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Research Article

Sohlh1 loss of function male and female infertility model impacts overall health beyond gonadal dysfunction in mice†

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Abstract

Reproductive longevity is associated with health outcomes. Early menopause, loss of ovarian function, and male infertility are linked to shorter lifespan and increased adverse health outcomes. Here we examined the extragonadal effects of whole animal loss of spermatogenesis and oogenesis specific basic helix–loop–helix 1 (*Sohlh1*) gene in mice, a well-described mouse model of female and male infertility. *Sohlh1* encodes a transcription factor that is primarily expressed in the male and female germline and regulates germline differentiation. The *Sohlh1* knockout mouse model, just like human individuals with *SOHLH1* loss of function, presents with hypergonadotropic hypogonadism and loss of ovarian function in females and impaired spermatogenesis in males, with a seemingly gonad restricted phenotype in both sexes. However, extragonadal phenotyping revealed that *Sohlh1* deficiency leads to abnormal immune profiles in the blood and ovarian tissues of female animals, sex-specific alterations of metabolites, and behavior and cognition changes. Altogether, these results show that *Sohlh1* deficiency impacts overall health in both male and female mice.

Summary Sentence
Sohlh1 deficiency affects systemic health beyond gonadal dysfunction in mice, inducing changes in immune cell populations, metabolism, and cognition.

Keywords: infertility, reproductive health, aging, primary ovarian insufficiency, Sohlh1, immune profile

Introduction

Epidemiological studies have revealed that both women's and men's reproductive health associates with overall health. In women, menopause usually occurs between 49 and 52 years of age and is marked by the hypothalamic–pituitary–ovarian axis being in the state of hypergonadotropic hypogonadism along with hypoestrogenism. Primary ovarian insufficiency (POI) is a condition that affects 1–5% of women and is defined by the cessation of menstruation before the age of 40 [1, 2]. Early menopause is linked to shorter life span, increased risk of diabetes mellitus, heart disease, cardiovascular disease, and osteoporosis [3–5]. Similarly, lower sperm counts are an independent predictor of comorbidity and mortality [6, 7]. Indeed, mounting evidence suggests an association between male infertility and life-threatening diseases including cancer, cardiovascular, autoimmune, and other chronic disorders [7, 8].

Multiple genetic traits including sex chromosome abnormalities and mutations in either autosomal recessive or dominant genes have been shown to cause different types of gonadal dysgenesis leading to infertility and shortened reproductive life span. Recent genome-wide association studies (GWASs) and whole-exome sequencing (WES) results have enabled the identification of genomic regions linked to POI and age at natural menopause [5, 9–11]. Interestingly, these reports underscored a role in reproductive health for a broad range of genes implicated in deoxyribonucleic acid (DNA) damage response (DDR). DDR-associated genes participate in several processes that ultimately preserve genomic stability by protecting against the accumulation of DNA damage, a major driver of aging [12]. Numerous genes involved in DNA repair have also been identified to have a role in male infertility [6, 13]. The enrichment of DDR genes in early menopause, POI, and male infertility could be indicative of reproductive aging being part of systemic aging. Additional evidence supporting a relationship between reproductive longevity and biological aging rate comes from a study examining methylation as a marker of epigenetic aging [14]. There is a negative correlation between epigenetic age of blood and age at natural menopause. Surgical menopause (bilateral oophorectomy) is associated with increased epigenetic age of blood and saliva [14], consistent with previously reported results suggesting that menopause itself may accelerate the aging process [15]. The precise mechanisms behind the effect of female and male

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infertility on overall health are not well understood. Hence, the debate as to whether gonadal function by itself is a determinant of an individual's life span, or whether specific variants in genes that affect gonadal function also affect somatic functions in various organs to accelerate somatic aging, remains controversial.

Several pathogenic variants in genes that appear to function preferentially in the gonads have been identified by WES in women with POI. Specifically, variants in genes that participate in folliculogenesis such as spermatogenesis and oogenesis specific basic helix–loop–helix 1 (*SOHLH1*), folliculogenesis specific bHLH transcription factor (*FIGLA*), LIM homeobox 8 (*LHX8*), forkhead box L2 (*FOXL2*), NOBOX oogenesis homeobox (*NOBOX*), growth differentiation factor 9 (*GDF9*), and bone morphogenetic protein 15 (*BMP15*) have been linked to ovarian dysgenesis and POI [5, 16]. *SOHLH1* encodes a transcription factor that is preferentially expressed in the female and male germline and regulates germline differentiation. Loss of function *Sohlh1* mice rapidly lose follicles in female mice and have impaired spermatogenesis in males [17, 18]. *SOHLH1* loss of function variants have been reported in women presenting with POI [19–22] and in men with non-obstructive azoospermia [23–26]. The impact of these pathogenic variants on overall health in affected individuals has not been studied to date. Mouse models that replicate human reproductive phenotypes can inform us about potential extragonadal effects in affected men and women.

In this study, we examined extragonadal effects of *Sohlh1* deficiency in female and male mice. The *Sohlh1* knockout (KO) mouse model, just like human individuals with *SOHLH1* loss of function [22, 23], presents with hypergonadotropic hypogonadism, loss of ovarian function in females, non-obstructive azoospermia in males, and a seemingly gonad restricted phenotype [17, 18, 27, 28]. We therefore used a *Sohlh1* KO mouse model and extensive extragonadal phenotyping via a Knockout Mouse Phenotyping Project facility pipeline to elucidate whether congenital *Sohlh1* loss of function disrupts various physiologic functions. Our data indicate that *Sohlh1* loss of function in mice leads to altered immune cell populations, metabolic functions, behavior, and cognition.

Materials and methods

Animal husbandry

Animal experiments were carried out on a C57BL/6/129S6/SvEv hybrid background. The *Sohlh1* mice used in this study have been previously described [18]. One mating pair of heterozygous *Sohlh1* mice was placed per cage at 6 weeks of age and inspected daily for presence of litters. All animal procedures were conducted at the University of California, San Francisco, unless otherwise stated. Animals were maintained under specific pathogen-free conditions in accordance with the regulatory standards of the NIH and the American Association of Laboratory Animal Care standards, and consistent with the University of California, San Francisco, Institution of Animal Care and Use Committee (IACUC approval: AN176170-03).

Genotyping

Mouse pups were weaned at 3 weeks of age and genotyped through multiplex polymerase chain reaction (PCR) using Amfisure PCR master mix (GenDepot). Primer set G1 (5′-GAGTCTCTGGCATTACGGGAT-3′) and G2 (5′-CTGAGTCTCAGGCTGAGGAG-3′), located in exons 1 and 2, respectively, amplify a 320-nucleotide wild type (WT) band. Primer set HPRT-2 (5′-GCAGTGTTGGCT GTATTTTCC-3′) and G3 (5′-CTGGAGCCCAAGAAGACA AG-3′), located in the hypoxanthine phosphoribosyltransferase (HPRT) cassette and intron 3, respectively, amplify a 220-bp mutant band. PCR was performed under the following cycle conditions: 95°C for 30 s; 60°C for 30 s; 72°C for 30 s; 72°C for 5 min with 35 cycles of amplification.

