Premature Ovarian Failure 20 (POF20 / MSH4-related Primary Ovarian Insufficiency): A Comprehensive Disease Characterization

Disease: Premature Ovarian Failure 20 (POF20) **Primary identifiers:** MONDO:0030975 · OMIM:619938 · UMLS:C5677011 · MedGen:1808256 · GARD:0025670 **Causal gene:** MSH4 (HGNC:7327; NCBI Gene 4438; Ensembl ENSG00000057468; UniProt O15457; gene-OMIM *602105; locus 1p31.1) Inheritance: Autosomal recessive (HP:0000007) Category:** Mendelian

> **Evidence-source key:** [H] human clinical; [M] model organism; [V] in-vitro/biochemical; [C] computational/database. Primary literature cited as PMIDs.

Summary

Premature Ovarian Failure 20 (POF20; MONDO:0030975, OMIM 619938) is a rare, Mendelian, **autosomal-recessive** form of primary/premature ovarian insufficiency (POI) caused by **biallelic loss-of-function variants in the meiosis-specific gene *MSH4* (MutS Homolog 4)**, located at chromosome 1p31.1. It was first delineated in 2017 by Carlosama and colleagues, who identified a homozygous donor splice-site variant in a consanguineous Colombian family PMID: 28541421. Multiple independent biallelic variants have since confirmed the gene–disease relationship across diverse cohorts.

Mechanistically, POF20 is a disease of **failed meiotic recombination**. MSH4 heterodimerizes with MSH5 to form the **MutSγ complex**, which binds and stabilizes Holliday-junction recombination intermediates and licenses the downstream MLH1–MLH3 (MutLγ) resolvase (with EXO1 and RFC-PCNA) to convert double Holliday junctions into crossovers. When MSH4 is lost, crossovers fail to form, homologous chromosomes cannot pair/synapse, meiosis I arrests, and oocytes are eliminated by apoptosis. The resulting depletion of the ovarian follicle

pool produces the clinical phenotype: **young-adult-onset secondary amenorrhea, female infertility, and hypergonadotropic hypogonadism** (elevated FSH and LH). Because the meiotic requirement is shared between sexes, the identical biallelic genotype causes **non-obstructive azoospermia (NOA)** in affected male relatives — the male counterpart is annotated as spermatogenic failure (SPGF2).

There is **no disease-modifying or curative therapy**. Management, per the 2024 ESHRE/ASRM/IMS evidence-based POI guideline, centers on **hormone replacement therapy (HRT)** until the average age of natural menopause (~51 years) to prevent the sequelae of premature estrogen deprivation (osteoporosis, cardiovascular disease, adverse cognitive/sexual health, increased all-cause mortality), together with bone-health monitoring and psychological support. Because meiosis is abolished, own-oocyte conception is essentially non-viable; **donor-oocyte (or donor-embryo) IVF** is the principal fertility option.

Key Findings

Finding 1 — POF20 is caused by biallelic loss-of-function variants in *MSH4* and is inherited autosomal-recessively [H]

POF20 maps unambiguously to the identifier set **OMIM:619938** / **MONDO:0030975** / **UMLS:C5677011** / **GARD:0025670**. The Monarch Initiative records a Causal-Gene-to-Disease association linking **HGNC:7327 (MSH4)** to POF20 with **autosomal recessive inheritance (HP:0000007)**. The disease was first described by Carlosama et al. (2017), who used whole-exome sequencing in a consanguineous Colombian family to identify a **homozygous donor splice-site mutation** causing skipping of exon 17 (p.Ile743_Lys785del). The variant segregated with POI and was absent in 135 fertile controls.

> *"Whole-exome sequencing of members of a Colombian family affected by POI allowed us to identify a novel homozygous donor splice-site mutation in the meiotic gene MSH4 (MutS Homolog 4)."* — PMID: 28541421
>
> *"Our study describes the first MSH4 mutation associated with POI."* — PMID: 28541421

Multiple independent biallelic variants have since confirmed the association, including reports from large POI gene-panel and exome cohorts PMID: 36099812, PMID: 36793102, PMID: 36373164, and PMID: 38175272.

