Otofaciocervical Syndrome (OFCS / OTFCS): A Comprehensive Disease Characteristics Report

Summary

Otofaciocervical syndrome (OFCS; also OTFCS) is a rare congenital malformation disorder of the branchial arches / pharyngeal pouches, arising from disruption of the **PAX–SIX–EYA–DACH developmental network (PSEDN)**. Its cardinal features are facial dysmorphism (long face, narrow nose), external/middle/inner-ear anomalies with predominantly conductive hearing loss, branchial/cervical defects (cervical fistulae, long neck), and a highly characteristic shoulder-girdle and vertebral malformation complex (sloping shoulders, low-set winged scapulae, clavicular anomalies), frequently accompanied by mild intellectual disability and short stature. Clinically it is difficult to separate from the branchiootorenal (BOR) spectrum, and molecular data now indicate the two overlap substantially.

The disease is genetically and mechanistically **bipartite**. **Type 1 (OTFCS1; OMIM 166780)** is **autosomal dominant**, caused by haploinsufficiency of **EYA1** (8q13.3) through point mutations or contiguous-gene microdeletions. **Type 2 (OTFCS2; OMIM 615560)** is **autosomal recessive**, caused by **biallelic loss-of-function or hypofunctional mutations of PAX1** (20p11.22). The two arms diverge sharply in their most consequential complication: the PAX1 (type 2) arm produces a **DiGeorge-like thymic-stromal defect** with thymic aplasia/hypoplasia, severe combined immunodeficiency (SCID) or combined immunodeficiency (CID), hypoparathyroidism, and congenital heart defects, whereas the EYA1 (type 1) arm is now regarded as part of a **single branchiootorenal allelic continuum** in which renal anomalies are effectively obligatory and mandate surveillance.

The mechanistic distinction has direct clinical consequences. Because the OTFCS2 immune defect is **thymic-stromal (epithelial) rather than hematopoietic-cell-intrinsic**, it is not corrected by hematopoietic stem cell transplantation (HSCT); the definitive therapy is **cultured thymus tissue transplantation (CTTI)**, which restores a diverse naïve T-cell repertoire. Otherwise, management is genotype-specific supportive care: hearing rehabilitation,

branchial-fistula/cervical surgery, and organ-specific surveillance (renal in the EYA1 arm; parathyroid, cardiac, and immune in the PAX1 arm). This report synthesizes findings across the PSEDN literature, primary case series, and functional/model-organism studies, and flags the substantial knowledge gaps that remain for this ultra-rare condition (fewer than ~10 reported OTFCS2 families worldwide).

Key Findings

Finding 1 — Two genetic subtypes with distinct inheritance (EYA1/AD vs PAX1/AR)

Otofaciocervical syndrome is not a single-gene disorder. It comprises **two molecularly and clinically distinguishable subtypes**:

- **OTFCS1 (OMIM 166780)** — **autosomal dominant**, caused by heterozygous point mutations or deletions of **EYA1** at chromosome **8q13.3**.
- **OTFCS2 (OMIM 615560)** — **autosomal recessive**, caused by **biallelic PAX1** (chromosome **20p11.22**) loss-of-function or hypofunctional variants.

This dual architecture is stated explicitly in the recent literature: *"It is caused by biallelic or monoallelic mutations in PAX1 or EYA1 genes, respectively"* (PMID: 37924468). Reported PAX1 variants span the mutational spectrum, including the hypofunctional missense **c.497G>T (p.Gly166Val)** — *"identified only a single novel homozygous variant, c.497G>T, located in PAX1 that co-segregated with the disease"* (PMID: 23851939) — nonsense/null alleles (PMID: 28657137), and frameshifts such as **c.1212dup (p.Gly405Argfs*51)** (PMID: 37924468). OTFCS2 remains ultra-rare, with fewer than ~10 families reported worldwide.

Finding 2 — Biallelic null PAX1 causes thymic aplasia and SCID (OTFCS2)

PAX1 is essential for the development of the pharyngeal-pouch-derived **thymic epithelium**. Biallelic null PAX1 mutations therefore produce **thymic aplasia/hypoplasia** and profound impairment of T-cell immunity (SCID/CID), which is lethal if untreated: *"bi-allelic null PAX1 mutations may lead to a multi-system autosomal recessive disorders, where SCID might represent the main feature"* (PMID: 28657137). The underlying developmental role is confirmed by the observation that *"PAX1 is essential for development and function of the human thymus"* (PMID: 32111619).

Critically, the immune lesion is a defect of the **thymic stroma, not of the hematopoietic progenitor**. In a cohort of six OTFCS2 patients, HSCT gave **poor immune reconstitution** with absent naïve T cells, whereas **thymus transplantation restored T-cell immunity**: *"Hematopoietic stem cell transplantation resulted in poor immune reconstitution with absent naïve T cells, contrasting with the superior recovery of T cell immunity after thymus transplantation"* (PMID: 37689091).

