SNP ranked second with a PP of 0.30 in UKBB, and ranked third with a PP of 0.13 in BBJ. Additionally, RNA and protein expressions were seen in multiple tissues related to inguinal hernia for both *EMX2*/*RAB11FIP2* genes as per the Human Protein Atlas. This locus could also be a causal variant, but further in-vitro studies will be necessary to understand its detailed functional role.

Lastly, we explored the relationship of the effect sizes of inguinal hernia-associated SNPs between BBJ and UKBB in order to investigate the differences of susceptibility across populations. We confirmed that risk alleles of inguinal hernia-associated variants in the UKBB data were shared in Japanese populations: 15 out of the 18 SNPs showed the same association directions and all 18 variants showed a strong correlation of effect size (binomial p=0•0075, Spearman rho = 0•49 with p=0•041, Fig. 5). The three variants with different directions of association between the two populations were rs2480924 in chromosome 9, rs12319548 in chromosome 12, and rs2076441 in chromosome 16. Risk alleles are rare in either population for rs2480924 and rs12319548, and the minor allele is opposite for the European and East Asian populations in the rs2076441 as per the 1000 Genomes Project. [62] We observed a strong trans-ethnic genetic-effect correlation (ρ_{ge} = 0•77, SE = 0•26). These results indicate strongly shared genetic components of inguinal hernia across these populations. Meta-analysis identified the SNPs with low RAF in

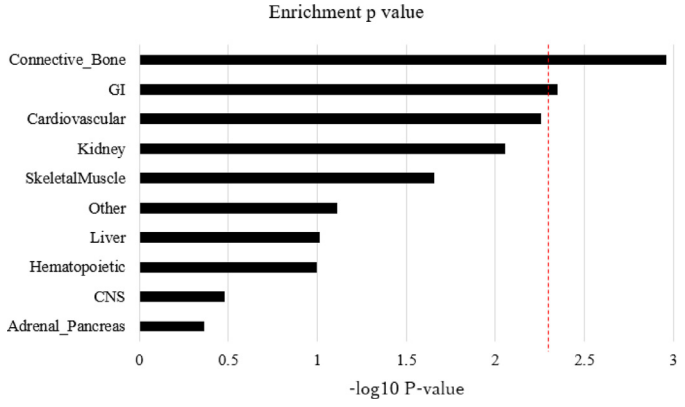

Fig. 3. P-value for the results of partitioned heritability analysis for the 10 different tissue types. Results are plotted as −log10 p values on the x-axis by 10 tissue types on the y-axis. The red dashed line represents the −log10 p value reaching the Bonferroni-corrected threshold.