> **Note on causal-gene correction:** Early in this investigation the gene was provisionally listed as MCM8. This was corrected to **MSH4**, which is the gene formally linked to OMIM 619938 / POF20. MCM8 defects cause a distinct POI entity (POF10 / OMIM 612885 — see PMID: 25873734, PMID: 32048466).

Finding 2 — Mechanism: MSH4 loss disrupts the MutSγ (MSH4–MSH5) complex, abolishing meiotic crossovers [V/H]

MSH4 heterodimerizes with MSH5 to form **MutSγ**, which binds and stabilizes Holliday-junction (HJ) recombination intermediates with the highest affinity among tested substrates (nanomolar K_d) PMID: 30467025. MutSγ licenses the **MLH1–MLH3 (MutLγ) endonuclease**, acting with EXO1 and RFC-PCNA, to resolve double Holliday junctions into crossovers PMID: 40319035, PMID: 32814904. Pathogenic MSH4 variants are typically loss-of-function or disrupt the conserved C-terminal ATPase/Walker-B domain.

> *"MSH4 and MSH5 encode components of the MutSγ complex, which stabilises Holliday junctions during meiotic recombination."* — PMID: 42386447
>
> *"Pathogenic variants are typically loss-of-function and/or disrupt conserved functional domains essential for meiotic recombination, in particular the C-terminal ATPase domain found in both MSH4 and MSH5."* — PMID: 42386447
>
> *"The endonuclease activity of MLH1-MLH3 (MutLγ) is stimulated by MSH4-MSH5 (MutSγ), EXO1, and RFC-PCNA to resolve meiotic recombination intermediates such as double Holliday junctions (HJs) into crossovers."* — PMID: 40319035

Loss of crossovers causes failure of homolog pairing/synapsis, meiotic arrest, apoptotic loss of oocytes, and streak/dysgenetic ovaries.

Finding 3 — *Msh4*-null mice recapitulate POF20; the mechanism is sex-shared (POI in females, azoospermia in males) [M/H]

Msh4-knockout mice are sterile in both sexes due to meiotic failure, with defective chromosome pairing/synapsis in prophase I. MSH4 acts in the same genetic pathway as MSH5.

> *"Disruption of the Msh4 gene in mice results in male and female sterility due to meiotic failure."* — PMID: 10809667
>
> *"MSH4 localization on chromosomes during the early stages of meiosis is essential for normal chromosome synapsis in prophase I and that it acts in the same pathway as MSH5."* — PMID: 10809667

In humans, the identical biallelic MSH4 defect produces POI in women and **non-obstructive azoospermia (NOA)** in their brothers within the same family, reflecting the shared meiosis-I requirement.

> *"inherited defects in meiosis I may result in common causes for both POI and NOA."* — PMID: 38175272

Finding 4 — MSH4 is loss-of-function-tolerant in heterozygotes, consistent with the recessive mechanism [C]

gnomAD v2 constraint metrics for MSH4 (ENSG00000057468) show it is **not depleted for predicted loss-of-function variants**: pLI ≈ 0 (7.8×10^{-34}), observed/expected LoF (oe_lof) = 0.93 (90% CI 0.80–1.10; LOEUF ≈ 1.10), obs_lof = 106 vs exp_lof = 113.7, lof_z = 0.61. Heterozygous LoF is therefore well tolerated — exactly what is expected for a gene that causes disease only when **biallelically** inactivated.

ClinVar lists 207 MSH4 records, of which 45 are classified pathogenic and 14 likely pathogenic. Reported pathogenic variant classes include canonical splice-site (exon-17 skipping; p.Ile743_Lys785del), nonsense (c.118C>T, p.Gln40*), frameshift, and missense affecting the conserved C-terminal ATPase/Walker-B domain.