Ovary collection

Ovaries were collected from individual mice at 7 weeks of age and stored in freezing media [fetal calf serum (FCS) (Gibco, #16140071), 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, #67685)] at −80°C until used for analysis. A total of 10 replicates, each comprised of pooled ovaries from five mice, were processed per experimental group (WT and KO). For each replicate, ovaries were transferred to 500 μL Roswell Park Memorial Institute (RPMI) medium (Thermo Fisher, #11875093) and minced into pieces. An equal volume of RPMI medium containing 35 U DNase I (Sigma-Aldrich, #10104159001), 250 U Collagenase I (Worthington Biochemicals, #LS004194), and 50 U Collagenase IV (Worthington Biochemicals, #LS004186) was added, and the ovaries were incubated at 37°C for 20 min with constant agitation. The digestion was interrupted by addition of 1 mL of fluorescence-activated cell sorting (FACS) buffer [PBS (Thermo Fisher, #14190250), 2% FCS, 2 mM EDTA (Life Technologies, #15575020), 0.1% sodium azide (Sigma-Aldrich, #S8032)], the suspension was filtered through a 35 μm cell strainer (Corning, #352235) into a fresh ice-cold tube and centrifuged at 1500 rpm for 5 min at 4°C. The pellet containing the cells was then resuspended in 1 mL FACS buffer. Before antibody staining, total cell number and viability were determined for each sample using Trypan blue.

Blood collection

Blood was collected in heparin-coated tubes by cardiac puncture of 7-week-old mice. It was then centrifuged for 10 min at 1500 rpm at room temperature and plasma was collected and stored at −80°C for hormonal assessment. The remaining pellet was resuspended in red blood cell lysis buffer (Millipore Sigma, #11814389001) according to the manufacturer's procedures. Cells were then resuspended in FACS buffer, filtered through a 100 μm filter (Sigma-Aldrich, #08-771-19), and centrifuged at 1500 rpm for 6 min. The resulting pellet, which contained leukocytes, was resuspended in freezing media (FCS + 10% DMSO) and stored at −80°C. For each experimental replicate, leukocytes from three mice were partially thawed at 37°C and an equivalent amount of RPMI was immediately added to dilute the freezing media up to a total volume of 15 mL. Cells were then centrifuged at 1500 rpm for 5 min at 4°C, resuspended in 1 mL of FACS buffer, and assessed for total cell number and viability using Trypan blue.

Histologic analysis

The following tissues were collected and fixed in 10% buffered formalin (Sigma-Aldrich, #50-00-0): reproductive tracts from 7-week-old WT and KO female mice, reproductive tracts from 16-week-old WT and KO male mice, and kidneys and livers from 16-week-old WT and KO male and female animals. Fixed tissues were then embedded in

paraffin, serially sectioned (5 μm thickness), and stained with hematoxylin and eosin or with Periodic Acid–Schiff reagent and hematoxylin as previously described [18]. Images were taken on a Nikon Eclipse E800 microscope. Germ cell cysts, primordial, primary, and secondary follicles were defined as described previously [18].

Plasma hormonal analysis

Hormonal analysis was performed by the Ligand Assay & Analysis Core, at the Center for Research in Reproduction, University of Virginia. Estradiol and progesterone measurements were performed on plasma samples from 50 WT and 50 KO 7-week-old female mice. Testosterone testing was performed on plasma samples from 6 WT and 5 KO 12-week-old male mice. All ELISA immunoassays for either estradiol (Calbiotech, #ES180S, Lot#ESG5996), progesterone (IBL America, #IB79183, Lot#23K060-2), or testosterone (IBL America, #IB79174, Lot#29K072) were carried out using the same kit lots to minimize batch effects.

Immunoprofiling by flow cytometry

In total, 200 μL of blood or ovary leukocyte cell suspensions were added to five wells of a 96-well plate and centrifuged at 1500 rpm for 5 min at 4°C. Cells were then resuspended in 200 μL of viability dye (Life Technologies, #L34966), transferred into a single well, and incubated for 20 min on ice. After incubation, the plate was centrifuged at 1500 rpm for 5 min at 4°C, and cells were washed twice with 200 μL of FACS buffer. A total of 70 μL of a 1:1 solution of FACS buffer:Brilliant Stain Buffer (BD Biosciences, #563794) containing antibodies targeting several immune cell-type specific antigens (Supplementary Table S1) were added to cells and incubated for 30 min on ice. Cells were then washed twice in FACS buffer, fixed in 1% PFA for 15 min at room temperature, and stored in FACS buffer for up to a week at 4°C in the dark until flow cytometry analysis.

Extragonadal phenotyping

WT and KO animals were analyzed at the Mouse Metabolic Phenotyping Center, UC Davis. A total of 10 WT and 14 KO mice were enrolled in a targeted phenotype pipeline. Enrollment began at 4 weeks and tests proceeded weekly for 16 weeks until necropsy. Housing conditions and testing protocols followed the Animal Welfare Act and the 2013 American Veterinary Medical Association (AVMA) Guidelines on Euthanasia and had prior approval from the UC Davis IACUC. Phenotypic evaluation of the mice was performed using standardized protocols from the International Mouse Phenotyping Consortium (IMPC) [29]. Supplementary Table S2 summarizes the 12-week pipeline and tests performed. These tests evaluated behavior and cognition (open field, light–dark, hole board, acoustic startle, and fear conditioning); locomotion (SHIRPA and open field); cardiovascular (electrocardiogram and heart weight), and metabolic [intraperitoneal glucose tolerance test (ipGTT), clinical chemistry with insulin, and complete blood count panels] functions; musculoskeletal domains (grip strength, dual-energy X-ray absorptiometry, and X-rays), and sensory (ophthalmological and auditory exams) assessment. Viability and body weight (BW) were monitored weekly. Some tests evaluated multiple domains, for example, the grip strength test evaluated neuromuscular function in addition to force; SHIRPA includes behavior, morphology, and functional assessments. All tests included full cohorts of WT (five females and five males) and KO (seven females and seven males) mice, except for the auditory brainstem analysis, which was performed on 2 WT (one male and one female) and four KO (two male and two female) animals.

Phenotypic comparison between *Sohlh1* and meiosis-specific KO genes

A set of 77 genes involved in meiosis and with a reported role in infertility or subfertility in males and/or females [30] were selected for phenotypic comparison. Phenotyping data was available for 41 out of the 77 genes in the IMPC database (www.mousephenotype.org) [29], which contains phenotype data for 8368 KO genes (data release version: 18.0). Of these 41 genes, four genes had a pre-weaning lethality phenotype, and 18 genes had an infertility phenotype in male and/or female mice, while no lethality or fertility phenotypes were noted for the other 19 genes in the database. We selected the 18 genes for which a fertility phenotype was described in the database and compared their associated phenotypes with those of *Sohlh1* KO mice.

Statistics

Significant differences between WT and KO datasets were determined using Student's *t*-test when the data were normally distributed and had equal variance. Otherwise, the nonparametric Mann–Whitney test was used. For all extragonadal phenotyping data, descriptive statistics and comparisons were generated by genotype and sex. Statistical analysis was done using data sets without outliers, which were defined as data 1.5 times the interquartile range below or above the low (Q1) and high (Q3) quartiles. Repeated measure data (BW, open field, and acoustic startle) were analyzed by ANOVA. A *P*-value <0.05 was considered statistically significant. Analysis was performed with GraphPad Prism 9 software.

Results

Sohlh1 deficiency leads to gonadal dysgenesis

Gross morphology measurements of WT and KO mice were comparable and are summarized in Supplementary Dataset. We examined BW from 4 to 16 weeks of age, as well as body length at 14 weeks of age, in both male and female mice. While no significant differences were observed in BW or body length between *Sohlh1* KO and WT female or male mice (Figure 1A and B), KO animals did show differences in the size of their gonads as compared to WT mice (Supplementary Figure S1). Consistent with our previous reports showing that *Sohlh1* depletion leads to defective gametogenesis [17, 18], we found that testes were significantly smaller in 16-week-old KO males (WT, $0.82 \pm 0.04\%$ BW; KO, $0.13 \pm 0.01\%$ BW; $P < 0.0001$) when compared to WT males (Supplementary Figure S1A and B) and histopathologic evaluation detected an absence of germ cells with interstitial cell hyperplasia (Supplementary Figure S1A). Similarly, ovaries of KO females were atrophied and smaller than WT at 7 weeks of age (Supplementary Figure S1C), and while presence of advanced antral follicles and primary follicles could be detected in WT ovaries, germ cells were entirely absent in the ovaries of KO animals. These observations mirror the human conditions of azoospermia and POI previously linked to *SOHLH1* deficiency [19–26].

