Supplementary Appendix

Supplement to: Dougherty MP, Poch AM, Chorich LP, et al. Unexplained female infertility associated with genetic disease variants. N Engl J Med 2023;388:1055-6. DOI: 10.1056/NEJMc2211539

This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Files

Dougherty et al. Unexplained Female Infertility Associated With Genetic-Disease Variants.

Supplementary Methods

Unexplained infertility:

Healthy couples without serious medical problems presented with infertility ≥1 year and met the following inclusion criteria for the Multiple Intrauterine Gestations from Ovarian Stimulation (AMIGOS) trial: normal uterine cavity with at least one patent fallopian tube, ≥9 menses/year, and a partner with a motile sperm count ≥5 million in the ejaculate.[1,2] Females ≥18 and ≤40 (mean age~32 years) had a physical exam and baseline labs on entry to the trial.[1]

Study cohort:

200 female DNA samples were randomly selected from the deidentified cohort of the 575 female AMIGOS study patients and subjected to WES (unfortunately, DNA was not available from male partners). The ethnicity is as follows: ~95% not Hispanic/Latino and 5% Hispanic/Latino. In terms of race, ~81% were White, 9% Black, 9% Asian and 1% American Indian or Alaskan Native. Comparison of our cohort to the two control populations is shown in Table S1.

Whole Exome Sequencing:

Deidentified genomic DNA was provided through the Reproductive Medicine Network from the AMIGOS trial[1]. DNA (2-3 µg/subject) was sent to the Yale Center for Genome Analysis for WES. DNA was sheared to a mean fragment length of ~220 bp by focused acoustic energy (Covaris E220). Blunt ends of the fragment were created followed by phosphorylation using T4 DNA polymerase and T4 polynucleotide kinase. Custom adapters were ligated to each fragment using T4 DNA ligase before amplification by Polymerase chain reaction (PCR).[3] Biotinylated DNA probes (IDT xGen Exome Panel) were synthesized, and hybridizations were performed at 65 °C for 16 hours. The captured fragments were PCR amplified then purified with AMPure XP beads. The Illumina NovaSeq 6000 S4 platform was used to create 100 bp reads. Burrows-Wheeler Aligner (BWA) was used to map sequence reads to the genome. The Genome Analysis Toolkit was used to call exome-wide variants, and Annovar and Variant Effect Predictor were used for variant annotation.[3] Average read depth for our cohort was 63, and 95.4% of exons were covered ≥20-fold.

Filtering variants:

High quality variants were filtered by gene, mapping quality (≥60), Combined Annotation Dependent Depletion (CADD) score ≥20[4] when applicable, type of variant (frameshift, stop-gained, splice site, missense), and allelic frequency ≤0.01 in the gnomAD database. Variants of uncertain significance (VUS) in MAG with CADD scores ≥ 20 were tabulated.

Variant confirmation:

P/LP variant calls from WES underwent confirmation with Sanger sequencing in triplicate.[3] P/LP calls were annotated using Clinvar and Varsome databases.

Statistical analysis:

Our primary outcome was the percent of women with P/LP variants in MAG compared to control databases, which reported secondary findings in 59 MAG from unselected patient populations—2% in the UK Biobank (UKB) and 2.5% in the NIH-funded Electronic Medical Records and Genomics (eMERGE) Network.[5,6] Power analysis at an alpha of 0.05 to detect a 4% (or 3-fold) difference in the prevalence of P/LP variants between unexplained infertility cohort and the UKB would require 141 patients at 80% power and 209 at 90% power. For the eMERGE population, samples of 165 and 242 were necessary at 80% and 90% power, respectively. Based upon this, we chose a sample size of 200. Statistical analysis was performed using Fischer's exact test.

Supplementary Discussion

We hypothesized that a genetic link exists between infertility and future medical illness. Utilizing WES from 197 DNA samples, we found that females with unexplained infertility have a 6.6% prevalence of P/LP variants in the 59 MAG known to cause serious medical illness. This amounted to a 2.5-3-fold higher prevalence of P/LP variants in the 59 MAG compared to two large, unselected patient populations.[5,6] The UK-based study of 49,960 unselected individuals showed a 2% prevalence in P/LP variants in MAG.[5] These findings were supported by the more diverse eMERGE population of 21,915 unselected individuals with a 2.5% detection rate of P/LP MAG variants.[6] MAG variants are highly penetrant and portend a substantial impact on health outcomes (Table S2; Table S3). Additionally, offspring have a 50% chance of inheriting these autosomal dominant (and one X-linked recessive) conditions, and those who inherit these genetic variants are also at serious risk.

