communications biology

Article

https://doi.org/10.1038/s42003-024-06875-2

Genome-wide association studies for pelvic organ prolapse in the Japanese population

Check for updates

Masatoshi Matsunami[1,31], Minako Imamura[1,2,31], Asuka Ashikari[3], Xiaoxi Liu[4,5], Kohei Tomizuka[4], Keiko Hikino[6], Kosei Miwa[7], Katsumi Kadekawa[8], Tetsuji Suda[3], The Biobank Japan project*, Koichi Matsuda[9,10], Minoru Miyazato[11], Chikashi Terao[4,5,12] ✉ & Shiro Maeda[1,2] ✉

Pelvic organ prolapse (POP) affects approximately 40% of elderly women, characterized by the descent of the pelvic organs into the vaginal cavity. Here we present the results of a genome-wide association study (GWAS) for susceptibility to POP comprising 771 cases and 76,625 controls in the Japanese population. We identified a significant association of *WT1* locus with POP in the Japanese population; rs10742277; odds ratio (OR) = 1.48, 95% confidence interval (CI), 1.29–1.68, $P = 6.72 \times 10^{-9}$. Subsequent cross-ancestry GWAS meta-analysis combining the Japanese data and previously reported European data, including 28,857 cases and 622,916 controls, identified *FGFR2* locus as a novel susceptibility locus to POP (rs7072877; OR = 1.06, 95% CI, 1.04–1.08, $P = 4.11 \times 10^{-8}$). We also observed consistent directions of the effects for 21 out of 24 European GWAS derived loci (binomial test $P = 2.8 \times 10^{-4}$), indicating that most of susceptibility loci for POP are shared across the Japanese and European populations.

Pelvic organ prolapse (POP) is characterized by a descent of the pelvic organs into the vaginal cavity, and the prevalence of POP patients with stage II or greater by POP-quantification (POP-Q), is estimated as 17% and 37% of aged women in the Japanese and Europeans, respectively[1–3]. The etiology of POP is considered multifactorial, and several risk factors, including vaginal childbirth, aging, obesity, and prior hysterectomy, have been established for the development of POP[2–5]. The degree of the symptoms in patients with POP, including discomfort, pain, voiding disorder, and walking difficulty, varies, and in some cases, these symptoms significantly reduce the quality of life of affected women[6–8]. Although POP is a highly prevalent condition, it may not always be symptomatic. For instance, in a study of 477 asymptomatic women undergoing annual examinations, 51% had prolapse to the level of the hymenal remnant or beyond[9,10]. In women with mild prolapse, nonsurgical management including pelvic floor muscle training, use of a vaginal pessary, and life-style managements, is useful in preventing further progression of POP. However, once POP develops to advanced stages, these nonsurgical procedures are often no longer successful, and these patients require surgical treatments to restore normal pelvic anatomy, eliminate POP symptoms, and normalize bowel, bladder, and sexual function[1]. Therefore, early detection and interventions including pelvic floor muscle training and weight control are important to prevent the development of advanced POP[6].

The contribution of genetic factors has been suggested, and the heritability of POP was estimated to be 43% in a twin study[11]. In 2020, a genome-wide association study (GWAS) of Icelandic and UK Biobank (UKBB) cohorts reported seven loci associated with POP[12]. Their expanded GWAS meta-analysis, comprising 28,086 cases and 546,291controls, identified additional 19 novel loci associated with POP in European

[1]Department of Advanced Genomic and Laboratory Medicine, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan. [2]Division of Clinical Laboratory and Blood Transfusion, University of the Ryukyus Hospital, Okinawa, Japan. [3]Department of Urology, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan. [4]Laboratory for Statistical and Translational Genetics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. [5]Clinical Research Center, Shizuoka General Hospital, Shizuoka, Japan. [6]Laboratory for Pharmacogenomics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. [7]Urogyne Center, Japanese Red Cross Gifu Hospital, Gifu, Japan. [8]Department of Urology, Okinawa Kyodo Hospital, Okinawa, Japan. [9]Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [10]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan. [11]Department of Systems Physiology, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan. [12]Department of Applied Genetics, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan. [31]These authors contributed equally: Masatoshi Matsunami, Minako Imamura. *A list of authors and their affiliations appears at the end of the paper. ✉e-mail: chikashi.terao@riken.jp; smaeda@med.u-ryukyu.ac.jp

populations[13]. Although a large-scaled GWAS for POP has been conducted in European populations, a GWAS for POP using populations other than Europeans has not yet been performed. Given the evidence of population-specific features of POP[14,15], a GWAS for POP using other ethnic groups, such as East Asians is necessary to further understand the genetic predisposition to POP.

In this study, we performed a GWAS meta-analysis for POP using two Japanese study sets to identify genetic loci associated with POP in the Japanese population. Additionally, we combined the results of our GWAS meta-analysis with those of a previously reported large-scaled European GWAS meta-analysis to identify additional novel loci that predispose individuals to POP.

Results

Meta-analysis of GWAS for POP in Japanese populations

We performed a GWAS for POP in Japanese participants registered in the Okinawa Bioinformation Bank (OBi) and Biobank Japan (BBJ) (Supplementary Fig. 1). In principal component analysis (PCA), we observed two Japanese sub-populations, namely the Hondo and Ryukyu clusters (Supplementary Fig. 2), as reported previously[16–18]. Therefore, we conducted a GWAS on four Japanese groups: BBJ-Hondo, BBJ-Ryukyu, OBi-Hondo, and OBi-Ryukyu. Then we combined all association data with an inverse-variance fixed-effects meta-analysis comprising 771 POP cases and 76,625 controls. No significant inflation was observed ($\lambda_{GC} = 1.01$, $\lambda_{GC}1000 = 1.007$, Supplementary Fig. 3). We identified a single nucleotide polymorphism (SNP) in *Wilms tumor 1 (WT1)* locus showing genome-wide significant association with POP (rs10742277; $P = 6.72 \times 10^{-9}$, odds ratio (OR) = 1.48, 95% confidence interval (CI), 1.29–1.68) (Fig. 1A, Table 1, Supplementary Data. 1, Supplementary Fig. 3A). The *WT1* locus has been previously reported as a susceptibility locus for POP in European populations[13], and the lead SNP (rs11031796) in a European study was in high linkage disequilibrium (LD) with rs10742277 ($r^2 = 0.84$, D' = 1 in 1000GJPT). The effect size (OR) of the *WT1* SNP for POP was significantly larger in the Japanese population than in the European population (rs10742277, OR = 1.48, 95%CI, 1.29–1.68 in the Japanese population, OR = 1.06, 95%CI, 1.04–1.08 in the European population[13], *P*-value for Cochran's Q heterogeneity test = 1.03×10^{-6}). In addition, a significant difference in the risk allele frequency (RAF) was observed among the Japanese sub-populations, Hondo and Ryukyu, and European populations [rs10742277-C: AF = 0.25–0.26, 0.20–0.21, and 0.629 in the Japanese Hondo controls, the Japanese Ryukyu controls, and the European population (1KG_EUR)] (Table 1). We further identified nine loci showing suggestive evidences for the association with POP in the Japanese ($5 \times 10^{-6} > P \geq 5 \times 10^{-8}$, Supplementary Data 1), none of these overlapped with loci previously identified in the European GWAS[12,13].