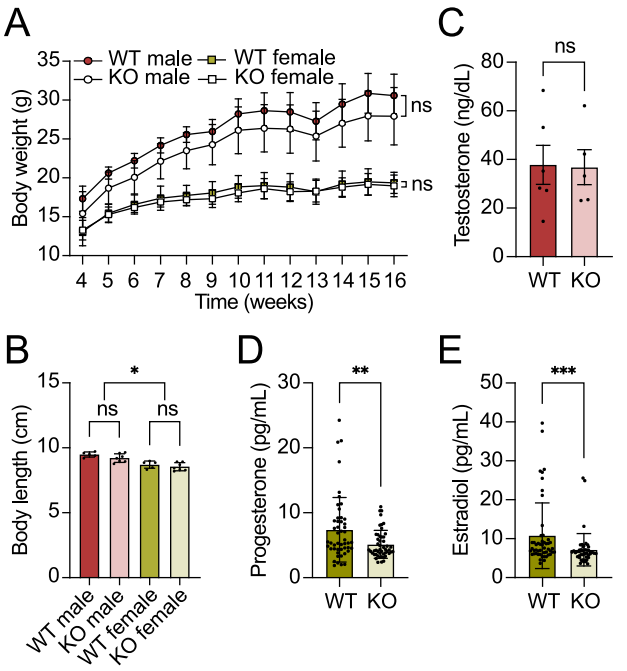

Figure 1. *Sohlh1* deficiency effect on BW, length, and sex hormones. (A) BW of WT ($n = 5$) and KO ($n = 7$) male and female mice plotted from 4 to 16 weeks of postnatal life. Data represents mean ± standard deviation (SD); ns, not significant. (B) Body length of WT ($n = 5$) and KO ($n = 7$) male and female mice measured at 14 weeks. Data represents mean ± SD. $^{*}P < 0.05$; ns, not significant (*t*-test). (C) Plasma concentration of testosterone at 12 weeks for WT and KO males. Bars represent mean of 5–6 replicates per sample and their SD. ns, not significant (*t*-test). (D–E) Plasma concentration of estrogen (D) and progesterone (E) at 7 weeks for WT and KO females. Shown are 47–50 replicates per sample, their mean and SD. $^{**}P < 0.01$, $^{***}P < 0.001$ (Mann–Whitney test).

We also tested whether the lack of functional gonads leads to reduced blood levels of sex hormones in KO mice. *Sohlh1* KO and WT male animals showed similar measurements of plasma testosterone (WT, 37.82 ± 19.6 ng/dL; KO, 36.84 ± 16.19 ng/dL; $P = 0.9312$) (Figure 1C), which is consistent with data from human patients carrying loss of function *SOHLH1* mutations whose testosterone levels were unaltered [24, 31]. However, in female animals, we found that the KO cohort had significantly lower levels of both estrogen (E2) (WT, 10.79 ± 8.42 pg/mL; KO, 7.18 ± 4.2 pg/mL; $P = 0.0007$) and progesterone (P4) (WT, 7.4 ± 4.98 ng/mL; KO, 5.13 ± 2.15 ng/mL; $P = 0.0063$) as compared to the WT group (Figure 1D and E), indicating that *Sohlh1* loss of function alters gonadal development and, in females, the hormonal milieu.

Sohlh1 deficiency disturbs the immune cell populations present in the blood and ovaries

The immune system plays an important role in ovarian physiology [32]. Defects in immune responses such as autoimmunity can alter ovarian homeostasis and detrimentally affect follicular dynamics, and in some cases associate with POI [33]. In a similar fashion, the decrease in ovarian-secreted hormones that accompanies the onset of menopause has been suggested to alter immunological profiles [34]. Having observed a decrease in E2 and P4 in KO females, we next examined if ovarian atrophy due to *Sohlh1* deletion impacts systemic immune cell composition in the blood as well as in the ovary.

We performed flow cytometry analysis to determine the immune cell populations present in the blood and ovaries of 7-week-old KO and WT female mice (Figure 2). Cells were labeled using antibodies targeting specific immune cell-type markers (Supplementary Table S1) allowing the identification of key immune cell populations (Supplementary Figure S2). While no differences were observed in the total number of blood cells between KO and WT mice (Figure 2A), the percentage of immune cells (CD45+) found in the blood of *Sohlh1* KO animals was significantly higher than that of the WT group (WT, 62.11 ± 3.76%; KO, 76.24 ± 4.6%; $P = 0.0288$) (Figure 2B). Specifically, when compared to WT mice, KO animals presented with an increased proportion of MCHII+ CD45R+ B cells (WT, 7.3 ± 1.2%; KO, 10.6 ± 0.9%; $P = 0.041$) that was accompanied by a significant decrease in CD90.2 T cells (WT, 50.8 ± 1.8%; KO, 40.6 ± 2.3%; $P = 0.0028$), which was potentially associated to the lower levels of CD4+ (WT, 27.2 ± 1.5%; KO, 21.5 ± 1.4%; $P = 0.0116$) and CD8+ (WT, 19.1 ± 0.5%; KO, 14.9 ± 1.0%; $P = 0.0013$) T cell populations (Figure 2C).

Consistent with the smaller size of *Sohlh1* KO ovaries (Supplementary Figure S1C), a lower number of total ovarian cells was recovered by flow cytometry in KO mice than in WT mice (WT, $8.77 \pm 6.0 \times 10^4$ cells; KO, $1.57 \pm 1.0 \times 10^4$ cells; $P = 0.0025$) (Figure 2D). However, no significant differences in the relative proportion of immune cells in the ovary were observed between WT and KO (Figure 2E). Interestingly, the immune profile of the ovaries displayed fewer differences between KO and WT mice than that of blood. Conversely to what we found in blood, an increase in CD90.2+ T cells (WT, 23.1 ± 1.5%; KO, 30.3 ± 2.8%; $P = 0.029$) was detected in the ovaries of KO animals (Figure 2F). This rise in T cells may have been driven by the higher levels of CD4- CD8- double negative (DNT) T cells found in the KO ovaries relative to the WT (WT, 10.2 ± 0.9%; KO, 16.0 ± 1.6%; $P = 0.0055$). Altogether, these data show that lack of *Sohlh1* leads to disruption of the immune profiles in blood and ovarian tissues.

Extragonadal phenotypes of *Sohlh1* KO mice

To better investigate the influence of *Sohlh1* deletion on overall mouse physiology, we performed a comprehensive phenotypic evaluation of WT and KO mice using a phenotyping pipeline designed by the IMPC [29]. *Sohlh1* deficiency had no significant effect on locomotion (open field test), cardiovascular function (electrocardiogram), or musculoskeletal domains (measured by grip strength, dual-energy X-ray absorptiometry, and X-rays) (Supplementary Dataset). We found mild but significant differences for multiple parameters that fell into metabolic function, sensory assessment, and behavior and cognition categories (Figure 3, Supplementary Dataset).

