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ORIGINAL ARTICLE

Multi-omics genetic prioritization reveals distinct molecular evidence profiles for candidate targets in female infertility

Jinxin Li[1], Shaorong Gao[2,*], Qingran Kong[1,*]

[1]Department of Gynecology and Obstetrics, West China Second University Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

[2]Department of Frontier Science Center for Stem Cell Research, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China

ABSTRACT

Background: Female infertility is heterogeneous, and genetically supported candidate targets remain limited. Although genome-wide association studies have identified infertility-associated loci, the effector molecules and molecular layers through which these signals act remain unclear. **Methods:** Using FinnGen female infertility (N14_FEMALEINFERT), we integrated Mendelian randomization (MR) with summary-data-based Mendelian randomization (SMR) using protein quantitative trait locus (pQTL), expression quantitative trait locus (eQTL), and methylation quantitative trait locus (mQTL) data, cross-layer colocalization, public trait lookup, and tractability assessment. **Results:** Multilayer analyses retained three candidate genes: interleukin 18 (IL18), RELT tumor necrosis factor receptor (RELT), and alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase (ABO). Colocalization revealed distinct molecular evidence profiles across the retained candidates. IL18 showed the clearest transcript-level evidence, with strong eQTL colocalization (posterior probability for a shared causal variant [PP.H4] = 0.833), whereas comparable plasma pQTL or mQTL colocalization support was not observed. RELT showed its strongest support at the methylation layer, with strong mQTL colocalization (PP.H4 = 0.863) and secondary protein-layer support across both pQTL resources. ABO showed supportive MR and protein-layer SMR evidence, but its signal could not be confidently assigned to a single molecular layer. Considering molecular localization, pleiotropy, and tractability, IL18 showed the most favorable translational profile, whereas RELT was retained as a regulatory candidate requiring cautious interpretation. **Conclusions:** Cross-layer integration prioritized IL18 and RELT as informative candidates for female infertility, with IL18 supported mainly at the transcript level and RELT at the methylation level. This multilayer framework clarified the molecular layer contributing the strongest evidence for each candidate and refined candidate prioritization in a biologically heterogeneous phenotype.

Keywords: female infertility, Mendelian randomization, multi-omics, colocalization, target prioritization

INTRODUCTION

Female infertility is a common reproductive health problem worldwide. WHO estimates that about one in six people experience infertility during their lifetime.[1] Infertility is also associated with substantial psychological distress and poorer quality of life.[2,3] In clinical practice, female infertility is usually classified as ovulatory, tubal, uterine, endometriosis-related, or unexplained.[4] However, these categories do not neces-

***Corresponding Author:**
Qingran Kong, Department of Gynecology and Obstetrics, West China Second University Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China.
Email: kqr721726@scu.edu.cn. https://orcid.org/0000-0002-5314-0627
Shaorong Gao, Department of Frontier Science Center for Stem Cell Research, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China.
Email: gaoshaorong@tongji.edu.cn. https://orcid.org/0000-0003-1041-3928
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sarily reflect distinct biological mechanisms. There is substantial heterogeneity both between and within diagnostic groups, and broad infertility phenotypes likely capture multiple underlying reproductive processes rather than a single mechanism.[4,5] As a result, clinical evaluation and management remain organized largely around conventional diagnostic categories rather than molecularly defined mechanisms.[4,6,7]

Genome-wide association studies (GWAS) have begun to clarify the genetic basis of infertility and related reproductive traits.[5] However, translating association signals into plausible biological targets remains difficult. Most risk variants lie in non-coding regions, the nearest gene is often not the causal effector, and locus-level association alone rarely identifies the molecule most relevant to disease biology.[8–10] This challenge is particularly important in female infertility, a clinically defined and biologically heterogeneous endpoint that likely captures several distinct reproductive processes.[4,5] As a result, when a locus is associated with infertility risk, it is often unclear which effector molecule should be prioritized and at which molecular layer provides the clearest shared-signal evidence.[8,9]

Molecular quantitative trait locus (QTL) resources help address this problem by linking disease-associated loci to different levels of genetic regulation. Protein QTL (pQTL), expression QTL (eQTL), and methylation QTL (mQTL) each reflect a different aspect of molecular regulation.[11–13] Integrative approaches such as Mendelian randomization (MR), summary-data-based Mendelian randomization (SMR), and colocalization can move beyond locus discovery. They can be used to ask which molecular feature is most likely to underlie the disease signal and whether the molecular and disease associations are consistent with a shared causal variant.[10,14–16] Not all molecular layers are equally informative. A locus may show signals across more than one layer, but the strongest support may be confined to only one, while signals in other layers may reflect linkage, horizontal pleiotropy, or more distal regulatory effects.[14–16] For a heterogeneous phenotype such as female infertility, this distinction is important because it can help separate broadly detectable associations from candidates with clearer molecular support.[5,8,16]

Here, we applied a layer-resolved genetic prioritization framework to female infertility. Using FinnGen female infertility as the primary endpoint, we examined evidence across protein, transcript, and methylation layers and then interpreted the retained signals together with pleiotropy and tractability. We aimed not only to list molecules associated with infertility risk, but also to define the molecular evidence profile of each candidate and identify the layer providing its clearest shared-signal support. By doing so, we sought to move from locus-level association toward more biologically anchored target prioritization in a heterogeneous reproductive phenotype.

METHODS

Study design

We analyzed FinnGen female infertility (N14_FEMALEINFERT) as the primary outcome in a stepwise genetic prioritization workflow designed to identify and prioritize candidate genes. The analytical framework comprised five components: source-specific MR screening in independent pQTL datasets; SMR across protein, transcript, and methylation layers; locus-level colocalization; supplementary MR sensitivity analyses with public variant-level trait lookup to contextualize pleiotropy; and qualitative assessment of druggability and therapeutic tractability. This workflow was used to identify proteins for multilayer follow-up, characterize the predominant pattern of molecular-layer support, and inform qualitative candidate prioritization (Figure 1).[14,15]

Outcome dataset

The primary outcome was female infertility from FinnGen release R12, corresponding to phenotype code N14_FEMALEINFERT.[17] This registry-based endpoint was analyzed as an aggregate female infertility phenotype rather than as a cause-specific diagnosis. According to the FinnGen endpoint definition, N14_FEMALEINFERT is based on infertility-related registry diagnoses under ICD-10 N97, with ICD-10 N97.4, denoting female infertility associated with male factors, excluded from the hospital-discharge definition. The GWAS summary statistics included 18,189 cases and 114,009 controls aligned to the Genome Reference Consortium Human Build 38. Summary statistics were harmonized into a common internal format before subsequent analyses. All analyses were based on summary-level data only.