> *"the mutation p.Ile743_Lys785del is predicted to lead to the ablation of the highly conserved Walker B motif of the ATP-binding domain, thus inactivating MSH4."* — PMID: 28541421

Finding 5 — Clinical phenotype: young-adult-onset secondary amenorrhea, infertility, hypergonadotropic hypogonadism [H]

Official HPO/OMIM annotations for OMIM:619938 (all from the index family, frequency 2/2 = 100%) are: **Young adult onset (HP:0011462); Secondary amenorrhea (HP:0000869); Female infertility (HP:0008222); Elevated circulating FSH (HP:0008232); Elevated circulating LH (HP:0011969); Autosomal recessive inheritance (HP:0000007).**

> *"characterized by an early cessation of menses and high FSH levels."* — PMID: 28541421

Broader literature indicates affected women predominantly present with secondary amenorrhea (often after initially normal menarche/puberty), with a phenotypic spectrum extending to primary amenorrhea at the severe end and milder **diminished ovarian reserve (DOR)** at the mild end.

> *"Affected individuals predominantly present with secondary amenorrhoea, with emerging evidence for milder phenotypes, including diminished ovarian reserve (DOR)."* — PMID: 42386447

Finding 6 — MSH4 is deeply conserved; functional models span yeast, worm, zebrafish, and mouse [M/C]

MSH4 orthologs exist across eukaryotes: mouse *Msh4* (Gene 55993), rat (295541), zebrafish *msh4* (559917), chicken (424725), dog (612681), cow (616664), chimpanzee (469357), macaque (705294), *S. cerevisiae* MSH4 (850545), *C. elegans* him-14/msh-4 (174157), and *Arabidopsis thaliana* MSH4 (827450). The mouse knockout validates the model.

> *"Disruption of the Msh4 gene in mice results in male and female sterility due to meiotic failure."* — PMID: 10809667

Finding 7 — Prognosis and management: non-progressive lifelong infertility with estrogen-deprivation sequelae; HRT + donor-oocyte IVF [H]

POF20 is not life-threatening but causes irreversible hypergonadotropic hypogonadism. Untreated premature estrogen deficiency increases risks of osteoporosis/fracture, cardiovascular disease, adverse cognitive and sexual-health outcomes, and all-cause mortality.

> *"Premature ovarian insufficiency can result in long-term decrements to all-cause mortality, bone density, cardiovascular health, sexual health, cognitive health, and body mass. Hormone replacement therapy has been demonstrated to reverse these long-term sequalae."* — PMID: 39448037

Management follows the 2024 ESHRE/ASRM/IMS evidence-based POI guideline.

> *"The current guideline provides 145 recommendations on symptoms, diagnosis, causation, sequelae and treatment of POI."* — PMID: 39652037

Finding 8 — Investigation complete: comprehensive 15-section characterization with corrected causal gene [C]

The verified identifier set and ontology annotations (below) anchor a complete 15-section characterization. MSH4 aliases **POF20** and **SPGF2** (the male spermatogenic-failure counterpart) were confirmed via mygene.info, and the Monarch Causal-Gene-to-Disease association (MSH4 → POF20, autosomal recessive) was verified.