Metabolic functions were assessed by ipGTT, clinical chemistry, and complete blood count panels. The glucose tolerance test measures the clearance of intraperitoneally injected glucose to detect disturbances in glucose metabolism. Although genotype did not have an effect on glucose clearance rates (Supplementary Dataset), plasma insulin levels measured at week 16 were found to be significantly lower in *Sohlh1* KO males when compared to WT males (WT, 23.5 ± 8.3 mIU/L; KO, 11.6 ± 4.6 mIU/L; $P = 0.0144$), while they remained unchanged between KO and WT females (Figure 3A). Notably, this decrease in insulin in KO males

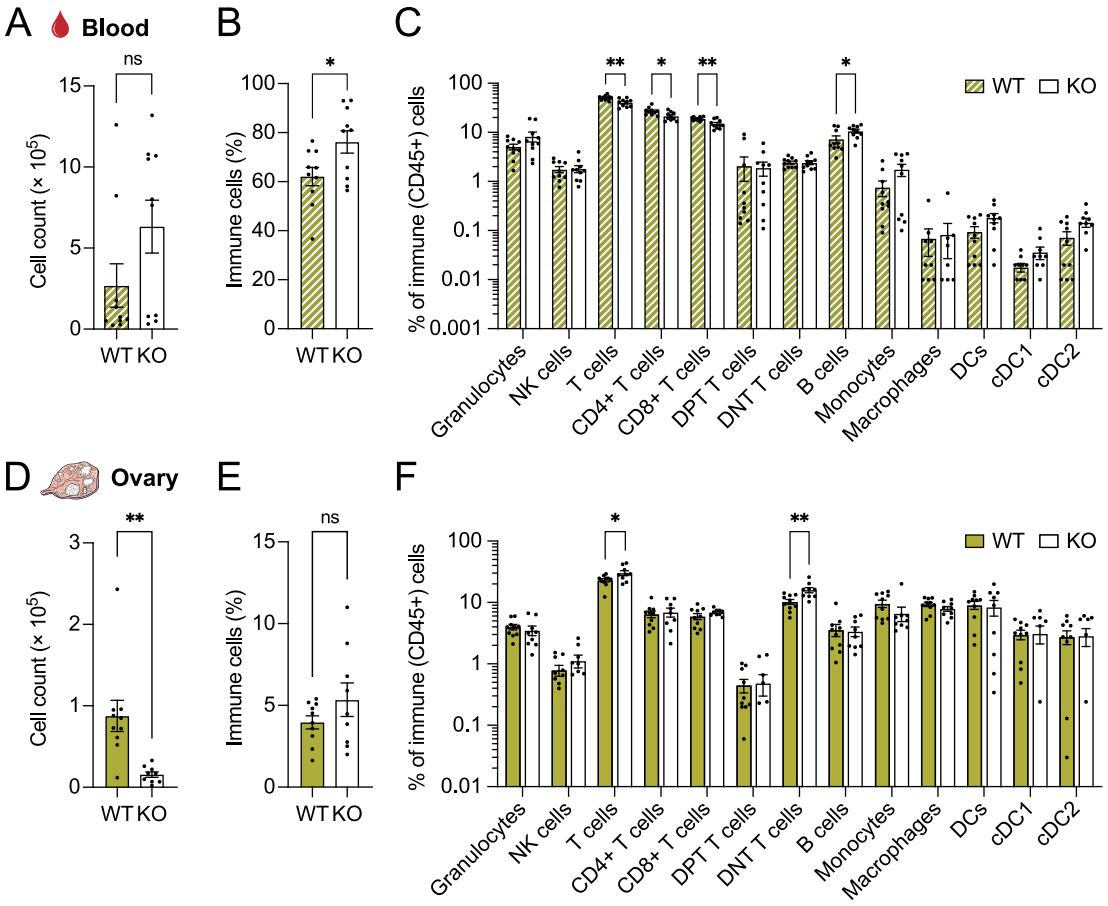

Figure 2. Immunoprofiling of blood and ovaries from WT and *Sohlh1* KO females. (A, D) Total number of cells recovered by flow cytometry in blood (A) and ovarian (D) samples from WT and KO mice. (B, E) Percentage of immune cells (CD45+) present in the blood (B) and ovary (E). (C, F) Proportion of immune cell types relative to the total number of CD45+ cells in each blood (C) and ovarian (F) sample. Shown are mean ± SD of 9–10 replicates per group. DNT, double negative; DPT, double positive; DCs, dendritic cells; cDC1, conventional type 1 dendritic cells; cDC2, conventional type 2 dendritic cells. $^{*}P < 0.05$, $^{**}P < 0.01$ (*t*-test).

was not accompanied by variations in the levels of blood glucose, which were comparable between KO and WT in both male and female mice (Figure 3B). *Sohlh1* KO females, but not KO males, displayed a significant genotype effect in a subset of clinical chemistry parameters. Compared to WT female mice, KO females had increased levels of serum albumin (WT, 3.1 ± 0.05 g/L; KO, 3.3 ± 0.17 g/L; $P = 0.01$), and total protein (WT, 5.3 ± 0.12 g/L; KO, 5.6 ± 0.23 g/L; $P = 0.0373$) (Figure 3C and D). Measurements of blood urea nitrogen (BUN) were also increased in KO females compared to WT females (WT, 14.3 ± 1.3 mg/dL; KO, 18.4 ± 3.4 mg/dL; $P = 0.0308$) (Figure 3E). While this could suggest impaired kidney function, histological examination of kidney tissue showed no morphology defects between genotypes (Figure 3F). KO females presented higher levels of plasma alanine aminotransferase (ALT) (WT, 37.7 ± 9.6 U/L; KO, 76.5 ± 35.8 U/L; $P = 0.042$) and aspartate aminotransferase (AST) activity (WT, 110.8 ± 47.2 U/L; KO, 236.7 ± 75.9 U/L; $P = 0.018$) than WT females, with comparable AST/ALT ratios (WT, 2.95 ± 1.13; KO, 3.46 ± 1.48; $P = 0.5284$) between genotypes (Figure 3G–I). This increase in AST and ALT activity may be related to the mild portal inflammatory phenotype, with immune cell infiltration and no signs of steatosis that we observed in the livers of *Sohlh1* KO females, but not in WT females nor in WT or KO males (Figure 3J). Complete blood count at 16 weeks of age showed comparable values for all parameters between WT and KO in both male and female mice (Supplementary Dataset).

Sensory evaluation included an eye examination and an auditory brainstem response test. Ophthalmological assessment revealed that all animals, regardless of their sex and genotype, presented with bilateral incipient cataracts. Additionally, KO males displayed a greater frequency of retinal dysplasia with abnormal retinal pigmentation (WT, 1/5 mice; KO, 7/7 mice) and vascularization (WT, 2/5 mice; KO, 5/7 mice) than WT male mice. Auditory examination indicated normal acoustic function in both WT and KO mice (Supplementary Dataset). Among the battery of tests used to assess behavior and cognition, significant differences were observed during fear conditioning testing. The fear conditioning test assesses the ability of mice to learn and remember associations between environmental stimuli (contextual and cued) and an aversive experience. Fear in response to these stimuli is quantified by freeze and locomotor activity and is used as a measure of conditioned learning/memory performance. Net movements in the arena in response to both contextual and cued stimuli were higher for KO females than for WT females, although only the response to a contextual stimulus was found to be significant (WT, 46.36 ± 26.03 a.u.; KO, 106.9 ± 32.32 a.u.; $P = 0.0112$) (Figure 3K), while no significant differences were observed between the movements of WT and KO males nor between