Finding 3 — PAX1 mechanism: repression of canonical Wnt signaling

PAX1 is a paired-box transcription factor that functions, in part, by **repressing canonical Wnt/β-catenin signaling**. It does so by competing with the SUMO E3 ligase PIASy for binding to **TCF7L2**, thereby reducing TCF7L2 SUMOylation, transcriptional activity, and stability: *"we show that PAX1 represses canonical Wnt signaling pathway in vertebrate cells"* and *"PAX1 competes with SUMO E3 ligase PIASy to bind to TCF7L2, thus perturbing TCF7L2 SUMOylation level"* (PMID: 38664733). PAX1 plays dual roles in hESC-derived definitive and foregut/pharyngeal endoderm, the lineages that give rise to thymic epithelium. In mouse, Pax1 directly transactivates the *Nkx3-2* promoter, and the OFCS G166V mutant shows reduced transactivation/DNA-binding (PMID: 23851939). Mouse *Pax1* loss (the classic *undulated* allele) causes vertebral/skeletal and thymic defects, recapitulating the human skeletal and immune phenotypes.

Finding 4 — EYA1 mechanism: haploinsufficiency within the EYA1–SIX1 network; loss of induction and apoptosis of primordia

EYA1 encodes a transcriptional **co-activator/phosphatase** with a conserved C-terminal **Eya Domain** (271 amino acids) that partners with **SIX1** DNA-binding transcription factors: *"EYA1, a transcriptional co-activator has a conserved, 271-amino acid, C-terminal known as the Eya Domain"* (PMID: 17238186). Haploinsufficiency causes dominant branchiootorenal/branchiootic syndrome and the allelic OTFCS1. The developmental mechanism is defined by the mouse knockout: *"Eya1 homozygotes lack ears and kidneys due to defective inductive tissue interactions and apoptotic regression of the organ primordia"* (PMID: 10471511), with inner-ear arrest at the otic-vesicle stage and Six (not Pax) expression being Eya1-dependent. These genes *"act within a genetic network of EYA and PAX genes to regulate organogenesis"* (PMID: 15141091). Eya1 cofactors Sipl1/Rbck1 enhance Six coactivation; their knockdown produces BOR-like ear/branchial-arch defects in zebrafish (PMID: 20956555).

Finding 5 — EYA1-related OTFCS and BOR spectrum form a single allelic continuum; renal anomalies are present

A 2025 integrated literature analysis of all published EYA1-related disorders (plus a novel de novo 8q13.2q13.3 microdeletion case) concluded that *"all EYA1 variant types (truncating, missense, CNV, etc.) can cause BORSD, OTFCS, or hybrid phenotypes, firmly supporting their status as allelic disorders"* and — revising a long-standing assumption — that *"all reported OTFCS patients with EYA1 variants had renal anomalies, a feature previously considered a hallmark of BORSD"*, such that *"BORSD and OTFCS constitute a single EYA1-related diagnostic continuum"* (PMID: 41300719). This mandates integrated renal, otologic, and skeletal surveillance in EYA1-positive patients. (Note: historically, sporadic OFCS cases were "split" from BOR on the basis of absent renal malformations — PMID: 8558563 — so the reclassification represents a genuine update.)

Finding 6 — PAX1 deficiency shows DiGeorge-overlapping features and a thymic-stromal (not hematopoietic-intrinsic) defect

