

Running title: High Frequency Actionable Exome Findings

## High Frequency Actionable Pathogenic Exome Mutations in an Average-Risk Cohort

Shannon Rego<sup>1,7</sup>, Orit Dagan-Rosenfeld<sup>1,7</sup>, Wenyu Zhou<sup>1</sup>, M. Reza Sailani<sup>1</sup>, Patricia Limcaoco<sup>1</sup>, Elizabeth Colbert<sup>3</sup>, Monika Avina<sup>1</sup>, Jessica Wheeler<sup>1</sup>, Colleen Craig<sup>3</sup>, Denis Salins<sup>1</sup>, Hannes L. Röst<sup>1</sup>, Jessilyn Dunn<sup>1,6</sup>, Tracey McLaughlin<sup>3</sup>, Lars M. Steinmetz<sup>1,4,5</sup>, Jonathan A. Bernstein<sup>2</sup>, Michael P. Snyder<sup>1,\*</sup>

1. Department of Genetics, Stanford University School of Medicine, Stanford, CA, 94305, USA
2. Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305, USA
3. Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
4. Stanford Genome Technology Center, Stanford University, Palo Alto, CA 94304, USA
5. European Molecular Biology Laboratory (EMBL), Genome Biology Unit, 69117 Heidelberg, Germany
6. Mobilize Center, Stanford University, Stanford, CA 94305 USA
7. These authors contributed equally to this work

\*Correspondence to: [mpsnyder@stanford.edu](mailto:mpsnyder@stanford.edu)

1    **Abstract**

2

3    Whole exome sequencing (WES) is increasingly utilized in both clinical and non-clinical  
4    settings, but little is known about the utility of WES in healthy individuals. In order to  
5    determine the frequency of both medically actionable and non-actionable but medically  
6    relevant exome findings in the general population we assessed the exomes of 70 participants  
7    who have been extensively characterized over the past several years as part of a longitudinal  
8    integrated multi-omics profiling study at Stanford University. We assessed exomes for rare  
9    likely pathogenic and pathogenic variants in genes associated with Mendelian disease in the  
10   Online Mendelian Inheritance in Man (OMIM) database. We used American College of  
11   Medical Genetics (ACMG) guidelines were used for the classification of rare sequence  
12   variants, and additionally we assessed pharmacogenetic variants. Twelve out of 70 (17%)  
13   participants had medically actionable findings in Mendelian disease genes, including 6 (9%)  
14   with mutations in genes not currently included in the ACMG's list of 59 actionable genes.  
15   This number is higher than that reported in previous studies and suggests added benefit from  
16   utilizing expanded gene lists and manual curation to assess actionable findings. A total of 60  
17   participants (89%) had non-actionable findings identified including 57 who were found to be  
18   mutation carriers for recessive diseases and 21 who have increased Alzheimer's disease risk  
19   due to heterozygous or homozygous *APOE* e4 alleles (18 participants had both). These results  
20   suggest that exome sequencing may have considerably more utility for health management in  
21   the general population than previously thought.

22

23

24    **Introduction**

25

26    Whole genome sequencing (WGS) and exome sequencing (WES) play increasingly important  
27    roles in providing molecular diagnoses for Mendelian disease (Manolio et al. 2013), as well  
28    as identifying potential driver mutations in patients with cancer. However, our understanding

1 of the extent to which WGS and WES can benefit healthy individuals is limited. While a few  
2 previous studies have attempted to elucidate the utility of WGS in healthy cohorts or  
3 individuals (Chen et al. 2012; Xue et al. 2012; Gonzalez-Garay et al. 2013; Dewey et al.  
4 2014; Dewey et al. 2015), more have identified “incidental” or “secondary” findings in  
5 disease cohorts—often cohorts with known or suspected genetic disease (Dorschner et al.  
6 2013; Lawrence et al. 2014; Tabor et al. 2014; Amendola et al. 2015; Jang et al. 2015;  
7 Jurgens et al. 2015). These studies have reached a wide range of conclusions regarding the  
8 rate at which Mendelian disease-causing mutations are identified, due in large part to  
9 significant differences in their approaches to variant filtering and curation and the use of gene  
10 lists to limit potential findings.