Molecular QTL datasets

Protein QTL datasets

Protein-level analyses used two independent plasma pQTL resources. The first was the deCODE SomaScan panel, which was used for source-specific MR screening, protein-level SMR, and colocalization analyses.[11] The second was the UK Biobank Pharma Proteomics Project (UKB-PPP) European Discovery panel, which was used as an independent resource for source-specific MR screening, protein-level SMR, and colocalization analyses.[18] Using two pQTL resources allowed us to assess whether protein-level signals were consistent across cohorts and proteomic platforms.[11] Detailed panel metadata and sample-size ranges are provided in the Supplementary Materials.

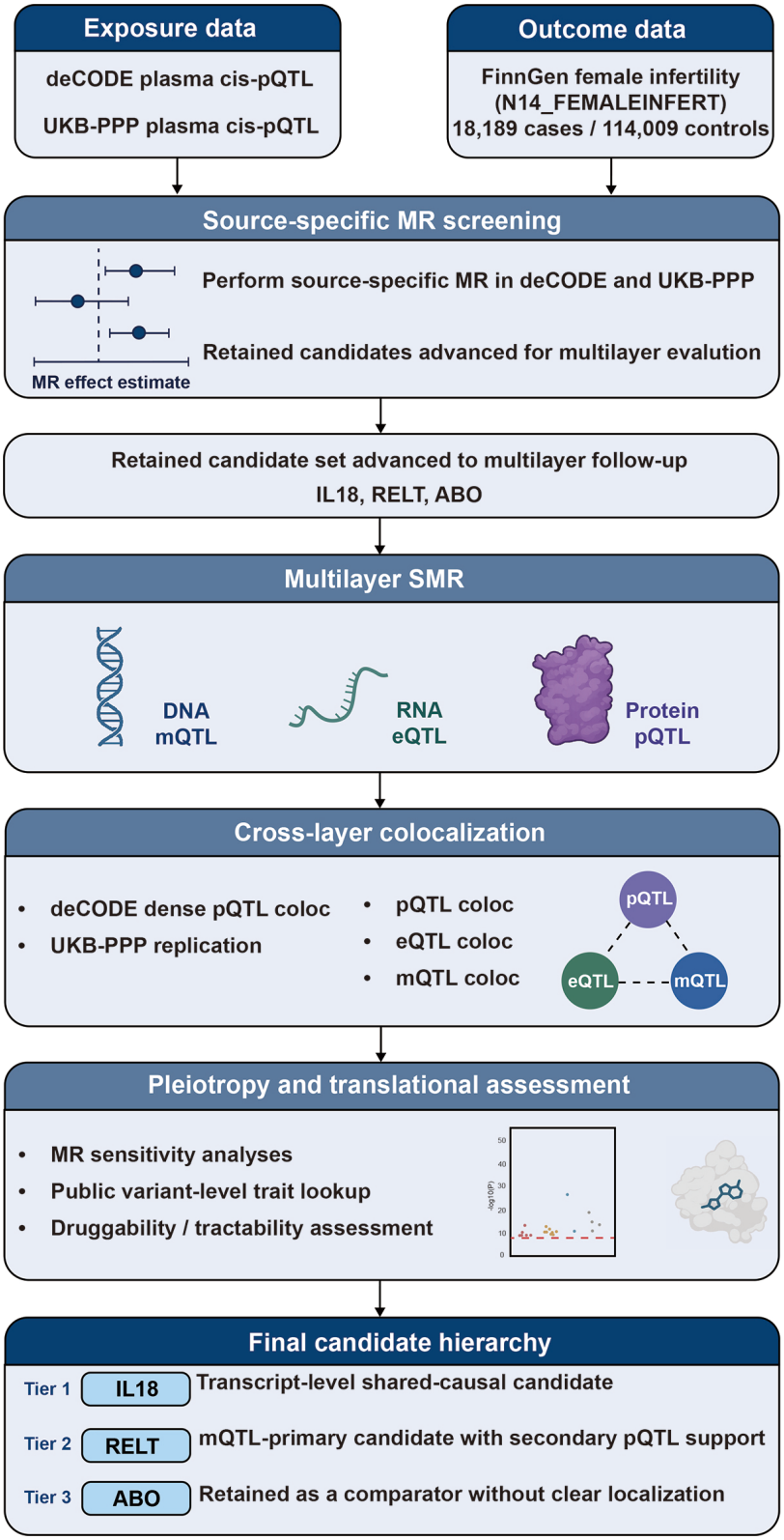

Figure 1. Study design and analytical workflow for multilayer genetic prioritization of candidate genes for female infertility. Multilayer SMR, colocalization, pleiotropy assessment, and therapeutic tractability evaluation followed plasma pQTL-based MR screening. SMR, summary-data-based Mendelian randomization; eQTL, expression quantitative trait locus; mQTL, methylation quantitative trait locus. IL18, interleukin 18; RELT, RELT tumor necrosis factor receptor; ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase.

eQTL and mQTL reference panels

Because tissue-specific QTL resources directly relevant to infertility were not consistently available, transcript- and methylation-level analyses were performed using curated eQTL and mQTL reference panels in Binary Efficient Summary Data format for SMR analysis. The underlying source resources and available panel information are described in the Supplementary Materials.[12,13] These panels were used for both SMR and colocalization analyses. For each retained locus, available eQTL and mQTL features were screened for evaluability, and one representative feature was selected for colocalization based on data completeness, variant overlap, interpretable SMR output, and association strength among the remaining evaluable features.

Source-specific Mendelian randomization screening

We performed source-specific MR screening separately in the deCODE and UKB-PPP pQTL resources, using FinnGen female infertility as the sole outcome.

Instrument selection and harmonization

Genetic instruments for each protein were selected from source-specific working pQTL tables at a significance threshold of $P < 5 \times 10^{-8}$. Instruments were restricted to cis-pQTL within ± 1 Mb of the encoded gene. Source-level linkage disequilibrium clumping had already been applied to the exposure tables using $r^2 < 0.001$ within a 10,000-kb window based on the 1000 Genomes Phase 3 European reference panel.[19] Palindromic variants with ambiguous strand orientation were removed during harmonization. Only directly overlapping harmonized variants were included in the analyses.