Table 4
Meta-analysis with UK Biobank data for inguinal hernia

Chr	position	SNP id (rs)	Gene	Ref/var	Risk allele frequency in Ctrl		Location	BBJ			UKBB			MANTRA	
					BBJ	UKBB		OR	95% CI	p-value	OR	95% CI	p-value	Log_{10} Bayes	posthg
1	9447189	rs2095906	*SPSB1 / LINC02606*	A/G	0.64	0.62	intergenic	1.05	0.98, 1.13	0.14	1.07	1.04, 1.10	1.00×10^{-7}	6.18	0.80
1	218524632	rs2799097	*TGFB2*	A/G	0.73	0.85	intron	1.07	0.99, 1.15	0.07	1.10	1.06, 1.14	2.01×10^{-8}	**7.01**	**0.33**
1	219675209	rs2820465	*LYPLAL1-AS1 / LOC107985272*	G/T	0.31	0.59	intron	0.99	0.92, 1.06	0.74	0.93	0.91, 0.95	6.70×10^{-9}	6.24	-0.09
2	25110962	rs6749170	*ADCY3*	A/G	0.44	0.46	intron	0.93	0.88, 0.99	0.03	0.94	0.92, 0.96	1.27×10^{-7}	6.25	-0.42
2	55976844	rs62167673	*PNPT1 / EFEMP1*	A/G	0.45	0.15	intergenic	1.07	1.00, 1.14	0.05	0.91	0.88, 0.93	4.01×10^{-9}	6.02	2.26
3	56149492	rs13091322	*ERC2*	A/G	0.15	0.31	intron	0.92	0.85, 0.9996	0.07	0.93	0.91, 0.96	2.39×10^{-7}	6.05	0.31
4	174616822	rs7686296	*RANP6 / LINC02269*	T/A	0.23	0.30	intergenic	1.07	0.98, 1.16	0.10	1.08	1.05, 1.11	1.67×10^{-8}	7.06	0.27
5	64451583	rs7702887	*ADAMTS6*	C/T	0.45	0.72	intron	1.05	0.98, 1.12	0.14	1.07	1.04, 1.11	7.15×10^{-8}	6.15	-0.08
5	121422560	rs995687	*LOX / ZNF474*	A/G	0.026	0.29	intergenic	1.05	0.85, 1.31	0.63	1.07	1.05, 1.11	4.68×10^{-8}	6.13	-0.03
6	6749069	rs9504915	*LOC101928004*	A/G	0.21	0.56	intron	1.01	0.93, 1.09	0.78	0.93	0.91, 0.95	7.55×10^{-9}	6.17	0.20
6	27699581	rs9393851	*TRV-CAC7-1 / GPR89P*	A/T	0.18	0.27	intergenic	1.03	0.94, 1.14	0.51	0.92	0.90, 0.95	3.48×10^{-9}	6.00	-0.03
6	117490664	rs1405212	*RNA5SP214 / VGLL2*	T/C	0.72	0.63	regulatory region (intergenic)	0.91	0.86, 0.97	0.01	0.94	0.92, 0.96	1.09×10^{-7}	**6.83**	**0.23**
6	143556810	rs6570551	*AIG1*	A/G	0.93	0.36	intron	1.08	0.94, 1.24	0.23	0.93	0.91, 0.95	8.31×10^{-9}	6.16	0.24
7	25692889	rs6943068	*LOC646588*	G/A	0.40	0.66	Non coding transcript variant	0.93	0.88, 0.99	0.03	0.94	0.92, 0.96	2.49×10^{-7}	**6.19**	**-0.22**
7	73503533	rs75566398	*LIMK1*	A/G	0.065	0.12	intron	0.99	0.87, 1.13	0.90	0.89	0.86, 0.92	8.00×10^{-10}	7.02	-0.21
8	25383344	rs4872325	*CDCA2 / EBF2*	G/T	0.77	0.72	intergenic	0.92	0.86, 0.99	0.03	0.93	0.91, 0.96	4.18×10^{-7}	6.02	0.01
9	16749020	rs4961753	*BNC2*	G/A	0.89	0.92	intron	1.02	0.91, 1.14	0.78	0.88	0.85, 0.92	5.54×10^{-9}	6.00	0.25
9	133037273	rs4837486	*HMCN2*	T/C	0.84	0.50	intron	0.95	0.88, 1.04	0.28	0.94	0.92, 0.96	7.31×10^{-8}	**6.07**	**0.16**
11	32296455	rs7940705	*THEM7P / WT1*	A/G	0.82	0.34	intergenic, non coding transcript variant	0.94	0.87, 1.02	0.17	0.93	0.91, 0.96	1.12×10^{-7}	6.07	0.04
13	51088052	rs183949	*DLEU1*	A/G	0.20	0.40	intron	1.08	0.99, 1.17	0.06	1.07	1.04, 1.10	9.08×10^{-8}	6.40	-0.01
14	54724054	rs2358483	*ATP5F1CP1 / CDKN3*	C/T	0.053	0.30	regulatory region (intergenic)	1.24	1.04, 1.47	0.00	1.07	1.04, 1.10	3.90×10^{-7}	**6.08**	**-0.04**
16	84856889	rs4783079	*CRISPLD2*	C/A	0.20	0.38	intron	1.05	0.96, 1.14	0.27	1.08	1.06, 1.11	5.91×10^{-11}	8.59	-0.15
17	12187295	rs8081231	*MAP2K4 / LINC00670*	T/C	0.13	0.31	intergenic	1.13	1.02, 1.25	0.01	1.08	1.05, 1.11	1.23×10^{-9}	8.70	0.89

Unreported loci are highlighted in bold.
Ctrl, control; BBJ, BioBank Japan; UKBB, UK Biobank; MANTRA, Meta-ANalysis of Transethnic Association studies; OR, odds ratio; CI, confidence interval; Chr, chromosome; Ref, reference allele; Var, variant allele; OR, odds ratio; CI, confidence interval; log_{10}BF, log-arithm of Bayes Factor; posthg, posterior probability of heterogeneity.