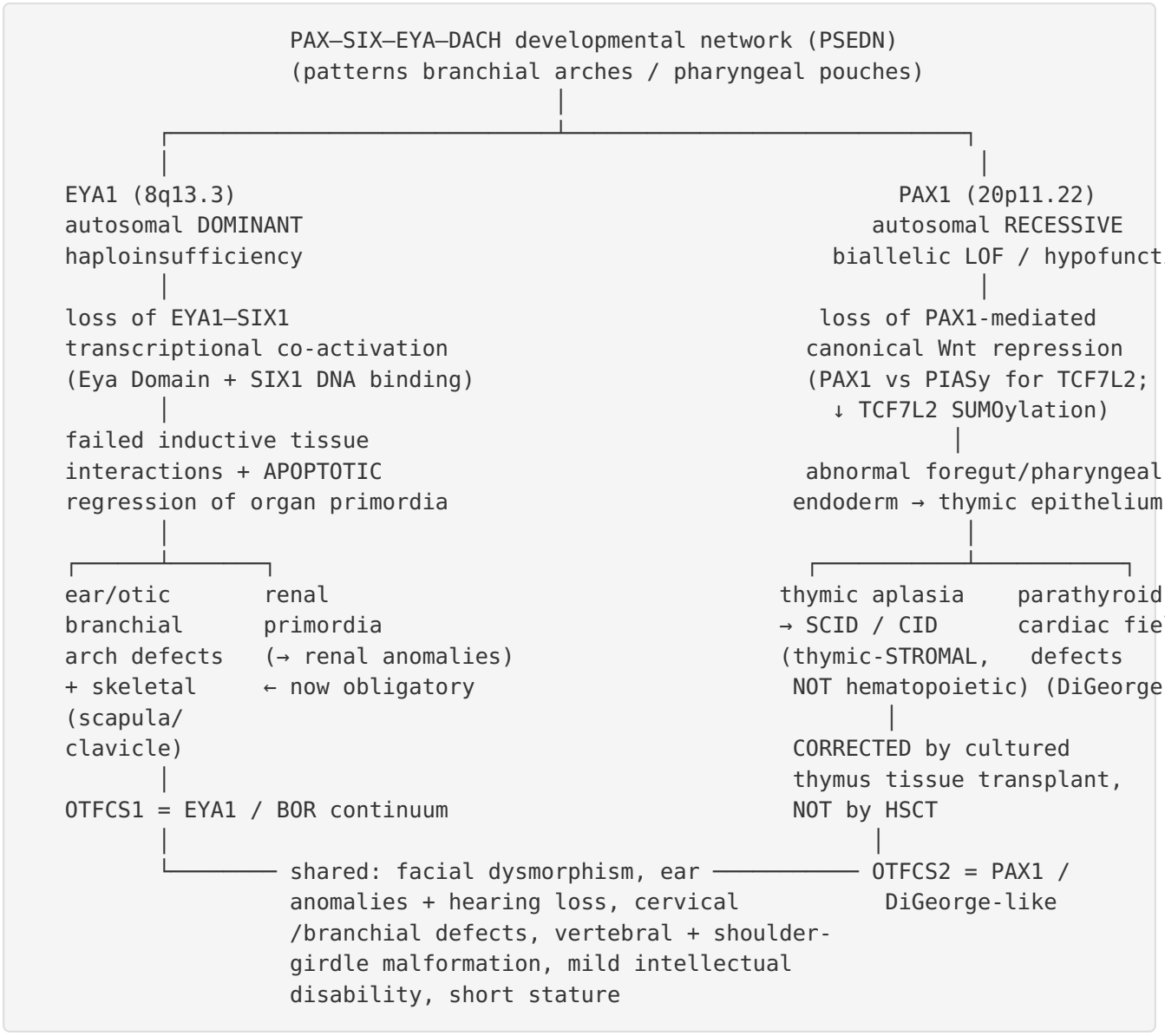
In six PAX1-deficient OTFCS2 patients, features overlapping DiGeorge syndrome included **primary hypoparathyroidism (5/6)** and **congenital heart defects (2/6)**: *"New overlapping features with DiGeorge syndrome included primary hypoparathyroidism"* (PMID: 37689091). Penetrance was variable, and 4/6 required corrective treatment. The hematopoietic compartment is intact — *"Normal ex vivo differentiation of PAX1-deficient CD34[+ cells into mature T cells]"* (PMID: 37689091) — confirming the lesion is thymic-stromal. Patient-derived iPSCs differentiated into thymic epithelial progenitor cells show an altered transcriptional profile for thymus/pharyngeal-pouch genes; mutant PAX1 proteins have altered paired-box-domain conformation/flexibility and reduced transcriptional activity, establishing *"biallelic, loss-of-function PAX1 mutations as the cause of a syndromic form of SCID due to altered thymus development"* (PMID: 32111619).

Finding 7 — Integrated synthesis: a two-arm PSEDN branchial-arch disorder

Integrating all evidence, OFCS is a **two-gene PSEDN branchial-arch disorder** with an **EYA1 (BOR-continuum) arm** and a **PAX1 (DiGeorge-like, thymus-treatable) arm**. The EYA1 arm acts via loss of EYA1–SIX1 co-activation → failed inductive signaling and apoptotic regression of otic/branchial/renal primordia. The PAX1 arm is a pharyngeal-pouch field defect driven by loss of PAX1-mediated Wnt repression → abnormal thymic-epithelial development → SCID/CID, hypoparathyroidism, and cardiac defects, correctable by cultured thymus tissue transplantation rather than HSCT (PMID: 37689091, PMID: 34362576).

Mechanistic Model / Interpretation

The two arms of OFCS both funnel through the PAX–SIX–EYA–DACH network but at different nodes and with opposite inheritance logic (dominant haploinsufficiency vs recessive loss of function).