MR analysis

For proteins with a single instrumental variant, causal estimates were calculated using the Wald ratio. For proteins with multiple independent variants, the primary MR method was inverse-variance weighted. Effect estimates were aligned to the exposure-increasing allele. Within each source-specific MR screening analysis, false discovery rate (FDR) correction was applied using the Benjamini–Hochberg procedure, with FDR < 0.05 considered statistically significant. Proteins with $P <$ 0.005 in at least one source-specific analysis were also considered eligible for downstream multilayer evaluation, including signals that did not meet the FDR threshold. The availability and completeness of the relevant molecular QTL data determined whether individual SMR and colocalization analyses were evaluable. Source-specific MR was used as an initial screening step, and no candidate ranking or priority-tier assignment was performed at this stage.

SMR analyses across molecular layers

We next performed SMR analyses across the protein, transcript, and methylation layers using SMR software version 1.4.0. Heterogeneity in dependent instruments was assessed with the heterogeneity in dependent instruments (HEIDI) test.[14] Protein-level SMR used pQTL summary statistics from the deCODE and UKB-PPP resources, whereas transcript- and methylation-level SMR used the corresponding reference eQTL and mQTL panels.[11–13] Across layers, support was evaluated jointly using the SMR association statistic and HEIDI compatibility. Results with $P_{SMR} < 0.05$ and $P_{HEIDI} > 0.05$ were interpreted as supported; results with $P_{SMR} < 0.05$ and $0.01 < P_{HEIDI} \leqslant 0.05$ were interpreted as borderline; and results with $P_{SMR} \geqslant 0.05$ or with $P_{SMR} < 0.05$ and $P_{HEIDI} \leqslant 0.01$ were interpreted as unsupported. Analyses without an interpretable SMR result, or nominal SMR associations for which the HEIDI test could not be evaluated, were classified as not evaluable. When multiple transcript or CpG features were available within a retained locus, the most informative feature was selected for colocalization. SMR results were then summarized to determine whether one or more molecular layers warranted locus-level follow-up and formal colocalization.[14]

Colocalization analyses

General strategy

We performed colocalization analyses to evaluate whether female infertility association signals and molecular QTL signals were consistent with a shared causal variant within each retained locus. Analyses were carried out separately for the pQTL, eQTL, and mQTL layers when suitable data were available.[15] Colocalization analyses were performed using the coloc R package under the coloc.abf framework, which assumes a single causal variant per locus.[15] Default coloc priors were used, with prior-sensitivity analyses described in the Supplementary Materials. The outcome was specified as a case-control trait, and the molecular trait was specified as a quantitative trait.

Locus definition and variant matching

For transcript- and methylation-layer analyses, molecular QTL loci were defined using the corresponding molecular-QTL coordinate system, and FinnGen variants were matched to molecular QTL variants by rsID and alleles before colocalization. Further details on locus construction, genome-build handling, and variant harmonization are provided in the Supplementary Materials.

Window definitions

Primary analyses generally used molecular-QTL-anchored ± 1-Mb regions centered on the relevant probe, molecular QTL feature, or locus anchor. For selected gene-layer pairs, additional ± 500 kb and ± 250

kb regions were examined as sensitivity analyses to assess robustness to regional definition. The ± 1 Mb result was used for the primary interpretation and manuscript display where evaluable. For ABO mQTL, a probe-centered ± 2 Mb fallback region was examined because no evaluable molecular QTL variants were available within the standard ± 1 Mb region; owing to the limited number of usable overlapping variants, this analysis was interpreted cautiously. Gene-layer pairs with insufficient molecular QTL data, incomplete summary statistics, or insufficient usable variant overlap were classified as not evaluable. Window-specific results and effective variant overlap are reported in Supplementary Table S5.[15]

Posterior probabilities and interpretation

For each analysis, posterior probabilities were recorded for the standard coloc hypotheses, with particular emphasis on posterior probability for hypothesis 3 (PP.H3), the posterior probability for two distinct causal variants, and posterior probability for hypothesis 4 (PP.H4), the posterior probability for a shared causal variant.[15] Colocalization support was classified as strong when PP.H4 $\geqslant$ 0.8, suggestive when 0.2 $\leqslant$ PP.H4 < 0.8, and inconclusive when PP.H4 < 0.2. Results with limited single nucleotide polymorphism (SNP) overlap, incomplete summary statistics, or no usable cis-QTL signal were classified as not evaluable. PP.H3 was used to identify cases in which the molecular QTL and infertility signals were more compatible with distinct causal variants than with a shared causal variant.

Cross-layer evidence interpretation

Colocalization results were compared across molecular layers to characterize the molecular evidence profile at each retained locus. Candidate-level interpretation considered the relative strength, evaluability, and robustness of shared-signal support across the protein, transcript, and methylation layers rather than relying on any individual analysis alone.[14,15]

MR sensitivity and pleiotropy assessment

Internal MR sensitivity analyses

Where instrument count permitted, supplementary MR sensitivity analyses were performed to assess the robustness of retained associations. Depending on instrument structure, these analyses included weighted median, MR-Egger, heterogeneity testing, Steiger directionality testing,[20] and leave-one-out analysis. When only a single instrumental variant was available, the sensitivity analysis was limited to a Wald-ratio re-estimate, and model-based sensitivity analyses were necessarily limited. Further details distinguishing the primary source-specific screening estimates from the sensitivity-model re-estimates are provided in the Supplementary Materials.

Public variant-level trait lookup and pleiotropy context

To better understand the broader trait context of retained loci, we queried publicly available variant-trait association resources, including the GWAS Catalog and the Open Targets Genetics platform.[21,22] Selected instrumental variants from retained loci were used to retrieve previously reported trait associations, which were then grouped into broad biological or clinical domains. This analysis was used for contextual public trait lookup rather than as a formal de novo phenome-wide association scan. These lookups were used to inform pleiotropy-aware interpretation and to provide context for prioritization, rather than to override the primary genetic evidence chain.[21,22]

Druggability and translational prioritization

After integrating MR, multilayer SMR, colocalization, and pleiotropy context, we performed a qualitative assessment of druggability and translational tractability for retained candidate genes.

Information sources

Target class and tractability-related features were summarized using publicly available molecular annotation resources, including UniProt,[23] the Open Targets Platform,[24] and curated literature where relevant.

Assessment dimensions

For each retained candidate gene, we summarized target class, target tractability, feasible therapeutic modality, clinical precedent, and specificity or pleiotropy context. Supporting annotations, including tractable family membership, clinical development stage, safety considerations, and direct target, pathway, or target-family-level therapeutic precedent, were used to inform these assessments.[24,25] For visualization, target tractability, therapeutic modality, clinical precedent, and specificity or pleiotropy context were each represented using an ordinal score from 0 to 3, with higher scores indicating a more favorable profile for candidate prioritization. These scores were used for qualitative visualization and contextual interpretation only, did not represent statistical effect estimates or uncertainty, and were not summed into a composite score.