Replication study for POP loci identified by the European GWAS

We searched for 26 previously reported loci[12,13] in the Japanese GWAS data. Of the 30 signals in 26 loci, 6 were monoallelic in the Japanese population, and the data for the remaining 24 SNPs were available in the Japanese GWAS data. Among them, four signals in three loci including *WT1*, *ADAMTS5*, and *GDF7-LDAH*, were significantly associated with POP in the Japanese ($P < 1.67 \times 10^{-3} = 0.05/30$), additional four signals showed nominal association ($1.67 \times 10^{-3} \leq P < 0.05$) (Supplementary Data 2,

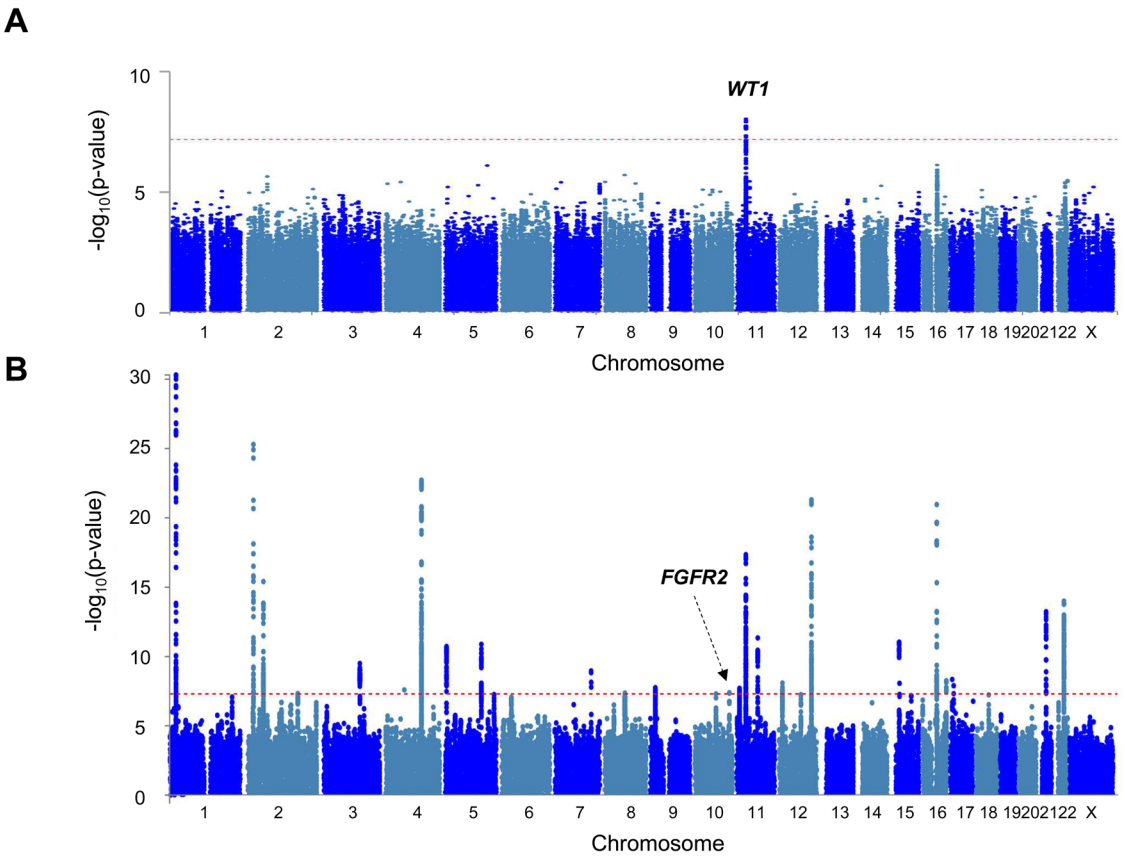

Fig. 1 | Manhattan Plots of genome-wide association studies (GWAS) for pelvic organ prolapse (POP). A GWAS meta-analysis for POP in Japanese populations Each plot indicates the individual association data of 9,862,117 SNPs in the GWAS meta-analysis combining four Japanese study sets (BBJ_Hondo, OBi_Hondo, BBJ_Ryukyu, OBi_Ryukyu; Total $N = 77,396$). **B** Cross ancestry GWAS meta-analysis for POP in Japanese and European populations Each plot indicates the individual association data of 5,417,787 SNPs in the GWAS meta-analysis, combining five study sets (BBJ_Hondo, OBi_Hondo, BBJ_Ryukyu, OBi_Ryukyu, European*; Total $n = 651,773$). Black arrow indicates a novel susceptibility locus for POP. The y-axis shows $-\log_{10} P$ values of association analysis of each SNP. The red line indicates the genome-wide significant threshold ($P = 5 \times 10^{-8}$). * Publicly available summary statistics of the GWAS data[13] were used.

Table 1 | Association of *WT1* locus with pelvic organ prolapse in the Japanese population

Lead SNP Chr; Position	Study	OR	95%CI	*P*	EAF		*N*	
EA/AA					case	control	case	control
rs10742277	BBJ_Hondo	1.60	(1.36–1.89)	1.50×10^{-8}	0.33	0.25	418	68,040
11;32452925	OBi_Hondo	1.16	(0.77–1.75)	0.49	0.31	0.26	128	387
C/G	BBJ_Ryukyu	1.21	(0.64–2.28)	0.57	0.25	0.21	29	5852
	OBi_Ryukyu	1.34	(1.003–1.78)	0.048	0.24	0.20	196	2346
	Meta-analysis	**OR**	**95%CI**	***P***	***P* het**	***I*²(%)**	***N***	
							case	**control**
		1.48	**(1.29–1.68)**	**6.72×10^{-9}**	**0.37**	**5.3**	**771**	**76,625**

Chr Chromosome, *Position* Chromosome Position GRCh37, *BBJ* BioBank Japan, *OBi* Okinawa Bioinformation Bank, *Hondo* Hondo cluster, *Ryukyu* Ryukyu cluster, *EA* Effect allele, *AA* Alternative allele, *OR* Odds Ratio, *CI* Confidential Interval, *P P*-value for association, *EAF* Effect allele frequency, *P* het: P-value for heterogeneity test, I^2 I^2 statistic for heterogeneity, *N* Sample size.

Table 2 | Association of *FGFR2* locus with pelvic organ prolapse in the Japanese and European populations

Lead SNP Chr; Position	Study	OR	95%CI	*P*	EAF		*N*	
EA/AA					case	control	case	control
rs7072877	BBJ_Hondo	1.16	(0.99 – 1.36)	0.072	0.791	0.765	418	68,040
10;123228660	OBi_Hondo	1.41	(0.90 – 2.19)	0.13	0.805	0.778	128	387
C/T	BBJ_Ryukyu	1.73	(0.92 – 3.25)	0.087	0.879	0.788	29	5852
	OBi_Ryukyu	1.07	(0.78 – 1.45)	0.68	0.839	0.821	196	2346
	European*	1.06	(1.04 – 1.08)	2.55×10^{-7}	0.780		28,086	546,291
Meta-analysis		**OR**		***P***	***P* het**	***I*² (%)**	***N***	
							case	**control**
		1.06	**(1.04 – 1.08)**	**4.11×10^{-8}**	**0.27**	**22.0**	**28,857**	**622,916**

Chr chromosome, *Position* Chromosome Position GRCh37, *BBJ* BioBank Japan, *OBi* Okinawa Bioinformation Bank, *Hondo* Hondo cluster, *Ryukyu* Ryukyu cluster, *EA* Effect allele, *AA* Alternative allele, *OR* Odds Ratio, *CI* Confidential Interval, *P P*-value for association, *EAF* Effect allele frequency, *P het P*-value for heterogeneity test, I^2 I^2 statistic for heterogeneity, N Sample size.
*Data from Pujol-Gualdo N. et al.[13].

Supplementary Fig. 4). Of the 24 signals evaluated, 21 showed the same direction of the effect with those in the European study (binomial test $P = 2.8 \times 10^{-4}$, suggesting that most of the susceptibility loci for POP are shared between the Japanese and European populations (Supplementary Data 2, Supplementary Fig. 5).

