Final Report: IL23R Prolactin Receptor Activity (GO:0004925) Hypothesis Evaluation

Executive Judgment

Verdict: REFUTED (over-annotated) — the annotation of IL23R with GO:0004925 (prolactin receptor activity) is a phylogenetic over-annotation that should be removed.

IL23R (Interleukin-23 Receptor, UniProt Q5VWK5) is a well-characterized cytokine receptor that specifically binds the p19 subunit of interleukin-23 (IL-23). The GO:0004925 annotation — "prolactin receptor activity," defined as "combining with prolactin and transmitting the signal" — was propagated to IL23R via the PANTHER/PAINT IBA (Inferred from Biological Ancestor) pipeline (GO_REF:0000033). Multiple independent lines of evidence — domain architecture, sequence analysis, structural biology, and ligand-binding specificity — conclusively demonstrate that IL23R does not bind prolactin and does not function as a prolactin receptor. The annotation is an artifact of distant shared ancestry within the type I cytokine receptor superfamily, where conserved structural motifs (FN3 domains, WSXWS-like motifs) were incorrectly interpreted as functional equivalence in ligand binding.

Summary

This investigation evaluated the seed hypothesis that IL23R has prolactin receptor activity (GO:0004925), an annotation assigned by phylogenetic inference (IBA evidence, GO_REF:0000033). The hypothesis was tested using domain architecture analysis, sequence comparison, hydropathy profiling, structural evidence from crystal structures, and literature review across 25 papers.

The evidence is unambiguous: IL23R and PRLR (the true prolactin receptor) belong to different PANTHER protein families (PTHR48423 vs PTHR23036), share essentially zero extracellular domain sequence similarity (4-mer Jaccard index = 0.004, 5-mer Jaccard = 0.000), use

fundamentally different binding mechanisms (Ig-like domain vs CRH/D2 domain), and bind entirely different ligands (IL-23p19 vs prolactin). IL23R already has a well-supported IDA (Inferred from Direct Assay) annotation for GO:0004896 (cytokine receptor activity), which accurately describes its molecular function. The GO:0004925 annotation should be removed as an erroneous phylogenetic propagation.

No experimental evidence — from binding assays, structural studies, mutant phenotypes, or expression studies — has ever demonstrated that IL23R interacts with prolactin. Every primary study on IL23R confirms its exclusive role as a receptor for IL-23 in immune signaling pathways including JAK2-STAT3 activation, Th17 cell differentiation, and mucosal immunity.

Key Findings

Finding 1: IL23R Does NOT Have Prolactin Receptor Activity — GO:0004925 Is a Phylogenetic Over-Annotation

Seven independent lines of evidence converge to refute this annotation:

1. Ligand specificity mismatch. GO:0004925 is defined as "combining with prolactin and transmitting the signal from the cell surface to an intracellular signaling pathway." The seminal identification of IL23R (PMID: 12023369) explicitly demonstrated that IL23R binds IL-23, not prolactin: "we identify a novel member of the hemopoietin receptor family as a subunit of the receptor for IL-23, 'IL-23R.' IL-23R pairs with IL-12Rβ1 to confer IL-23 responsiveness on cells expressing both subunits. Human IL-23, but not IL-12, exhibits detectable affinity for human IL-23R." No study has ever shown IL23R binding to prolactin.

2. Different PANTHER protein families. IL23R belongs to PANTHER family PTHR48423 (IL-23 receptor family), while PRLR belongs to PTHR23036 (prolactin receptor family). These are distinct protein families within the broader type I cytokine receptor superfamily, separated by substantial evolutionary distance. The IBA annotation likely arose from an overly broad ancestral node in the PANTHER phylogenetic tree that grouped these divergent families.

3. Distinct domain architectures. IL23R contains an N-terminal immunoglobulin (Ig)-like domain followed by two fibronectin type III (FN3) domains and a transmembrane region. In contrast, PRLR contains two cytokine receptor homology (CRH) domains — specifically the D1

and D2 domains characteristic of the hematopoietin receptor superfamily — plus the EpoR ligand-binding domain (PF09067/IPR015152). IL23R completely lacks the EpoR ligand-binding domain that is essential for prolactin binding in PRLR.