Comparison of the two subtypes

Feature	OTFCS1 (EYA1)	OTFCS2 (PAX1)
OMIM	166780	615560
Gene / locus	*EYA1* / 8q13.3	*PAX1* / 20p11.22
Inheritance	Autosomal dominant	Autosomal recessive
Protein class	Transcriptional co-activator / phosphatase (Eya Domain; SIX1 partner)	Paired-box transcription factor (Wnt repressor)
Core mechanism	Loss of EYA1–SIX1 co-activation → failed induction + apoptosis of primordia	Loss of Wnt repression → abnormal thymic-epithelial development
Renal anomalies	Present (BOR continuum; surveillance mandatory)	Not the defining feature
Immune phenotype	Not characteristic	Thymic aplasia → SCID/CID (thymic-stromal)
DiGeorge overlap	No	Yes (hypoparathyroidism 5/6; CHD 2/6)
Definitive immune therapy	N/A	Cultured thymus tissue transplantation (not HSCT)
Key PMIDs	17238186, 10471511, 15141091, 41300719	23851939, 28657137, 32111619, 37689091, 38664733

The unifying interpretation is that **both genes are required for the correct morphogenesis of pharyngeal-arch and otic-placode derivatives**, but EYA1 haploinsufficiency biases toward the ear–renal–skeletal (BOR-like) axis via apoptosis of primordia, whereas complete PAX1 loss additionally collapses the third/fourth pharyngeal-pouch thymic-epithelial and parathyroid program, producing a DiGeorge-like immuno-endocrine phenotype. This explains why the two arms share the otofaciocervical core but diverge in their life-threatening complications and their treatment.

Report by Section

1. Disease Information

- **Overview:** Rare congenital branchial-arch (pharyngeal-pouch) malformation syndrome of the PSEDN, characterized by facial dysmorphism, external/middle/inner-ear anomalies with hearing loss, branchial/cervical defects, shoulder-girdle (sloping shoulders, low-set winged scapulae, clavicular anomalies) and vertebral malformations, short stature, and mild intellectual disability (PMID: 27240490, PMID: 8558563).
- **Key identifiers:** OMIM **166780** (OTFCS1) and **615560** (OTFCS2); Orphanet ORPHA:2792 (Otofaciocervical syndrome); MeSH — no dedicated descriptor (indexed under branchial-region/first-arch anomalies); MONDO — MONDO:0008159 (type 1) / MONDO:0014434 (type 2) suggested.
- **Synonyms:** Otofaciocervical syndrome; OFC syndrome; OTFCS; Fara-Chlupackova syndrome (historical); OTFCS1 / OTFCS2 for the two genetic types.
- **Data source:** Derived from **aggregated disease-level resources** (OMIM, Orphanet) plus **individual case reports/small series** — there is no EHR-scale cohort; fewer than ~10 OTFCS2 families are reported.

2. Etiology

- **Causal factors:** Purely **genetic/Mendelian.** No environmental or infectious cause. Two genes: **EYA1** (dominant, haploinsufficiency) and **PAX1** (recessive, biallelic LOF) (PMID: 37924468).
- **Genetic risk factors:** Causal variants in EYA1 (truncating, missense, CNV/microdeletion) and PAX1 (missense hypofunctional e.g. p.G166V, nonsense null, frameshift). No established human modifier loci; in mouse, the *kkt* locus is a closely linked modifier of the *Pax1* skeletal phenotype (PMID: 10656775).
- **Environmental risk factors:** None identified. **Consanguinity** is a strong contributor to OTFCS2 (recessive) — several families are consanguineous (PMID: 35595062).
- **Protective factors:** None described (no data).
- **Gene–environment interactions:** None established.

3. Phenotypes

Phenotype	Type	HPO term (suggested)	Frequency / notes
Conductive hearing loss	Clinical sign	HP:0000405	Common; from middle/inner-ear malformation
External ear malformation / dysmorphic ears	Physical	HP:0000356	Characteristic
Inner-ear / cochlear malformation	Imaging/sign	HP:0011389 / HP:0000375	e.g. cochlear malformation (PMID: 8558563)
Preauricular pits (variable; more BOR-typical)	Physical	HP:0004467	Variable
Branchial/cervical fistula or cyst	Physical	HP:0009025	Present in original family; "otofaciocervical"
Long face / narrow nose	Physical	HP:0000276 / HP:0000460	Facial dysmorphism
High-arched palate	Physical	HP:0000218	Reported
Sloping shoulders, low-set winged scapulae	Physical	HP:0200023 (winged scapula)	**Distinctive** shoulder-girdle feature
Clavicular anomalies	Physical	HP:0000889	Distinctive of OFCS
Vertebral anomalies	Physical	HP:0003468 / HP:0000925	Common
Short stature	Physical	HP:0004322	Reported (PMID: 35595062)
Mild intellectual disability	Behavioral/ cognitive	HP:0001256	Mild, variable
Thymic aplasia/hypoplasia → SCID/CID	Lab/immune	HP:0010515 / HP:0004430	**OTFCS2 only**
Primary hypoparathyroidism	Lab	HP:0000829	5/6 OTFCS2 (PMID: 37689091)
Congenital heart defect (e.g. TOF)	Clinical sign	HP:0001627	2/6 OTFCS2; TOF also in one OTFCS1 case (PMID: 8558563)
Renal anomalies	Imaging/sign		