Cross-ancestry meta-analysis of GWAS for POP in Japanese and European populations

Next, we performed a cross-ancestry meta-analysis combining the Japanese GWAS data and previously reported European GWAS data for POP[13] (Fig. 1B, Supplementary Fig. 3B). No significant inflation was observed ($\lambda_{GC} = 1.120$, $\lambda_{GC}1000 = 1.002$, Supplementary Fig. 3B). A GWAS meta-analysis with a sample size of 651,773 identified 25 genome-wide significant associations with POP, including the *FGFR2* locus which has not been previously reported as a POP susceptibility locus (rs7072877 located approximately 9 kbp downstream of *FGFR2;* $P = 4.11 \times 10^{-8}$, OR = 1.06, 95% CI 1.04–1.08, Table 2, Supplementary Data 3, Fig. 1B). No heterogeneity in the effect of rs7072877 (*P*- het > 0.05) was observed among the studies.

Expression/splicing quantitative trait (eQTL/sQTL) analysis and summary data-based Mendelian randomization (SMR) analysis

To uncover the molecular mechanisms of the novel locus, *FGFR2*, on susceptibility to POP, we examined the association between the lead SNP (rs7072877) and gene expression in various tissues using the publicly available eQTL database, GTEx (Genotype tissue expression), (Supplementary Data 4). We found that carrying rs7072877-C (the risk allele for POP) was significantly associated with decreased *FGFR2* expression in three vascular tissues; $P = 2.7 \times 10^{-19}$ in the tibial artery, $P = 3.2 \times 10^{-14}$ in the aorta, $P = 5.2 \times 10^{-6}$ in the coronary artery). The eQTL lead SNPs, rs12255289 in tibial artery, rs10788184 in aorta and coronary artery, were in high LD with rs7072877 ($r^2 = 0.85$–0.88 in 1KG CEU) and their regional plots suggested co-localization of association signals for POP and eQTL for *FGFR2* (Fig. 2, Supplementary Fig. 6).

Using summary data-based Mendelian randomization (SMR) analysis and the heterogeneity in dependent instruments (HEIDI) test[19], we intended to identify associations between *FGFR2* expression and POP using summary data from eQTL studies and GWAS, followed by a heterogeneity test to see if *FGFR2* expression and the POP were affected by the same underlying causal variant[19]. The results of the SMR/HEIDI test suggested significant association between *FGFR2* expression and POP (*P*-SMR = 2.8×10^{-6} in the tibial artery, *P*-SMR = 8.6×10^{-6} in the aorta, *P*-SMR = 4.5×10^{-4} in the coronary artery, Supplementary Data 5), and POP susceptibility and *FGFR2* expression were likely affected by the same causal variant (*P*-value of HEIDI test ≥ 0.05, Supplementary Data 5).

We also examined the eQTL of the *WT1* locus, in which a genome wide significant association was observed in the Japanese GWAS (Please see Supplementary Note for details). Because there might be some differences in the LD structure of *WT1* locus between the Japanese and European populations (Supplementary Fig. 7) and the available eQTL data were mainly for Europeans, we used cross-ancestry GWAS data for the SMR/HEIDI test. We identified a significant association between the POP risk

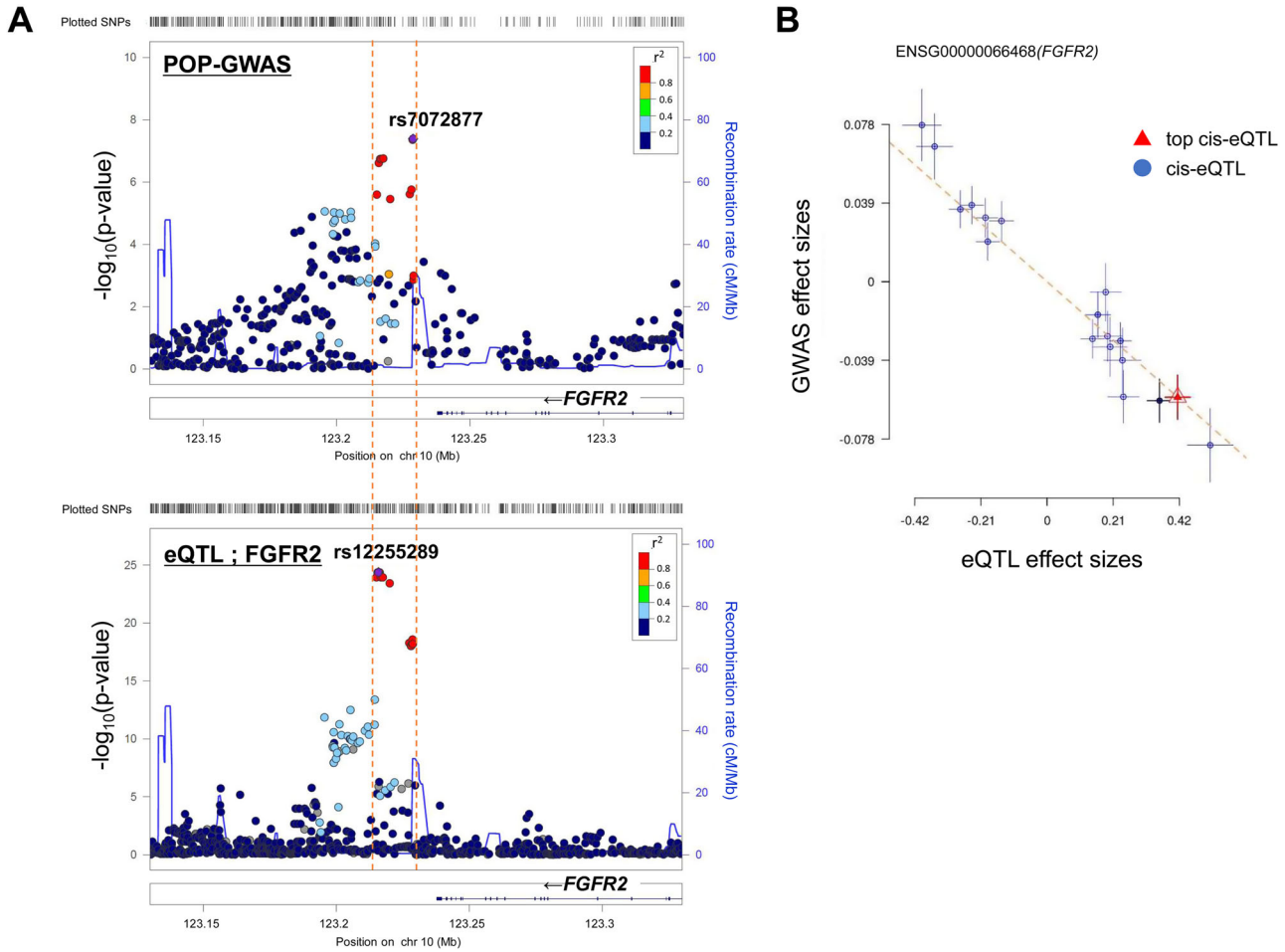

Fig. 2 | Regional association plots of *FGFR2* locus in the cross-ancestry genome-wide association study (GWAS) meta-analysis and expression quantitative trait loci (eQTL) analysis. A Results of the association study for POP (upper panel) and eQTL for *FGFR2* expression (lower panel) in the tibial artery. Each plot shows $-\log_{10}$ *P*-values. The variants with the most significant associations (lead SNPs) within this locus, rs7072877 for the association study and rs12255289 for the eQTL, are shown as purple diamonds. The colours in the other plots indicate the extent of linkage disequilibrium (LD) (hg19/1KGP 2014 EUR), as shown in the inset to the lead variants. The estimated recombination rates from the 1KGP 2014 EUR reference are indicated by the blue lines. **B** Comparison of the effect sizes of variants used for the HEIDI test between GWAS and eQTL data for the tibial artery. Orange dashed line indicates the expected beta (x and y) based on eQTL lead SNP (red triangles). Vertical and horizontal bars represent standard errors of the effects of individual SNPs.

allele in *WT1* (rs11031796-G, proxy for rs10219425-A, $r^2 = 1$ in 1KG CEU) and increased *WT1-AS* expression in the heart (atrial appendage) (Supplementary Data 4). The lead SNPs for the eQTL in the heart (rs3858454) and GWAS (rs10219425) were in high LD ($r^2 = 0.82$ in 1KG CEU) (Supplementary Fig. 8), and the result of the SMR/HEIDI test suggested their co-localization (Supplementary Data 5).

