Genetic Screening of a Reportedly Healthy Population for Familial Hypercholesterolemia, Hereditary Breast and Ovarian cancer syndrome, and Lynch syndrome

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Introduction

The timely diagnosis of disease is essential for increasing positive health outcomes for patients and their families. Advances in next-generation sequencing have made genomic testing more affordable and have facilitated increased discussion around improving population health through genomic approaches. The identification of individuals and families who are unaware of their increased risk and carry pathogenic variants in disease-associated genes could significantly reduce morbidity and mortality. The CDC's Office of Public Health Genomics (OPHG) has noted that nearly 2 million people in the United States are at increased risk for adverse health outcomes due to genetic variants which predispose them to three disorders: hereditary breast and ovarian cancer syndrome (HBOC), Lynch syndrome (LS), or familial hypercholesterolemia (FH). The OPHG has determined that early detection in these individuals would have a significant positive impact on public health based on available evidence-based guidelines and recommendations.

Healthy Screening: the CDC Tier 1 Panel

Disorder	Genes Tested	Disease Risk
Hereditary Breast and Ovarian Cancer Syndrome (HBOC)	• *BRCA1* • *BRCA2*	increased risk for breast cancer, ovarian cancer (including fallopian tube and primary peritoneal cancers), prostate cancer, pancreatic cancer, and melanoma
Lynch syndrome (LS)	• *MLH1* • *MSH2* • *MSH6* • *PMS2* • *EPCAM* deletions	increased risk for colorectal cancer, and cancers of the endometrium, ovary, stomach, small bowel, urinary tract, biliary tract, brain (usually glioblastoma), skin (sebaceous adenomas, sebaceous carcinomas, and keratoacanthomas), pancreas, and prostate
Familial hypercholesterolemia (FH)	• *APOB* • *LDLR* • *LDLRAP1* • *PCSK9*	increased risk of premature cardiovascular events such as angina, myocardial infarction, and stroke due to high cholesterol levels

Tier 1 genomic applications are defined by the CDC's Office of Public Health Genomics (OPHG) as those having significant potential for a positive impact on public health based on available evidence-based guidelines and recommendations. (https://www.cdc.gov/)

For consideration

A Careful understanding of variant classification and the relationship of variants to mechanism of disease is essential in being able to return accurate and appropriate findings.

- For the APOB gene, only pathogenic autosomal dominant gain-of-function (GOF) variants are associated with FH.
- For the APOB gene, pathogenic autosomal recessive loss-of-function (LOF) variants are associated with hypobetalipoproteinemia.
- For the PCSK9 gene, only pathogenic autosomal dominant gain-of-function (GOF) variants are associated with FH.
- The LDLRAP1 gene is associated with autosomal recessive FH.

Variant classification for population studies requires increased stringency to account for unclear penetrance and expressivity.

- FH has the advantage of routine chemistry which can aid in the classification of variants. A careful

CONCLUSIONS

Of the 6,871 individuals tested, 131 individuals were found to have diagnostic findings for at least one of the three disorders tested (1.9%; ~1 in 52 individuals tested).

- The prevalence of a diagnostic CDC Tier 1 finding in this reportedly healthy population may be more common than expected.

Of the 131 individuals with positive results, 48 were associated with FH (36.6%), 58 were associated with HOBC (44.3%), and 28 were associated with LS (21.4%).

- Screening of presumably healthy populations facilitates diagnoses of disorders for which early detection results in improved health outcomes.

The genetic results for individuals with positive findings include diagnostic results in the following genes: 33 LDLR (25.2%), 13 APOB (9.9%), 2 LDLRAP1 (1.5%), 31 BRCA2 (23.6%), 27 BRCA1 (20.6%), 15 PMS2 (11.4%), 6 MSH6 (4.6%), 5 MSH2 (3.8%) and 2 MLH1 (1.5%).

- Genetic results help to inform of disease risk and response to treatment, leading to positive health outcomes for patients and their families.

Of the individuals tested, three individuals had results consistent with homozygous FH (two LDLR variants), one individual had results consistent with FH and LS (APOB + PMS2), one individual had results consistent with FH and HBOC (LDLR + BRCA1), and one individual with two variants in HBOC genes (BRCA1 + BRCA2), and one individual had biallelic loss-of-function variants in the APOB gene, consistent with APOB-related familial hypobetalipoproteinemia.