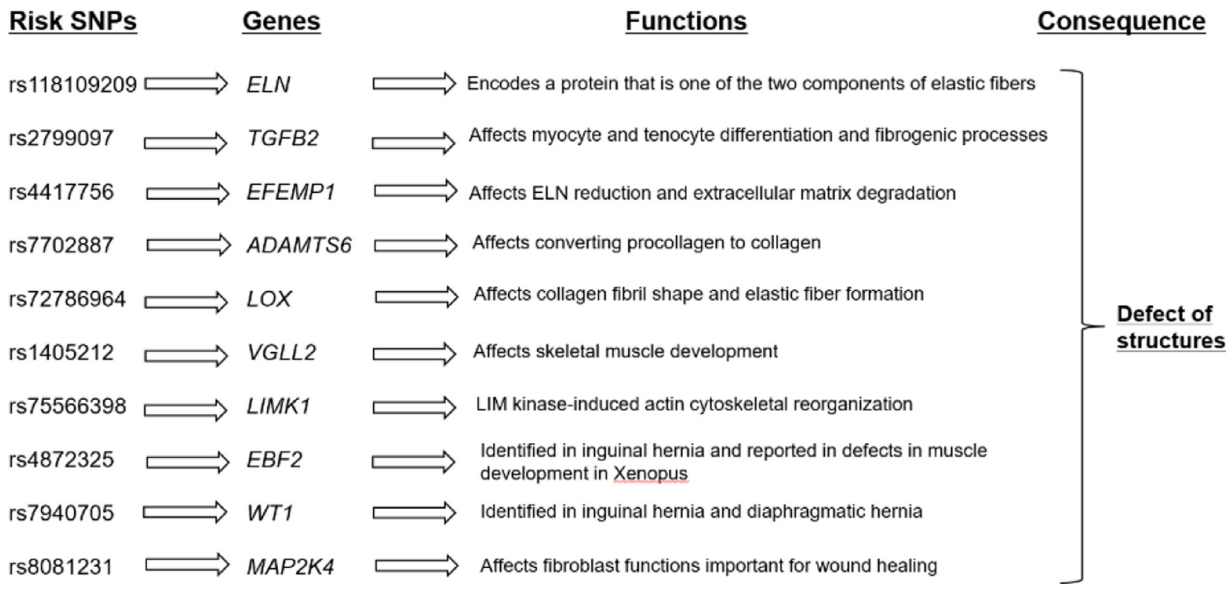

Fig. 4. Significant loci identified in this study which can be biologically explained in development of inguinal hernia. The flows of each risk SNP, its gene name, the functions of the gene, and the consequence of the gene function which is expected to cause inguinal hernia are shown.

either population, successfully increasing the power of detecting associated SNPs (Supplemental Fig. 6a). Meta-analysis identified the SNPs with lower MAF in the data from UKBB as significant, which again increased the power of detecting associated SNPs (Supplemental Fig. 6b). Additionally, we found a population-specific variant in *ELN* locus in each population (Fig 2]. [62] The lead SNP in the BBJ is not seen in Europeans; A=0•9742 and T=0•0258 in East Asians but A=1•0000 and T=0•0000 in Europeans; conversely the lead SNP in the UKBB is not seen in East Asians; A=1•0000 and T=0•0000 in East Asians but A=0•9085, T=0•0915 in Europeans as per 1000 Genomes. [62] When conducting finemapping analyses for each of those loci, the lead SNP in the BBJ data was the only SNP contained in the 95% credible sets for causal signal in the region with a PP of 0.999993. For the lead locus in UKBB, two SNPs were contained in the 95% credible sets, of which the top ranked SNP with a PP of 0.94 was the lead SNP in the region. These indicate that population-specific variants in these two populations share the same associations with inguinal hernias, highlighting the critical role of elastin in inguinal hernias.

4. Discussion

We conducted the first large-scale GWAS for inguinal hernia in the Japanese population and successfully identified a locus, the closest gene of which was *ELN* at the GWAS significant level. Downstream analyses support the importance of elastin and show strong evidence of extracellular components, especially elastic fibers, involved in the development of inguinal hernias. We also found five unreported susceptibility loci for inguinal hernia in the meta-analysis and showed a strong shared genetic architecture across different populations, which implicates the generalizability of our findings.

Elastin is a component of elastic fibers in the extracellular matrix. [63] A network analysis conducted by Jorgenson et al., showed potential interactions between the *ELN* gene and the *EFEMP1* gene(23), one of the GWAS significant loci in this current study. Additionally, a recent study also showed the decreased expression of ELN in direct inguinal hernia in the transversalis fascia [71]. In our study, the lead SNP has no overlap in H3K27ac, H3k4me1, H3k4me3 and DNaseI hypersensitivity clusters per UCSC browsers. [57] Thus, the functions of the lead variant or tightly linked variant(s) should be evaluated in hernia-specific tissues in future studies.