In addition, we searched for sQTL using the GTEx Portal browser and identified rs11031796 as a significant sQTL for splicing events at chr11:32452131:32453262 in the uterus, ovary, vagina and visceral adipose tissues (Supplementary Data 6). An extended sQTL search analyzing a set of sQTL summary statistics [GTEx_V8_cis_sqtl_summary(hg19);V8release] (please see Supplementary Note for details) revealed that rs11031796 was significantly associated with additional alternative splicing events other than chr11:32452131:32453262 (Supplementary Data 7, Supplementary Fig. 9). We observed a general trend that carrying POP risk allele were associated with increased splicing events at chr11:32435846:32452021, chr11:32452131:32453262 and chr11:32452131:32457915, and decreased splicing events at chr11:32435846:32437070, chr11:32435846:32437845 and chr11:32437197:32437845, which may increase the *WT1-AS* isoforms 206, 211, 212, and decrease the *WT1-AS* isoforms 201, 205, 208, although splice sites with statistically significant association were different by the tissues, (Supplementary Data 7, Supplementary Fig. 9). The results of the SMR/HEIDI test indicated not all but some of sQTL signals had significant association with POP (p-SMR < 0.05, Supplementary Data 7), and POP susceptibility and splicing at *WT1-AS* were likely affected by the same causal variant (*P*-value of HEIDI test ≥ 0.05, Supplementary Data 7). Regional plots of sQTL loci with p-SMR < 0.05 and p-HEIDI ≥ 0.05 are shown in Supplementary Fig. 10.

Gene-based analyses using MAGMA

Next, we performed a gene-based association analysis using MAGMA[20,21] with data from a Japanese GWAS meta-analysis. The analysis identified that *WT1* was significantly associated with POP ($P < 2.55 \times 10^{-6} = 0.05/19{,}589$, Supplementary Data 8). Because the gene-based association between the *WT1* gene and POP was no longer significant after including the effect of the lead SNP (rs10742277) as covariate in the GWAS ($P = 0.24$, Supplementary Data 8), the significant association of *WT1* with POP may simply reflect the effect of rs10742277. A gene-set analysis identified that a gene set, namely "Intracellular sphingolipid homeostasis," was significantly enriched in POP susceptibility genes ($P < 2.94 \times 10^{-6} = 0.05/17{,}012$, Supplementary Data 9). We also performed tissue enrichment analyses using MAGMA; however no significant enrichment was observed (Supplementary Fig. 11A).

A gene-based association analysis using a cross-ancestry GWAS meta-analyses data combining Japanese and European populations identified 21

genes significantly associated with POP in total including three genes that are not located within the previously reported POP susceptibility loci: *FMNL2*, *AC104809.3* (a.k.a. *CROCC2*) and *DMRT2*, ($P < 2.68 \times 10^{-6} = 0.05/18{,}689$, Supplementary Data 10). A gene-set analysis identified that three gene sets, namely "Skeletal muscle development," "Cartilage development," and "Connective tissue development," were significantly enriched in POP susceptibility genes ($P < 2.94 \times 10^{-6} = 0.05/17{,}007$, Supplementary Data 11). Tissue enrichment analyses indicated that the expression of POP susceptibility genes identified by the gene-based analysis was significantly enriched in nine tissues, notably the pelvic organ tissues including the uterus, cervix, ovary, and bladder (Supplementary Fig. 11B)

Genetic correlations between POP and other traits in the Japanese population

We assessed the genetic overlap between POP and other traits by evaluating the genetic correlations using LD score regression (LDSC)[22]. We incorporated the results of a previously reported Japanese GWAS for quantitative traits, including body mass index (BMI)[23], systolic and diastolic blood pressures (SBP, DBP)[24], inguinal hernia (IH)[25], and type 2 diabetes (T2D)[26]. We did not observe any significant genetic overlap between POP and other traits, which was likely due to the modest sample size of the Japanese POP_GWAS, although the direction of the effect of POP was consistent with that of IH, T2D, higher SBP, DBP, or BMI (Rg > 0, Supplementary Data. 12).

Discussion

In this study, we performed a GWAS meta-analysis of POP in 771 Japanese patients with POP and 76,625 controls and identified the *WT1* locus as susceptibility to POP in the Japanese population. After combining Japanese GWAS and previously reported European GWAS data[13], we additionally identified a novel locus, namely *FGFR2*, for susceptibility to POP with a genome-wide significant association ($p < 5 \times 10^{-8}$).

This is the first GWAS for POP in a non-European population. Although novel POP susceptibility loci with genome-wide significant associations were not identified in the Japanese GWAS, we identified the most significant association at the *WT1* locus, which was not significant in the initial European GWAS[12]. Risk allele frequencies of *WT1* were significantly different by populations; rs10742277-C: AF = 0.629 in Europeans and 0.25–0.26 in the Japanese Hondo, and 0.20–0.21 in controls for Japanese Ryukyu. The effect size of rs10742277-C in the Japanese population was significantly larger than that in the European population, suggesting that the *WT1* locus is more relevant for Japanese women. Since the sample size in this study is smaller than those in the European studies especially for case groups, the possibility for Winners Curse effect might exist[27]. Then, we performed four analyses for correcting the Winners Curse effect; Conditional Likelihood (CL), empirical Bayes (EB), bootstrap methods (boot), and FDR Inverse Quantile Transformation (FIQT) method implemented in the R package "winnerscurse"[27]. The effect sizes (β-estimates) of rs10742277 in the Japanese were decreased after the corrections (Supplementary Data. 13); therefore, the effect size of this locus in the Japanese might be overestimated in the original GWAS, although the corrected effect sizes are considered still larger than European populations.

Although the involvement of *WT1* antisense RNA (*WT1-AS*) in POP susceptibility has not yet been reported, our results of eQTL/sQTL analyses suggest a possible model that the effect of causal variant in *WT1* on POP susceptibility is mediated by the increased expression of *WT1-AS* and altered relative abundance of *WT1-AS* splicing isoforms, that is consistent with the observed SMR/HEIDI results[19]. *WT1-AS* is a non-cording RNA located upstream of the *WT1*; these two genes are bi-directionally transcribed from the same promoter region. Although previous studies have extensively reported the role of *WT1-AS* in different types of cancer[28,29], the physiological role of *WT1-AS* in non-tumor cells remains unclear. *WT1-AS* co-expresses/co-localizes with *WT1* and can bind to both WT1 mRNA and protein to regulate the WT1 protein expression/abundance in tumor cells[28,30]. WT1 is a transcription factor that plays an important role in genitourinary system development as well as an inhibitory role in the development and progression of Wilms tumor[31–33]. Although the precise molecular mechanisms remain unclear, it is assumed that increased *WT1-AS* expression, alternatively spliced *WT1-AS* isoforms, or both may affect WT1 protein expression/abundance in various tissues, including the pelvic organs, which might contribute to POP susceptibility.