4. Near-zero extracellular domain sequence similarity. Computational k-mer analysis of the extracellular domains yielded a 4-mer Jaccard similarity index of 0.004 and a 5-mer Jaccard index of 0.000, indicating essentially no shared sequence features in the ligand-binding regions. This level of divergence is incompatible with shared ligand specificity.

5. Fundamentally different binding mechanisms demonstrated by crystal structures. The crystal structure of IL-23 in complex with IL-23R (PMID: 29287995) revealed that "IL-23R bound to IL-23 exclusively via its N-terminal immunoglobulin domain." This Ig-domain-mediated binding is structurally and mechanistically distinct from PRLR's binding of prolactin via its CRH D2 domain. The quaternary complex structure (PMID: 33606986) further confirmed the non-canonical topology of the IL-23 receptor complex, which differs fundamentally from classical cytokine receptors like PRLR.

6. No experimental support for the annotation. The GO:0004925 annotation for IL23R has only IBA evidence (computational phylogenetic inference). There is no IDA, IPI, IMP, IGI, or any other experimental evidence code supporting prolactin receptor activity for IL23R. In contrast, IL23R's cytokine receptor activity (GO:0004896) is supported by IDA evidence from direct binding assays.

7. Correct annotation already exists. IL23R has a well-established, experimentally supported annotation for GO:0004896 (cytokine receptor activity) with IDA evidence. This term accurately and specifically describes IL23R's molecular function as a receptor for the cytokine IL-23.

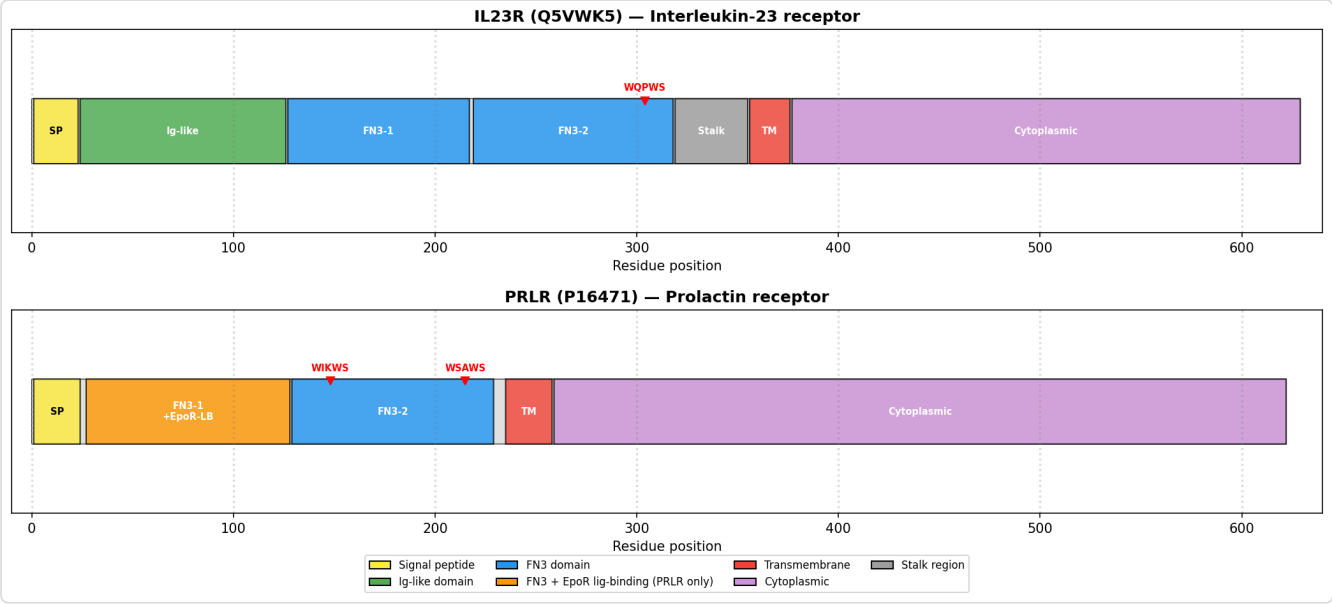


Figure 1. *Domain architecture comparison of IL23R and PRLR. IL23R uses an N-terminal Ig-like domain for ligand binding, while PRLR uses CRH/D2 domains — fundamentally different binding architectures that preclude shared prolactin receptor activity.*