Most MAG variants identified in our population (9/14=64%) were associated with hereditary cancers and CVD, consistent with prior findings in women with prior infertility.[7-11] In one recent secondary analysis of a large multicenter, randomized clinical trial there was an independent association between infertility and mortality among females.[8] The $\geq$10 year follow up showed a 10% increase in all-cause mortality during the study period in women with infertility.[8] In infertile women who were otherwise at low-risk for cancer, there was an observed 23% increased risk of death due to cancer, which was predominantly due to a 2.6-fold increase occurrence of breast cancer.[8] We propose that this increase in breast cancer is due at least in part to genetic causes, as we found four women (2%) with P/LP variants in *BRCA1/2*. Another four-year follow-up study showed a 32% increased risk of death from any cause (adjusted hazard ratio of 1.32).[12] This increased risk was seen across all ages, races and ethnicities, known comorbidities and modes of delivery.[12] Furthermore, this risk was seen with infertility diagnosis and testing, but not for treatment, implicating the diagnosis of infertility for increased risk of poor medical outcomes.[12]

There is limited data regarding an underlying mechanism by which infertility may presage mortality and medical co-morbidities despite known associations. However a proposed mechanism does exist for *BRCA1* and *BRCA2*.[13,14] There is increasing evidence that infertility shares a common physiologic pathway with cancer genes as *BRCA2* is known to play an important role for homologous recombination in meiosis[15] and *BRCA1* is involved in meiotic synapsis and crossover.[16,17] Additionally, both BRCA1 and BRCA2 play an essential role in double strand break repair, which has been associated with ovarian aging in addition to cancer risk.[14,17,18] Disruption of this pathway could contribute to the earlier age of menopause and lower ovarian reserve seen in patients affected by *BRCA1* or *BRCA2* variants.[14,17,19,20] In our study, there was more than a 7-fold increased likelihood of having a P/LP *BRCA1* or *BRCA2* variant with unexplained infertility. While this alone does not prove a genetic cause for the increased risk of death due to cancer in infertile females,[8,12,21] it provides supporting evidence and illustrates the need for further investigation.

An increased risk of CVD has also been associated with infertility. This was largely attributed to decreased estradiol exposure in patients with diminished ovarian reserve and primary ovarian insufficiency (POI),[10,11,22] or metabolic syndromes resulting from polycystic ovary syndrome.[9] A considerable number of P/LP variants related to CVD were identified in 3% (6/197) women, which included familial hypercholesterolemia, arrhythmias, cardiomyopathy, and increased risk of aortic dissection. A common pathway connecting CVD and infertility has yet to be discovered. It is possible that infertility is only a biomarker for future medical illness. P/LP variants were also identified in inborn errors in metabolism (Fabry disease), and other miscellaneous disorders including malignant hyperthermia, and myopathy. All described variants pose considerable health

risks (Table S2, Table S3). Even if a common pathway cannot be confirmed, a strong association between infertility and future disease can still assist in early detection and intervention.[23]

In addition to P/LP variants in MAG, 10.7% of women with unexplained infertility demonstrated P/LP variants in genes resulting in conditions not considered medically actionable. These disorders include >20 autosomal dominant conditions (Table S3; Table S5), many of which are severe, life altering or debilitating disorders. An *ANAX11* P variant was identified in one subject which portends a 90% risk for developing amyotrophic lateral sclerosis (ALS), a severe neurodegenerative disease. Additionally, two patients were found to each have a P variant in a gene associated with cancer outside of the 59 medically actionable genes (*ATM* and *RAD51C*). We found P/LP variants in several other neurologic disorders (Alzheimer disease, neuropathy, early onset Parkinson disease, ophthalmic disease) as well as other conditions (Table S3; Table S5). Two disorders, hypogonadotropic hypogonadism and POI, also result in infertility making them particularly interesting.