In the cross-ancestral GWAS, we identified a novel POP locus, rs7072877, which is located approximately 9 kbp downstream of *FGFR2*. The results of eQTL analysis suggested a possible model that the effect of causal variant in *FGFR2* on susceptibility to POP was mediated by reduced *FGFR2* expression, that was consistent with the observed SMR/HEIDI results. *FGFR2* (Fibroblast growth factor receptor 2) is one of the four FGFR family members (FGFR1-4) that encode transmembrane receptor tyrosine kinases[34]. It plays an essential role in the regulation of osteoblast differentiation, proliferation and apoptosis, and is required for normal skeleton development[35]. Pathogenic gain-of-function variants of *FGFR2* cause syndromic craniosynostosis, including Crouzon syndrome, Pfeiffer syndrome, Apert syndrome, and Jackson-Weiss syndrome[36–38], whereas loss-of-function variants of *FGFR2* are responsible for Lacrimo-auriculo-dento-digital (LADD) syndrome[39,40]. Somatic mutations, structural amplifications, and fusions in *FGFR2* have been observed in multiple types of cancer[34]. However, to our knowledge, there have been no reports suggesting a role for *FGFR2* in POP susceptibility. Cumulative findings from animal studies suggested that *fgfr2* played a crucial role in the formation and maintenance of connective tissues[41–43]. In addition, *FGFR2* was identified as one of candidate genes for cervical artery dissections (CAD) coexisted with Ehlers-Danlos syndrome (EDS)-III like connective tissue alterations by a linkage analysis[44]. Although further studies are required to verify the causality of altered *FGFR2* expression in connective tissues on POP susceptibility, depletion of *FGFR2* in the connective tissues supporting the vagina and pelvic organs or in the muscles in the pelvic floor may be proposed as one of the mechanisms contributing to the pathophysiology of POP. Notably, the GTEx eQTL analysis identified significant correlation between rs7072877 and *FGFR2* expression only in three vascular tissues out of 49 tissues, which is in line with the statement by Pujol-Gualdo et al.[13] that many GWAS associations previously reported by European study (*WT1, KLF13, DUSP16, MAFF, VCL*, and *LDAH*) suggested a link between metabolic and cardiovascular health and POP[13].

Our study has some limitations. First, the sample size of case group in Japanese GWAS was small. Second, detailed clinical information, such as the number of vaginal childbirths, prior hysterectomy, smoking status, history of constipation, and asthma was not available for all participants. As these are considered important confounders of POP risk, the results of our Japanese GWAS could have been influenced by these factors. Lastly, possible misclassification of case and control samples may reduce the statistical power, since controls of the OBi study were recruited from the general population and the diagnosis of POP in BBJ was based on existing medical records in multiple medical institutions. BBJ cases were selected from participants with POP or a past history of POP based on self-reports or medical records for underwent surgeries or other interventions (i.e., pessary). Therefore, several POP cases might be misclassified as controls, especially early-stage or asymptomatic cases. In the OBi, all POP cases were recruited by medical specialists for POP, and diagnosis of POP was made based on POP Quantification (POP-Q) system. As a result, most of the cases were surgical cases (95.4%) and symptomatic POP cases with stage 3 or higher in POP-Q system (91.8%). Because relatively advanced stages of POP patients were analyzed as cases in both cohorts, it is suggested that considerable phenotypic heterogeneity between the two cohorts did not exist in this study.

In conclusion, we conducted Japanese GWAS and subsequent cross-ancestral GWAS meta-analysis. We identified *WT1* as a significant susceptibility locus for POP in the Japanese population. By combining our Japanese GWAS data with previously reported European GWAS data, we identified a novel locus, *FGFR2*, which contributes to POP susceptibility. Our study provides valuable insights into the etiology of POP, however an

analysis with a larger sample size is essential to clarify the genetic architecture of POP in the Japanese population.

Materials and Methods

Participants, genotyping and quality control

Okinawa Bioinformation Bank (OBi). We recruited patients with POP ($n = 328$) who visited the outpatient clinic of the urology department of University of the Ryukyus Hospital, Okinawa Kyodo Hospital, and Urogyne Center of Japanese Red Cross Gifu Hospital between 2016 and 2023. Female patients diagnosed with POP (cystocele, uterine prolapse, vaginal prolapse) were invited to participate in this study. Participants with uncured cancer or those under 40 years of age were excluded. Each subject participated in an interview and underwent a clinical examination and Pelvic Organ Prolapse Quantification (POP-Q) system assessment (stage 0: 0%, stage 1: 0%, stage 2: 8.2%, stage 3: 77.5%, stage 4: 14.3%). We selected controls ($n = 2750$) from female participants aged ≥ 40 years from the general population registered in the Okinawa Bioinformation Bank (OBi)[17,18]. Demographic origins of individuals were surveyed for some of the participants in the OBi Project; information about the birthplace (islands) of their four grandparents was obtained by questionnaire for some participants.

Genomic DNA was extracted from peripheral leukocytes or saliva using standard procedures. All individuals were genotyped using the Illumina Asian Screening Array (Illumina,Inc, San Diego, CA, USA) (655,904 SNPs ;autosomes: 629,779 SNPs and X chromosome: 26,125 SNPs). As a quality control (QC) of samples, we excluded individuals with (1) sample call rates < 0.98, (2) individuals having a shared identity-by-descent ($\hat{\pi} \geq 0.95$), (3) sex mismatch between genotypic and phenotypic information, and (4) outliers from the East Asians identified by a principal component analysis, using genotyped samples and the three major reference populations (Africans, Europeans and East Asians) from the International HapMap Project. For the QC of SNPs, we excluded SNPs with (1) call rate < 0.98, (2) genotype distributions were not in accordance with Hardy–Weinberg equilibrium ($p < 1 \times 10^{-6}$), and (3) minor allele frequency (MAF) < 0.01. We used Plink ver.1.9 and v2.0 software for this QC process[45] As a result, 3057 participants (324 cases and 2733 controls) and 328,400 SNPs (autosome: 312,566 SNPs and X chromosome: 15,834 SNPs) passed these QCs.

Filtered genotyping data were phased using Eagle v2.3[46]. Genotype imputation was performed by minimac4[47] using the 1KGP3 + JEWEL_3K (1000 genomes phase 3 + 3256 Japanese WGS) as the reference panel for autosomes and X-chromosome)[48]. We selected SNPs with MAF ≥ 0.005 and imputation quality Rsq ≥ 0.7 for the analyses.

BioBank Japan. The BBJ is a hospital-based disease cohort which consists of DNA samples and clinical data from patients with 47 target diseases[49]. Clinical information, including comorbidities and past medical history, of each participant was registered in BBJ by doctors-in-charge[49]. All individuals in BBJ were genotyped using Illumina Infinium Omni Express, Human Exome, Infinium Omni Express Exome v1.0, or Infinium Omni Express Exome v1.2 (Illumina Inc). We defined POP cases as follows.1) patients with cystocele, uterine prolapse, or vaginal prolapse as comorbidities or 2) patients having past medical history for cystocele, uterine prolapse, or vaginal prolapse. Controls were female participants with no history of POP. Participants aged less than 40 years were excluded, and 450 cases and 75,761 controls were analyzed in this study.

For the QC of samples, we excluded individuals with (1) sample call rates < 0.98, (2) genetically identical to others, (3) sex mismatch between genotypic and phenotypic information, and (4) outliers from East Asians identified by principal component analysis using genotyped samples and the three major reference populations (Africans, Europeans and East Asians) from the International HapMap Project. For the QC of SNPs, we excluded SNPs with (1) call rate < 0.99, (2) p-values for Hardy–Weinberg equilibrium (HWE) test < 1×10^{-6}, and (3) minor allele frequency (MAF) < 0.01. Plink v.19 software was used for this QC process[45]. A total of 503,998 QCed SNPs (autosomes: 496,226 SNPs and X chromosome: 7772 SNPs) were used for further analyses.