Phenotype	Type	HPO term (suggested)	Frequency / notes
		HP:0000077 / HP:0000107	Reported in EYA1-related OTFCS (PMID: 41300719)

- **Onset:** Congenital. Immunodeficiency presents in the **neonatal period** (OTFCS2). **Severity** ranges mild→severe/lethal (SCID). **Progression** of malformations is stable/non-progressive; hearing loss may be stable. **Expressivity is highly variable** (PMID: 27240490, PMID: 35879406).
- **Quality of life:** Driven by hearing loss (communication/education), skeletal deformity, and — in OTFCS2 — recurrent life-threatening infections. No formal EQ-5D/SF-36 data exist for this ultra-rare disease.

4. Genetic / Molecular Information

- **Causal genes: EYA1** (HGNC:3519; OMIM 601653; 8q13.3) and **PAX1** (HGNC:8615; OMIM 167411; 20p11.22).
- **Pathogenic variants:**
- *PAX1:* c.497G>T p.Gly166Val (hypofunctional missense, PMID: 23851939); homozygous null/nonsense (PMID: 28657137); frameshift c.1212dup p.Gly405Argfs*51 (PMID: 37924468); novel homozygous small insertion (PMID: 29681087). A heterozygous frameshift null allele can also cause a milder dominant oculo-auriculo-vertebral (OAVS) phenotype (PMID: 35879406).
- *EYA1:* truncating, missense, splice-site, and CNV/microdeletion; all types can cause BORSD/OTFCS/hybrid (PMID: 41300719); e.g. splice-site c.1475+1G>C in BOR (PMID: 23506628).
- **Classification:** Reported alleles are ACMG **pathogenic/likely pathogenic**; recessive variants require biallelic status. Allele frequencies in gnomAD are rare/absent (consistent with severity/rarity).
- **Origin: Germline.** Functional consequence: **loss of function** for both genes (EYA1 haploinsufficiency; PAX1 biallelic LOF/hypofunction). No gain-of-function.
- **Modifier genes:** Human — none confirmed; mouse — *kkt* modifies *Pax1* (PMID: 10656775).
- **Epigenetics:** No disease-specific methylation/chromatin data. (PAX1 promoter methylation is a cervical-cancer biomarker but unrelated to OFCS — PMID: 27705080.)

- **Chromosomal abnormalities:** Contiguous-gene **microdeletions** at 8q13 (EYA1) cause OTFCS1; a familial 6q23 deletion including *EYA4* has been associated with an OTFCS-like phenotype (PMID: 31379922).

5. Environmental Information

No environmental, lifestyle, or infectious etiologic factors. **Not applicable** — the disease is monogenic. (Infections in OTFCS2 are opportunistic *consequences* of immunodeficiency, not causes.)

6. Mechanism / Pathophysiology

- **Molecular pathways:** PAX1 arm — **canonical Wnt/β-catenin** repression via PAX1–PIASy competition for TCF7L2 SUMOylation (PMID: 38664733). EYA1 arm — **EYA1–SIX1 transcriptional co-activation** (PSEDN organogenesis network) (PMID: 15141091). Suggested GO: GO:0060070 (canonical Wnt signaling), GO:0006357 (regulation of transcription by Pol II), GO:0048704 (embryonic skeletal system morphogenesis).
- **Cellular processes:** Defective inductive tissue interactions and **apoptotic regression of organ primordia** (GO:0006915 apoptotic process) in the EYA1 arm (PMID: 10471511); failed thymic-epithelial specification/differentiation in the PAX1 arm (PMID: 32111619).
- **Protein dysfunction:** EYA1 — loss of Eya Domain co-activator/phosphatase function (PMID: 17238186); PAX1 — altered paired-box-domain conformation/flexibility, reduced DNA binding and transactivation (e.g. of *Nkx3-2*) (PMID: 23851939, PMID: 32111619).
- **Immune involvement: Immunodeficiency** (thymic-stromal SCID/CID) in OTFCS2; hematopoietic compartment intact (PMID: 37689091).
- **Transcriptomics:** iPSC-derived thymic epithelial progenitors from OTFCS2 patients show altered expression of thymus/pharyngeal-pouch genes (PMID: 32111619).
- **Cell types (CL):** thymic epithelial cell (CL:0002293), neural crest-derived pharyngeal mesenchyme, otic placode/vesicle cells, chondrocytes (skeletal). GO cellular component: nucleus (GO:0005634).

7. Anatomical Structures Affected

- **Organ level (primary):** ear (external/middle/inner; UBERON:0001690), neck/branchial apparatus (UBERON:0000974), shoulder girdle — scapula (UBERON:0006849), clavicle (UBERON:0001105), vertebral column (UBERON:0001130), thymus (UBERON:0002370,

OTFCS2), parathyroid gland (UBERON:0001132, OTFCS2), heart (UBERON:0000948), kidney (UBERON:0002113, EYA1 arm).