P/LP variants were identified in 6.6% in MAG and 10.7% of genes not considered MAG. We also identified 196 rare VUS in in 46 of the 59 MAG, which either have a high impact variant call (splice site, frameshift, stop gained) or were missense variants with a CADD score $\geq$20 (Table S4). Most variants are involved in genes for cancer and CVD. VUS will require *in vitro* studies to determine pathogenicity. Nonetheless, it is likely that at least several VUS could be shown to be deleterious, which would increase the detection rate.

There are several barriers to understanding how infertility is associated with future medical illness. One is through investigating all patients with infertility, instead of well-defined patient populations. Our findings are likely only relevant to females with unexplained infertility. We cannot conclude that women with polycystic ovary syndrome, tubal disease, or endometriosis will have the same risks, although these causes of infertility should also be interrogated in future studies. Likewise, the frequency of P/LP variants in MAG of males is currently unknown since we did not have their DNA available.

Our study is not without limitations. The increased prevalence of P/LP variants in unexplained infertility was largely seen in White women. More diverse population studies will be needed, although MAG variants in eMERGE displayed similar prevalence in Whites vs. Nonwhites after exclusion of a known *HFE* variant common in Whites.[6] To determine the true prevalence of P/LP variants in patients with unexplained infertility, *in vitro* analysis of the VUS is necessary. Also, the control studies did not exclude patients with infertility. While this would only increase the significance of our findings, it still could affect the true increase in P/LP variants between fertile and infertile populations. Furthermore, patient information from the AMIGOS trial is deidentified without detailed family history or long term follow up. However, these variants are known to be highly penetrant and have been found to have a substantial impact on health outcomes.

The strength of this study is the relatively large sample size and the rigorous screening process for the AMIGOS clinical trial. We used an unbiased approach of WES to identify genetic disorders[24,25] on a well-characterized group of females with unexplained infertility and found a significantly higher prevalence of P/LP variants in MAG than unselected individuals from large data sets.[5,6] Additionally, these variants were confirmed by Sanger sequencing, which was not reported in the control studies.[5,6] To our knowledge, this is the first study that identified an increased prevalence of disease-causing genetic variants in females with unexplained infertility. Although only 6.6% of subjects had a P/LP variant in a MAG, the finding of ~11% with additional P/LP variants in non-MAG requires further study. We cannot routinely recommend WES for women with unexplained infertility at this time. Our findings support the notion that the higher incidence of future medical illness in women with unexplained infertility may have a genetic component.

Race/Ethnicity	Our cohort (%)	21,915 patient trial (%)	49960 patient trial (%)
White	160 (81.2)	14480 (66.0)	46762 (93.6)
Black	17 (8.6)	3279 (14.9)	744 (1.5)
Hispanic	9 (4.5)	1666 (7.6)	Not reported
Asian	9 (4.5)	1497 (6.8)	270 (0.5)
American Indian	2 (1.0)	77 (0.4)	0 (0)

Table S1. Race and ethnicity of the participants in the study population and the control populations. ~4% of patients in the trial of 21,950 unselected individuals did not respond[6] and ~5% of the race/ethnicity of patients in study with 49,960 patients are unknown.[5]