11

12 In 2015 the American College of Medical Genetics and Genomics (ACMG) published  
13 guidelines to standardize the classification of genomic sequence variants (Richards et al.  
14 2015). These guidelines reinforce the necessity of expert manual curation for accurate variant  
15 classification. However, manual curation is labor intensive and has been estimated to take  
16 nearly an hour per variant (Dewey et al. 2014). Most previous studies assessing medically  
17 relevant WGS and WES findings have classified variants using in-silico predictors or by  
18 matching variants against publicly available databases. However, avoiding the step of expert  
19 variant curation significantly impairs the ability to accurately classify variants, as in-silico  
20 predictors lack accuracy and current publicly available databases for human genomic variants  
21 contain variants that are incorrectly classified as disease-causing (Dewey et al. 2014);  
22 (Thusberg et al. 2011; Vail et al. 2015; Masica and Karchin 2016). Most previous studies also  
23 restricted their analyses by searching for variants in a limited list of genes. However,  
24 restricting the search for medically relevant variants to a targeted gene list—for example, the  
25 commonly used list of 59 genes compiled by the ACMG—limits findings to only a fraction of  
26 genes associated with Mendelian disease (Green et al. 2013; Kalia et al. 2017). Thus, studies  
27 that perform a comprehensive analysis of Mendelian risk in generally healthy individuals  
28 using ACMG guidelines have not been performed.

1

2 In this research study we examine the utility of WES for the general population by using  
3 established guidelines to perform an in-depth search for variants with potential medical  
4 significance in a group of 70 unrelated adult volunteers enrolled in a longitudinal wellness  
5 study. Our analysis included variants in all genes previously associated with Mendelian  
6 genetic diseases in the Online Mendelian Inheritance in Man (OMIM) database  
7 (<http://www.omim.org>) or on the list of 59 ACMG genes. In addition, we assessed  
8 pharmacogenetic variants. We found a number of medically relevant variants that lie in genes  
9 other than those on the ACMG list. These results were reported back to participants by a  
10 genetic counselor in accordance with their expressed preferences for the types of results they  
11 would like to receive.

12

13

## 14 **Results**

15

### 16 *Participant Demographics*

17 The exomes of 70 participants were analyzed. The participants were all generally healthy at  
18 the time of enrollment, with the exception of four diabetics, three of whom were previously  
19 diagnosed and being treated, and one with diabetes detected at the time of enrollment due to  
20 an HbA1c  $\geq 6.5\%$ . Twenty out of 70 participants (29%) were pre-diabetic (defined by a  
21 HbA1c between 5.7% and 6.5%), which is similar to the general population prevalence of  
22 pre-diabetes (National Diabetes Statistics Report 2014). Participant characteristics are  
23 summarized in Table 1. They represented a range of self-reported ethnic backgrounds,  
24 including 48 Caucasian, 8 Southeast Asian, 6 Indian, 5 African-American, and 3 Hispanic  
25 participants. Thirty-six participants were men and 34 were women. Their ages ranged from 34  
26 to 76 years old with a median age of 57. Fifty-five participants consented to make their  
27 sequences public—they are available at <http://ihmpdcc.org/resources/osdf.php>. These plus  
28 the remaining will be made available in dbGAP upon acceptance of the manuscript.