The encoded protein of *TGFB2* is a secreted ligand of the TGF-beta, reported to be associated with inguinal hernia formation. [68] rs2799097, the lead SNP in the *TGFB2* gene is the most likely causal variant, given the enrichment in histone marks as well as the results of finemapping. Additionally, previous studies show the association of the isoforms of TGF-beta with inguinal hernia and TGF-beta 2 itself with involvements in fibroblasts in various tissues and myocytes. [23,68,72-75] The other significant loci in the meta-analysis includes the four loci identified in the previous non-UKBB GWAS, [23] namely, *EFEMP1, WT1, EBF2* and *ADAMTS6*. The associations of those loci showed the same directions of signals as in the BBJ data though they did not reach GWAS significant levels (p-values ranged from 0•093 to $1•5 \times 10^{-6}$). These indicate the shared genetic components of inguinal hernias not only between BBJ and UKBB, but across other populations. In addition, multiple genes have functions consistent with the development of inguinal hernia (Fig.4] and the enrichment in the components and curated gene sets related to extracellular matrix and elastic fiber formation. Additionally, we found enrichment in focal adhesion pathways consistent with the causal mechanism of inguinal hernia, a pathway which functions through bundles of actin filaments anchored to the integrin family's transmembrane receptors via a multi-molecular complex of junctional plaque proteins. [76] For all significant loci in our analysis, we further investigated by finemapping in order to prioritize potentially functional SNPs. By doing this, we were able to identify some loci which are not only the lead loci but also the putative causal loci. Elucidating the causative loci and mechanical pathways sow good seeds for future research.

We showed the shared genetic architecture (Fig.5], which was further supported by the trans-ethnic genetic correlation as stated above. The genetic feature of the top locus in our BBJ GWAS was similar to the UKBB; both have significant loci in or near the *ELN* gene. Understanding the existence of similar polygenic architecture of inguinal hernia across different populations will enhance the design of future larger studies, and facilitate further investigations of possible unknown loci in this phenotype.

Our study shows the enrichment not only in connective tissues but also GI tissues, and additionally shows strong mutation effects in the epithelial cells in the GI tract (Fig.3, Supplemental Table 3 and 6). An inguinal hernia occurs when the abdominal contents, including gastrointestinal tract, pass down through the inguinal canal; thus, there could be a tendency to have more inguinal hernias with the