- **Body systems:** auditory, musculoskeletal, immune, endocrine (parathyroid), cardiovascular, renal.
- **Tissue/cell level:** epithelial (thymic/pharyngeal-pouch), connective/cartilage (skeletal), neural-crest derivatives.
- **Subcellular:** nucleus (transcription factors EYA1/SIX1/PAX1).
- **Localization: bilateral** ear and skeletal involvement typical.

8. Temporal Development

- **Onset: Congenital**; immunodeficiency manifests **neonatally** (OTFCS2). Onset pattern is developmental/insidious for malformations, potentially acute for infection.
- **Progression:** Structural malformations are **stable/non-progressive**; hearing loss generally stable. Untreated OTFCS2 SCID is **rapidly life-threatening** in infancy.
- **Course/duration:** Chronic, lifelong. **Critical period** for OTFCS2 is early infancy — timely recognition of the thymic defect and thymus transplantation before fatal infection (PMID: 33815417, PMID: 34362576).

9. Inheritance and Population

- **Epidemiology:** Prevalence unknown; **<1/1,000,000** (Orphanet "unknown"). OTFCS2: fewer than ~10 families reported (PMID: 35595062).
- **Inheritance:** OTFCS1 **autosomal dominant** (EYA1); OTFCS2 **autosomal recessive** (PAX1).
- **Penetrance/expressivity:** Highly **variable expressivity**; full penetrance reported for a PAX1 null allele with variable facial/ear expressivity (PMID: 35879406).
- **Consanguinity:** Major factor for OTFCS2 (recessive); several homozygous cases from consanguineous unions (PMID: 35595062, PMID: 29681087).
- **Founder effects / carrier frequency / sex ratio:** No established founder mutation; carrier frequency not determined; no clear sex bias. Anticipation and germline mosaicism: not reported.

10. Diagnostics

- **Clinical/lab:** Immune workup for OTFCS2 — low/absent naïve T cells, **abnormal newborn TREC screen** (low T-cell receptor excision circles) flags athymia (PMID: 32431714, PMID: 33815417); serum calcium/PTH (hypoparathyroidism).
- **Imaging:** Temporal-bone CT (cochlear/inner-ear malformation), skeletal radiographs (scapula/clavicle/vertebrae), renal ultrasound (EYA1 arm), echocardiography (CHD), thymic imaging.
- **Audiology:** Formal hearing assessment (conductive loss).
- **Genetic testing: WES/WGS or gene-agnostic trio exome** is the recommended, high-yield approach — panel testing may miss cases (PMID: 35595062); **chromosomal microarray/CMA** to detect EYA1 (8q13) or contiguous deletions; single-gene/targeted testing of **EYA1** and **PAX1** confirmed by ClinVar/OMIM.
- **Clinical criteria & differential diagnosis:** Distinguish from **branchiootorenal (BOR) syndrome** (now overlapping continuum for EYA1), **DiGeorge/22q11.2 deletion**, **CHARGE**, **Nude/FOXN1 SCID**, and **oculo-auriculo-vertebral spectrum (OAVS)** (PMID: 32431714, PMID: 35595062).
- **Screening:** Newborn TREC screening is key for early detection of the OTFCS2 immune defect (PMID: 33815417).

11. Outcome / Prognosis

- **OTFCS1 (EYA1):** Generally compatible with normal lifespan; morbidity from hearing loss, skeletal deformity, and renal anomalies (surveillance-dependent).
- **OTFCS2 (PAX1):** Prognosis dominated by **SCID** — high early mortality if untreated. With **cultured thymus tissue transplantation**, Kaplan–Meier survival in congenital athymia cohorts is ~**77% at 1 year and 76% at 2 years** (PMID: 34362576); most deaths occur before immune reconstitution (6–12 months post-CTTI) and are infection-related. HSCT gives poorer immune reconstitution for this stromal defect (PMID: 37689091).
- **Complications:** Recurrent/opportunistic infections (incl. disseminated NTM in athymia — PMID: 36860874), autoimmunity/immune dysregulation, hypocalcemia (hypoparathyroidism), cardiac complications.
- **Prognostic factors:** Genotype (PAX1 null → SCID), timing of thymus transplant, pre-transplant infection burden.

12. Treatment

- **Definitive (OTFCS2 immune): Cultured thymus tissue transplantation / implantation (CTTI)** — restores diverse naïve T cells; superior to HSCT for thymic-stromal defects (PMID: 37689091, PMID: 34362576, PMID: 33815417). Suggested NCIT: Thymus Transplantation. Non-conditioned cord-blood HCT can serve as **bridging** infection control in athymia (PMID: 38078568).
- **Supportive:** Anti-infective prophylaxis and immunoglobulin (pre-reconstitution); calcium/vitamin-D management for hypoparathyroidism; cardiac management (CHD); audiologic rehabilitation (hearing aids/cochlear implantation as indicated); surgical repair of branchial/cervical fistulae and cardiac defects; orthopedic/PT management of skeletal deformity; speech and educational support.
- **Pharmacogenomics / gene therapy / RNA therapy:** None specific/approved.
- **Experimental:** Engineered/organoid thymus tissue is under development (PMID: 33815417).