Priority tiers

Retained candidate genes were assigned to translational priority tiers based on combined evidence from genetic support, mechanistic localization, pleiotropic context, and tractability features. Tier 1 designation reflected relatively coherent genetic support, clearer layer-specific colocalization, and a comparatively favorable tractability and pleiotropy profile. Lower tiers reflected greater uncertainty in mechanistic localization, broader pleio-

tropic context, or weaker translational tractability.

Evidence integration framework

Final candidate interpretation was based on five evidence domains: source-specific MR support, cross-layer SMR support, the predominant pattern of molecular-layer support from colocalization, pleiotropy context from public trait lookup, and therapeutic tractability. This integration helped separate loci with convergent cross-layer support from those with weaker or less clearly localized evidence.[14,15,24]

Statistical analysis

MR and sensitivity analyses were performed in R version 4.4.1 using relevant packages, including TwoSampleMR version 0.7.4. Additional data retrieval, preprocessing, and result collation were performed in Python version 3.10. SMR analyses were conducted using SMR version 1.4.0, and colocalization analyses were performed using coloc version 5.2.3.[14,15] All *P* values were interpreted according to the relevant analysis stage. FDR correction was applied during source-specific MR screening. Unless otherwise stated, all statistical tests were two-sided.

RESULTS

Source-specific MR screening identified candidates for multilayer follow-up

Using FinnGen female infertility (N14_FEMALEINFERT) as the primary outcome, we performed source-specific MR across the deCODE and UKB-PPP pQTL resources in 18,189 cases and 114,009 controls (n = 132,198). Among the protein-source pairs shown in Figure 2, only a small number showed evidence that remained notable after source-specific FDR correction. The strongest UKB-PPP associations among the retained candidates were observed for IL18 and ABO, whereas RELT was retained for downstream multilayer clarification despite not meeting the FDR threshold. Among the retained candidates, IL18 showed the most robust protective association (OR = 0.816, 95% CI: 0.749-0.889, $P = 3.47 \times 10^{-6}$, FDR = 0.005), whereas ABO showed a risk-increasing association (OR = 1.083, 95% CI: 1.042-1.126, $P = 5.38 \times 10^{-5}$, FDR = 0.041). RELT showed a protective association in UKB-PPP (OR = 0.896, 95% CI: 0.846-0.949, $P = 1.63 \times 10^{-4}$, FDR = 0.059) together with suggestive support in deCODE (OR = 0.510, 95% CI: 0.324-0.803, P = 0.00361; Figure 2, Table 1). RELT was retained because it met the prespecified nominal screening criterion in both pQTL resources, despite not reaching the source-specific FDR threshold. Paired immunoglobulin-like type 2 receptor beta (PILRB) emerged in the initial screen but was not retained because downstream molecular support did not converge. We therefore carried forward IL18, RELT, and ABO for multilayer follow-up.

Multilayer SMR revealed distinct molecular evidence profiles across retained candidate genes

Source-specific MR estimates for the retained candidate gene set are summarized in Figure 3A. IL18 was not represented in the deCODE resource because no eligible cis-pQTL instrument was available, whereas the 95% confidence interval for the deCODE ABO estimate extended beyond the plotted range. SMR analyses across pQTL, eQTL, and mQTL layers further distinguished the retained signals (Figure 3B). RELT showed support across all three molecular layers, with pQTL support in both UKB-PPP (P_{SMR} = 0.00147, P_{HEIDI} = 0.640) and deCODE pQTL (P_{SMR} = 0.00361, P_{HEIDI} = 0.995), together with supportive eQTL and mQTL results; the strongest signal was observed at the methylation layer (P_{SMR} = 0.000182, P_{HEIDI} = 0.440). IL18 differed in its support profile: protein-layer support was confined to UKB-PPP (P_{SMR} = 0.0381, P_{HEIDI} = 0.0571), the eQTL signal showed strong association but only borderline HEIDI compatibility (P_{SMR} = 0.000140, P_{HEIDI} = 0.0272), and the mQTL layer remained supportive. ABO showed more limited multilayer support. The UKB-PPP pQTL result was supported, whereas the deCODE pQTL result showed SMR association but failed the HEIDI test, the transcript layer was unsupported, and the methylation layer showed no SMR support. These results suggested that the retained candidate genes showed distinct cross-layer molecular evidence profiles and therefore required locus-level clarification by colocalization.

Colocalization resolved IL18 and RELT as higher-priority but mechanistically distinct candidates

Colocalization analyses further sharpened the distinction among the retained candidate genes. IL18 showed its clearest shared-signal support at the transcript layer, where eQTL colocalization met the strong-evidence threshold (PP.H4 = 0.833, PP.H3 = 0.094; Figure 4). By contrast, the UKB-PPP pQTL and mQTL analyses did not show comparable support, and the deCODE pQTL analysis was not evaluable. RELT showed a different configuration. Its clearest support was at the methylation layer, where mQTL colocalization was strong (PP.H4 = 0.863, PP.H3 = 0.041). Protein-layer colocalization was weaker but present across both pQTL resources, whereas transcript-level colocalization remained inconclusive. These patterns supported two mechanistically distinct higher-priority candidates: IL18 as a transcript-predominant candidate with strong shared-signal support and RELT as an mQTL-primary locus with secondary, suggestive protein-layer support. By contrast, ABO remained a retained upstream signal without

Table 1: Integrated genetic evidence summary for retained candidate loci

Gene	Tier	OR (95% CI)	*P*	FDR	SMR support	eQTL coloc	mQTL coloc	Pleiotropy
IL18	Tier 1	0.816 (0.749-0.889)	3.47×10^{-6}	0.005	pQTL: S eQTL: B mQTL: S	Strong	Inconclusive	Sparse public annotation
RELT	Tier 2	0.896 (0.846-0.949)	1.63×10^{-4}	0.059	pQTL: S eQTL: S mQTL: S	Inconclusive	Strong	Caution (broad)
ABO	Tier 3	1.083 (1.042-1.126)	5.38×10^{-5}	0.041	pQTL: S eQTL: *U* mQTL: U	Inconclusive	Not evaluable	Caution (broad)

OR, odds ratio; FDR, false discovery rate; MR, Mendelian randomization; SMR, summary-data-based Mendelian randomization; pQTL, protein quantitative trait locus; eQTL, expression quantitative trait locus; mQTL, methylation quantitative trait locus; S, supported; B, borderline; U, unsupported.