No.	Gene	Cytoband	HGVS coding	Protein Change	Variant type	Affected Exon	Amino Acid Affected	GnomAD Frequency (%)	ACMG call	Race
	Cancer genes									
1	*BRCA1*	17q21.31	c.1953dup	p.Lys652GlufsTer21	Frameshift	10 of 24	651 of 1885	0	Likely Pathogenic	Caucasian
2	*BRCA2*	13q13.1	c.2857dup	p.Asn986LysfsTer2	Frameshift	11 of 27	986 of 3419	0	Pathogenic	Caucasian
3	*BRCA2*	13q13.1	c.2957dup	p.Trp1692MetfsTer3	Frameshift	11 of 27	1692 of 3419	0.002	Pathogenic	Caucasian
4	*BRCA2**	13q13.1	c.10095_10096insT	p.Ser3366Ter	Stop gained	27 of 27	3366 of 3419	0.3	Likely Pathogenic	Caucasian
	Connective tissue disorder genes									
5	*MYH11*	16p13.11	c.1593_1596dup	p.Asn533ThrfsTer50	Frameshift	14 of 42	532 of 1980	0	Likely Pathogenic	Caucasian
	Cardiomyopathy genes									
6	*GLA*	Xq22.1	c.644A>G	p.Asn215Ser	Missense	5 of 7	215 of 430	0.001	Pathogenic	Caucasian
	Arrythmia genes									
7	*PKP2*	12p11.21	c.1951C>T	p.Arg651Ter	Stop gained	9 of 14	651 of 882	0	Pathogenic	Caucasian
8	*KCNQ1*	11p15.5	c.573_577del	p.Arg192CysfsTer91	Frameshift	3 of 16	191 of 677	0.002	Pathogenic	Caucasian
9	*SCN5A*	3p22.2	c.3784C>T	p.Arg1262Ter	Stop gained	21 of 27	1262 of 1963	0	Pathogenic	Caucasian
10	*SCN5A*	3p22.2	c.3746G>A	p.Arg1249Gln	Missense	21 of 27	1249 of 1963	0.001	Likely Pathogenic	Caucasian
	Miscellaneous genes									
11	*RYR1*	19q13.2	c.957+5_957+29del	-	Splice site	Deletion in intron 10 of 105	-	0.041	Likely Pathogenic	Caucasian
12	*RYR1**	19q13.2	c.325C>T	p.Arg109Trp	Missense	4 of 106	109 of 5039	0.083	Pathogenic	Caucasian
13	*APOB*	2p24.1	c.13028_13029del	p.Tyr4343CysfsTer3	Frameshift	29 of 29	4343 of 4564	0.001	Likely Pathogenic	Asian
14	*CACNA1S*	1q32.1	c.5104C>T	p.Arg1702Ter	Stop gained	41 of 44	1702 of 1874	0.02	Likely Pathogenic	Black

Table S2: Comprehensive list of pathogenic (P)/likely pathogenic (LP) variants that were identified through WES and confirmed by Sanger sequencing. Those marked with asterisks were in the same patient.

P variants have a >99% certainty of pathogenicity,[26] and LP variants have >90% certainty of pathogenicity in known disease-causing genes.[26] Identification of these variants does not require *in vitro* analysis due to proven or likely pathogenicity. The list of MAG originally consisted of 56 genes in version 1 (v.1.0)[27], which was then updated to 59 MAG (v.2.0),[28,29] and recently updated to 73 genes (v.3.0).[30,31] We utilized v.2.0 since control UKB[5] and eMERGE[6] databases did also.

The American College of Medical Genetics and Genomics (ACMG) defines MAG variants, also called "secondary findings",[27] as variants that must have proven pathogenicity, a high likelihood of causing disease (high penetrance) and have clinical relevance with available preventative or therapeutic options. Disorders of MAG consist of cancer, cardiovascular diseases (CVD), inborn errors of metabolism, and miscellaneous.[27-29] These genes predominantly exhibit autosomal dominant (AD) inheritance as well as several X-linked disorders capable of affecting females.