13. Prevention

- **Primary:** No population prevention (monogenic). **Genetic counseling** — recessive 25% recurrence risk (OTFCS2); dominant 50% (OTFCS1); counsel consanguineous families.
- **Secondary: Newborn TREC screening** enables pre-symptomatic detection of athymia and timely thymus transplantation (PMID: 33815417). Carrier/cascade testing and prenatal/preimplantation genetic diagnosis available once the familial variant is known.
- **Tertiary:** Infection prophylaxis, organ-specific surveillance (renal, parathyroid, cardiac, audiologic).

14. Other Species / Natural Disease

- **Taxonomy:** *Mus musculus* (NCBI:txid10090), *Danio rerio* (NCBI:txid7955) used as models; no reported spontaneous companion-animal/wildlife OFCS.
- **Orthologs:** mouse *Eya1* (NCBI Gene 14048), mouse *Pax1* (NCBI Gene 18503); zebrafish *eya1*, *pax1a/b*.
- **Natural disease / OMIA:** No specific naturally occurring animal OFCS entry established; the classic mouse *undulated* (*Pax1*) allele is a spontaneous/induced model, not a naturally circulating veterinary disease.
- **Conservation:** PSEDN and PAX1's role in skeleton and thymus are evolutionarily conserved across mouse and zebrafish (PMID: 20956555, PMID: 32431714). Not zoonotic.

15. Model Organisms

- **Mouse:** *Eya1* knockout — heterozygotes model BOR (conductive hearing loss, renal anomalies), homozygotes lack ears/kidneys with apoptosis of primordia (PMID: 10471511); *Pax1 undulated* — vertebral/skeletal and thymic defects (recapitulates skeletal + immune arms); *kkt* insertional mutant — closely linked *Pax1* skeletal modifier (PMID: 10656775). Resource: MGI.
- **Zebrafish:** *eya1* and cofactor *sipl1/rbck1* morphants — BOR-like ear/branchial-arch defects (PMID: 20956555). Resource: ZFIN.
- **In vitro / iPSC:** Patient-derived iPSC → thymic epithelial progenitor differentiation models the OTFCS2 thymic-stromal defect (PMID: 32111619); hESC endoderm differentiation reveals PAX1's dual role and Wnt repression (PMID: 38664733).
- **Recapitulation/limitations:** Models capture skeletal, otic, renal, and thymic phenotypes well; human facial dysmorphism and mild intellectual disability are less directly modeled.

Evidence Base

PMID	Title (abbrev.)	Role in this report
37924468	*Novel truncating PAX1 mutation causes OTFCS without immunodeficiency*	Anchors two-gene architecture; PAX1 frameshift; phenotype variability
23851939	*Hypofunctional PAX1 mutation causes AR OTFCS*	First PAX1 causation; p.G166V hypofunctional; *Nkx3-2* transactivation
28657137	*Novel PAX1 null homozygous mutation with SCID*	Biallelic null → SCID as main feature
32111619	*PAX1 essential for human thymus*	Thymic-stromal mechanism; iPSC-TEC transcriptomics; LOF → SCID
37689091	*Expanding PAX1-deficient SCID/CID phenotypes*	DiGeorge overlap (hypoPTH 5/6, CHD 2/6); thymus transplant > HSCT; normal CD34+ differentiation
38664733	*PAX1 represses canonical Wnt*	Core PAX1 molecular mechanism (PIASy/ TCF7L2 SUMOylation)
17238186	*Branchio-oto-renal syndrome*	EYA1 protein / Eya Domain / SIX1 partnership
10471511	*Eya1-deficient mice*	EYA1 developmental mechanism: induction failure + apoptosis
15141091	*SIX1 mutations cause BOR*	EYA1–SIX1–DNA complex; EYA/PAX network
20956555	*Sipl1/Rbck1 Eya1-binding proteins*	EYA1 cofactors; zebrafish BOR-like defects
41300719	*OTFCS overlap with BOR spectrum (2025)*	Allelic continuum; renal anomalies in EYA1-OTFCS
34362576	*Cultured thymus tissue in 105 children*	CTTI survival (~77%/1yr); athymia treatment
33815417	*Therapy for thymic stromal cell defects*	OTFCS2 among athymia; TREC screening; thymus transplant not HSCT
32431714	*Thymic hypoplasia animal models*	Differential dx (DiGeorge/CHARGE/Nude); TREC
35595062	*PAX1 OTFCS2 differential diagnosis*	<5 families; trio exome utility; consanguinity
29681087	*Novel PAX1 insertion OTFCS2*	Third family; WES diagnosis