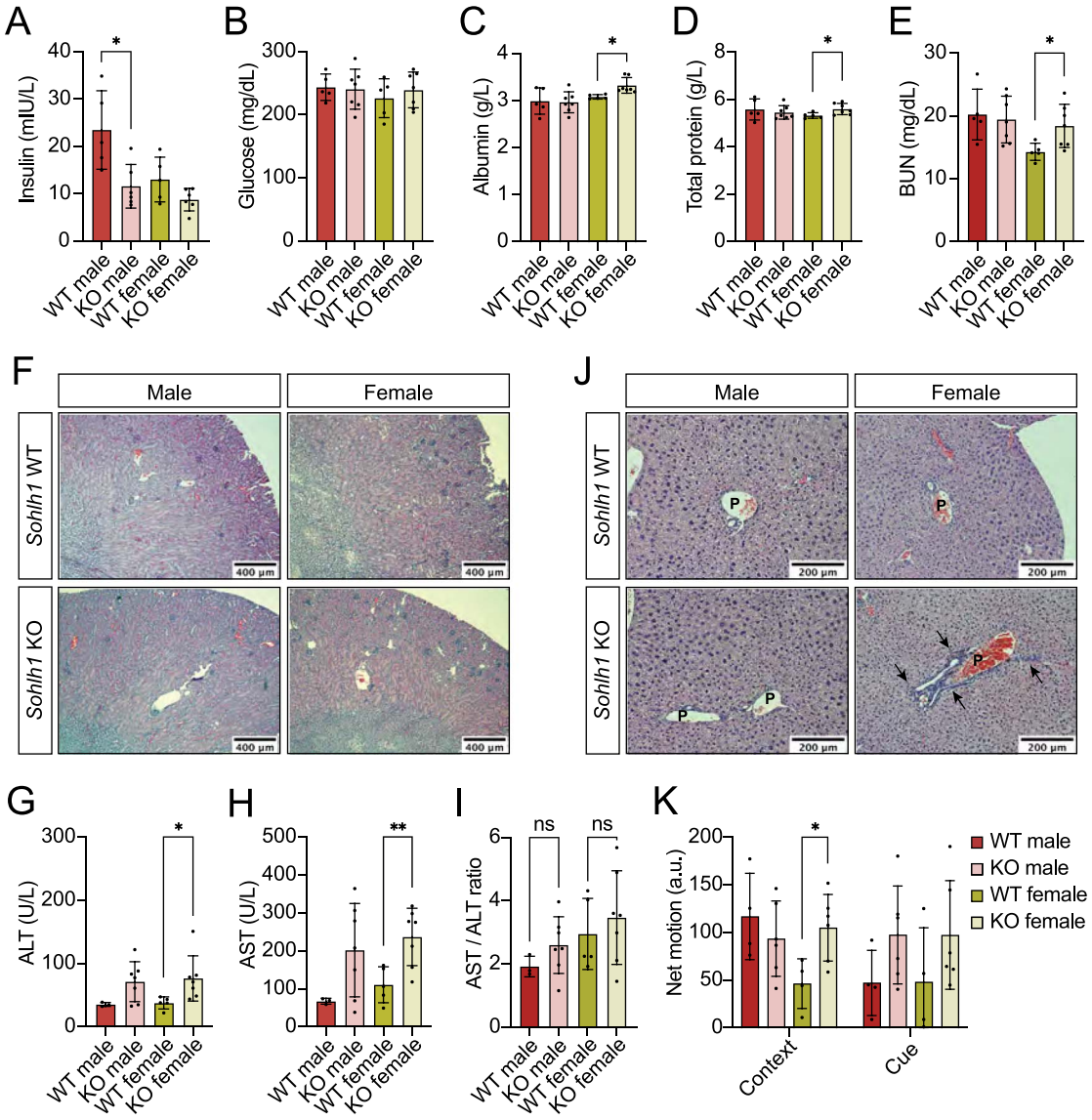

Figure 3. Extragonadal phenotyping of WT and *Sohlh1* KO mice. (A–E) Plasma concentration of insulin (A), glucose (B), albumin (C), total protein (D), BUN (E) in WT ($n = 5$) and KO ($n = 7$) male and female mice measured at 15–16 weeks of age. Bars represent mean and SD. $^*P < 0.05$ (*t*-test). (F) Representative images of kidney sections from 16-week-old WT and KO male and female mice stained with hematoxylin and eosin. (G–I) Plasma ALT (G) and AST activity (H), and AST/ALT ratio (I) for WT ($n = 3$–5) and KO ($n = 7$) male and female mice measured at 15–16 weeks of age. Data represents mean ± SD. $^*P < 0.05$; $^{**}P < 0.01$; ns, not significant (*t*-test). (J) Representative images of liver sections from 16-week-old WT and KO male and female mice stained with hematoxylin and eosin. Images show liver parenchyma and portal tracts (P). Arrows indicate portal inflammation with mild periportal activity in *Sohlh1* KO females. (K) Fear conditioning test. Net motion in response to cued and contextual cues for 11-week-old WT ($n = 4$) and KO ($n = 6$) mice. Data represents mean ± SD. $^*P < 0.05$ (*t*-test); a.u., arbitrary units.

the freezing times of WT and KO (Supplementary Dataset). These observations may indicate that KO female mice have diminished associative learning compared to WT females.

Discussion

Epidemiological studies have shown that early menopause and loss of ovarian function are strongly correlated with shorter life span and elevated risk of metabolic, cardiovascular, and osteoporosis disease, among others [3–5]. GWAS and WES studies have identified 290 loci that associate with age at natural menopause [9], some of which overlap with the >100 genes currently linked to POI [35, 36]. This significant genetic heterogeneity may associate with genotype-specific clinical presentations including different extragonadal phenotypes. For example, loss of genes primarily expressed in the gonads (such as meiosis-specific genes that are only expressed in the germline) may result in loss of gonadal function but little extragonadal manifestations beyond those related to hypogonadism. Conversely, genes expressed in both soma and germline may affect a variety of extragonadal organ systems directly, causing phenotypic heterogeneity. Reproductive biologists tend to focus on examining reproductive sequelae of genetic alterations often ignoring other phenotypes and organ involvements. It is therefore of great interest to determine if specific genotypes that cause gonadal dysfunction also associate with specific extragonadal phenotypes.

Previous mouse studies have suggested that *Sohlh1* phenotype is restricted to the gonads and its expression pattern seems limited to the germline [17, 18]. However, subsequent

immunohistochemical studies have shown that *Sohlh1* is expressed in the brain, iris, ciliary body, and retina, digestive tract as well as muscle [37]. Although antibody-based expression assays are limited due to false positive or negative staining, ribonucleic acid (RNA) expression studies have detected *Sohlh1* transcripts in the brain, in addition to gonads [38]. We therefore decided to use a well-described mouse model of POI, a *Sohlh1* loss of function mouse, to study its effect on several physiologic states by examining gross and histological morphology, cardiovascular, metabolic, and hormonal functions, neurological assessment of behavior, locomotion, and cognitive and sensory functions, as well as musculoskeletal evaluation and immunological profiling.

The main findings of this study show that the lack of *Sohlh1* affects the overall health of the mouse. Our results showed that *Sohlh1* KO female mice have different immune profiles than WT, with a higher proportion of total immune cells in the blood of KO animals. B cells were observed to be higher in KO blood, while T cells were present at a lower proportion in the blood and at a higher proportion in the ovaries of KO animals (Figure 2). Considering the growing body of evidence underscoring the role of sex hormones as modulators of the immune response [39, 40], it is possible that these differences in immune cell populations are associated with the significantly lower levels of both E2 and P4 hormones found in *Sohlh1* KO serum plasma (Figure 1). Consistently, increased levels of B cells in bone marrow and spleen have also been reported in ovariectomized mice, where E2 deficiency was found to induce B-cell lymphopoiesis [41, 42]. While several reports using ovariectomized mice, as well as studies in postmenopausal women, also described an expansion of T cells [43–46], we observed the opposite in the blood of *Sohlh1* KO animals. In mice, a causal role of T cell activity in ovariectomy-induced bone loss has been demonstrated [46, 47]. Interestingly, bone composition measurements and serum levels of calcium and alkaline phosphatase (Supplementary Dataset), which are known markers of bone remodeling, were comparable between WT and *Sohlh1* KO. However, it is yet unclear if these observations and the decrease in blood T cells in *Sohlh1* KO mice are related. Altogether our data shows an altered immune profile in *Sohlh1* KO animals with differences in T cell levels compared to ovariectomized mice.