Num.	Gene	Genetic disorder	Penetrance and Risk	PMID
1	*BRCA1*	Breast/Ovarian/Pancreatic Cancer	Breast (40-87%)[1] Ovarian (16-86%)[1] Pancreatic (2.5%)[2]	28632866[1] 35077220[2]
2	*BRCA2*	Breast/Ovarian/Pancreatic Cancer	Breast (27-84%)[1] Ovarian (13-32%)[1] Pancreatic (2.5%)[2]	28632866[1] 35077220[2]
3	*MYH11*	Aortic dissection	17%	17666408
4	*GLA*	Fabry Disease (cardiac, cerebrovascular, and renal)	Neuropathic pain (64%), Renal impairment (33%) and ESRD (1%), TIA or stroke (27%), Tinnitus and hearing loss (47%), Gastrointestinal symptoms (53%)	15025684
5	*PKP2*	Arrhythmogenic right ventricular dysplasia/ cardiomyopathy	Arrhythmogenic right ventricular dysplasia/ cardiomyopathy (11-47%)	17010805
6	*KCNQ1*	Familial atrial fibrillation, Long QT syndrome	Long QT syndrome (73%), Sudden death (9.5%)	12702160
7	*SCN5A*	6 different cardiac arrythmias; Brugada Syndrome	Syncope (22-30%)[1,2], Sudden cardiac death (10-20%)[1,2]	27472692[1] 27566755[2]
8	*RYR1*	Central core disease of muscle malignant hyperthermia	Penetrance of malignant hyperthermia (40.6%)	31206373
9	*APOB*	Familial hypercholesterolemia	Nearly all patients have hepatic steatosis 5-10% develop severe hepatic steatosis with occasional progression to cirrhosis	33983694
10	*CACNA1S*	Hypokalemic periodic paralysis	Hypokalemic periodic paralysis (84-100%) characterized by low potassium, myopathy, and recurrent episodic paralysis	15098604
11	*ALPL*	Hypophosphatasia	Likely complete penetrance. Dental caries, bone pain and fractures, premature loss of teeth	20301329
12	*ANXA11*	Amyotrophic lateral sclerosis breast, colon and ovarian cancer associations	Known penetrance for ALS genes is ~90%	23941283
13	*ATM*	Breast cancer	60% risk breast cancer by age 80, 5-9-fold increased risk of all cancer in women	35354106
14	*BEST1*	Vitelliform Macular Dystrophy	Slow progressive visual impairment (>70%)	20301346
15	*C1QTNF5*	Late onset macular degeneration	Fully penetrant. Visual difficulties in 40's and lose their eyesight after >60 years of age	24531000
16	*CFHR5*	C3 Glomerulopathy	Hematuria (80%)[1], Hypertension (50%)[1], ESRD development within 10 years of diagnosis (70%)[2]	27490940[1] 30692664[2]
17	*CLCN7*	Osteopetrosis in late childhood/adolescence	Penetrance (60-90%), fractures (60-80%) with a mean of 3 fractures per person, hearing and vision loss (19%)	20301306
18	*DUSP6*	Hypogonadotropic hypogonadism	Penetrance unknown	32389901
19	*ERCC6*	Premature ovarian insufficiency	Unknown penetrance	26218421
20	*GBA*	Early Onset Parkinson Disease	Penetrance 6-14%. Onset before 50 years of age with higher likelihood of cognitive impairment	20301402
21	*IMPG2*	Adult-onset macular dystrophy	Macular vitelliform dystrophy was noted in 69% of patients. Mean age of onset is 43 years of age	25085631
22	*KCNJ11*	Transient diabetes of the newborn Permanent Diabetes of the newborn Non-insulin dependent diabetes	Highly penetrant.[1] 50% of patient who develop transient neonatal diabetes will develop diabetes later in life.[2] 20% have associated neurologic features[1]	133529164[1] 7446535[2]
23	*MEF2A*	Coronary Artery Disease	High, but likely incomplete penetrance	15931371
24	*PKD1*	Polycystic kidney disease	Almost 100% penetrance with almost all patients having ESRD by age 70. Average age of onset for ESRD is 55 years of age.	20301424
25	*PSEN1*	Alzheimer's disease	With no family history of Alzheimer's disease (15-25%) with a family history of Alzheimer's disease (30-50%)	20301340
26	*RAD51C*	Breast/Ovarian Cancer	Breast (30%) Ovarian (20-23%)	32107557
27	*SLC25A4*	Progressive external ophthalmoplegia, cardiomyopathy	Variable penetrance	20301382
28	*TTN*	Hereditary Myopathy with Early Respiratory Failure	Likely complete penetrance. Progressive myopathy presenting between 30-50 years old. Often require respiratory support.	24575448
29	*TTR*	Neuropathy, Cardiac amyloidosis	Overall disease risk by age 70 (36-91%) Cardiac amyloidosis (>66%)	20301373
30	*VWF*	von Willebrand Disease type 2	Fully penetrant. Manifests as excessive bleeding with surgery	20301765

Table S3. List of genes with P/LP variants identified in the study population. Numbers 1-10 document the medically actionable genes. Numbers 11-30 denote other P/LP variants identified.