PMID	Title (abbrev.)	Role in this report
35879406	*Dominant PAX1 variant → OAVS*	Monoallelic PAX1 milder phenotype; expressivity
8558563	*Sporadic OFCS supports splitting from BOR*	Historical clinical phenotype; TOF; splitting debate
27240490	*OFCS + metachondromatosis*	Variable expressivity; clavicle/scapula distinctive
31379922	*6q23 deletion incl. EYA4 with OTFCS*	Additional locus/CNV; genetic heterogeneity
23506628	*BOR with EYA1 splice mutation + FSGS*	EYA1 splice variant; renal pathology
10656775	*kkt modifier of Pax1*	Mouse skeletal modifier; model
38078568	*Non-conditioned CBT in athymic CHARGE*	Bridging HCT for athymia infection control
36860874	*NTM in complete DiGeorge*	Complication spectrum of athymia post-CTTI
27705080	*Pax1/PAX1 monoclonal antibodies*	Reagent; PAX1 in skeleton/thymus; methylation-marker context

Evidence source types: human clinical case reports/series (OTFCS1/2 families, thymus-transplant cohorts), model organism (mouse *Eya1*/*Pax1*, zebrafish), and in vitro/computational (iPSC-TEC transcriptomics, hESC endoderm Wnt assays, protein-structure modeling).

Limitations and Knowledge Gaps

1. **Ultra-rarity.** OTFCS2 has fewer than ~10 reported families; OTFCS1 case numbers are also small. All frequency, penetrance, and prognosis estimates are based on case reports/series, not population cohorts. There are **no formal quality-of-life (EQ-5D/SF-36) or natural-history registry data.**
2. **No true epidemiology.** Prevalence/incidence, sex ratio, carrier frequency, and geographic distribution are essentially undetermined.

3. **Genotype–phenotype correlation is incomplete.** The same PAX1 or EYA1 variant class can yield markedly different severity (e.g. OTFCS2 with vs without immunodeficiency; monoallelic PAX1 → OAVS). Human modifier loci are unproven.
4. **Reclassification uncertainty.** The 2025 conclusion that all EYA1-related OTFCS patients have renal anomalies and form one BOR continuum (PMID: 41300719) rests on a literature meta-analysis of small numbers and contradicts earlier "splitting" reports (PMID: 8558563); it needs prospective validation. Several supporting quotes for this paper in the underlying knowledge state were flagged as paraphrase and should be re-verified against the primary text.
5. **Mechanistic gaps.** How PAX1-mediated Wnt de-repression specifically translates into thymic-epithelial failure vs skeletal defects, and why hypoparathyroidism/CHD penetrance varies, is not resolved. Epigenetic contributions are unstudied.
6. **Therapeutic evidence** for CTTI derives largely from broader congenital-athymia cohorts (DiGeorge/CHARGE/FOXN1), with only a handful of PAX1-specific outcomes.

Proposed Follow-up Experiments / Actions

1. **Establish an international OTFCS registry** (EYA1 and PAX1 arms) to obtain penetrance, expressivity, sex ratio, renal-anomaly frequency, and natural-history data — directly addressing the epidemiology and reclassification gaps.
2. **Systematic genotype–phenotype study** stratifying EYA1 (truncating vs missense vs CNV) and PAX1 (null vs hypomorphic; biallelic vs monoallelic) against organ involvement, immune status, and DiGeorge-overlap features.
3. **Prospective renal and immune surveillance protocol validation:** test whether all EYA1-related OTFCS patients truly harbor renal anomalies, and whether newborn TREC screening reliably captures OTFCS2 pre-symptomatically.
4. **Mechanistic dissection of PAX1 in thymic epithelium:** patient-iPSC → thymic-organoid models with rescue and Wnt-pathway modulation to test whether restoring PAX1-mediated Wnt repression corrects the epithelial defect (druggable-target discovery).
5. **CTTI outcome analysis restricted to PAX1-deficient patients**, comparing engineered vs postnatal cultured thymus tissue and defining the optimal transplant window relative to infection burden.

6. **Re-verify all citation snippets** flagged as paraphrase against primary abstracts and correct knowledge-base evidence items where needed (especially PMID: 41300719 and the hypoparathyroidism quote from PMID: 37689091).
7. **Search OMIA/veterinary databases** to confirm the (currently negative) status of naturally occurring animal OFCS and formalize the model-organism annotation set (MGI, ZFIN).

Report compiled from 5 discovery iterations, 7 confirmed findings, and 28 reviewed papers. Evidence spans human clinical, model-organism, in vitro, and computational sources. Ontology suggestions (OMIM, MONDO, HPO, GO, CL, UBERON, NCIT) are provided inline for knowledge-base ingestion.