The extragonadal phenotyping showed mild but significant differences between WT and *Sohh1* KO mice for multiple parameters that fell into the categories of metabolic functions, tissue weights, sensory assessment, and behavior and cognition (Figure 3, Supplementary Figure S1). Alterations of the metabolic functions in *Sohlh1* KO were sex specific. On the one hand, 16-week-old KO females had higher concentrations of albumin, total protein, BUN, and ALT and AST activities compared to WT females. This was accompanied by mild portal inflammation in the livers of KO females. KO males, on the other hand, presented with lower plasma insulin levels than WT males. This sexual dimorphism in some metabolic functions may be related to the unchanged levels of plasma testosterone in *Sohlh1* KO mice, since testosterone is known to play a key role in carbohydrate, fat, and protein metabolism [48]. Differences were also seen in specific tissues in *Sohlh1* KO mice. *Sohlh1* KO animals had significantly smaller gonads in both males and females (Supplementary Figure S1), consistent with our previous findings [17, 18]. Within the sensory assessment category, ophthalmological examination revealed a greater frequency of abnormal retinal pigmentation and vascularization in *Sohlh1* KO males than in WT males. Finally, among the battery of tests used to assess behavior and cognition, significant differences were observed in associative learning (lower in KO females). While mouse *Sohlh1* transcript has been described to be preferentially expressed by germ cells, messenger RNA (mRNA) expression in the brain neurons has also been reported [38]. Similarly, human *SOHLH1* mRNA can be detected in the nervous system [49]. Therefore, it remains to be investigated whether the behavioral phenotype observed in *Sohlh1* KO female mice is related to *Sohlh1* deficiency in brain neurons. Other tests performed as part of the phenotyping pipeline showed that *Sohlh1* loss of function has no significant effect on locomotion or cardiovascular function.

We also searched the IMPC database for extragonadal phenotypes in other genes known to function primarily in the germline. We identified 18 meiosis-specific genes that have an infertility phenotype and extragonadal phenotyping recorded in the database (Table 1). *Sohlh1* KO mice shared at least one phenotype with 7 out of 18 genes examined: ocular defects (*Mlh1, Setx, Sun1, Syce1, Trip13*), increased leukocyte cell number (*Kash5, Sun1*), increased levels of AST (*Rec8, Mlh1*) and ALT [*Mlh1* (data not shown)], and increased BUN levels [*Mlh1* (data not shown)]. Phenotypic differences between *Sohlh1* KO and these 18 genes were also noticed. Among the 18 genes phenotyped, pathologies in bone structure and composition, in grip strength, in body mass amount and composition, or in locomotor activity were identified in a subset of genes, but not found in *Sohlh1* KO animals. Decreased associative learning was a *Sohlh1*-specific phenotype not reported among these 18 genes. Two main factors should be considered when comparing extragonadal phenotypes of infertility-related genes. First, genetic background has been shown to influence behavioral and molecular characteristics of mouse strains [50–52], and second, extragonadal gene expression can lead to alterations in somatic tissues and, as a result, influence systemic health independently of reproductive impairment. The overlap between the phenotypes of our *Sohlh1* KO and of those seven other genes may suggest shared effects of gonadal dysfunction on overall health, or simply a common pathway due to disrupted hormonal production. Hormonal supplementation studies across different KO genotypes will help resolve this question in the future. Moreover, extensive phenotyping of mice null for infertility-related genes that are expressed exclusively in the gonads or that are conditionally inactivated in that tissue will help to resolve if the observed phenotypes are due to direct genetic deficiency in extragonadal tissues as opposed to gonadal dysfunction.

Our studies may have implications for patients with *SOHLH1*-associated infertility. A previous study in four affected sisters from two unrelated consanguineous Turkish families with POI showed that they presented with primary amenorrhea, lack of secondary sex characteristics, delayed bone age and, in one of the two families, short stature [22]. Hormone replacement therapy with cyclic estrogen and progesterone resulted in breast development, menstrual cycles, and increased height. However, no mention is made of other phenotypic features. In one of the two families with *SOHLH1*-related POI, short stature was observed [22]. Mouse females deficient in *Sohlh1* were not shorter than WT females and the two sisters of the other affected family did not present with short stature [22]. Therefore, it is possible that short stature is related to other homozygous loci in

Table 1. Phenotypes associated with depletion of *Sohlh1* and meiosis-specific genes. Sown are the phenotypes associated with *Sohlh1* and/or with at least two meiotic infertility-related genes as found in the IMPC database

Phenotype	*Sohlh1* KO	Meiosis-specific Genes
Male infertility	✓	*4930447C04Rik, Ccnb1ip1, Cntd1, Cul4a, Fbxo47, Kash5, Mlh1, Mlh3, Rad21l, Rec8, Setx, Spata22, Stag3, Sun1, Syce1, Syce2, Trip13, Zcwpw1*
Female infertility	✓	*4930447C04Rik, Ccnb1ip1, Cntd1, Kash5, Mlh3, Spata22, Stag3, Sun1, Syce1, Syce2, Trip13*
Small testis	✓	*Cntd1, Cul4a, Mlh1, Setx, Spata22, Stag3, Sun1, Syce2, Zcwpw1*
Abnormal testis morphology	✓	*Ccnb1ip1, Cntd1, Cul4a, Mlh1, Spata22, Syce2, Zcwpw1*
Decreased bone mineral density	—	*4930447C04Rik, Spata22, Stag3, Syce1, Syce2, Trip13*
Abnormal eye morphology and vasculature	✓	*Mlh1, Setx, Sun1, Syce1, Trip13*
Decreased bone mineral content	—	*4930447C04Rik, Spata22, Stag3, Syce2, Trip13*
Decreased grip strength	—	*4930447C04Rik, Stag3, Syce1, Syce2, Trip13*
Decreased lean body mass	—	*4930447C04Rik, Stag3, Sun1, Syce2, Trip13*
Decreased locomotor activity	—	*Ccnb1ip1, Kash5, Sun1, Syce1, Trip13*
Increased total body fat amount	—	*4930447C04Rik, Stag3, Sun1, Syce2, Trip13*
Abnormal bone structure	—	*Cntd1, Spata22, Trip13, Trip13*
Abnormal epididymis morphology	✓	*Ccnb1ip1, Mlh1, Syce2, Zcwpw1*
Increased fasting circulating glucose level	—	*Cul4a, Stag3, Syce1, Trip13*
Increased circulating HDL cholesterol level	—	*Kash5, Stag3, Trip13*
Abnormal ovary morphology	✓	*Ccnb1ip1, Syce2*
Abnormal pancreas morphology	—	*Spata22, Zcwpw1*
Decreased lymphocyte cell number	NA	*Setx, Sun1*
Enlarged lymph nodes	—	*Ccnb1ip1, Zcwpw1*
Improved glucose tolerance	—	*Setx, Syce1*
Increased circulating aspartate transaminase level	✓	*Mlh1, Rec8*
Increased leukocyte cell number	✓	*Kash5, Sun1*
Increased monocyte cell number	—	*Setx, Sun1*
Increased neutrophil cell number	—	*Setx, Sun1*
Increased thigmotaxis	—	*Syce1, Trip13*

NA, not available.

this consanguineous family. Similarly, *SOHLH1* variants have also been described in infertile men. One particular heterozygous *SOHLH1* variant was identified in Japanese and Korean males presenting with non-obstructive azoospermia due to spermatogenesis defects [23, 24]. Interestingly, a recent study detected a *SOHLH1* homozygous mutation in a Chinese man diagnosed with severe oligozoospermia, and it described the presence of this variant in heterozygosity in three patients with teratozoospermia and normal sperm counts [26]. Beyond elevated FSH levels, no other phenotypic features were reported for these patients [23, 24, 26]. Thus, there is an unmet need for further, deeper phenotyping of patients with *SOHLH1* deficiency, especially as related to our mouse findings of retinal and behavioral problems. Based on our data, where several sex-specific phenotypic alterations due to the *Sohlh1* genotype were observed, and on the fact that many genes that cause POI also result in azoospermia, it is critical to carefully study the extragonadal effects of infertility in both sexes.