1

2 Table 1: Participant Demographics

	<b>Range (Median)</b>
<b>Age</b>	34-76 (57)
<b>Ethnicity</b>	<b>No. of Participants (% of cohort)</b>
Caucasian	48 (69%)
Southeast Asian	8 (11%)
Indian	6 (9%)
African-American	5 (7%)
Hispanic	3 (4%)
<b>Gender</b>	<b>No. of Participants (% of cohort)</b>
Male	36 (51%)
Female	34 (49%)

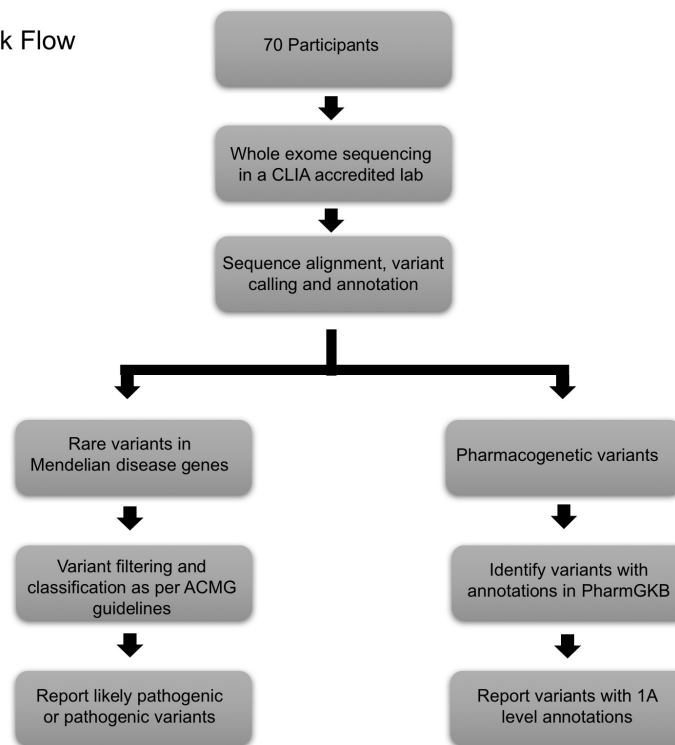
3

4

5 *Exome Results*

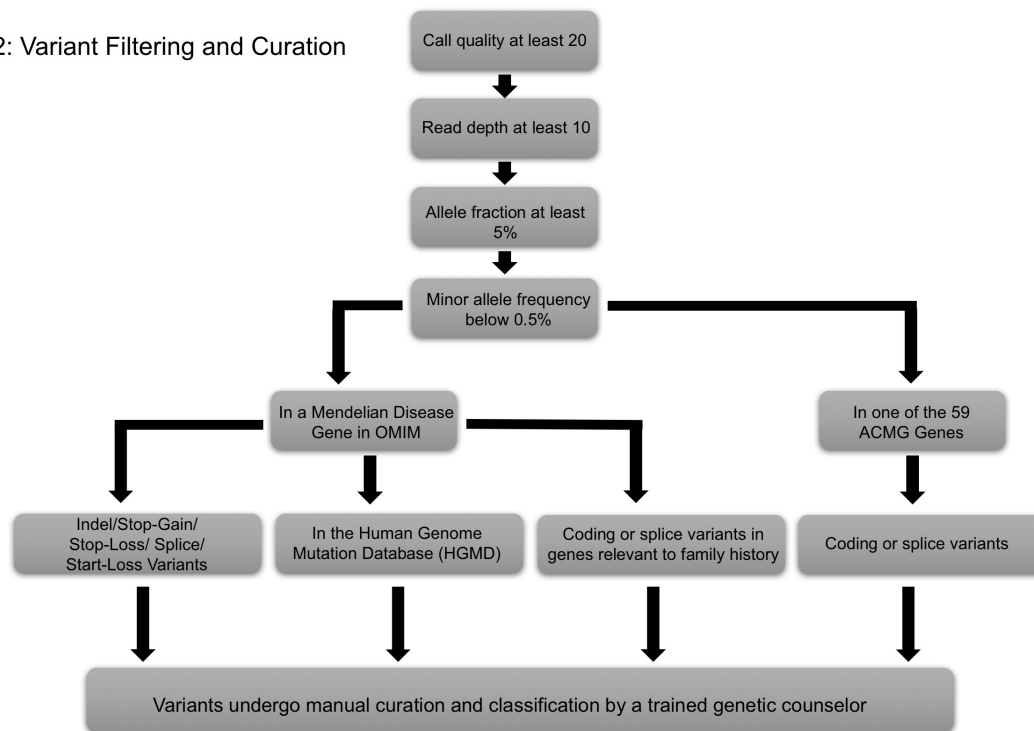
6 The gene coding regions were sequenced using an enhanced exome sequencing strategy that  
7 provides comprehensive coverage of coding regions as well as additional genomic regions of  
8 interest (Patwardhan et al. 2015) (see methods). See methods and Figure 1 for work flow. A  
9 range of 149,311 to 262,804 variants was called per exome. Following the filtering steps  
10 described in Figure 2, a total of 1,452 variants were reviewed and further filtered manually as  
11 described in methods. A total of 668 variants (an average of 9.5 per participant) underwent  
12 manual curation using ACMG guidelines (Richards et al. 2015). Of these, 48 variants were  
13 classified as pathogenic and 96 as likely pathogenic. The remainder were classified as  
14 variants of unknown significance (VUS), likely benign, or benign. The details of variant  
15 classification are presented in Table 2.