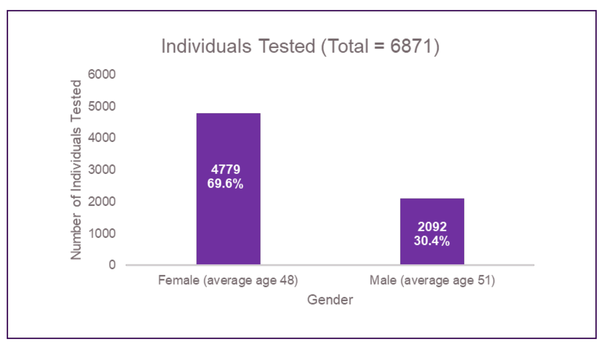
The benefits associated with a genetic diagnosis illustrate the need for increased genetic screening of the general population for these disorders.

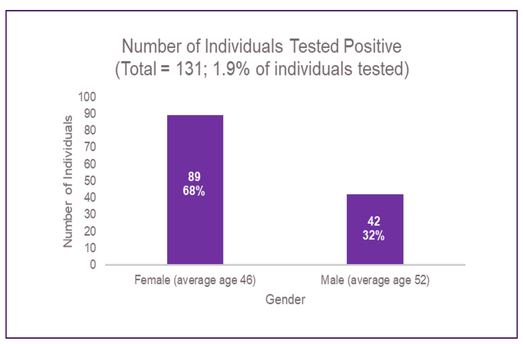
Methods

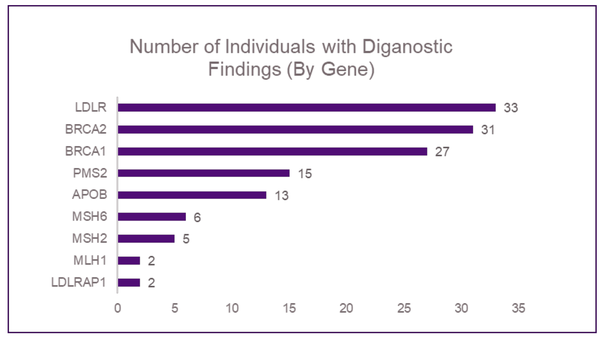
Next-generation panel sequencing was performed on 6,871 reportedly healthy adults for the three disorders covered by the CDC's OPHG recommendations for genetic screening (HBOC, LS, and FH). Genomic screening of these disorders encompasses the following 11 genes: *APOB, LDLR, PCSK9, LDLRAP1, BRCA1, BRCA2, EPCAM, MLH1, MSH2, MSH6,* and *PMS2*.

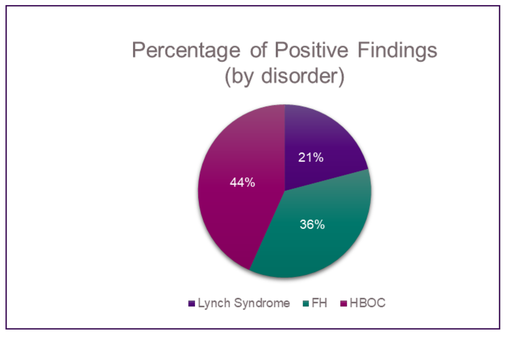
The identification of variants of uncertain significance in individuals with no personal history of disease is not clinically useful. Given the lack of clinical utility and literature which suggests that variants of uncertain significance should not be returned in individuals with no evidence of a phenotype, our laboratory has implemented a policy of returning only pathogenic and likely pathogenic variants for panel testing of healthy individuals; variants of uncertain significance are not returned.