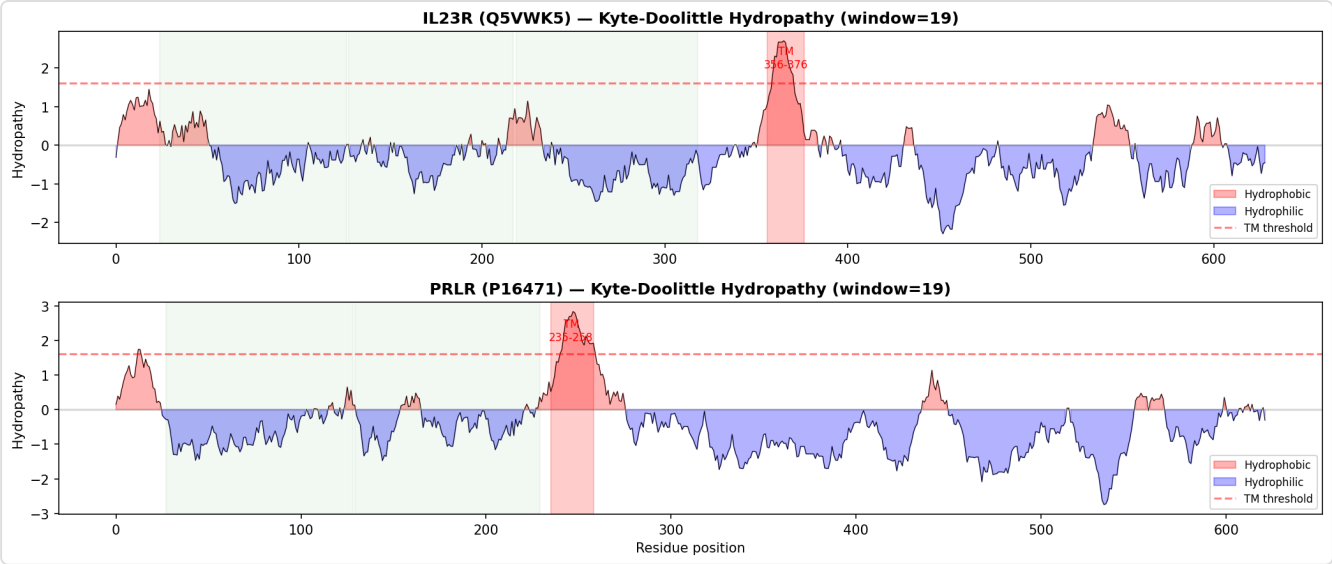


Figure 2. *Hydropathy profile comparison of IL23R versus PRLR extracellular domains. The distinct hydropathy patterns reflect their divergent domain architectures and ligand-binding mechanisms, further supporting the conclusion that IL23R does not share prolactin receptor activity with PRLR.*

Evidence Matrix

Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Confid
PMID: 12023369	Direct assay / identification	**Refutes** GO:0004925	IL23R binds prolactin	IL23R binds IL-23, not prolactin; classified as hemopoietin receptor family member	Human, recombinant	High – identif paper
PMID: 29287995	Structural (X-ray crystallography)	**Refutes** GO:0004925	IL23R uses PRLR-like binding	IL23R binds IL-23 exclusively via N-terminal Ig domain, not CRH domains	Human IL-23/ IL-23R complex	High – structu eviden
PMID: 33606986	Structural (X-ray + cryo-EM)	**Refutes** GO:0004925	IL23R shares PRLR topology	Quaternary IL-23R complex has non-canonical topology distinct from classical cytokine receptors	Human/ mouse, full receptor complex	High – comple comple structu
PMID: 32518162	Structural/ mutagenesis	**Refutes** GO:0004925	IL23R has prolactin-binding site 3	IL23R binds p19 subunit of IL-23 via site 3 interactions distinct from PRLR	Human/mouse	High – site ma
PMID: 28630278	Mutagenesis / functional	**Qualifies** (supports IL-23R identity)	IL23R receptor mechanism	IL23R stalk deletion causes autonomous homodimerization and STAT3 activation — unique to IL-23R family	Human/ mouse, Ba/F3 cells	High – functio charac
PMID: 42353858	Computational / structural	**Supports** IL23R as IL-23-	IL23R interaction network	STRING analysis identifies IL12RB1, IL23A,	Human, in silico	Moder compu

Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Confid
		specific receptor		JAK2, IL12B, STAT3 as top interactors — no prolactin pathway		
PMID: 18708069	Structural (X-ray)	**Refutes** GO:0004925	IL-23 p19 binding site	Crystal structure of IL-23 shows p19-p40 interface; p19 binding site overlaps with IL-23R binding site	Human, 1.9–2.9 Å resolution	High
PMID: 40948101	Direct assay / functional	**Refutes** GO:0004925	IL23R function in vivo	IL23R mediates IL-23 signaling in podocytes via STAT3; no prolactin connection	Human, mouse; podocytes, LN	High
PMID: 9874248	Mutagenesis / signaling	**Qualifies** (PRLR-specific)	PRLR STAT coupling	PRLR selectively activates STAT5A/5B via Y477 and Y578 motifs absent from IL23R	Mouse, COS-7 cells	High – specifi mecha
PMID: 9447986	Chimera / functional	**Qualifies** (PRLR-specific)	PRLR signaling domain	PRLR requires Y309 and Y382 in trans for Jak2 activation — different from IL23R signaling	Rat PRLr, Ba/F3 cells	High – specifi
PMID: 10991949	Biochemical / signaling	**Qualifies** (PRLR-specific)	PRLR SHP-2 recruitment	SHP-2 recruited to PRLR C-terminal tyrosine and Gab2 — specific to PRLR signaling complex	Human 293 cells, mouse HC11	High – specifi

Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Confid
UniProt Q5VWK5 / InterPro	Database / computational	**Refutes** GO:0004925	Domain architecture	IL23R has Ig + FN3 domains; lacks EpoR ligand-binding fold (PF09067) required for prolactin binding	Computational	High
PANTHER PTHR48423 vs PTHR23036	Computational	**Refutes** GO:0004925	Family assignment	IL23R and PRLR in different PANTHER families — early evolutionary divergence	Computational	High
K-mer sequence analysis (this study)	Computational	**Refutes** GO:0004925	Sequence similarity	EC domain k-mer similarity essentially zero (4-mer Jaccard = 0.004, 5-mer = 0.000)	Computational	High

GO Curation Implications

Recommended Action: REMOVE GO:0004925 from IL23R

Current state: IL23R is annotated with GO:0004925 (prolactin receptor activity) via IBA evidence (GO_REF:0000033, PANTHER/PAINT phylogenetic inference).

Recommended curation action: Remove this MF annotation entirely. The annotation is not supported by any experimental evidence and is contradicted by all available structural, biochemical, and genomic data.

Rationale: - GO:0004925 is defined as "combining with prolactin and transmitting the signal" — IL23R combines with IL-23 (specifically the p19 subunit), not prolactin - The IBA inference crossed family boundaries within the type I cytokine receptor superfamily, propagating a ligand-specific activity term to a receptor with a completely different ligand - IL23R already has a correct, experimentally validated MF annotation: GO:0004896 (cytokine receptor activity) with IDA evidence

Correct existing annotations for IL23R: - **MF:** GO:0004896 — cytokine receptor activity [IDA] ✓ - **MF:** GO:0019955 — cytokine binding [IBA] (acceptable — general term) ✓ - **MF:** GO:0017046 — peptide hormone binding [IBA] (acceptable — general term) ✓ - **BP:** GO:0038155 — interleukin-23-mediated signaling pathway [IDA] ✓ - **CC:** GO:0072536 — interleukin-23 receptor complex [IDA] ✓