Figure 1: Work Flow



1  
2  
3

Figure 2: Variant Filtering and Curation



4  
5  
6

1 Table 2: Variant Classifications

Variant Call	Number of Variants (average per participant)
Pathogenic	48 (0.7)
Likely Pathogenic	96 (1.4)
Variant of Unknown Significance (VUS)	445 (6.6)
Likely Benign	66 (0.9)
Benign	13 (0.2)
Reviewed and not classified <sup>a</sup>	784 (11.2)

2

3 <sup>a</sup>Variants were not classified if viewing the aligned reads suggested the variant was an artifact, if variants in that  
4 gene are expected to cause serious, highly penetrant disease at a young age and which the participant did not have  
5 the associated phenotype (variants were only removed when the patient had a genotype that would be expected to  
6 cause disease were the variant pathogenic—i.e. homozygous for a recessive disease or heterozygous for a  
7 dominant disease), or when they were observed in more than 0.5% of a subpopulation in the Exac or 1000  
8 Genomes databases but passed the upstream MAF filter because the overall population MAF was lower than 0.5%

9

10 As expected, the vast majority of likely pathogenic and pathogenic variants identified in the  
11 cohort were located in genes associated with autosomal recessive diseases, and participants  
12 were therefore considered mutation carriers who, in most cases, were unlikely to manifest  
13 symptoms. However, actionable pathogenic or likely pathogenic variants were identified 12  
14 participants (see Figure 3). These variants were primarily in genes associated with autosomal  
15 dominant disease, although one pathogenic variant was in *MUTYH* (MIM: 604933)—a gene  
16 which is associated with autosomal recessive *MUTYH*-associated polyposis (MIM:  
17 608456)—but for which carriers are known to be at increased lifetime colon cancer risk  
18 (5.6% for female heterozygotes and 7.2% for male heterozygotes by age 70; higher for  
19 patients with a first degree relative with colon cancer) (Win et al. 2014). Due to this increased  
20 risk, the National Comprehensive Cancer Network (NCCN) has issued screening guidelines  
21 for heterozygous mutation carriers (National Comprehensive Cancer Network 2016).  
22 Therefore, we considered this variant actionable. The actionable variants lie in 10 distinct  
23 genes (Table 3) and include five variants classified as pathogenic with strong evidence  
24 suggestive of a causative role in disease as per ACMG classification guidelines; five  
25 classified as likely pathogenic; and one variant that was identified in two individuals was  
26 classified as a risk allele. The risk allele—in the *APC* gene (MIM: 611731)—is a well-studied  
27 founder mutation in the Ashkenazi Jewish population that the NCCN has described as a