Mechanistic Model / Interpretation

Causal chain — from mutation to clinical manifestation

```
1. Biallelic LoF variant in MSH4 (splice / nonsense / frameshift / ATPase-domain missense)
        │  results in
        ▼
2. Absent or non-functional MSH4 protein  →  cannot heterodimerize with MSH5
        │  leads to
        ▼
3. Loss of the MutSγ (MSH4–MSH5) complex  →  Holliday junctions not stabilized   [inferred: bioc
        │  results in
        ▼
4. MLH1–MLH3 (MutLγ) resolvase not licensed  →  double Holliday junctions not resolved   [infer
        │  leads to
        ▼
5. Meiotic crossovers fail to form  →  homologous chromosomes do not pair/synapse   [demonstrate
        │  results in
        ▼
6. Meiosis-I arrest  →  apoptotic elimination of germ cells / oocytes   [inferred from model/cor
        │  leads to
        ▼
7. Depletion of the ovarian follicle pool  →  streak/dysgenetic ovaries
        │  results in
        ▼
8. Hypergonadotropic hypogonadism (loss of estradiol/inhibin feedback → ↑FSH, ↑LH)
        │  manifests as
        ▼
9. CLINICAL: young-adult secondary amenorrhea + female infertility (POF20)

   ── BRANCH (same genotype, male relatives) ──
7b. Spermatogenic meiotic arrest → non-obstructive azoospermia (SPGF2)
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Steps 3–4 are **inferred from biochemical and model-organism data** (yeast MutSγ binding assays, mouse knockout synapsis defects) rather than demonstrated directly in human ovary. Steps 1, 2, 8, and 9 are established in human patients.

Upstream vs downstream

Position	Event	Evidence type
Upstream (initiating)	Biallelic MSH4 LoF	Human genetics (P 28541421)
Upstream	MutSγ complex loss	Biochemistry (P 30467025)
Midstream	Crossover failure, synapsis failure	Mouse model (P 10809667)
Downstream	Oocyte apoptosis, follicle depletion	Inferred / comparative
Downstream (clinical)	Hypergonadotropic hypogonadism, amenorrhea, infertility	Human clinical (P 28541421)

Ontology annotation set

Category	Terms
Disease	MONDO:0030975; OMIM:619938; UMLS:C5677011; MedGen:1808256; GARD:0025670
Gene/Protein	HGNC:7327 (MSH4); NCBI 4438; Ensembl ENSG00000057468; UniProt O15457
Phenotype (HPO)	HP:0000869 (secondary amenorrhea); HP:0008222 (female infertility); HP:0008232 (elevated FSH); HP:0011969 (elevated LH); HP:0011462 (young adult onset); HP:0000815 (hypergonadotropic hypogonadism); HP:0008209 (premature ovarian insufficiency); HP:0000786 (amenorrhea); HP:0000007 (AR inheritance)
Biological process (GO)	GO:0007131 (reciprocal meiotic recombination); GO:0007127 (meiosis I); GO:0006298 (mismatch repair)
Cellular component (GO)	GO:0000795 (synaptonemal complex); GO:0005713 (recombination nodule); GO:0005634 (nucleus); GO:0000794 (condensed nuclear chromosome)
Molecular function (GO)	GO:0005524 (ATP binding); GO:0030983 (mismatched DNA binding); GO:0140664 (ATP-dependent DNA damage sensor activity); GO:0003690 (dsDNA binding)
Anatomy (UBERON)	UBERON:0000992 (ovary)
Cell type (CL)	CL:0000023 (oocyte); CL:0000020 (spermatogonium) / CL:0000019 (sperm) in males
Treatment (NCIT)	NCIT:C62002 (HRT); NCIT:C617 (estrogen); NCIT:C713 (progestin); NCIT:C15354 (IVF); NCIT:C92755 (oocyte donation)

Section-by-Section Report

1. Disease Information

POF20 is a rare monogenic form of **primary/premature ovarian insufficiency (POI)** — loss of normal ovarian function before age 40 — defined by its causal gene *MSH4*. Key identifiers: **OMIM 619938, MONDO:0030975, UMLS C5677011, MedGen 1808256, GARD 0025670.** There is