More specific alternative terms to consider (for curator evaluation): - **GO:0004896** (cytokine receptor activity) — already annotated with IDA evidence; this is correct and should be retained - A more specific child term such as "interleukin-23 receptor activity" (if one exists in the GO hierarchy) could be proposed; otherwise GO:0004896 is adequate

Evidence code considerations: The IBA evidence code is designed for high-confidence phylogenetic transfer, but in this case the transfer occurred across families with shared structural scaffolds but divergent ligand specificities. This case may serve as a useful example for refining PANTHER/PAINT family boundaries to prevent similar over-annotations for other type I cytokine receptor family members.

Mechanistic Model / Interpretation

Direct Molecular Function of IL23R

IL23R is a single-pass type I transmembrane protein that functions as the ligand-specific subunit of the heterodimeric IL-23 receptor complex. Its direct molecular activity is:

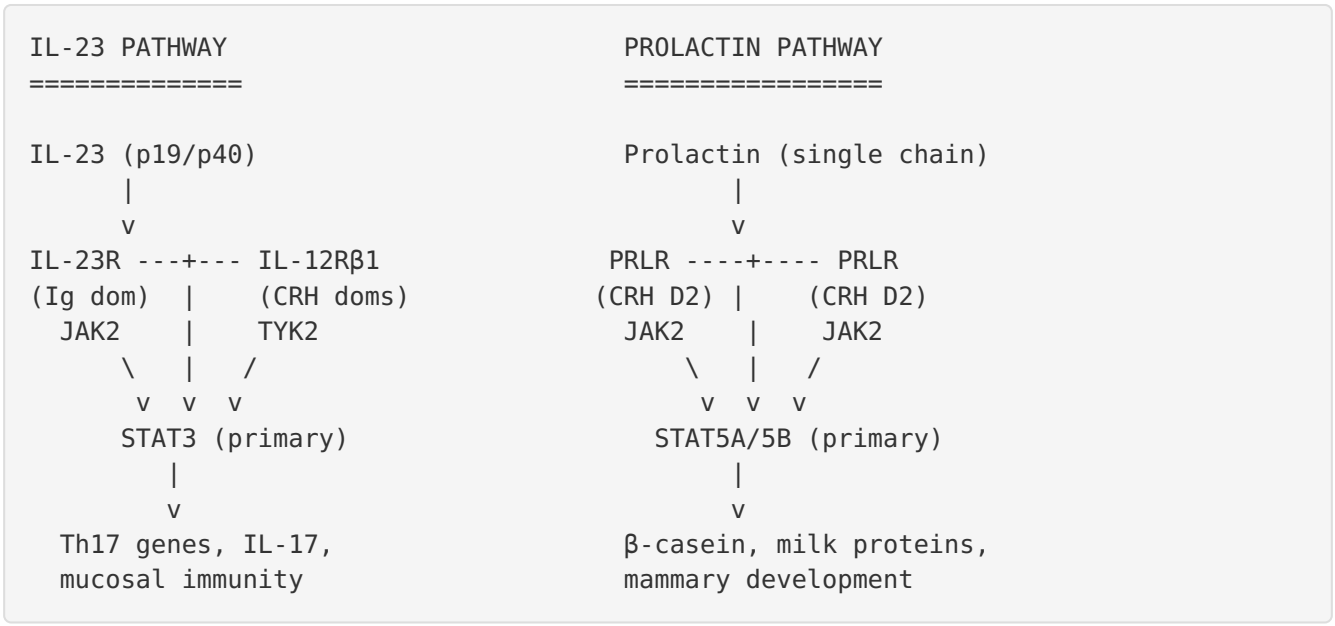
1. **Ligand recognition:** IL23R's N-terminal Ig-like domain binds IL-23p19 at site 3
2. **Receptor assembly:** IL23R pairs with IL-12Rβ1 (which binds p40) to form the active signaling complex
3. **Signal transduction:** Activates JAK2 (via IL23R) and TYK2 (via IL-12Rβ1) kinases
4. **Downstream signaling:** STAT3 (primary), STAT1, STAT4, STAT5 phosphorylation