Genotype imputation was performed as described previously in ref. 25. In brief, we phased the genotyping data of autosomal chromosomes using SHAPEIT2 (version2.837), and then imputed with minimac4 (version 2.0.1)[47] using a reference panel consisting of 3256 Japanese WGS from BBJ and 2,504 individuals from the 1KGP (phase3v5). We selected SNPs for analyses with MAF ≥ 0.005 and imputation quality Rsq ≥ 0.7.

GWAS and GWAS meta-analysis

We first performed PCA using direct genotyped data of autosomal SNPs in BBJ and OBi individually to define the Japanese subpopulations; the Hondo (literally translated as main-islands) cluster for the Japanese Archipelago and the Ryukyu cluster for the Ryukyu Archipelago (Supplementary Fig. 2). The Association with POP was analyzed using the imputed gene dosage data for each of the four independent populations: BBJ_Hondo, BBJ_Ryukyu, OBi_Hondo, and OBi_Ryukyu. We applied the generalized mixed model implemented in SAIGE version 1.0.5[50] for the association analysis, which controls for case–control imbalance. Age, and PC1-3 were included as covariates. SNPs with MAF < 0.005 and imputation quality Rsq < 0.7 were excluded from association analyses.

GWAS data from the four study groups (BBJ_Hondo, BBJ_Ryukyu, OBi_Hondo, and OBi_Ryukyu) were subsequently combined by meta-analysis using the METAL program[51]. A fixed-effect model with inverse-variance weighted was used for all meta-analyses. The heterogeneity of effects across studies was assessed using Cochran's Q-test implemented in METAL.

We also conducted a cross-ancestry meta-analysis by combining our Japanese GWAS (BBJ_Hondo, BBJ_Ryukyu, OBi_Hondo, and OBi_Ryukyu) with a publicly available European POP GWAS[13]. European GWAS summary statistics were obtained from https://www.ebi.ac.uk/gwas/studies/GCST90102470. We retained the SNPs present in all studies for the cross-ancestry GWAS meta-analysis.

Gene-based association analysis and gene-set analysis

Gene-based association analyses and gene-set analyses were conducted using MAGMA v1.08[20], implemented in FUMA v1.6.0[21]. We used the summary statistics of the Japanese meta-analysis for 7,496,192 SNPs and cross ancestral GWAS meta-analysis for 5,417,787 SNPs with high imputation quality ($r^2 \geq 0.7$). SNPs for which association data were available for all the study groups were used for the analyses. SNPs in the Japanese and the cross ancestral GWAS were mapped to 19,589 and 18,689 protein-coding genes (hg19 build) respectively, and gene-based *P*-values were calculated. Linkage disequilibrium (LD) from the 1000 G Phase 3 ASN and ALL was used for the Japanese and cross-ancestry GWAS data respectively. Window sizes were set between 3 kb upstream and 1 kb downstream of genes. The statistical significance of the gene-based analysis was set at a Bonferroni-corrected $P < 2.55 \times 10^{-6}$ (0.05/19,589) for the Japanese study, and $P < 2.68 \times 10^{-6}$ (0.05/18,689) for the cross ancestral study. In the gene-set analysis, 17,012 (Japanese) and 17,007 (cross ancestry) gene sets (curated gene sets and GO terms) from MsigdB v7.0 were examined; therefore, statistical significance was set at $P < 2.94 \times 10^{-6}$ (0.05/17,012 or 17,007).

Expression/ splicing quantitative trait (eQTL/sQTL) analysis

We assessed cis-eQTL and sQTL effects by searching publicly available data as of November 2023: GTEx portal v8 (https://gtexportal.org/home/). In addition, a set of sQTL summary statistics [GTEx_V8_cis_sqtl_summary(hg19);V8release] was retrieved from the SMR portal site (https://yanglab.westlake.edu.cn/software/smr/#Overview as of November 2023).

Summary data-based Mendelian randomization (SMR) analysis /heterogeneity in dependent instruments (HEIDI) test

Co-localization and causality between genome-wide associations and gene expression regulation were analyzed using the SMR/HEIDI test[19]. We

analyzed the co-localization and causality of two POP GWAS loci, *WT1* and *FGFR2*, with their significant eQTL/sQTL identified by a GTEx search. The cross-ancestral GWAS meta-analysis for POP in this study was used as GWAS data set. We obtained the eQTL/sQTL datasets [#V8 release of the GTEx eQTL/sQTL summary data], from the SMR Portal site: https://yanglab.westlake.edu.cn/software/smr/#DataResource. Genotyping data from the 1KGP EUR population (release 20130502 phase3) were used as the LD reference. *P*-value of HEIDI test (*P*-HEIDI) ≥ 0.05 was considered that the GWAS signal and eQTL/sQTL were co-localized, and *P*-value of SMR test (*P*-SMR) < 0.05 was considered disease susceptibility was mediated by gene expression/altered splicing events. SMR locus plots were created using R-script as described by Zhu et al.[19].

Genetic correlation

We conducted bivariate LD score regression[22] to quantify genetic correlations between POP and other diseases, such as type 2 diabetes[26] and inguinal hernias[25], and 59 quantitative traits including anthropometric, metabolic, kidney-related, haematological and blood pressure traits[23,24]. For the regression analysis, we used the population-specific LD score (1000 G ASN 2014) and summary statistics of high-quality common SNPs present in the HapMap 3 reference panel for each available trait or disease. Significant genetic correlations were defined as $P < 0.05$.

Correction for an effect size using the Winner's Curse methods

To evaluate the possible bias on effect size of rs10742277 in Japanese GWAS, we calculated corrected effect size using the Winner's Curse methods. We applied four correcting methods implemented in the R package "winnerscurse"[27]. The package includes Conditional Likelihood (CL) methods[52], Empirical Bayes (EB) method[53], FDR Inverse Quantile Transformation (FIQT) method[54], and Bootstrap (boot) method[27]. For analysis using CL methods, we used 25 variants with genome-wide significant ($p < 5 \times 10^{-8}$) association in Japanese POP GWAS. For other three methods, EB, FIQT and boot, we used 7,496,192 SNPs which association data was available from all of four Japanese sub-group. Effect sizes in European GWAS were used as "replication set". We calculated adjusted beta as well as adjusted standard error with 100 bootstrap replicates for EB, FIQT and boot. Since standard error estimation using bootstrap was not available for adjusted beta estimated by CL method, we calculated adjusted 95% CI instead of standard error. The estimated mean square error (MSE) of SNPs were calculated to evaluate the estimation of each method.

Softwares

The Manhattan and quantile- quantile plots were generated using FUMA v1.6.0[21], and regional association plots were generated using LocusZoom[55].

Statistics and reproducibility

GWAS for POP in Japanese population (OBi and BBJ) were performed using a generalized mixed model implemented in SAIGE version 1.0.5[50] including age and top three PC as covariates. Association data was combined with an inverse variance fixed-effects meta-analysis using METAL[51]. Significance threshold of the GWAS meta-analysis was $P < 5.0 \times 10^{-8}$ accounting for multiple testing. Gene-based association analyses and Gene-set analysis were conducted using MAGMA v1.08[20], implemented in FUMA v1.6.0[21] as of October 2023. We applied Bonferroni-corrected significance thresholds for analyses with MAGMA as shown in Supplementary Data 8–11. The eQTL and sQTL analyses were conducted using the GTEx portal v8 (http://www.gtexportal.org/home/) as of November 2023. SMR/HEIDI test was performed using SMR version 1.3.1[19]. Significance threshold of SMR/HEIDI test was $P < 0.05$. LDSC (version 1.0.1)[22] was applied to estimate SNP-based heritability and genetic correlation. Significance threshold of LDSC was $P < 0.05$. For all analyses using case-control sample in Japanese (BBJ and OBi) and European[13], the sample sizes are provided in Supplementary Fig. 1.