Genes related to cancer phenotypes	Number of VUS (58)
STK11	1
BRCA1	5
BRCA2	6
TSC2	8
RB1	1
WT1	2
PTEN	1
BMPR1A	2
RET	3
TSC1	4
PMS2	3
APC	7
MLH1	4
VHL	2
MSH6	4
MSH2	2
MUTYH	3

Genes related to inborn errors in metabolism and miscellaneous phenotypes	
Genes related to inborn errors in metabolism	Number of VUS (2)
GLA	2
Genes related miscellaneous phenotypes	Number of VUS (43)
RYR1	13
ATP7B	9
APOB	10
CACNA1S	11

Genes related to cardiovascular phenotypes	Number of VUS (93)
DSG2	8
DSC2	2
PKP2	3
KCNQ1	1
DSP	11
SCN5A	9
RYR2	11
TMEM43	1
TINNI3	1
ACTC1	1
MYL2	1
MYH7	2
MYBPC3	12
LMNA	1
MYL3	1
TNNT2	1

PCSK9	**1**
COL3A1	**4**
FBN1	**9**
TGFBR1	**1**
TGFBR2	**1**
ACTA2	**1**
SMAD3	**1**
MYH11	**9**

Table S4: 196 variants of uncertain significance (VUS) in 46 medically actionable genes with a CADD score >20 were identified. All were present at a frequency of <0.01 in the gnomAD database. The genes are listed here with the number of VUS for each gene in parentheses.

No.	Gene	Cytoband	HGVS Coding	Protein change	Variant type	Affected Exon	Gnomad Frequency (%)	ACMG Call	Race
1	*ALPL*	1p36.12	c.1363G>A	p.Gly455Ser	Missense	12 of 12	0.041	P	Caucasian
2	*ANXA11*	10q22.3	c.112G>A	p.Gly38Arg	Missense	38 of 506	0.008	P	Caucasian
3	*ATM*	11q22.3	c.3538del	p.Val1180Ter	Stop gained	1180 of 3057	0	P	Caucasian
4	*BEST1*	11q12.3	c.404C>T	p.Ala135Val	Missense	4 of 9	0.04	P	Caucasian
5	*C1QTNF5*	11q23.3	c.-2366+1G>A	-	Splice site	-	0.003	P	Caucasian
6	*CFHR5*	1q31.3	c.678del	p.Glu226AspfsTer7	Frameshift	5 of 10	0.001	P	Black
7	*CLCN7*	16p13.3	c.2299C>T	p.Arg767Trp	Missense	24 of 25	0	P	Caucasian
8	*DUSP6*	12q21.33	c.566A>G	p.Asn189Ser	Missense	2 of 3	0.068	P	Caucasian
9	*ERCC6*	10q11.23	c.3607_3608insGGGCTGGCTGCTTAAGGTCCACCTTA	p.Lys1203ArgfsTer33	Frameshift	18 of 21	0	P	Caucasian
10	*GBA*	1q22	c.1226A>G	p.Asn409Ser	Missense	10 of 12	0.27	P	Black
11	*IMPG2*	3q12.3	c.3023-6_3030dup	p.Ala1011PhefsTer2	Stop gained	15 of 19	0.002	P	Caucasian
12	*KCNJ11*	11p15.1	c.185C>T	p.Thr62Met	Missense	1 of 753	0	P	Caucasian
13	*MEF2A*	15q26.3	c.836C>T	p.Pro279Leu	Missense	8 of 11	0.118	LP	Caucasian
14	*PKD1*	16p13.3	c.12391G>T	p.Glu4131Ter	Stop gained	45 of 46	0.003	P	Asian
15	*PSEN1*	14q24.2	c.617G>C	p.Gly206Ala	Missense	7 of 12	0.051	P	Caucasian
16	*RAD51C*	17q22	c.577C>T	p.Arg193Ter	Stop gained	4 of 9	0.004	P	Caucasian
17	*SLC25A4*	4q35.1	c.523del	p.Gln175ArgfsTer38	Frameshift	2 of 4	0	P	Caucasian
18	*TTN*	2q31.2	c.73254_73255del	p.Glu24419IlefsTer2	Stop gained	276 of 313	0.017	LP	Caucasian
19	*TTR*	18q12.1	c.424G>A	p.Val142Ile	Missense	4 of 4	0.59	P	Black
20	*VWF**	12p13.31	c.2561G>A	p.Arg854Gln	Missense	20 of 52	0.9	P	Caucasian*

Table S5: AD pathogenic variants not included in the 59 MAG genes, which could affect health. 20 P/LP variants were identified in 21 individuals.