no POF20-specific ICD code; it is captured under POI/premature menopause codes (ICD-10 **E28.31**, premature ovarian failure; ICD-11 **5A80.1**) and MeSH *Primary Ovarian Insufficiency* (D016649). **Synonyms/alternative names:** Premature Ovarian Failure 20; MSH4-related POI; ovarian insufficiency due to MSH4 deficiency. The male allelic counterpart is **Spermatogenic Failure 2 (SPGF2)**. Information is derived from **aggregated disease-level resources** (OMIM, Monarch, ClinVar) and a small number of **individual-patient reports/case series** rather than EHR-scale datasets.

2. Etiology

Causal factor: entirely **genetic** — biallelic (homozygous or compound heterozygous) loss-of-function variants in *MSH4*. **Genetic risk factors:** the only established risk factor is carriage of two pathogenic MSH4 alleles; **consanguinity** substantially raises risk (the index family was consanguineous Colombian; PMID: 28541421). Digenic/oligogenic contributions have been reported, including digenic MSH4+MSH5 variants PMID: 36793102. **Environmental risk factors:** none specific to POF20 — this is a Mendelian disease; general POI environmental contributors (chemotherapy, radiation, smoking) are not causal here. **Protective factors:** none genetic identified; general estrogen-protective lifestyle factors do not modify the underlying meiotic defect. **Gene–environment interactions:** not established; the phenotype is driven by the germline genotype.

3. Phenotypes

Phenotype	HPO term	Type	Onset	Frequency
Secondary amenorrhea	HP:0000869	Clinical sign	Young adult	2/2 (100%) index family
Female infertility	HP:0008222	Clinical	Young adult	High
Elevated circulating FSH	HP:0008232	Lab abnormality	Young adult	2/2 (100%)
Elevated circulating LH	HP:0011969	Lab abnormality	Young adult	2/2 (100%)
Hypergonadotropic hypogonadism	HP:0000815	Lab/clinical	Young adult	High
Premature ovarian insufficiency	HP:0008209	Clinical	<40 y	Defining
Young adult onset	HP:0011462	Modifier	16–40 y	Typical

Severity is **variable** (primary amenorrhea → secondary amenorrhea → DOR); the course is **stable/non-progressive** once ovarian reserve is lost. **Quality-of-life impact:** substantial — infertility, estrogen-deficiency symptoms (vasomotor, genitourinary, mood), and long-term bone/cardiovascular/cognitive risks; POI broadly impairs QoL and psychological well-being (PMID: 40377060).

4. Genetic / Molecular Information

Causal gene: *MSH4* (OMIM *602105; HGNC:7327; locus 1p31.1; chr1:75,796,882–75,913,242, GRCh38; NCBI Gene 4438; UniProt O15457). Variant spectrum: canonical splice-site (exon-17 skipping, p.Ile743_Lys785del — PMID: 28541421); nonsense (c.118C>T, p.Gln40 —* PMID: 38175272); frameshift; and missense in the conserved C-terminal ATPase/Walker-B domain (PMID: 42386447). **Classification:** ClinVar — 45 pathogenic, 14 likely pathogenic of 207 records. **Allele frequency:** pathogenic alleles are rare/absent in gnomAD; MSH4 is LoF-tolerant in heterozygotes (pLI≈0, LOEUF≈1.10). **Origin:** germline. **Functional consequence:** loss of

function (recessive). **Modifier genes:** MSH5 (digenic reports); co-occurring meiotic-gene variants may modulate severity. **Epigenetic/chromosomal abnormalities:** none specific to POF20 (though POI generally has epigenetic contributors — PMID: 40620015).

5. Environmental Information

Not applicable as a cause — POF20 is monogenic. No toxins, lifestyle factors, or infectious agents are implicated in its etiology. (Environmental/iatrogenic exposures cause *other* POI subtypes but not MSH4-related disease.)

6. Mechanism / Pathophysiology

See the **Mechanistic Model** section above for the ordered causal chain. In brief: MSH4 LoF → loss of MutSγ (MSH4–MSH5) → failure to stabilize Holliday junctions → failure to license MutLγ (MLH1–MLH3)/EXO1/RFC-PCNA → no crossovers → synapsis/pairing failure → meiosis-I arrest → oocyte apoptosis → follicle depletion → hypergonadotropic hypogonadism → amenorrhea/infertility. Key pathway: **meiotic homologous recombination** (not Wnt/MAPK/mTOR). Cellular process: **apoptosis of germ cells** following meiotic arrest. Protein dysfunction: **loss of ATPase-dependent HJ-binding function**. Cell types: oocytes (CL:0000023); subcellular site: synaptonemal complex (GO:0000795). Male branch → spermatocyte arrest → azoospermia.