Ethics

All participants provided written informed consent before their enrolment in this study. The study protocol conformed to the provisions of the Declaration of Helsinki and was approved by the ethics committees of University of the Ryukyus (Nishihara, Japan) for Medical and Health Research Involving Human Subjects (Approval No 21-1826-08-02-00, 17-171-03-01-00), and the RIKEN Center for Integrative Medical Sciences (Yokohama, Japan) (Approval No. RIKEN-Y-2022-068). All ethical regulations relevant to human research participants were followed.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The summary statistics of Japanese GWAS for POP is available from Database Center for Life Science (DBCLS) website (https://humandbs.dbcls.jp/en/: Research ID: hum0467.v1.gwas.v1).

Received: 11 April 2024; Accepted: 10 September 2024;
Published online: 30 September 2024

References

1. Barber, M. D. Pelvic organ prolapse. *BMJ* **20**, i3853 (2016).
2. Kato, J. et al. Pelvic organ prolapse and Japanese lifestyle: prevalence and risk factors in Japan. *Int. Urogynecol. J.* **33**, 47–51 (2022).
3. Swift, S. et al. Pelvic Organ Support Study (POSST): the distribution, clinical definition, and epidemiologic condition of pelvic organ support defects. *Am. J. Obstet. Gynecol.* **192**, 795–806 (2014).
4. Vergeldt, T. F., Weemhoff, M., IntHout, J. & Kluivers, K. B. Risk factors for pelvic organ prolapse and its recurrence: a systematic review. *Int. Urogynecol. J.* **26**, 1559–1573 (2015). Review.
5. Giri, A., Hartmann, K. E., Hellwege, J. N., Velez Edwards, D. R. & Edwards, T. L. Obesity and pelvic organ prolapse: a systematic review and meta-analysis of observational studies. *Am. J. Obstet. Gynecol.* **217**, 11–26 (2017). Review.
6. Ashikari, A., Suda, T. & Miyazato, M. Collagen type 1A1, type 3A1, and LOXL1/4 polymorphisms as risk factors of pelvic organ prolapse. *Bmc. Res. Notes* **14**, 15 (2021).
7. Ellerkmann, R. M. et al. Correlation of symptoms with location and severity of pelvic organ prolapse. *Am. J. Obstet. Gynecol.* **185**, 1332–1338 (2001).
8. Jelovsek, J. E. & Barber, M. D. Women seeking treatment for advanced pelvic organ prolapse have decreased body image and quality of life. *Am. J. Obstet. Gynecol.* **194**, 1455–1461 (2006).
9. Raju, R. & Linder, B. J. Evaluation and Management of Pelvic Organ Prolapse. *Mayo. Clin. Proc.* **96**, 3122–3129 (2021).
10. Swift, S. E., Tate, S. B. & Nicholas, J. Correlation of symptoms with degree of pelvic organ support in a general population of women: what is pelvic organ prolapse? *Am. J. Obstet. Gynecol.* **189**, 372–379 (2003).
11. Altman, D., Forsman, M., Falconer, C. & Lichtenstein, P. Genetic influence on stress urinary incontinence and pelvic organ prolapse. *Eur. Urol.* **54**, 918–922. (2008).
12. Olafsdottir, T. et al. Genome-wide association identifies seven loci for pelvic organ prolapse in Iceland and the UK Biobank. *Commun. Biol.* **17**, 129 (2020). Mar.
13. Pujol-Gualdo, N. et al. Advancing our understanding of genetic risk factors and potential personalized strategies for pelvic organ prolapse. *Nat. Commun.* **13**, 3584 (2022).
14. Hendrix, S. L. et al. Pelvic organ prolapse in the Women's Health Initiative: gravity and gravidity. *Am. J. Obstet. Gynecol.* **186**, 1160–1166 (2002).

15. Cheung, R. Y. K. et al. Pelvic organ prolapse in Caucasian and East Asian women: a comparative study. *Ultrasound Obstet. Gynecol.* **53**, 541–545 (2019).
16. Yamaguchi-Kabata, Y. et al. Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. *Am. J. Hum. Genet.* **83**, 445–456 (2008).
17. Matsunami, M. et al. Fine-Scale Genetic Structure and Demographic History in the Miyako Islands of the Ryukyu Archipelago. *Mol. Biol. Evol.* **38**, 2045–2056 (2021).
18. Liu, X. et al. Natural Selection Signatures in the Hondo and Ryukyu Japanese Subpopulations. *Mol. Biol. Evol.* **40**, msad231 (2023).
19. Zhu, Z. et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat. Genet.* **48**, 481–487 (2016).
20. de Leeuw, C. A., Mooij, J. M., Heskes, T. & Posthuma, D. MAGMA: generalized gene-set analysis of GWAS data. *PLoS. Comput. Biol.* **11**, e1004219 (2015).
21. Watanabe, K., Taskesen, E., van Bochoven, A. & Posthuma, D. Functional mapping and annotation of genetic associations with FUMA. *Nat. Commun.* **8**, 1826 (2017).
22. Bulik-Sullivan, B. et al. An atlas of genetic correlations across human diseases and traits. *Nat. Genet.* **47**, 1236–1241 (2015).
23. Akiyama, M. et al. Genome-wide association study identifies 112 new loci for body mass index in the Japanese population. *Nat. Genet.* **49**, 1458–1467 (2017).
24. Kanai, M. et al. Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. *Nat. Genet.* **50**, 390–400 (2018).
25. Hikino, K. et al. Susceptibility loci and polygenic architecture highlight population specific and common genetic features in inguinal hernias: genetics in inguinal hernias. *EBioMedicine* **70**, 103532 (2021).
26. Suzuki, K. et al. Identification of 28 new susceptibility loci for type 2 diabetes in the Japanese population. *Nat. Genet.* **51**, 379–386 (2019).
27. Forde, A. et al. Review and further developments in statistical corrections for Winner's Curse in genetic association studies. *PLoS Genet* **19**, e1010546 (2023).
28. Zhang, Y. et al. Long Non-coding Wilms Tumor 1 Antisense RNA in the Development and Progression of Malignant Tumors. *Front. Oncol.* **10**, 35 (2020).
29. Jia, M. et al. WT1-AS/IGF2BP2 Axis Is a Potential Diagnostic and Prognostic Biomarker for Lung Adenocarcinoma According to ceRNA Network Comprehensive Analysis Combined with Experiments. *Cells* **11**, 25 (2021).
30. Dallosso, A. R. et al. Alternately spliced WT1 antisense transcripts interact with WT1 sense RNA and show epigenetic and splicing defects in cancer. *RNA* **13**, 2287–2299 (2007).
31. Pritchard-Jones, K. et al. The candidate Wilms' tumour gene is involved in genitourinary development. *Nature* **346**, 194–197 (1990).
32. Parenti, R. et al. Wilms' tumor 1 (WT1) protein expression in human developing tissues. *Acta Histochem.* **117**, 386–396 (2015). Review.
33. Hsu, S. Y. et al. Wilms' tumor protein WT1 as an ovarian transcription factor: decreases in expression during follicle development and repression of inhibin-alpha gene promoter. *Mol. Endocrinol.* **9**, 1356–1366 (1995).
34. Katoh, M. Fibroblast growth factor receptors as treatment targets in clinical oncology. *Nat. Rev. Clin. Oncol.* **16**, 105–122 (2019).
35. Su, N., Jin, M. & Chen, L. Role of FGF/FGFR signaling in skeletal development and homeostasis: learning from mouse models. *Bone Res.* **2**, 14003 (2014). Review.
36. Wang, Y. et al. FGFR2 Mutation p.Cys342Arg Enhances Mitochondrial Metabolism-Mediated Osteogenesis via FGF/FGFR-AMPK-Erk1/2 Axis in Crouzon Syndrome. *Cells* **11**, 3129 (2022).
37. Holmes, G. et al. Midface and upper airway dysgenesis in FGFR2-related craniosynostosis involves multiple tissue-specific and cell cycle effects. *Development* **145**, dev166488 (2018).
38. Wilkie, A. O. et al. Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome. *Nat. Genet.* **9**, 165–172 (1995).
39. Rohmann, E. et al. Mutations in different components of FGF signaling in LADD syndrome. *Nat. Genet.* **38**, 414–417 (2006).
40. Lew, E. D. et al. Structural basis for reduced FGFR2 activity in LADD syndrome: Implications for FGFR autoinhibition and activation. *Proc. Natl Acad. Sci. Usa.* **104**, 19802–19807 (2007).
41. Cool, S. M. et al. Temporal expression of fibroblast growth factor receptors during primary ligament repair. *Knee. Surg. Sports Traumatol. Arthrosc.* **12**, 490–496 (2003).
42. Nichol, P. F. et al. Conditional mutation of fibroblast growth factor receptors 1 and 2 results in an omphalocele in mice associated with disruptions in ventral body wall muscle formation. *J. Pediatr. Surg.* **6**, 90–96 (2011).
43. Wernlé, K. K. et al. Loss of Fgfr1 and Fgfr2 in Scleraxis-lineage cells leads to enlarged bone eminences and attachment cell death. *Dev. Dyn.* **252**, 1180–1188 (2023).
44. Wiest, T. et al. Genetic analysis of familial connective tissue alterations associated with cervical artery dissections suggests locus heterogeneity. *Stroke* **37**, 1697–1702 (2006).
45. Chang, C. C. et al. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* **4**, 7 (2015).
46. Loh, P. R. et al. Reference-based phasing using the Haplotype Reference Consortium panel. *Nat. Genet.* **48**, 1443–1448 (2016).
47. Das, S. et al. Next-generation genotype imputation service and methods. *Nat. Genet.* **48**, 1284–1287 (2016).
48. Terao, C. et al. Population-specific reference panel improves imputation quality and enhances locus discovery and fine-mapping Research Square (preprint) https://www.researchsquare.com/article/rs-3194976/v1.
49. Nagai, A. et al. Overview of the BioBank Japan Project: Study design and profile. *J. Epidemiol.* **3S**, S2–S8 (2017).
50. Zhou, W. et al. Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. *Nat. Genet.* **50**, 1335–1341 (2018).
51. Willer, C. J. et al. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* **26**, 2190–2191 (2010).
52. Ghosh, A. et al. Estimating odds ratios in genome scans: an approximate conditional likelihood approach. *Am. J. Hum. Genet* **82**, 1064–1074 (2008).
53. Ferguson, J. P. et al. Empirical Bayes correction for the Winner's Curse in genetic association studies. *Genet. Epidemiol.* **37**, 60–68 (2013).
54. Bigdeli, T. B. et al. A simple yet accurate correction for winner's curse can predict signals discovered in much larger genome scans. *Bioinformatics* **32**, 2598–2603 (2016).
55. Pruim, R. J. et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* **26**, 2336–2337 (2010).