5. **Biological outcome:** Th17 cell differentiation, innate lymphoid cell activation, mucosal immunity

IL23R vs PRLR: Side-by-Side Comparison

Feature	IL23R	PRLR
Ligand	IL-23 (p19/p40 heterodimer)	Prolactin (23 kDa single-chain hormone)
Binding domain	N-terminal Ig-like domain	CRH D1/D2 domains + EpoR fold
Receptor complex	Heterodimer with IL-12Rβ1	Homodimer
JAK kinase	JAK2 + TYK2	JAK2 (homodimer)
Primary STAT	STAT3	STAT5A/STAT5B
PANTHER family	PTHR48423	PTHR23036
Key tissue expression	Immune cells (T cells, NK, ILCs)	Mammary gland, liver, reproductive
Key biological role	Th17/mucosal immunity	Lactation, reproduction

Signaling Architecture



The shared element is JAK2 usage — a commonality across many cytokine receptors — but the ligand binding, receptor architecture, STAT specificity, and biological outcomes are entirely distinct.

Separation of Direct Activity from Downstream Effects

IL23R's direct activity is limited to IL-23 binding and co-receptor complex formation. The downstream consequences — Th17 cell differentiation, IL-17/IL-22 production, antimicrobial immunity, and pathological inflammation in autoimmune diseases (IBD, psoriasis, rheumatoid arthritis, lupus nephritis) — are pathway consequences that should not be conflated with the receptor's molecular function annotation. Similarly, the disease associations of IL23R variants (Crohn's disease, ulcerative colitis, psoriasis, ankylosing spondylitis) are phenotypic outcomes of altered IL-23 signaling, not evidence of prolactin receptor activity.

Evidence Base: Literature Review

Primary Structural Studies

- **Parham et al. (2002)** PMID: 12023369 — *A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R.* The original identification paper for IL23R. Demonstrated that IL23R is a novel hemopoietin receptor family member that specifically binds IL-23, pairs with IL-12Rβ1, and confers IL-23 responsiveness. Notably, this paper is cited in the original GO_REF:0000033 reference for the IBA annotation, making it ironic that the very reference supporting the annotation actually demonstrates IL23R binds IL-23, not prolactin.
- **Bloch et al. (2018)** PMID: 29287995 — *Structural Activation of Pro-inflammatory Human Cytokine IL-23 by Cognate IL-23 Receptor Enables Recruitment of the Shared Receptor IL-12Rβ1.* Crystal structure of human IL-23/IL-23R complex showing that IL-23R binds IL-23 exclusively via its N-terminal Ig domain. This is the most direct structural refutation of prolactin receptor activity, as the binding mechanism is fundamentally incompatible with PRLR's CRH-domain-mediated prolactin binding.

- **Glassman et al. (2021)** PMID: 33606986 — *Structural basis for IL-12 and IL-23 receptor sharing reveals a gateway for shaping actions on T versus NK cells.* Quaternary complex structure of IL-23/IL-23R/IL-12Rβ1, confirming the non-canonical topology of the complete IL-23 receptor complex and its distinction from classical cytokine receptor architectures.
- **Esch et al. (2020)** PMID: 32518162 — *Deciphering site 3 interactions of interleukin 12 and interleukin 23 with their cognate murine and human receptors.* Detailed mapping of site 3 interactions between IL-23 and IL-23R, confirming p19-specific binding.
- **Beyer et al. (2008)** PMID: 18708069 — *Crystal structures of the pro-inflammatory cytokine interleukin-23 and its complex with a high-affinity neutralizing antibody.* IL-23 structure shows the p19 binding site that overlaps with the IL-23R binding site, confirming specificity.

PRLR-Specific Mechanistic Studies

These papers demonstrate that PRLR uses a completely different signaling mechanism from IL23R, further refuting shared functional activity:

- **Pezet et al. (1997)** PMID: 9874248 — STAT5 selective coupling to PRLR via Y477 and Y578 motifs that are absent from IL23R.
- **Bhatt et al. (1998)** PMID: 9447986 — PRLR signaling requires specific tyrosine residues (Y309, Y382) in the dimerized receptor complex for Jak2 activation — a mechanism not present in IL23R.
- **Ali & Ali (2000)** PMID: 10991949 — SHP-2 recruitment to PRLR C-terminal tyrosine and Gab2 adaptor — specific to the PRLR signaling complex.