7. Anatomical Structures Affected

Primary organ: ovary (UBERON:0000992), typically streak/dysgenetic. **Body system:** endocrine/reproductive (hypothalamic-pituitary-ovarian axis). **Secondary involvement (from estrogen deficiency):** skeleton (osteoporosis), cardiovascular system, CNS (cognition/mood), genitourinary tissues. **Tissue/cell:** germ cells — oocytes (CL:0000023); in males, spermatocytes/spermatogonia. **Subcellular:** nucleus (GO:0005634), synaptonemal complex (GO:0000795), condensed nuclear chromosome (GO:0000794). **Lateralization:** bilateral ovarian involvement.

8. Temporal Development

Onset: young adult (HP:0011462), typically after apparently normal puberty/menarche; **insidious/chronic** onset of oligo-/amenorrhea. **Progression:** the primary defect is developmental (meiotic failure in fetal/early life depletes the oocyte pool); it presents clinically as **non-progressive, stable** ovarian insufficiency once reserve is exhausted. **Disease course:** chronic, lifelong. **Remission:** none spontaneous; sporadic ovulation is rare. **Critical period:** the meiotic window is prenatal/perinatal — by the time of diagnosis the germ-cell pool is already depleted, limiting fertility-preservation opportunities to early-diagnosed cases.

9. Inheritance and Population

Inheritance: autosomal recessive (HP:0000007). **Penetrance:** appears high in biallelic carriers but formally incompletely characterized (small case numbers). **Expressivity:** variable (primary amenorrhea ↔ DOR). **Epidemiology:** POI overall affects ~**3.5%** of women (PMID: 39652037); MSH4/POF20 is a rare monogenic subset — in targeted-panel/exome POI cohorts, MSH4 accounts for a small percentage of solved cases (PMID: 36793102, PMID: 36373164, PMID: 36099812). **Consanguinity/founder effects:** homozygous cases enriched in consanguineous families. **Carrier frequency:** low; MSH4 LoF alleles are tolerated in heterozygotes (gnomAD). **Sex:** females present with POI, males with NOA — same genotype, sex-specific manifestation.

10. Diagnostics

Clinical/lab: elevated FSH (ESHRE 2024: a single FSH >25 IU/L suffices for POI diagnosis), elevated LH, low estradiol, low AMH; pelvic ultrasound shows small ovaries/reduced antral follicle count. **Genetic testing:** POI gene panels or whole-exome sequencing identify biallelic MSH4 variants — the definitive diagnostic for POF20. WES/panel testing is high-yield in familial/consanguineous POI (PMID: 36099812). Karyotype and FMR1 premutation testing are performed to exclude common POI causes. **Differential diagnosis:** Turner syndrome, FMR1 premutation, autoimmune oophoritis, other monogenic POI (FOXL2, NOBOX, MCM8/9, MSH5, HFM1, SYCE1, STAG3). **Screening:** cascade genetic testing of at-risk relatives (including male siblings for azoospermia risk).

11. Outcome / Prognosis

Survival: not directly life-threatening; however untreated POI is associated with **increased all-cause mortality** and cardiovascular/skeletal morbidity (PMID: 39448037, PMID: 20938225). **Morbidity:** infertility, osteoporosis/fracture risk, cardiovascular disease, cognitive and sexual-health effects, vasomotor symptoms. **Recovery:** ovarian function is irreversibly lost; HRT reverses many estrogen-deprivation sequelae but not fertility. **Prognostic factors:** younger age at ART and milder (DOR) phenotype associate with better reproductive prognosis (PMID: 40936058).

12. Treatment

No disease-modifying/curative therapy exists. Management is supportive and hormonal:

Intervention	NCIT	Purpose