RESULTS









Detailed Positive Findings

Individual	Gene	DNA Change	Protein Change	Zygosity	Classification
1	APOB	c.10238del	-	Heterozygous	Pathogenic
7	APOB	c.10238del	-	Homozygous	Pathogenic
2	APOB	c.10579C>T	p.Arg3527Trp	Heterozygous	Pathogenic
6	APOB	c.10579C>T	p.Arg3527Trp	Homozygous	Pathogenic
82	APOB	c.10579C>T	p.Arg3527Trp	Heterozygous	Pathogenic
15	APOB	c.10580G>A	p.Arg3527Gln	Heterozygous	Pathogenic
27	APOB	c.10580G>A	p.Arg3527Gln	Heterozygous	Pathogenic
30	APOB	c.10580G>A	p.Arg3527Gln	Heterozygous	Pathogenic
86	APOB	c.10580G>A	p.Arg3527Gln	Heterozygous	Pathogenic
97	APOB	c.10580G>A	p.Arg3527Gln	Heterozygous	Pathogenic
106	APOB	c.10580G>A	p.Arg3527Gln	Heterozygous	Pathogenic
114	APOB	c.10580G>A	p.Arg3527Gln	Heterozygous	Pathogenic
122	APOB	c.10580G>A	p.Arg3527Gln	Heterozygous	Pathogenic
87	LDLR	c.1103G>A	p.Cys368Tyr	Heterozygous	Pathogenic
34	LDLR	c.1247G>A	p.Arg416Gln	Heterozygous	Likely Pathogenic
66	LDLR	c.1359-1G>A	-	Heterozygous	Pathogenic
38	LDLR	c.1414G>T	p.Asp472Tyr	Heterozygous	Likely Pathogenic
39	LDLR	c.1444G>A	p.Asp482Asn	Heterozygous	Pathogenic
131	LDLR	c.1444G>A	p.Asp482Asn	Heterozygous	Pathogenic
24	LDLR	c.1567G>A	p.Val523Met	Heterozygous	Pathogenic
73	LDLR	c.1690A>C	p.Asn564His	Heterozygous	Likely Pathogenic
93	LDLR	c.1691A>G	p.Asn5645er	Heterozygous	Likely Pathogenic
126	LDLR	c.172G>T	p.Glu58Ter	Heterozygous	Likely Pathogenic
58	LDLR	c.1747C>T	p.His583Tyr	Heterozygous	Pathogenic
72	LDLR	c.1747C>T	p.His583Tyr	Heterozygous	Pathogenic
123	LDLR	c.1747C>T	p.His583Tyr	Heterozygous	Pathogenic
26	LDLR	c.1897C>T	p.Arg633Cys	Heterozygous	Pathogenic
70	LDLR	c.1898G>A	p.Arg633His	Heterozygous	Likely Pathogenic
88	LDLR	c.2054C>T	p.Pro685Leu	Heterozygous	Pathogenic
73	LDLR	c.2397_2405del	p.Val800_Leu802del	Heterozygous	Likely Pathogenic
19	LDLR	c.241C>T	p.Arg81Cys	Heterozygous	Likely Pathogenic
65	LDLR	c.241C>T	p.Arg81Cys	Heterozygous	Likely Pathogenic
74	LDLR	c.313+1G>A	-	Heterozygous	Pathogenic
67	LDLR	c.337G>A	p.Glu113Lys	Heterozygous	Likely Pathogenic
59	LDLR	c.631C>T	p.His211Tyr	Heterozygous	Pathogenic
12	LDLR	c.662A>G	p.Asp221Gly	Heterozygous	Pathogenic
117	LDLR	c.662A>G	p.Asp221Gly	Heterozygous	Pathogenic
124	LDLR	c.680_686delinsCGGTATACC	-	Heterozygous	Likely Pathogenic
111	LDLR	c.681C>A	p.Asp227Glu	Heterozygous	Pathogenic
81	LDLR	c.6del	-	Heterozygous	Pathogenic
107	LDLR	c.718G>A	p.Glu240Lys	Heterozygous	Likely Pathogenic
4	LDLR	c.798T>A	p.Asp266Glu	Homozygous	Pathogenic
33	LDLR	c.862G>A	p.Glu288Lys	Heterozygous	Likely Pathogenic
92	LDLR	c.862G>A	p.Glu288Lys	Heterozygous	Pathogenic
120	LDLR	c.862G>A	p.Glu288Lys	Heterozygous	Pathogenic
28	LDLR	c.917C>T	p.Ser306Leu	Heterozygous	Pathogenic
23	LDLR	c.97C>T	p.Gln33Ter	Heterozygous	Pathogenic
3	LDLRAP1	c.406C>T	p.Gln136Ter	Heterozygous	Pathogenic
5	LDLRAP1	c.406C>T	p.Gln136Ter	Homozygous	Pathogenic