Table 2: Tractability and translational context of retained candidate genes

Gene	Tier	Target class	Target tractability	Therapeutic modality	Clinical precedent	Translational note
IL18	Tier 1	Secreted cytokine	High	Ligand-neutralizing biologic or pathway-directed intervention	Clinical-stage pathway-level precedent	Strongest translational rationale transcript-level lead
RELT	Tier 2	TNFR-superfamily receptor	Moderate	Antibody-accessible receptor modulation	Family-level precedent; no direct RELT program identified	Regulatory candidate; caution due to broader pleiotropy
ABO	Tier 3	Glycosyltransferase	Low	No clearly actionable modality identified	No direct ABO-targeted program identified	Background comparator; not a near-term development priority

TNFR, tumor necrosis factor receptor.

convincing layer localization: pQTL colocalization was at most suggestive, transcript-level colocalization was inconclusive, and the mQTL analysis was not considered evaluable because SNP overlap was insufficient, with only five usable overlapping variants available in the fallback region. This result was therefore not interpreted as evidence against colocalization. Colocalization therefore refined the retained set into mechanistically distinct candidates rather than uniformly validating all retained signals.

Public trait lookup refined pleiotropy interpretation

Public variant-level trait lookup provided additional context for interpreting pleiotropy among the retained candidate genes (Figure 5). To facilitate visual interpretation, labels in Figure 5 were limited to representative non-protein and non-metabolite trait associations rather than all retrieved associations. IL18 had fewer reported cross-trait associations in the queried resources than RELT and ABO; however, this relative sparsity may partly reflect differences in instrument number and database coverage and should not be interpreted as evidence of absent pleiotropy. RELT showed associations across several trait domains, including cardiometabolic, hematologic, respiratory, and other systemic traits, suggesting that its MR signal should be interpreted with caution rather than as a fertility-specific effect. ABO showed a broad and highly significant public trait background, including molecular-trait and systemic associations, supporting its interpretation as a lower-specificity pleiotropic comparator. These patterns indicated clear differences in the reported public trait context among the retained candidate genes.

Integrated evidence supported a three-tier prioritization framework

We next integrated genetic evidence, pleiotropy context, and therapeutic tractability to assign the retained candidates to three tiers (Figure 6; Tables 1 and 2). IL18 was placed in Tier 1 because it combined the strongest MR evidence, transcript-level colocalization, limited public trait associations, and a tractable secreted-cytokine target class. RELT was placed in Tier 2. Its main support came from the methylation layer, with additional but weaker protein-layer evidence, while its broader public trait profile warranted more cautious interpretation. ABO was placed in Tier 3 because, although it was retained in the upstream MR screen, it showed no convincing localization to a single molecular layer and had a broad public trait background. This tiered framework therefore separated IL18 as the leading transcript-level candidate, RELT as a regulatory candidate requiring pleiotropy-aware interpretation, and ABO as a retained background signal.

DISCUSSION

Multilayer genetic triangulation generated distinct molecular evidence profiles across the three retained candidate genes. Across MR, SMR, colocalization, public trait lookup, and therapeutic tractability, IL18 showed the most coherent overall support. RELT also remained of interest, although its interpretation was less straight-

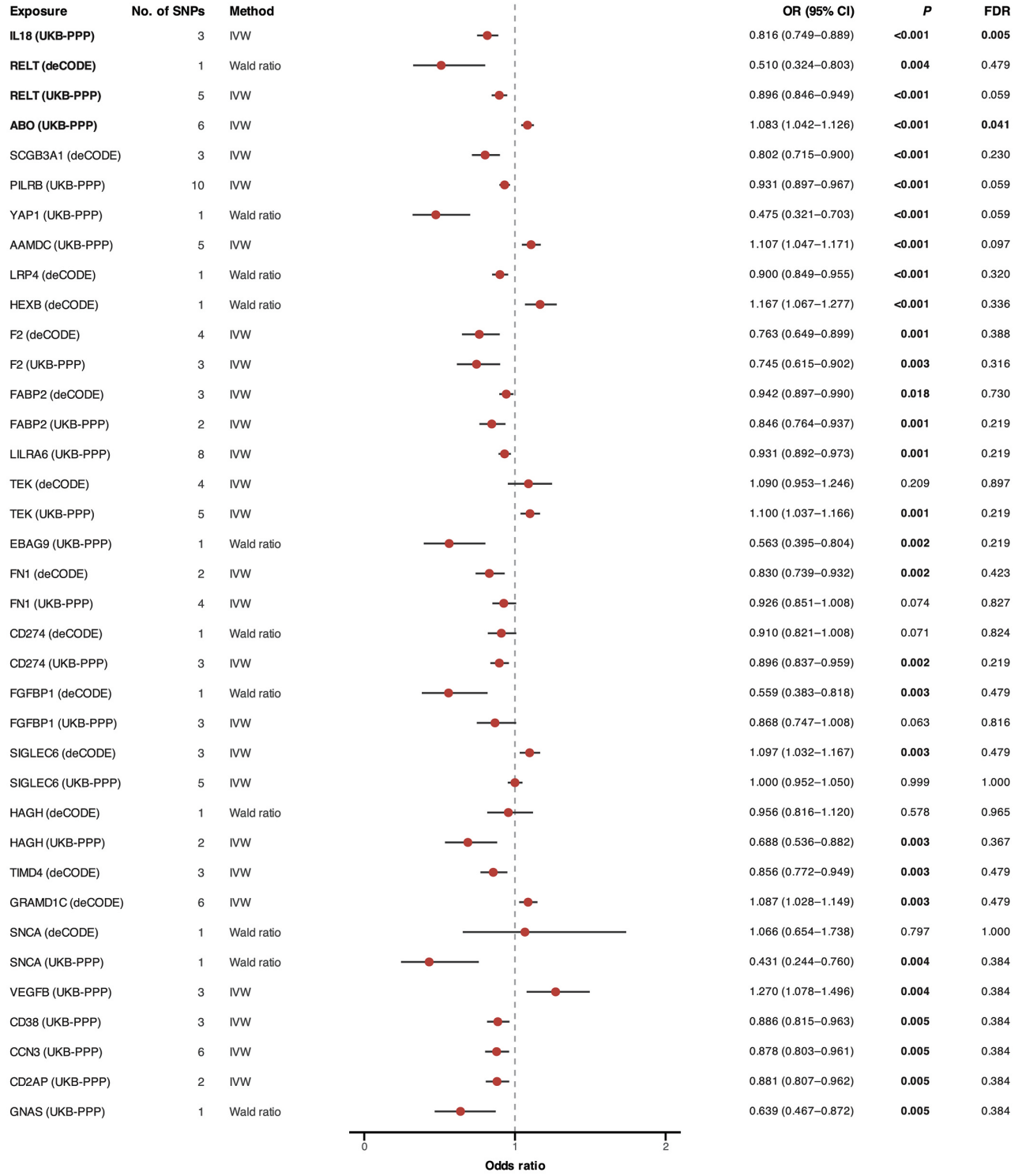

Figure 2. Source-specific Mendelian randomization estimates across circulating proteins for female infertility. Points and horizontal lines represent ORs and 95% CIs, respectively. IL18, RELT, and ABO were retained for downstream analyses. The dashed vertical line indicates OR = 1. Source-specific nominal *P* values and within-source false discovery rates are shown on the right; Other *P* values are rounded to three decimal places. UKB-PPP, UK Biobank Pharma Proteomics Project; pQTL, protein quantitative trait locus; IL18, interleukin 18; RELT, RELT tumor necrosis factor receptor; ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase.