Hormone replacement therapy (estradiol + progestogen)	NCIT:C62002	Symptom control; bone/CV/cognitive protection until ~age 51
Estrogen	NCIT:C617	Core HRT component
Progestin	NCIT:C713	Endometrial protection
In vitro fertilization	NCIT:C15354	Fertility (with donor oocytes)
Oocyte donation	NCIT:C92755	Principal fertility option

Adjuncts: calcium/vitamin D, DXA bone-density monitoring, psychological support (2024 ESHRE/ASRM/IMS guideline, 145 recommendations — PMID: 39652037). Own-oocyte IVF is generally non-viable because meiosis is abolished; **donor-oocyte/donor-embryo IVF** is the mainstay. No gene therapy or pharmacogenomic strategy is currently applicable.

13. Prevention

Primary prevention is not possible (germline Mendelian disease). **Secondary/tertiary prevention:** early diagnosis enables timely HRT to prevent osteoporosis and cardiovascular complications, and (if diagnosed before pool exhaustion) consideration of fertility preservation. **Genetic counseling** is central: autosomal-recessive recurrence risk (25% for future siblings of a proband), carrier testing of partners in consanguineous unions, and counseling of male relatives regarding azoospermia risk. **Preimplantation/prenatal genetic testing** is feasible for families with a known biallelic genotype.

14. Other Species / Natural Disease

MSH4 is **deeply conserved**. Orthologs: mouse *Msh4* (Gene 55993; taxid 10090), rat (295541), zebrafish *msh4* (559917), chicken (424725), dog (612681), cow (616664), chimpanzee (469357), macaque (705294), *S. cerevisiae* MSH4 (850545; taxid 4932), *C. elegans* him-14/msh-4 (174157; taxid 6239), *Arabidopsis thaliana* MSH4 (827450). The meiotic recombination function is conserved from yeast to mammals. No specific naturally occurring companion-animal disease is catalogued for MSH4, but the shared mechanism makes cross-species meiotic sterility phenotypes directly comparable.

15. Model Organisms

Model	Type	Phenotype recapitulation
Mouse *Msh4*-null	Mammalian knockout	Male + female sterility; prophase-I synapsis failure — faithfully recapitulates POF20/SPGF2 (PMID: 10809667)
***S. cerevisiae* MSH4**	Yeast	Crossover/recombination defects; biochemistry of MutSγ–HJ binding (PMID: 30467025)
***C. elegans* him-14/msh-4**	Invertebrate	Meiotic crossover failure, chromosome missegregation
Zebrafish *msh4*	Vertebrate	Meiotic model (ortholog available)

Model strengths: the mouse knockout captures the core sterility/meiotic-failure phenotype and the sex-shared mechanism. **Limitations:** rodent ovarian-aging/reserve dynamics differ from humans, and human milder (DOR) phenotypes are harder to model. Resources: MGI, ZFIN, SGD, WormBase.

Evidence Base

PMID	Title / focus	Role in this report	Evidence type
28541421	*Homozygous donor splice-site mutation in MSH4 causes POI*	**Foundational** — first MSH4–POI link; variant, phenotype, Walker-B ablation	Human clinical/ genetic
42386447	*MSH4 and MSH5 variants in POI: review and case study*	Variant spectrum (LoF/ATPase); phenotype spectrum (2° amenorrhea → DOR)	Review + case
38175272	*Homozygous nonsense MSH4 variant → POI and NOA*	Sex-shared meiosis-I mechanism; nonsense variant	Human clinical/ genetic
10809667	*Msh4 knockout mouse*	Model organism; synapsis role; same pathway as MSH5	Mouse model
30467025	*MutSγ–DNA conformational changes*	MutSγ binds HJs with nanomolar affinity	In vitro biochemistry
40319035	*EXO1 promotes MLH1-MLH3 endonuclease*	MutSγ licenses MutLγ to resolve dHJs into crossovers	In vitro/ biochemistry
32814904	*Regulation of MLH1-MLH3 endonuclease in meiosis*	Downstream crossover resolution	Review/ biochemistry
36099812	*Genetic landscape of large POI cohort*	Confirms MSH4 causal role; diagnostic yield	Human cohort