Individual	Gene	DNA Change	Protein Change	Zygosity	Classification
14	BRCA1	c.135-1G>T	-	Heterozygous	Pathogenic
100	BRCA1	c.1687C>T	p.Gln563Ter	Heterozygous	Pathogenic
42	BRCA1	c.181T>G	p.Cys61Gly	Heterozygous	Pathogenic
64	BRCA1	c.181T>G	p.Cys61Gly	Heterozygous	Pathogenic
96	BRCA1	c.181T>G	p.Cys61Gly	Heterozygous	Pathogenic
53	BRCA1	c.211A>G	p.Arg71Gly	Heterozygous	Pathogenic
85	BRCA1	c.211A>G	p.Arg71Gly	Heterozygous	Pathogenic
50	BRCA1	c.2199del	-	Heterozygous	Likely Pathogenic
89	BRCA1	c.2457del	-	Heterozygous	Pathogenic
40	BRCA1	c.2679_2682del	-	Heterozygous	Pathogenic
112	BRCA1	c.3400G>T	p.Glu1134Ter	Heterozygous	Pathogenic
126	BRCA1	c.3619A>T	p.Lys1207Ter	Heterozygous	Likely Pathogenic
95	BRCA1	c.4035del	-	Heterozygous	Pathogenic
13	BRCA1	c.5080G>T	p.Glu1694Ter	Heterozygous	Pathogenic
79	BRCA1	c.5095C>T	p.Arg1699Trp	Heterozygous	Pathogenic
47	BRCA1	c.5123C>A	p.Ala1708Glu	Heterozygous	Pathogenic
84	BRCA1	c.5266dup	-	Heterozygous	Pathogenic
9	BRCA1	c.5332+1G>C	-	Heterozygous	Pathogenic
102	BRCA1	c.5368del	-	Heterozygous	Likely Pathogenic
80	BRCA1	c.5434C>G	p.Pro1812Ala	Heterozygous	Pathogenic
127	BRCA1	c.5558dup	-	Heterozygous	Pathogenic
46	BRCA1	c.68_69del	-	Heterozygous	Pathogenic
51	BRCA1	c.68_69del	-	Heterozygous	Pathogenic
56	BRCA1	c.68_69del	-	Heterozygous	Pathogenic
57	BRCA1	c.68_69del	-	Heterozygous	Pathogenic
69	BRCA1	c.68_69del	-	Heterozygous	Pathogenic
83	BRCA1	c.798_799del	-	Heterozygous	Pathogenic
21	BRCA2	c.1238del	-	Heterozygous	Pathogenic
60	BRCA2	c.2507del	-	Heterozygous	Likely Pathogenic
116	BRCA2	c.2507del	-	Heterozygous	Likely Pathogenic
20	BRCA2	c.3103G>T	p.Glu1035Ter	Heterozygous	Pathogenic
103	BRCA2	c.3264dup	-	Heterozygous	Pathogenic
105	BRCA2	c.3264dup	-	Heterozygous	Pathogenic
110	BRCA2	c.3264dup	-	Heterozygous	Pathogenic
35	BRCA2	c.3847_3848del	-	Heterozygous	Pathogenic
52	BRCA2	c.3847_3848del	-	Heterozygous	Pathogenic
54	BRCA2	c.3847_3848del	-	Heterozygous	Pathogenic
121	BRCA2	c.3847_3848del	-	Heterozygous	Pathogenic
48	BRCA2	c.4284dupT	p.Gln1429fs	Heterozygous	Pathogenic
37	BRCA2	c.4398_4402del	-	Heterozygous	Pathogenic
11	BRCA2	c.4631delA	p.Asn1544fs	Heterozygous	Pathogenic
130	BRCA2	c.5073dup	-	Heterozygous	Pathogenic
104	BRCA2	c.5215del	-	Heterozygous	Likely Pathogenic
41	BRCA2	c.5238dup	p.Asn1747Ter	Heterozygous	Pathogenic
109	BRCA2	c.5828del	-	Heterozygous	Pathogenic
43	BRCA2	c.5864C>A	p.Ser1955Ter	Heterozygous	Pathogenic
49	BRCA2	c.5946del	-	Heterozygous	Pathogenic
75	BRCA2	c.658_659del	-	Heterozygous	Pathogenic
77	BRCA2	c.658_659del	-	Heterozygous	Pathogenic
100	BRCA2	c.658_659del	-	Heterozygous	Pathogenic
119	BRCA2	c.6757_6758del	-	Heterozygous	Pathogenic
25	BRCA2	c.6938-1G>C	-	Heterozygous	Likely Pathogenic
76	BRCA2	c.7069_7070del	-	Heterozygous	Pathogenic
31	BRCA2	c.7558C>T	p.Arg2520Ter	Heterozygous	Pathogenic
44	BRCA2	c.7618-1G>A	-	Heterozygous	Pathogenic
101	BRCA2	c.7618-1G>A	-	Heterozygous	Pathogenic
108	BRCA2	c.7618-1G>A	-	Heterozygous	Pathogenic
55	BRCA2	c.8904del	-	Heterozygous	Pathogenic