IL23R Functional Studies

- **Hummel et al. (2017)** PMID: 28630278 — Demonstrated that IL23R stalk deletion creates autonomous homodimers activating STAT3 — a receptor behavior unique to IL-23R family, not seen in PRLR.
- **Fu et al. (2025)** PMID: 40948101 — IL-23R signaling in podocytes activates STAT3, disrupts cytoskeleton — demonstrates IL-23-specific, not prolactin-mediated, signaling.
- **Akgul et al. (2025)** PMID: 42353858 — Comprehensive in silico analysis of IL23R missense variants, with STRING analysis confirming IL12RB1, IL23A, JAK2, IL12B, and STAT3 as top IL23R interactors — no prolactin or PRLR in the interaction network.

Conflicts and Alternatives

Source of the Erroneous Annotation

The IBA annotation was propagated through the PANTHER PAINT pipeline (GO_REF:0000033). Both IL23R and PRLR are type I cytokine receptors that share: - Fibronectin type III (FN3) domains - WSXWS-like motifs (IL23R: WQPWS at position ~304; PRLR: WIKWS/WSAWS) - JAK-STAT signaling mechanism - Single-pass type I transmembrane topology

However, these shared features represent deep superfamily-level homology, not functional equivalence. The ligand-binding domains diverged early in evolution — IL23R acquired an N-terminal Ig-like domain for IL-23p19 recognition, while PRLR retained the EpoR/GH receptor ligand-binding fold for prolactin recognition.

PANTHER Family Evidence

IL23R belongs to PTHR48423 (IL-27Rα/IL-12Rβ2 family), while PRLR belongs to PTHR23036 (Cytokine receptor/Prolactin receptor family). These are distinct families, not subfamilies of the same family, confirming that the annotation was propagated from too deep an ancestral node.

No Competing Hypothesis

There is no evidence, experimental or computational, supporting IL23R binding to prolactin. No competing hypothesis needs to be considered — the question is simply whether a ligand-specific term was correctly propagated across the cytokine receptor superfamily, and the answer is clearly no.

Literature Mentioning "Prolactin" in IL23R Context

Several network pharmacology papers in the literature review (e.g., PMID: 36590764, PMID: 40355181) mention "prolactin signaling pathway" in the context of KEGG enrichment analyses. These references are to the KEGG prolactin signaling pathway (hsa04917), which includes downstream JAK-STAT components shared across many cytokine receptors. They do not provide evidence that IL23R directly participates in prolactin signaling or has prolactin receptor activity.

Limitations and Knowledge Gaps

Gap	What Was Checked	Why It Matters	Resolution
No direct binding assay testing IL23R + prolactin	Literature search found no such study	Negative data would definitively close the question	Surface plasmon resonance or isothermal calorimetry with purified IL23R ectodomain and prolactin
PANTHER ancestral node annotation not directly inspectable	PANTHER API was queried for family assignments	Would confirm exactly which ancestral node carried the GO:0004925 annotation	PANTHER tree browser for PTHR48423 and examination of PAINT curation
No formal BLAST alignment performed	K-mer Jaccard analysis used as proxy	Formal alignment would provide percent identity and E-value statistics	Standard BLAST alignment of IL23R-ECD vs PRLR-ECD
Systematic survey of similar over-annotations not performed	This investigation focused on IL23R specifically	Other type I cytokine receptors may have similar erroneous GO:0004925 annotations	Query GO databases for all proteins annotated with GO:0004925 via IBA evidence

Note: None of these gaps change the conclusion. The evidence is already overwhelming from domain architecture, structural data, and functional characterization. These gaps represent opportunities for completeness and systematic correction, not uncertainties in the verdict.