Num	Gene	Disorder	Disease Prevalence	Precent Disease That is Genetic	Percent Genetic Due to This Gene	Prevalence of pathogenic variants for gene in the general populations (%)	PMID
1	*ALPL*	Hypophosphatasia	Moderate: 1/6,300 Severe: 1/300,00	100%	47%	0 – 0.025	29659871
2	*ANXA11*	Amyotropic lateral sclerosis	4-6/100,000	5-10%	1.1%	0 – 0.088	36162820
3	*ATM*	Hereditary breast & ovarian cancer	Breast:12% Ovarian: 1.5% Male breast: 0.1%	5-10%	1%	0 – 0.069	29719442 34493284 33406487
4	BEST1	Vitelliform macular dystrophy-2; Vitreoretinocoroid opathy	1/5,500	?	?	0 – 0.038	20301346
5	*C1QTNF5*	Late onset macular degeneration	8.7%	?	?	0.0049 – 0.44	24531000
6	*CFHR5*	C3 glomerulopathy	2/1,000,000	25%	4-16%	0.0099 – 0.95	33096866
7	*CLCN7*	Osteopetrosis	1/1,000,000-1/5,000,000	50-80%	75%	0 – 0.020	33105733 20301306
8	*DUSP6*	Normosmic hypogonadotropic hypogonadism; Kallmann syndrome	1/8,000 males 1/40,000 females	50%	1%	0– 0.0035	23643382 32389901
9	*ERCC6*	Primary ovarian insufficiency	1-3%	20-25%	0.5%	0 – 0.15	26218421
10	*GBA*	Adult-onset Parkinson disease	1-/1,000	10-15%	13.7%	0 – 0.022	17875915 28150045
11	IMPG2	Adult-onset macular dystrophy-5	0.05% before 50 12% after age 80	?	8%	0 – 0.022	25085631 25085631
12	*KCNJ11*	NIDDM	6.28%	20%-80%	26-64%	0 – 0.076	32175717 30169531 23961321
13	*MEF2A*	Coronary artery disease	1.72% Worldwide 5-10% USA	40% to 60%	1.93%	0 – 0.00091	32742886 15861005 17301730
14	*PKD1*	Autosomal dominant polycystic kidney disease	1/1,000	98%	78%	0 – 0.049	29326913 30135240
15	*PSEN1*	Early Onset Alzheimer disease	1-2/1,000	10-15%	20-70%	0 – 0.015	29599933 28350801 10441572
16	*RAD51C*	Breast and ovarian cancer	Breast:12% Ovarian: 1.5% Male breast: 0.1%	5-10%	0.23-0.45%	0 – 0.082	29719442 33406487
17	*SLC25A4*	Progressive external ophthalmoplegia; Mitochondrial DNA depletion syndrome 12A (cardiomyopathic type)	1.2/100,000	?	?	0 – 0.0055	20301382
18	*TTN*	Hereditary myopathy with early respiratory failure	?	?	5%	0 – 2.1	26701604
19	*TTR*	Neuropathy with cardiac amyloidosis	1/1,000,000	100%	8%	0 – 0.0029	32456532
20	*VWF*	Von Willebrand type 2	1/107,000	15-30%	60%	0 – 0.097	Obstet Gynecol 2013;122:1368–73 30279008

Table S6: The frequency of P/LP variants for the non-MAG genes in the general population is shown above. The prevalence of pathogenic variants in the general population was collected using the Varsome database.