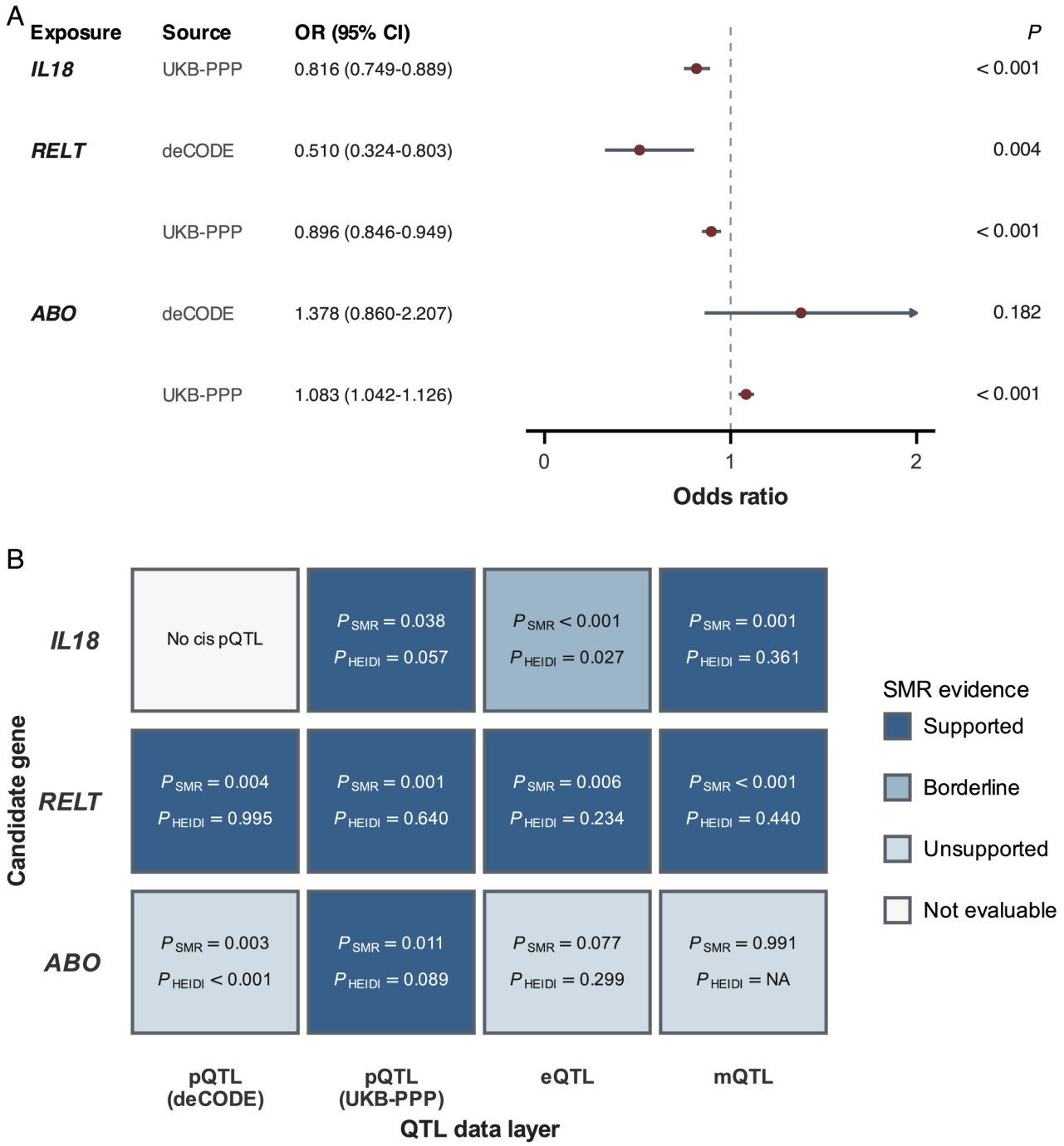

Figure 3. Source-specific MR and multilayer SMR evidence for the retained candidate gene set. (A) Source-specific MR estimates for IL18, RELT, and ABO. Points and horizontal lines represent ORs and 95% CIs, respectively. (B) SMR and HEIDI results across the indicated molecular QTL layers. MR, Mendelian randomization; SMR, summary-data-based Mendelian randomization; pQTL, protein quantitative trait locus; HEIDI, heterogeneity in dependent instruments; UKB-PPP, UK Biobank Pharma Proteomics Project; eQTL, expression quantitative trait locus; mQTL, methylation quantitative trait locus. IL18, interleukin 18; RELT, RELT tumor necrosis factor receptor; ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase.

forward. By contrast, ABO was more appropriately interpreted as a comparator than as a leading signal. Rather than pointing to a single shared infertility mechanism, these results indicate that even a small retained set can resolve into biologically and analytically distinct categories.[8,9]

We further compared IL18, RELT, and ABO with the 25 infertility loci reported by Venkatesh *et al.* in a recent large-scale genome-wide association study meta-