1 moderate risk allele for colon cancer, and has issued screening guidelines for heterozygous  
2 carriers of this mutation (Boursi et al. 2013; Liang et al. 2013; National Comprehensive  
3 Cancer Network 2016). In total, 12 of the 70 individuals in the cohort (17%) had medically  
4 actionable likely pathogenic or pathogenic variants identified (see Table 3 for the complete  
5 list of actionable variants). Of the 12 variants, six reside in the 59 genes reported as actionable  
6 in the most recent ACMG guidelines regarding incidental findings (Green et al. 2013; Kalia et  
7 al. 2017). These include heterozygotes for likely pathogenic and pathogenic mutations in the  
8 highly penetrant cancer risk genes *BRCA1* (MIM:113705), which is associated with  
9 hereditary breast and ovarian cancer (MIM: 604370) and *SDHB* (MIM: 185470), which is  
10 associated with hereditary paraganglioma and pheochromocytoma (MIMs: 115310, 171300).  
11 The remaining six variants reside in genes that are not included in the ACMG guidelines but  
12 that are associated with medically actionable disease as defined in the methods.

13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29



1 Table 3: Medically Actionable Exome Findings

	Gene	Inheritance	Genbank Transcript	Mutation	Variant Call/Associated Disease	ACMG Evidence (Richards et al. 2015)
Genes on the ACMG List <sup>a</sup>	<i>SDHB</i>	Dominant	NM_003000	c.72_73insA; p.Q25fs	Likely pathogenic for hereditary paraganglioma and pheochromocytoma	PSV1 PM2
	<i>SDHB</i>	Dominant	NM_003000	c.137G>A; p.R46Q	Likely pathogenic for hereditary paraganglioma and pheochromocytoma	PS3 (Kim et al. 2015; Saxena et al. 2016) PS4 (Gimenez-Roqueplo et al. 2002; Benn et al. 2003; Kimura et al. 2014) <sup>d</sup> PP3 PP5
	<i>APC</i> (2)	Dominant	NM_001127510	c.3920T>A; p.I1307K <sup>c</sup>	Risk allele for colon cancer	PS3 (Laken et al. 1997) PS4 (Frayling et al. 1998; Woodage et al. 1998; Stern et al. 2001; Boursi et al. 2013)
	<i>BRCA1</i>	Dominant	NM_007294	c.4689C>G; p.Y1563*	Pathogenic for hereditary breast and ovarian cancer	PSV1 PS4 (Shih et al. 2002; Turkovic et al. 2010; Pern et al. 2012) <sup>d</sup> PM2
	<i>MUTYH</i>	Recessive <sup>b</sup>	NM_012222	c.724C>T; p.R242C	Pathogenic for familial adenomatous polyposis (increased colon cancer risk in carriers)	PS3 (Ruggieri et al. 2013; Komine et al. 2015) PS4 (Miyaki et al. 2005; Olschwang et al. 2007; Ruggieri et al. 2013) <sup>d</sup> PM2 PM3 (Ruggieri et al. 2013)
	Genes not on the ACMG List <sup>a</sup>	<i>ABCC8</i>	Dominant or Recessive	NM_000352	c.1562G>A; p.R521Q	Likely pathogenic for AD hyperinsulinemia
<i>HNFI1A</i>		Dominant	NM_000545	c.476G>A; p.R159Q	Pathogenic for maturity-onset diabetes of the young (MODY)	PS3 (Tonooka et al. 2002) PS4 (Vaxillaire et al. 1997; Yamada et al. 1999; Bazalova et al. 2010) PM2 PP1(Costa et al. 2000) PP3
<i>PROS1</i>		Dominant	NM_000313	c.586A>G; p.K196E	Pathogenic for protein S deficiency	PS3 (Hayashi et al. 1994; Banno et al. 2015) PS4 (Hayashi et al. 1994; Kimura et al. 2006; Miyata et al. 2009; Ikejiri et al. 2010; Hayakawa et al. 2011; Neki et al. 2011; Huang et al. 2016) PP5
<i>CHEK2</i>		Dominant	NM_001005735	c.1281dupA; p.E500fs	Likely pathogenic for <i>CHEK2</i> -related cancers, including breast cancer	PSV1 PM2
<i>RBM20</i>		Dominant	NM_001134363	c.1898C>T; p.P633L	Likely pathogenic for dilated cardiomyopathy	PM1 (Li et al. 2010) PM2 PP2 PP3
<i>SLC7A9</i>		Dominant or Recessive	NM_014270	c.544G>A; A182T	Pathogenic for AD cystinuria	PS3 (Font et al. 2001) PS4 (Font et al. 2001; Halbritter et al. 2015)

2 <sup>a</sup>The American College of Medical Genetics and Genomics (ACMG) created a gene list to guide the return of  
3 incidental findings for patients undergoing WES/WGS (Green et al. 2013; Kalia et al. 2017).