These results provide valuable information regarding the consequences of infertility due to the *Sohlh1* genotype on overall health. Future studies will expand on this phenotyping and include other genes commonly encountered in genetic forms of infertility. Our studies also suggest that deep phenotyping of human patients is likely to lead to additional phenotypic findings which may be of relevance to the affected individuals.

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Supplementary material

Supplementary material is available at *BIOLRE* online.

Conflict of interest

The authors have declared that no conflict of interest exists.

Author contributions

Conceptualization, A.R.; Methodology, B.R.-A., S.B., M.R.-E.; Formal analysis, M.R.-E., B.R.-A.; Investigation, M.R.-E., B.R.-A., S.B.; Writing—original draft, M.R.-E., B.R.-A., A.R.; Writing—review & editing, M.R.-E., S.B., A.R.; Visualization, M.R.-E.; Resources, A.R.; Supervision, A.R.; Project administration, A.R.; Funding acquisition, A.R.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol* 1986; **67**:604–606.
2. Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, Cifkova R, de Muinck K-SS, Hogervorst E, Janse F, Liao L, Vlaisavljevic V, *et al.* ESHRE guideline: management of women

with premature ovarian insufficiency. *Hum Reprod* 2016; **31**: 926–937.
3. Louwers YV, Visser JA. Shared genetics between age at menopause, early menopause, POI and other traits. *Front Genet* 2021; **12**:676546.
4. Rajkovic A, Pangas S. Ovary as a biomarker of health and longevity: insights from genetics. *Semin Reprod Med* 2017; **35**: 231–240.
5. Desai S, Rajkovic A. Genetics of reproductive aging from gonadal dysgenesis through menopause. *Semin Reprod Med* 2017; **35**: 147–159.
6. Punjani N, Lamb DJ. Canary in the coal mine? Male infertility as a marker of overall health. *Annu Rev Genet* 2020; **54**:465–486.
7. del Giudice F, Kasman AM, Ferro M, Sciarra A, de Berardinis E, Belladelli F, Salonia A, Eisenberg ML. Clinical correlation among male infertility and overall male health: A systematic review of the literature. *Investig Clin Urol* 2020; **61**:355–371.
8. Jensen TK, Jacobsen R, Christensen K, Nielsen NC, Bostofte E. Good semen quality and life expectancy: A cohort study of 43,277 men. *Am J Epidemiol* 2009; **170**:559–565.
9. Ruth KS, Day FR, Hussain J, Martínez-Marchal A, Aiken CE, Azad A, Thompson DJ, Knoblochova L, Abe H, Tarry-Adkins JL, Gonzalez JM, Fontanillas P, *et al.* Genetic insights into biological mechanisms governing human ovarian ageing. *Nature* 2021; **596**: 393–397.
10. Day FR, Ruth KS, Thompson DJ, Lunetta KL, Pervjakova N, Chasman DI, Stolk L, Finucane HK, Sulem P, Bulik-Sullivan B, Esko T, Johnson AD, *et al.* Large-scale genomic analyses link reproductive aging to hypothalamic signaling, breast cancer susceptibility and BRCA1-mediated DNA repair. *Nat Genet* 2015; **47**: 1294–1303.
11. Jiao X, Ke H, Qin Y, Chen Z-J. Molecular genetics of premature ovarian insufficiency. *Trends Endocrinol Metabolism* 2018; **29**: 795–807.
12. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013; **153**:1194–1217.
13. Gunes S, Al-Sadaan M, Agarwal A. Spermatogenesis, DNA damage and DNA repair mechanisms in male infertility. *Reprod Biomed Online* 2015; **31**:309–319.
14. Levine ME, Lu AT, Chen BH, Hernandez DG, Singleton AB, Ferrucci L, Bandinelli S, Salfati E, Manson JE, Quach A, Kusters CDJ, Kuh D, *et al.* Menopause accelerates biological aging. *Proc Natl Acad Sci* 2016; **113**:9327–9332.
15. Shuster LT, Gostout BS, Grossardt BR, Rocca WA. Prophylactic oophorectomy in premenopausal women and long-term health. *Menopause Int* 2008; **14**:111–116.
16. Yatsenko SA, Rajkovic A. Genetics of human female infertility. *Biol Reprod* 2019; **101**:549–566.
17. Ballow D, Meistrich ML, Matzuk M, Rajkovic A. Sohlh1 is essential for spermatogonial differentiation. *Dev Biol* 2006; **294**: 161–167.
18. Pangas SA, Choi Y, Ballow DJ, Zhao Y, Westphal H, Matzuk MM, Rajkovic A. Oogenesis requires germ cell-specific transcriptional regulators Sohlh1 and Lhx8. *Proc Natl Acad Sci* 2006; **103**: 8090–8095.
19. Zhao S, Li G, Dalgleish R, Vujovic S, Jiao X, Li J, Simpson JL, Qin Y, Ivanisevic M, Ivovic M, Tancic M, Al-Azzawi F, *et al.* Transcription factor SOHLH1 potentially associated with primary ovarian insufficiency. *Fertil Steril* 2015; **103**:548–553.e5.
20. Jolly A, Bayram Y, Turan S, Aycan Z, Tos T, Abali ZY, Hacihamdioglu B, Coban Akdemir ZH, Hijazi H, Bas S, Atay Z, Guran T, *et al.* Exome sequencing of a primary ovarian insufficiency cohort reveals common molecular etiologies for a spectrum of disease. *J Clin Endocrinol Metab* 2019; **104**:3049–3067.
21. Bouilly J, Beau I, Barraud S, Bernard V, Azibi K, Fagart J, Fèvre A, Todeschini AL, Veitia RA, Beldjord C, Delemer B, Dodé C, *et al.* Identification of multiple gene mutations accounts for a new genetic architecture of primary ovarian insufficiency. *J Clin Endocrinol Metab* 2016; **101**:4541–4550.
22. Bayram Y, Gulsuner S, Guran T, Abaci A, Yesil G, Gulsuner HU, Atay Z, Pierce SB, Gambin T, Lee M, Turan S, Bober E, *et al.* Homozygous loss-of-function mutations in *SOHLH1* in patients with nonsyndromic hypergonadotropic hypogonadism. *J Clin Endocrinol Metab* 2015; **100**:E808–E814.
23. Choi Y, Jeon S, Choi M, Lee M, Park M, Lee DR, Jun K-Y, Kwon Y, Lee O-H, Song S-H, Kim J-Y, Lee K-A, *et al.* Mutations in SOHLH1 gene associate with nonobstructive azoospermia. *Hum Mutat* 2010; **31**:788–793.
24. Nakamura S, Miyado M, Saito K, Katsumi M, Nakamura A, Kobori Y, Tanaka Y, Ishikawa H, Yoshida A, Okada H, Hata K, Nakabayashi K, *et al.* Next-generation sequencing for patients with non-obstructive azoospermia: implications for significant roles of monogenic/oligogenic mutations. *Andrology* 2017; **5**:824–831.
25. Song B, Zhang Y, He X, Du W, Ruan J, Zhou F, Wu H, Zha X, Xie X, Ye L, Wei Z, Zhou P, *et al.* Association of genetic variants in SOHLH1 and SOHLH2 with non-obstructive azoospermia risk in the Chinese population. *Eur J Obstetrics Gynecol Reprod Biol* 2015; **184**:48–52.
26. Liu M, Yang Y, Wang Y, Chen S, Shen Y. The mutation c.346-1G > A in SOHLH1 impairs sperm production in the homozygous but not in the heterozygous condition. *Hum Mol Genet* 2022; **31**:1013–1021.
27. Shin Y-H, Ren Y, Suzuki H, Golnoski KJ, Ahn H, Mico V, Rajkovic A. Transcription factors SOHLH1 and SOHLH2 coordinate oocyte differentiation without affecting meiosis I. *J Clin Investig* 2017; **127**:2106–2117.
28. Suzuki H, Ahn HW, Chu T, Bowden W, Gassei K, Orwig K, Rajkovic A. SOHLH1 and SOHLH2 coordinate spermatogonial differentiation. *Dev Biol* 2012; **361**:301–312.
29. Groza T, Gomez FL, Mashhadi HH, Muñoz-Fuentes V, Gunes O, Wilson R, Cacheiro P, Frost A, Keskivali-Bond P, Vardal B, McCoy A, Cheng TK, *et al.* The international mouse phenotyping consortium: comprehensive knockout phenotyping underpinning the study of human disease. *Nucleic Acids Res* 2023; **51**:D1038–D1045.
30. Xie C, Wang W, Tu C, Meng L, Lu G, Lin G, Lu L-Y, Tan Y-Q. Meiotic recombination: insights into its mechanisms and its role in human reproduction with a special focus on non-obstructive azoospermia. *Hum Reprod Update* 2022; **28**:763–797.
31. Cannarella R, Condorelli RA, Paolacci S, Barbagallo F, Guerri G, Bertelli M, la Vignera S, Calogero AE. Next-generation sequencing: toward an increase in the diagnostic yield in patients with apparently idiopathic spermatogenic failure. *Asian J Androl* 2021; **23**: 24–29.
32. Bukovsky A, Caudle MR. Immunoregulation of follicular renewal, selection, POF, and menopause in vivo, vs. neo-oogenesis in vitro, POF and ovarian infertility treatment, and a clinical trial. *Reprod Biol Endocrinol* 2012; **10**:97.
33. Silva CA, Yamakami LYS, Aikawa NE, Araujo DB, Carvalho JF, Bonfá E. Autoimmune primary ovarian insufficiency. *Autoimmun Rev* 2014; **13**:427–430.
34. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. *Hum Reprod Update* 2005; **11**: 411–423.
35. Yang Q, Mumusoglu S, Qin Y, Sun Y, Hsueh AJ. A kaleidoscopic view of ovarian genes associated with premature ovarian insufficiency and senescence. *FASEB J* 2021; **35**:21753.
36. França MM, Mendonca BB. Genetics of ovarian insufficiency and defects of folliculogenesis. *Best Pract Res Clin Endocrinol Metab* 2022; **36**:101594.
37. Zhang X, Liu R, Su Z, Zhang Y, Zhang W, Liu X, Wang F, Guo Y, Li C, Hao J. Immunohistochemical study of expression of Sohlh1 and Sohlh2 in normal adult human tissues. *PLoS One* 2015; **10**:e0137431.
38. Baldarelli RM, Smith CM, Finger JH, Hayamizu TF, McCright IJ, Xu J, Shaw DR, Beal JS, Blodgett O, Campbell J, Corbani LE, Frost PJ, *et al.* The mouse gene expression database (GXD): 2021 update. *Nucleic Acids Res* 2021; **49**:D924–D931.