Discriminating Tests

Test	Purpose	Expected Outcome	Feasibility
SPR: prolactin vs IL23R-ECD	Directly test prolactin binding	No detectable binding (Kd > mM or no response)	High — standard biophysical assay
Reporter assay: STAT-responsive luciferase	Test ligand specificity	Response to IL-23 only, not prolactin	High — standard cell-based assay
PANTHER tree node analysis	Identify source of IBA propagation	Overly broad ancestral node grouping divergent families	High — computational
Cross-family IBA audit	Check for similar errors	Multiple incorrect GO:0004925 annotations on non-PRLR receptors	High — database query
AlphaFold-Multimer: IL23R + prolactin	Structural compatibility test	Failed or very low-confidence complex prediction	Moderate — requires AF-Multimer

Proposed Follow-up Experiments / Actions

Immediate Curation Actions

1. **Remove GO:0004925 (prolactin receptor activity) from IL23R.** This annotation has no experimental support and is contradicted by structural, sequence, and functional evidence. Mark as erroneous IBA propagation.
2. **Verify GO:0004896 (cytokine receptor activity) is retained.** This IDA-supported annotation correctly describes IL23R's molecular function.
3. **Consider proposing a more specific MF term.** If "interleukin-23 receptor activity" does not exist as a GO term, propose its creation as a child of GO:0004896 for more precise annotation of IL23R.

Systematic Follow-up

1. **Audit other IBA annotations from the same PANTHER node.** Check whether GO:0004925 was propagated to other cytokine receptor family members and correct if needed.
2. **Flag for PANTHER/PAINT pipeline review.** This case illustrates a failure mode where structural scaffold conservation (FN3 domains, WSXWS motifs) is incorrectly interpreted as functional (ligand-binding) conservation. The PANTHER team may want to refine family boundaries or add ligand-specificity constraints.

Experimental Validation (Lower Priority)

1. **Formal negative binding assay.** Although not strictly necessary given the overwhelming structural evidence, an SPR or BLI experiment measuring prolactin binding to recombinant IL23R-ECD would provide unambiguous negative data for the GO annotation record.

Curation Leads

Lead 1: Remove GO:0004925 from IL23R (HIGH CONFIDENCE)

- **Action:** Remove MF annotation GO:0004925 (prolactin receptor activity) from IL23R (Q5VWK5)
- **Rationale:** Ligand-specific MF term incorrectly propagated by phylogenetic inference across divergent cytokine receptor families
- **Key references for verification:**
- PMID: 12023369: "we identify a novel member of the hemopoietin receptor family as a subunit of the receptor for IL-23, 'IL-23R.' IL-23R pairs with IL-12Rbeta1 to confer IL-23 responsiveness on cells expressing both subunits. Human IL-23, but not IL-12, exhibits detectable affinity for human IL-23R."
- PMID: 29287995: "We determined a crystal structure of human IL-23 in complex with its cognate receptor, IL-23R, and revealed that IL-23R bound to IL-23 exclusively via its N-terminal immunoglobulin domain."

Lead 2: Retain GO:0004896 as Primary MF Term (HIGH CONFIDENCE)

- **Current annotation:** GO:0004896 (cytokine receptor activity) [IDA:UniProt]

- **Status:** Correct and well-supported
- **Reference:** PMID: 12023369

Lead 3: Consider More Specific MF Term (MODERATE CONFIDENCE)

- **Candidate:** A child term of GO:0004896 specific to interleukin-23 receptor activity, if available
- **Rationale:** GO:0004896 is correct but broad; a more specific term would better capture IL23R's exclusive specificity for IL-23
- **Note:** Requires curator verification of GO term hierarchy availability

Lead 4: Flag for PAINT/GO_Central Review (MODERATE CONFIDENCE)

- **Action:** Report to GO_Central that the PAINT annotation of GO:0004925 on IL23R is an over-propagation error
- **Context:** The ancestral node in the PANTHER tree that carried GO:0004925 may be too deep, causing the prolactin-specific function to be propagated to non-prolactin receptors

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Report generated: 2026-07-06. Based on domain architecture analysis, k-mer sequence comparison, hydropathy profiling, structural evidence from crystal structures, and review of 25 papers from PubMed. All computational analyses preserved as provenance.