1 <sup>b</sup>The *MUTYH* gene is included on the ACMG gene list, however, as per ACMG guidelines only compound  
2 heterozygous or homozygous mutations in this gene should be reported as incidental findings (Green et al. 2013).  
3 Heterozygotes for *MUTYH* mutations are, however, at increased risk for developing colon cancer, and the National  
4 Comprehensive Cancer Network (NCCN) recommends increased screening for mutation carriers (Win et al. 2014;  
5 National Comprehensive Cancer Network 2016).

6 <sup>c</sup>This *APC* mutation does not cause traditional familial adenomatous polyposis, but rather, has been shown to  
7 increase risk for colon cancer, and the NCCN recommends increased surveillance for carriers of this specific  
8 mutation (Boursi et al. 2013; Liang et al. 2013; National Comprehensive Cancer Network 2016).

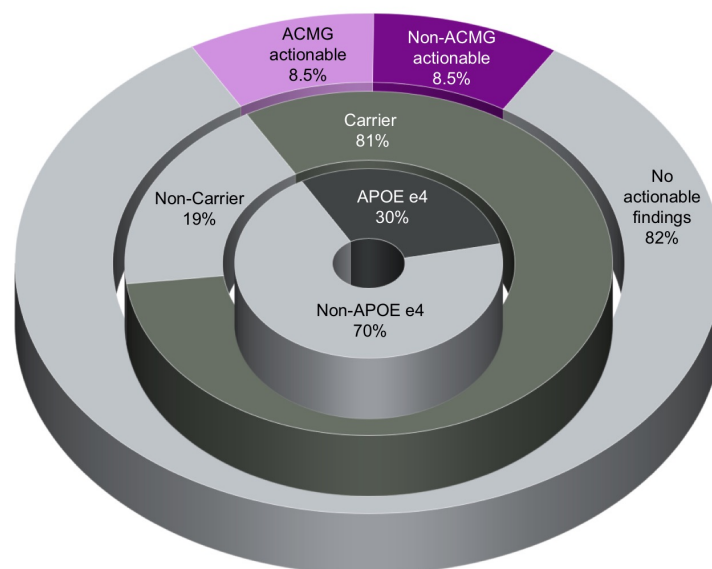
9 <sup>d</sup>This evidence was used as “moderate” level evidence as per ACMG guidelines.

10 (2) indicates that two participants were heterozygous for this mutation.

11 All variants in table 3 were found in heterozygous state.

12

Figure 3: Actionable and non-actionable exome findings



- Heterozygous for a likely pathogenic or pathogenic actionable variant from the ACMG list
- Heterozygous for a likely pathogenic or pathogenic actionable variant not on the ACMG list
- Heterozygous carriers of likely pathogenic or pathogenic variants in genes implicated in recessive disease
- Heterozygous or homozygous for the APOE e4 allele

13

14

### 15 *Patients with Personal or Family Medical History Consistent with their Mutation*

16 At least two individuals have personal or family medical histories consistent with the  
17 presence of their mutations. A 46-year-old female with elevated glucose and family history of  
18 diabetes was found to be heterozygous for a likely pathogenic *HNF1A* (MIM: 142410)  
19 mutation. *HNF1A* mutations cause autosomal dominant maturity onset diabetes of the young

1 (MODY [MIM: 600496]), a form of monogenic diabetes that is often misdiagnosed as type 1  
2 or type 2 diabetes, as was the case in this participant, who was incorrectly diagnosed with  
3 type 2 diabetes in her early 30s. Compared to type 2 diabetes, diabetes caused by *HNF1A*  
4 mutations is considerably more responsive to sulphonylurea drugs. Early recognition of  
5 *HNF1A*-MODY and subsequent initiation of sulphonylurea therapy may reduce the incidence  
6 of diabetic complications (Shepherd et al. 2009; Bacon et al. 2016). The participant, who was  
7 currently managing her diabetes with a combination of three non-sulphonylurea oral  
8 medications, was referred to endocrinology to discuss potential changes to her treatment plan,  
9 and her three children also underwent genetic testing for the mutation to inform diabetes  
10 screening regimens.