Acknowledgements

The authors thank all participating doctors, staff, and patients from the collaborating institutes for providing the DNA samples. We thank Ms. Naoko Miyagawa, RIKEN Center for Integrative Medical Sciences for her technical assistance. This work was partly supported by a grant for Project for Whole Implementation to Support and Ensure the Female Life—Wise from the Japan Agency for Medical Research and Development (AMED) to MM (Grant Number JP22gk0210025), and a grant from the promotion project of innovation-eco system collaborating organization from the Okinawa prefecture to SM.

Author contributions

Conception and Design: Minoru Miyazato, Shiro Maeda. Resources: OBi; Asuka Ashikari, Kosei Miwa, Katsumi Kadekawa, Tetsuji Suda, Minoru Miyazato, Shiro Maeda, BBJ: Chikashi Terao, Koichi Matsuda. Data curation: Masatoshi Matsunami, Asuka Ashikari, Xiaoxi Liu, Kohei Tomizuka, Keiko Hikino. Analysis – Genome wide association studies: Masatoshi Matsunami, Xiaoxi Liu. Analysis – Gene base, gene set analyses: Minako Imamura. Analysis –Genetic correlation: Masatoshi Matsunami. Analysis – eQTL/sQTL: Minako Imamura. Analysis- SMR/HEIDI: Masatoshi Matsunami, Xiaoxi Liu. Supervising and data interpretation: Chikashi Terao, Minoru Miyazato, Shiro Maeda, Writing – original draft: Masatoshi Matsunami, Minako Imamura. Writing – review & editing: Chikashi Terao, Minoru Miyazato, Shiro Maeda. All authors approved the final manuscript. Shiro Maeda is the guarantor of the work and as such had full access to all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s42003-024-06875-2.

Correspondence and requests for materials should be addressed to Chikashi Terao or Shiro Maeda.

Peer review information *Communications Biology* thanks Triin Laisk and the other, anonymous, reviewers for their contribution to the peer review of this work. Primary Handling Editors: Aylin Bircan, Laura Rodriguez Perez. A peer review file is available.

The Biobank Japan project

Koichi Matsuda[9,10], Takayuki Morisaki[9,10], Yukinori Okada[13], Yoichiro Kamatani[14], Kaori Muto[9], Akiko Nagai[9], Yoji Sagiya[10], Natsuhiko Kumasaka[9], Yoichi Furukawa[9], Yuji Yamanashi[9], Yoshinori Murakami[9], Yusuke Nakamura[9], Wataru Obara[15], Ken Yamaji[16], Kazuhisa Takahash[17], Satoshi Asai[18,19], Yasuo Takahashi[19], Shinichi Higashiue[20], Shuzo Kobayashi[20], Hiroki Yamaguchi[21], Yasunobu Nagata[21], Satoshi Wakita[21], Chikako Nito[22], Yu-ki Iwasaki[23], Shigeo Murayama[24], Kozo Yoshimori[25], Yoshio Miki[26], Daisuke Obata[27], Masahiko Higashiyama[28], Akihide Masumoto[29], Yoshinobu Koga[29] & Yukihiro Koretsune[30]

[13]Department of Genome Informatics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan. [14]Laboratory of Complex Trait Genomics, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan. [15]Department of Urology, Iwate Medical University, Iwate, Japan. [16]Department of Internal Medicine and Rheumatology, Juntendo University Graduate School of Medicine, Tokyo, Japan. [17]Department of Respiratory Medicine, Juntendo University Graduate School of Medicine, Tokyo, Japan. [18]Division of Pharmacology, Department of Biomedical Science, Nihon University School of Medicine, Tokyo, Japan. [19]Division of Genomic Epidemiology and Clinical Trials, Clinical Trials Research Center, Nihon University. School of Medicine, Tokyo, Japan. [20]Tokushukai Group, Tokyo, Japan. [21]Department of Hematology, Nippon Medical School, Tokyo, Japan. [22]Laboratory for Clinical Research, Collaborative Research Center, Nippon Medical School, Tokyo, Japan. [23]Department of Cardiovascular Medicine, Nippon Medical School, Tokyo, Japan. [24]Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan. [25]Fukujuji Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan. [26]The Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Tokyo, Japan. [27]Center for Clinical Research and Advanced Medicine, Shiga University of Medical Science, Shiga, Japan. [28]Department of General Thoracic Surgery, Osaka International Cancer Institute, Osaka, Japan. [29]Iizuka Hospital, Fukuoka, Japan. [30]National Hospital Organization Osaka National Hospital, Osaka, Japan.