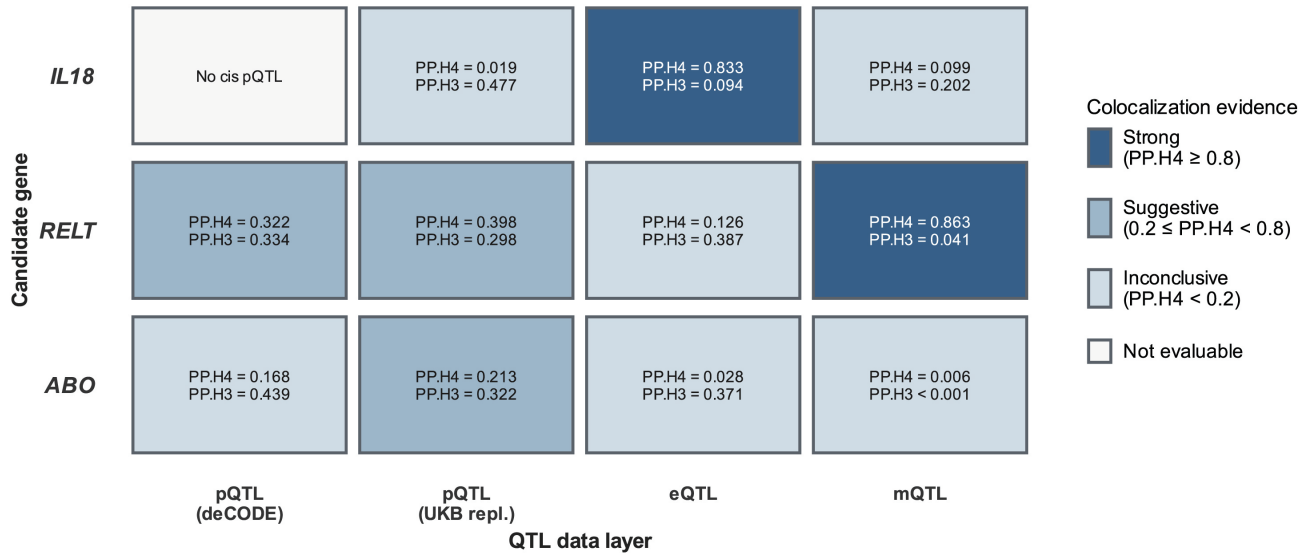

Figure 4. Cross-layer colocalization summary for retained candidate genes. Tiles summarize colocalization results across the indicated pQTL, eQTL, and mQTL datasets. Cell labels report PP.H4 and PP.H3, and fill color indicates the overall colocalization category: strong, suggestive, inconclusive, or not evaluable. PP.H4, posterior probability for a shared causal variant, PP.H3, posterior probability for two distinct causal variants, pQTL, protein quantitative trait locus; UKB-PPP, UK Biobank Pharma Proteomics Project; eQTL, expression quantitative trait locus; mQTL, methylation quantitative trait locus; IL18, interleukin 18; RELT, RELT tumor necrosis factor receptor; ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase.

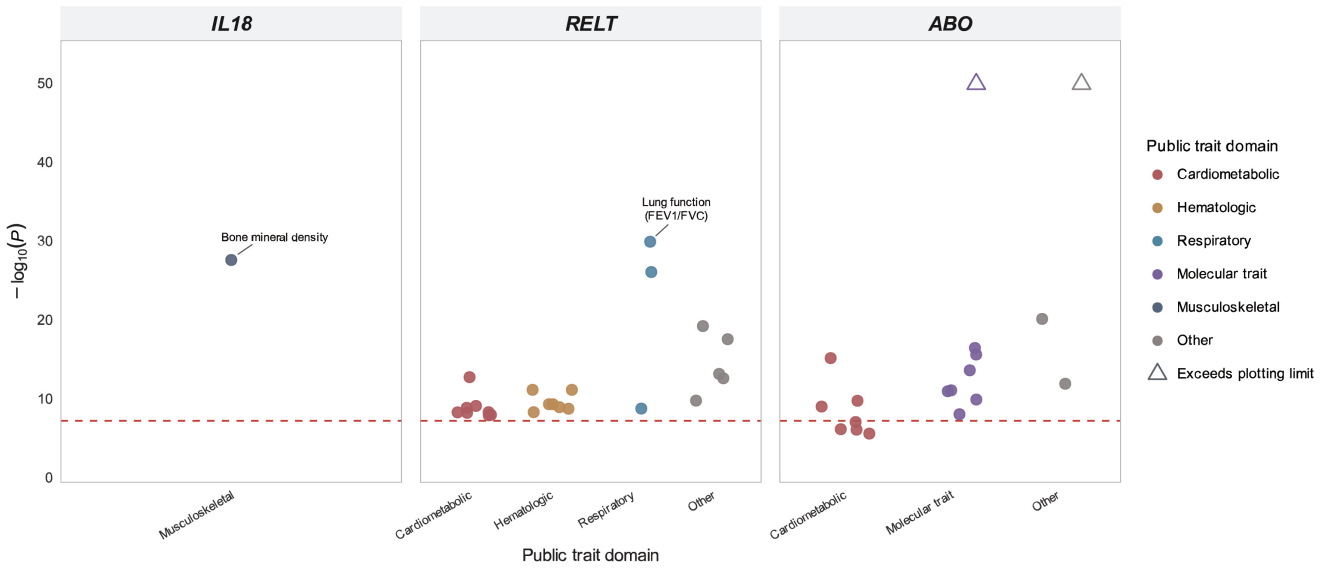

Figure 5. Public trait lookup context for instrument variants in retained candidate genes. Each point represents a reported public trait association for an instrument variant mapped to IL18, RELT, or ABO, retrieved from the GWAS Catalog or Open Targets Genetics. Associations are grouped by public trait domain and plotted as $-\log_{10}(P)$. The red dashed line indicates the conventional genome-wide significance threshold ($P = 5 \times 10^{-8}$). Open triangles denote associations with $-\log_{10}(P)$ values exceeding the plotting limit and are displayed at $-\log_{10}(P) = 50$. IL18, interleukin 18; RELT, RELT tumor necrosis factor receptor; ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase; GWAS, genome-wide association studies.

analysis.[5] None of the three retained candidate genes matched the reported mapped genes or fell within the corresponding lead-variant regions. This suggests that the present multi-omics prioritization did not simply recapitulate previously reported genome-wide significant infertility loci but provided complementary QTL-informed evidence for candidate target prioritization.

Among the retained candidate genes, IL18 showed the most consistent pattern of evidence. It had the strongest MR signal and the clearest shared-signal support at the transcript level. By contrast, the available protein-level results were not backed by convincing colocalization. Overall, the evidence supported IL18 more strongly at the transcript level than at the protein level. This