11

12 Another participant with a family history of dilated cardiomyopathy (DCM [MIM: 613172])  
13 was identified to be heterozygous for a likely pathogenic *RBM20* (MIM: 613171) variant. The  
14 variant has not been previously reported as associated with DCM but was prioritized for  
15 curation as a result of the participant's family history. The variant is in a hotspot for DCM-  
16 associated mutations located in the RS domain of *RBM20* and is located in a codon adjacent  
17 to a series of five codons previously reported in DCM cases (Li et al. 2010). Due to family  
18 history, as well as low-normal ejection fraction on echocardiogram revealed due to follow up  
19 subsequent to genomic analysis, the participant began taking blood pressure lowering  
20 medications as a preventative measure. The participant was referred to cardiovascular  
21 genetics clinic for follow up.

22

### 23 *Non-medically actionable findings*

24 A total of 60 participants (89% of the cohort) were identified to have non-actionable findings  
25 (including carriers for recessive conditions and/or *APOE* e4 allele carriers—see Figure 3). In  
26 57 participants we identified 133 likely pathogenic and pathogenic variants in genes that  
27 cause autosomal recessive diseases (see supplemental table 1 for a complete list of likely  
28 pathogenic and pathogenic variants). Most of these variants convey no health risks to carriers

1 beyond reproductive risks, but there are exceptions. In addition to the *MUTYH* mutation  
2 discussed earlier, pathogenic heterozygous *GBA* (MIM: 606463) mutations were identified in  
3 three participants. *GBA* mutations cause autosomal recessive Gaucher disease (MIMs:  
4 608013, 230800, 230900, 231000, 231005), but like individuals affected with Gaucher  
5 disease, heterozygous mutation carriers are also at significantly increased risk for Parkinson's  
6 disease (MIM: 168600)(Tayebi et al. 2003; Halperin et al. 2006; Alcalay et al. 2014). In  
7 addition, 21 participants were identified to be heterozygous or homozygous for the *APOE*  
8 (MIM: 107741) e4 allele, which is associated with significantly increased lifetime risk for  
9 developing Alzheimer's disease (MIM: 104310) (Corder et al. 1993; Bertram et al. 2010).  
10 The *APOE* genotype was only disclosed in two cases where participants specifically inquired  
11 about it and had opted to receive both actionable and non-actionable findings on their consent  
12 form.

13

#### 14 *Pharmacogenetic variants*

15 In addition to disease causing variants we also assessed participant exomes for variants  
16 impacting response to drugs. Level 1A variants in PharmGKB (<https://www.pharmgkb.org>)  
17 have high confidence for affecting drug dose and/or side effects. The 70 exomes were  
18 examined for 28 rsIDs with level 1A classifications (extracted from pharmgkb.org in May  
19 2017) (see supplemental table 2 for a list of rsIDs). A range of 1 to 6 level 1A variants were  
20 identified per participant, with a median of 3 variants. Well-known examples include several  
21 variants in *CYP2C19* that are associated with altered metabolism or risk of side effects for  
22 drugs such as Clopidogrel and Amitriptyline, including rs9923231 and rs4244285 (Whirl-  
23 Carrillo et al. 2012). Thus, overall, the majority of our participants received potentially useful  
24 pharmacogenetic information.