Num	Gene	White	African/ African American	Latino	Asian	Other
1	*ALPL*	0.025	0.025	0.023	0.025	0
2	*ANXA11*	0.021	0.044	0.067	0.11	0.016
3	*ATM*	0.28	0.068	0.19	0.29	0.20
4	BEST1	0.020	0.0062	0.017	0.042	0.033
5	*C1QTNF5*	0.094	0.44	0.035	0.093	0.099
6	*CFHR5*	0.040	0.074	0.075	0.98	0.21
7	*CLCN7*	0.034	0.0062	0.012	.0098	0.016
8	*DUSP6*	0.002	0	0	0.0035	0
9	*ERCC6*	0.26	0.093	0.064	0.11	0.033
10	*GBA*	0.055	0	0.0058	0.042	0.016
11	IMPG2	0.037	0.025	0.020	0.10	0
12	*KCNJ11*	0	0	0	0	0
13	*MEF2A*	0.00091	0	0	0	0
14	*PKD1*	0.028	0.0062	0.012	0.060	0.017
15	*PSEN1*	0.019	0.0062	0.015	0.0054	0
16	*RAD51C*	0.089	0.031	0.012	0.16	0.065
17	*SLC25A4*	0.0044	0	0.0029	0.0055	0
18	*TTN*	1.3	2.2	0.63	1.2	0.59
19	*TTR*	0.0026	0	0.0029	0	0
20	*VWF*	0.22	0.093	0.038	0.36	0.16

Table S7: The percent prevalence of pathogenic variants in the general population shown as subgroups by race and ethnicity. This data was collected using the Varsome database. (Of note, the white population is a combined group of Ashkenazi Jewish, Finnish, and European non-Finnish from the Varsome data base). The Asian population was determined by combining east and south Asian subsets from the Varsome data base.

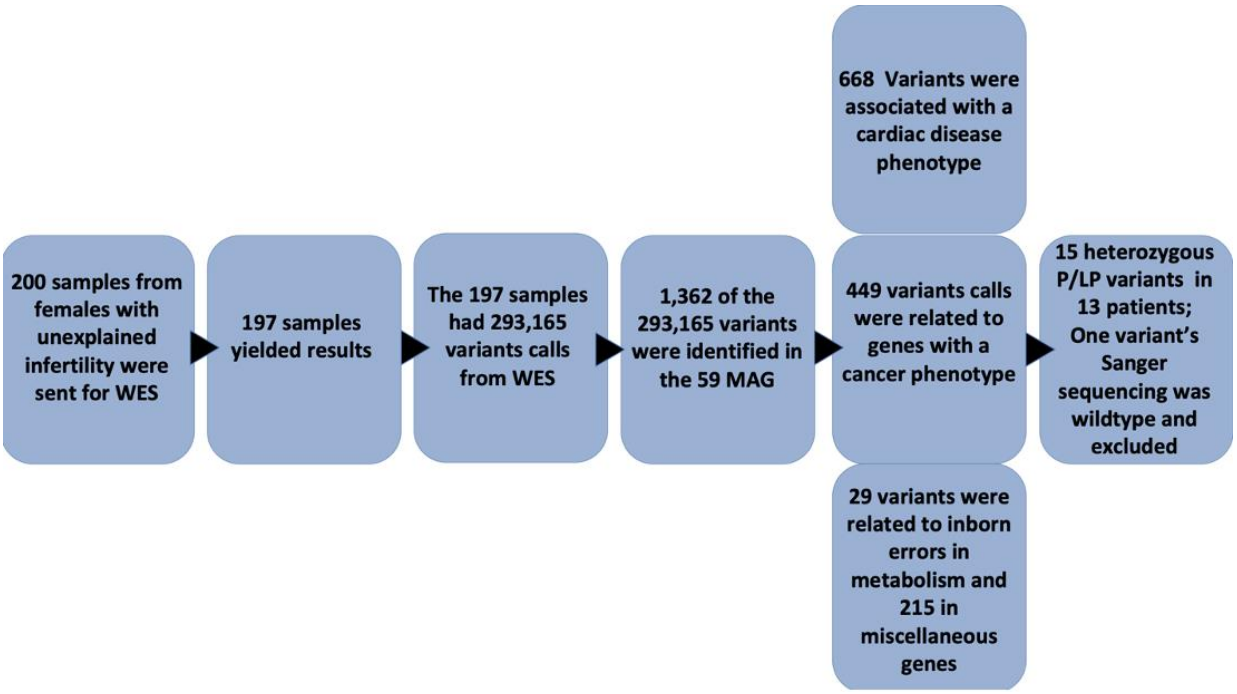

Figure S1. Filtering for P/LP variants in the 59 medically actionable genes

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