39. Bereshchenko O, Bruscoli S, Riccardi C. Glucocorticoids, sex hormones, and immunity. *Front Immunol* 2018; **9**:1332.
40. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016; **16**:626–638.
41. Masuzawa T, Miyaura C, Onoe Y, Kusano K, Ohta H, Nozawa S, Suda T. Estrogen deficiency stimulates B lymphopoiesis in mouse bone marrow. *J Clin Investig* 1994; **94**:1090–1097.
42. García-Pérez MA, Noguera I, Hermenegildo C, Martínez-Romero A, Tarín JJ, Cano A. Alterations in the phenotype and function of immune cells in ovariectomy-induced osteopenic mice. *Hum Reprod* 2006; **21**:880–887.
43. Adeel S, Singh K, Vydareny KH, Kumari M, Shah E, Weitzmann MN, Tangpricha V. Bone loss in surgically ovariectomized premenopausal women is associated with T lymphocyte activation and thymic hypertrophy. *J Invest Med* 2013; **61**:1178–1183.
44. Yu M, Pal S, Paterson CW, Li JY, Tyagi AM, Adams J, Coopersmith CM, Weitzmann MN, Pacifici R. Ovariectomy induces bone loss via microbial-dependent trafficking of intestinal TNF+ T cells and Th17 cells. *J Clin Investig* 2021; **131**:e143137.
45. D'Amelio P, Grimaldi A, di Bella S, Brianza SZM, Cristofaro MA, Tamone C, Giribaldi G, Ulliers D, Pescarmona GP, Isaia G. Estrogen deficiency increases osteoclastogenesis up-regulating T cells activity: A key mechanism in osteoporosis. *Bone* 2008; **43**:92–100.
46. Cenci S, Toraldo G, Weitzmann MN, Roggia C, Gao Y, Qian WP, Sierra O, Pacifici R. Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN- -induced class II transactivator. *Proc Natl Acad Sci* 2003; **100**:10405–10410.
47. Gao Y, Qian WP, Dark K, Toraldo G, Lin ASP, Guldberg RE, Flavell RA, Weitzmann MN, Pacifici R. Estrogen prevents bone loss through transforming growth factor β signaling in T cells. *Proc Natl Acad Sci USA* 2004; **101**:16618–16623.
48. Kelly DM, Jones TH. Testosterone: a metabolic hormone in health and disease. *J Endocrinol* 2013; **217**:R25–R45.
49. Carithers LJ, Ardlie K, Barcus M, Branton PA, Britton A, Buia SA, Compton CC, Deluca DS, Peter-Demchok J, Gelfand ET, Guan P, Korzeniewski GE, *et al.* A novel approach to high-quality postmortem tissue procurement: The GTEx project. *Biopreserv Biobank* 2015; **13**:311–319.
50. Logue SF, Owen EH, Rasmussen DL, Wehner JM. Assessment of locomotor activity, acoustic and tactile startle, and prepulse inhibition of startle in inbred mouse strains and F1 hybrids: Implications of genetic background for single gene and quantitative trait loci analyses. *Neuroscience* 1997; **80**:1075–1086.
51. Sultana R, Ogundele OM, Lee CC. Contrasting characteristic behaviours among common laboratory mouse strains. *R Soc Open Sci* 2019; **6**:190574.
52. Holmes A, Wrenn CC, Harris AP, Thayer KE, Crawley JN. Behavioral profiles of inbred strains on novel olfactory, spatial and emotional tests for reference memory in mice. *Genes Brain Behav* 2002; **1**:55–69.