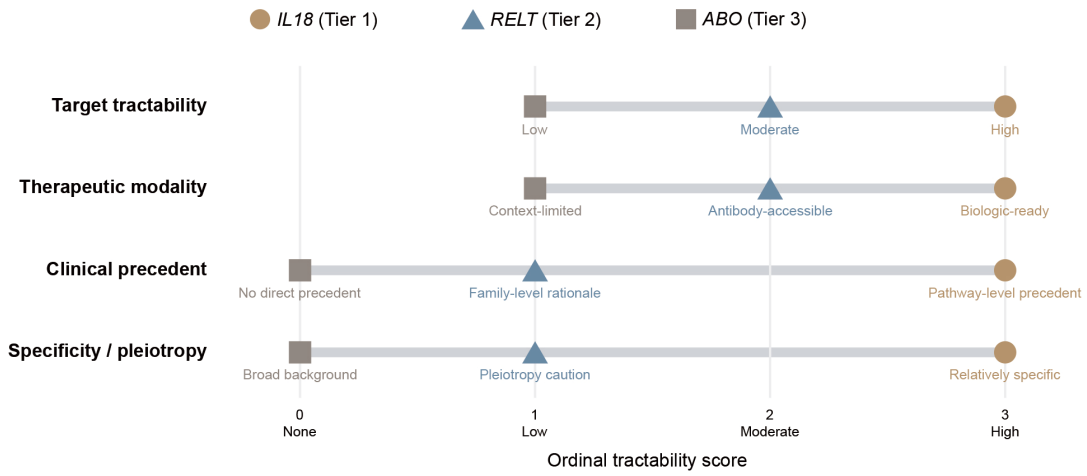

Figure 6. Therapeutic tractability and specificity profile of retained candidate genes. Ordinal scores summarize target tractability, therapeutic modality, clinical precedent, and specificity/pleiotropy context for IL18, RELT, and ABO. Higher scores indicate a more favorable translational profile. Gray horizontal bars indicate the observed score range across the three retained candidates. Text labels indicate the assigned category for each candidate within each dimension. IL18, interleukin 18; RELT, RELT tumor necrosis factor receptor; ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase.

distinction supports biological prioritization but should not yet be interpreted as target validation. RELT showed a different pattern. Its strongest evidence came from the methylation layer, where colocalization was more consistent than at the protein or transcript level. Protein-level colocalization was suggestive across both pQTL resources but was weaker than the methylation-layer evidence. RELT is therefore more appropriately viewed as a regulatory candidate, distinct from IL18 in both layer localization and pleiotropic background. This interpretation is consistent with the broader biology of RELT as an immune-associated member of the tumor necrosis factor receptor superfamily, rather than with a narrowly localized infertility-specific signal.[26,27] More broadly, immune dysregulation at the endometrial interface has been implicated in selected infertility-related settings, supporting cautious interpretation of immune-linked candidates in reproductive phenotypes.[28]

ABO should be interpreted more conservatively. Although ABO remained detectable in the primary screen, downstream analyses did not assign its signal convincingly to a single molecular layer, and its public trait background was broad. This pattern is more consistent with broad systemic biology, including coagulation, inflammation, glycosylation, and carbohydrate-related processes, than with a reproductive-specific infertility mechanism. For that reason, ABO was less informative as a prioritized infertility target and more useful in showing why multilayer follow-up is necessary. A statistically retained protein signal is not automatically a biologically focused target candidate. In this study, ABO illustrated this clearly: once colocalization, pleiotropy, and tractability were considered together, the case for prioritization became much weaker. Accordingly, the public trait-lookup analysis was treated as contextual evidence for pleiotropy, rather than as an independent criterion for ranking the retained candidates.[8,16]

Several limitations should be noted. First, although N14_FEMALEINFERT is defined using infertility-related registry codes, it remains an aggregate endpoint, and residual diagnostic heterogeneity or control misclassification may dilute subtype-specific genetic effects and reduce the resolution of colocalization analyses.[4,5] Second, the pQTL resources were not fully interchangeable across all loci, and not every molecular layer could be evaluated equally well for every candidate. Third, the strongest retained signals were not uniformly supported by protein-level colocalization. In addition, the available eQTL and mQTL reference panels were not fully matched to infertility-relevant tissues. Some colocalization results also need cautious interpretation because effective SNP overlap was limited. The public trait-lookup step further depended on existing database annotation rather than on a newly conducted systematic phenome-wide analysis. Interpretation of RELT was also limited because the primary deCODE MR estimate was based on a single instrument, which restricted the available sensitivity analyses.[11–13,15,18,21,22]

Overall, the data support tiered prioritization rather than definitive target nomination. Within this framework, IL18 showed the most favorable translational profile, RELT remained a plausible but more pleiotropy-sensitive regulatory candidate, and ABO was retained mainly as a background or contrast signal. More broadly, the key question is not only whether a locus is associated with female infertility, but also which molecular layer

carries the strongest support. This layer-specific perspective may also be useful for genetically guided target prioritization in other heterogeneous reproductive phenotypes.[8,9]

CONCLUSION

Multi-omics genetic triangulation prioritized IL18 and RELT as the two better-supported candidates for female infertility. IL18 showed the clearest evidence at the transcript level and the most favorable translational profile. RELT was supported mainly at the methylation level but required more cautious interpretation because of its broader pleiotropic background. ABO remained a background signal without clear support from a single molecular layer. Compared with pQTL-based Mendelian randomization alone, the cross-layer framework provided a clearer view of the cross-layer molecular evidence underlying each signal and refined candidate prioritization in this heterogeneous reproductive phenotype.

DECLARATIONS

Supplementary information

Supplementary materials are only available at the official site of the journal (www.hksmp.com).

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None.

Author contributions

Li JX: Conceptualization, Methodology, Software, Formal analysis, Data curation, Visualization, Writing—Original draft. Gao SR: Conceptualization, Supervision, Writing—Review and Editing. Kong QR: Conceptualization, Supervision, Project administration, Writing—Review and Editing. All authors reviewed and approved the final manuscript.

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Not applicable.

Conflict of interest

The authors declare no competing interest.

Use of large language models, AI and machine learning tools

During the preparation of this work, the authors used ChatGPT by OpenAI for language polishing and editing assistance. The tool was not used to generate data, perform statistical analyses, or draw scientific conclusions. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the manuscript.

Data availability statement

All data used in this study were derived from publicly available summary-level datasets. The female infertility GWAS outcome data were obtained from FinnGen release R12 for phenotype code N14_FEMALEINFERT and are available through the FinnGen results browser and download portal (https://www.finngen.fi/en/access_results). Plasma pQTL data were obtained from the deCODE Genetics summary data resource (https://www.decode.com/summarydata/) and the UK Biobank Pharma Proteomics Project resource (https://registry.opendata.aws/ukbppp/). eQTL and mQTL summary resources used for SMR and colocalization analyses were obtained from publicly available reference panels described in the Methods and Supplementary Materials. Public variant-trait associations and tractability annotations were obtained from the GWAS Catalog (https://www.ebi.ac.uk/gwas/), Open Targets Genetics (https://genetics.opentargets.org/), Open Targets Platform (https://platform.opentargets.org/), and UniProt (https://www.uniprot.org/). The derived results supporting the findings of this study are included in the manuscript and Supplementary Materials. Additional processed results are available from the corresponding author upon reasonable request.

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