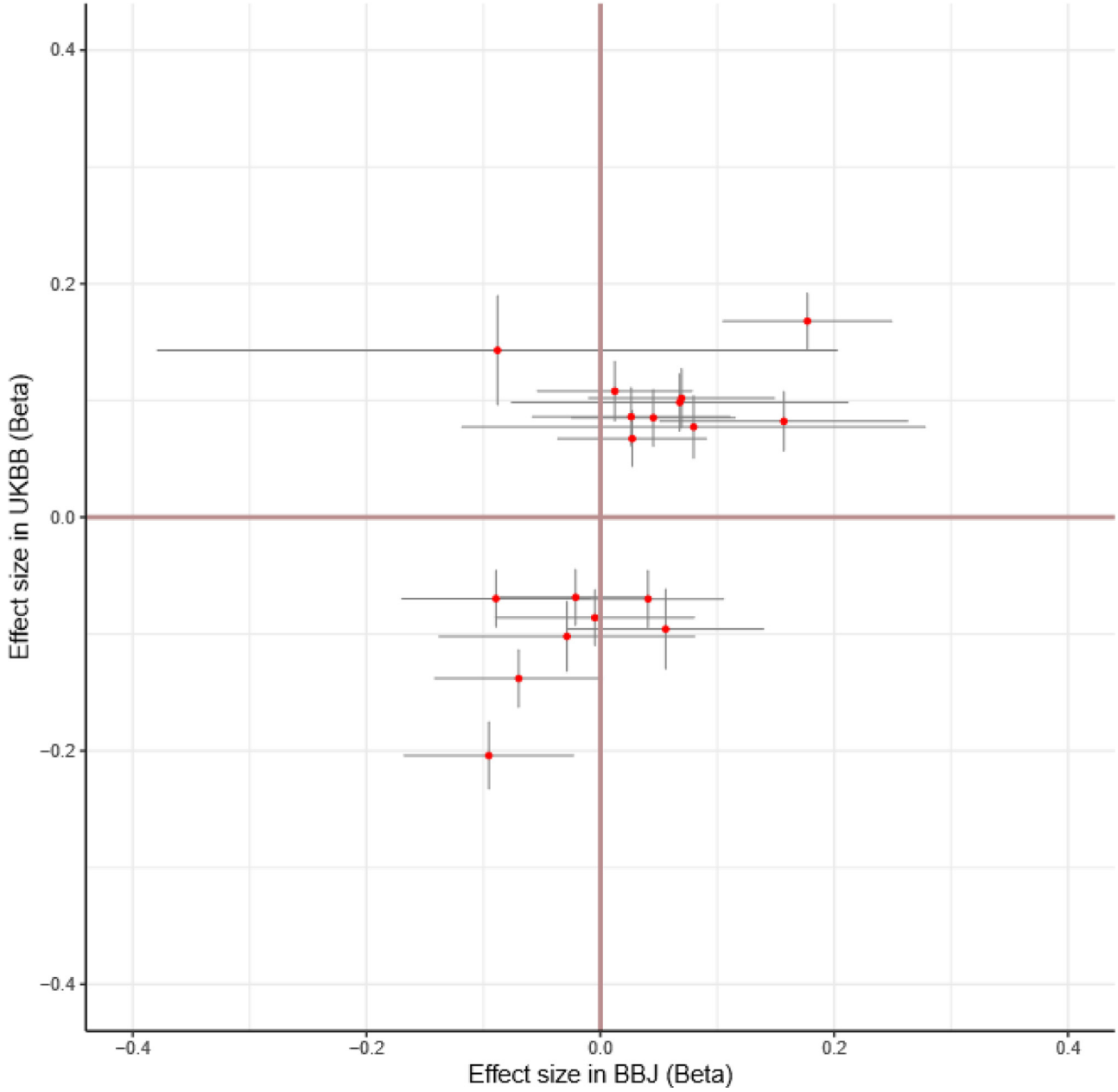

Fig. 5. Relationship of inguinal hernia-associated SNPs reaching GWAS significance between the populations. X-axis shows the beta coefficients in the BBJ data. Y-axis shows the beta coefficients in the UKBB data. The bars crossing the red dots represent the SEs of the beta coefficients.

existence of abnormalities of the structures supporting the position of the gastrointestinal tract. Furthermore, TWAS showed overlap in the skin of suprapubic regions. These could be supported by the GWAS significant locus in our study. Mutations of the *ELN* gene might lead to collapse of the transcriptional network responsible to dermal elastin, loose skin, and lead to protrusion of the inguinal hernia. Additionally, our enrichment analyses showed the enrichment in the connective and GI tissues which reached the Bonnferroni-corrected threshold, but not for the skeletal musles. These could be due to the fact that the data for muscle tissues used in this analysis were from the Roadmap Epigenomics Project [77] and did not represent low abdominal skeletal muscles. [62] Further investigations are needed to confirm these results.

Our study bears multiple limitations. First, biochemical analyses using actual patient samples would make our findings much more robust; however, unfortunately the BioBank Japan Project did not possess these specimens. Thus, we utilized the results of the gene expression data which are publicly available. Future studies should definitely consider doing those valuable analyses. Second, given that the cases were selected from past medical histories of the 47 target diseases in BBJ, the details of the inguinal hernias, including direct/indirect, and medial/lateral were not available. The differences of indirect hernia and direct hernia is not only in the way the hernias occur but also in the underlying pathology as stated by Somuncu et al. [78] However, the results of previous GWAS indicated the underlying significant loci across different subtypes of inguinal hernia. [23] Thus, we believe that our conclusions should not be affected by the unavailability of such data. Also, there is the possibility of included possible future cases, those who may develop inguinal hernia in the future, as controls. However, this is the nature of case-control studies, and it does not affect our conclusions. Additionally, the closest gene of the rs118109209 was *ELN*, and *ELN* is only a gene with the SNP in the moderate LD with the lead variant (Supplemental Fig. 7). ELN could be affected by this variant, given the roles of *ELN* as

mentioned above; however, future in-vitro studies are required in order to confirm this. Lastly, we did not investigate sex-stratified analysis due to the small sample size in females. Future studies will ideally investigate this aspect further, given the differences in heritability and prevalence of inguinal hernia between sexes.

As a summary, we have presented further insights into the genetic mechanisms of inguinal hernias and our findings pave a path to future research and a potential to improve treatment for patients with inguinal hernias.

Declaration of Competing Interest

The authors declare no conflict of interest.

Contributors

K.H., M.K., and C.T. designed the study. K.H., and K.T. analyzed the data. K.H., and C.T. interpreted the data. K.H. wrote the manuscript. M.K., X.L., Yu.Mo., Ta.Mo., Yo.Mu., B.J.P., Ta.Mu., and C.T. performed critical revision. The underlying data have been verified by K.H., K.T., and C.T. All authors read and approved the final version of the manuscript.

Data Sharing Statement

Full GWAS results will be available via the website of the Japanese ENcyclopedia of GEnetic associations by Riken (JENGER, http://jenger.riken.jp/en/).

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2021.103532.

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