36793102	*NGS of 500 POI patients*	Digenic MSH4+MSH5; compound-het confirmation	Human cohort
36373164	*Genetic screening in ovarian dysfunction*	MSH4 splice variants; expands spectrum	Human cohort
40936058	*Genetic variants in DOR/POI & ART outcomes*	Meiotic variants → poor ART; younger age better	Human cohort
39652037	*2024 ESHRE/ASRM/IMS POI guideline*	Diagnosis (FSH>25) and management framework	Guideline
39448037	*HRT in radiation-induced POI*	Estrogen-deprivation sequelae; HRT reverses them	Review/clinical
20938225	*Skeletal effect of natural early menopause*	Bone/mortality risks of early estrogen loss	Review

PMID	Title / focus	Role in this report	Evidence type
25873734	*MCM8 mutations → gonadal failure*	Distinguishes POF10 (MCM8) from POF20 (MSH4)	Human genetic

Consistency: All independent lines of evidence — human genetics (multiple families/cohorts), the mouse knockout, and yeast/worm biochemistry — converge on a single coherent model: MSH4 LoF → MutSγ failure → crossover loss → meiotic arrest → ovarian insufficiency. No contradictory findings were identified.

Limitations and Knowledge Gaps

1. **Small patient numbers.** Core phenotype frequencies (e.g., 100% secondary amenorrhea/elevated FSH) derive from the 2-sibling index family; broader cohorts are needed to establish robust penetrance and expressivity estimates.
2. **No direct human ovarian histology/omics.** The oocyte-apoptosis and follicle-depletion steps are inferred from mouse and comparative data, not demonstrated in human MSH4-deficient ovary. No POF20-specific transcriptomic, proteomic, metabolomic, or single-cell datasets exist.
3. **Penetrance/expressivity.** The genotype–phenotype relationship (why some carriers show primary amenorrhea vs milder DOR) and the role of modifier/digenic alleles (MSH5) are incompletely resolved.
4. **Epidemiology.** No POF20-specific prevalence/incidence figures exist; only the broad POI prevalence (~3.5%) and small panel-based contributions are available.
5. **No disease-modifying therapy or trials.** All treatment evidence is generic POI management; no MSH4-targeted therapeutics or NCT-registered trials specific to POF20.
6. **Epigenetic/environmental modifiers** of the meiotic phenotype are unstudied in this subtype.

Proposed Follow-up Experiments / Actions

1. **Aggregate an international MSH4-POI cohort** (GeneMatcher, large POI consortia) to quantify penetrance, expressivity, genotype–phenotype correlations, and sex-specific outcomes (POI vs NOA).
2. **Functional validation of VUS**: minigene splicing assays and MutSγ complex-formation / HJ-binding assays to reclassify uncertain MSH4 missense variants per ACMG/AMP.
3. **Patient-derived iPSC → germ-cell / ovarian organoid models** to directly observe meiotic arrest and oocyte apoptosis in the human MSH4-deficient context, filling the inferred mechanistic gap.
4. **Single-cell transcriptomics of residual ovarian tissue / model germ cells** to define the apoptotic and granulosa-cell responses downstream of meiotic failure.
5. **Early-diagnosis fertility-preservation protocols**: identify biallelic carriers before pool exhaustion to evaluate oocyte/ovarian-tissue cryopreservation windows.
6. **Structured genetic counseling pathway** including cascade testing of male siblings (azoospermia risk) and partner carrier screening in consanguineous families.
7. **Longitudinal HRT outcome study in genetically defined POF20** to confirm that estrogen-deprivation sequelae are mitigated as in general POI.

Report compiled from a 5-iteration autonomous investigation: 8 confirmed findings, 34 papers reviewed. The causal gene was corrected from MCM8 to MSH4 during the investigation. Evidence integrates human clinical/genetic reports, mouse knockout models, yeast/invertebrate biochemistry, and the 2024 ESHRE/ASRM/IMS POI guideline.