Individual	Gene	DNA Change	Protein Change	Zygosity	Classification
113	MLH1	c.350C>T	p.Thr117Met	Heterozygous	Pathogenic
63	MLH1	c.676C>T	p.Arg226Ter	Heterozygous	Pathogenic
118	MSH2	c.1566C>A	p.Tyr522Ter	Heterozygous	Likely Pathogenic
8	MSH2	c.1915C>T	p.His639Tyr	Heterozygous	Pathogenic
29	MSH2	c.2131C>T	p.Arg711Ter	Heterozygous	Pathogenic
78	MSH2	c.901A>T	p.Lys301Ter	Heterozygous	Likely Pathogenic
91	MSH2	c.998G>A	p.Cys333Tyr	Heterozygous	Pathogenic
125	MSH6	c.1115G>A	p.Trp372Ter	Heterozygous	Pathogenic
62	MSH6	c.1346T>C	p.Leu449Pro	Heterozygous	Pathogenic
128	MSH6	c.2504del	-	Heterozygous	Pathogenic
22	MSH6	c.3261del	-	Heterozygous	Pathogenic
71	MSH6	c.3867_3870dup	-	Heterozygous	Likely Pathogenic
10	MSH6	c.4001G>A	p.Arg1334Gln	Heterozygous	Pathogenic
45	PMS2	c.137G>T	p.Ser46Ile	Heterozygous	Pathogenic
94	PMS2	c.137G>T	p.Ser46Ile	Heterozygous	Pathogenic
98	PMS2	c.137G>T	p.Ser46Ile	Heterozygous	Pathogenic
99	PMS2	c.137G>T	p.Ser46Ile	Heterozygous	Pathogenic
129	PMS2	c.137G>T	p.Ser46Ile	Heterozygous	Pathogenic
17	PMS2	c.1A>T	p.Met1?	Heterozygous	Likely Pathogenic
18	PMS2	c.247_250dup	-	Heterozygous	Pathogenic
90	PMS2	c.400C>T	p.Arg134Ter	Heterozygous	Pathogenic
16	PMS2	c.706-2A>G	-	Heterozygous	Likely Pathogenic
68	PMS2	c.736_741delCCCCCTinsTGTGTGTGAAG	-	Heterozygous	Pathogenic
32	PMS2	c.736_741delinsTGTGTGTGAAG	-	Heterozygous	Pathogenic
61	PMS2	c.736_741delinsTGTGTGTGAAG	-	Heterozygous	Pathogenic
115	PMS2	c.736_741delinsTGTGTGTGAAG	-	Heterozygous	Pathogenic
30	PMS2	c.88C>T	p.Gln30Ter	Heterozygous	Pathogenic
36	PMS2	c.989-1G>T	-	Heterozygous	Pathogenic

Special Notes:

Individual 30: pathogenic variants in both *APOB* and *PMS2*
Individual 100: pathogenic variants in both *BRCA1* and *BRCA2*
Individual 126: likely pathogenic variants in both *BRCA1* and *LDLR*
Individual 4: homozygous pathogenic *LDLR* variant consistent with HoFH
Individual 6: homozygous pathogenic *APOB* variant consistent with HoFH
Individual 73: compound heterozygous *LDLR* variants consistent with HoFH
Individual 5: homozygous *LDLRAP1* variant consistent with FH
Individual 7: homozygous LoF variant in *APOB* consistent with hypobetalipoproteinemia