25

26

#### 27 **Discussion**

28 Previous studies have found that approximately 1-5% of individuals will have actionable

1 incidental or secondary findings identified on exome sequencing (Johnston et al. 2012;  
2 Dorschner et al. 2013; Lawrence et al. 2014; Amendola et al. 2015; Jurgens et al. 2015). The  
3 substantially higher rate of actionable findings found in our cohort (17%) suggests that  
4 employing manual variant curation and expanded gene lists may enhance the accurate  
5 identification of actionable variants in exomes—information that can ultimately lead to  
6 improved treatment, screening, or prevention. Our study also highlights the fact that many  
7 individuals can receive useful risk information from genome and or exome sequencing.

8

### 9 *The ACMG List and Beyond*

10 A number of previous studies of the utility of WGS or WES in healthy individuals have  
11 limited their search for clinically relevant findings to the list of 56 genes first compiled by the  
12 ACMG in 2013 and later revised to include 59 genes in 2016 (Green et al. 2013; Kalia et al.  
13 2017). It is recommended that incidental findings from genes on this list be returned to  
14 patients regardless of the indication for WGS/WES, as pathogenic/likely pathogenic variants  
15 in these highly penetrant genes lead to high risk for serious preventable and/or treatable  
16 diseases. The wide range of percentages (1-5%) of actionable findings reported in previous  
17 studies may in part be explained by the lack of widely accepted standardized guidelines for  
18 interpretation of genomic variants prior to 2015 (Richards et al. 2015). Although variants in  
19 the 59 ACMG genes are actionable and therefore clinically relevant, they represent only a  
20 fraction of genes known to cause Mendelian disease in humans.

21

22 Among the criticisms of the ACMG list is the lack of agreement among experts on the  
23 ACMG panel that compiled the list over which findings should be reported, as well as the  
24 existence of numerous other genes in which mutations can cause highly penetrant and  
25 treatable or preventable disease (Burke et al. 2013; Holtzman 2013). Our findings, which  
26 include several medically actionable pathogenic or likely pathogenic variants in genes not  
27 included in the latest version of the ACMG list, support this concern. In addition to mutations  
28 in genes on the ACMG list we identified pathogenic or likely pathogenic variants in six other

1 genes not on the ACMG list that have medical relevance, including two (*HNFI1A* and *RBM20*)  
2 in which participants had personal and/or family medical history consistent with  
3 pathogenicity.

4  
5 In addition to *HNFI1A* and *RBM20* mutations, we identified mutations in other noteworthy  
6 genes. As previously noted, one participant was found to carry a likely pathogenic *MUTYH*  
7 mutation. Current ACMG guidelines for the reporting of incidental findings recommend only  
8 reporting compound heterozygote or homozygous mutations in *MUTYH*, as *MUTYH*-  
9 associated polyposis is considered a recessive disease (Green et al. 2013; Kalia et al. 2017).  
10 However, heterozygote carriers of *MUTYH* mutations are at an increased risk of colon cancer  
11 and the National Comprehensive Cancer Network (NCCN) has recommended carriers  
12 undergo more frequent colonoscopies starting at an earlier age than the general population  
13 recommendations (National Comprehensive Cancer Network 2016). In one male participant  
14 we identified a pathogenic *CHEK2* mutation, which leads to a dominantly-inherited high  
15 lifetime risk for cancers including breast and colorectal (Meijers-Heijboer et al. 2002; Xiang  
16 et al. 2011). *CHEK2* is not on the ACMG list, but NCCN recommends increased cancer  
17 screening starting at younger ages for mutation carriers (National Comprehensive Cancer  
18 Network 2017). Identification of such mutations can also alert family members to their  
19 potential cancer risk, which for female relatives of our participant found to carry his same  
20 mutation would include a significant (potentially more than two-fold) increased breast cancer  
21 risk (*CHEK2* Breast Cancer Case-Control Consortium 2004). Both of these participants were  
22 referred to a cancer genetics clinic for follow up.

23  
24 Additionally, a female participant found to be heterozygous for a pathogenic *PROS1* mutation  
25 is at increased risk for thrombophilia due to protein S deficiency, which leads to preventative  
26 treatment in some patients, particularly those who already have a family history of thrombotic  
27 events (De Stefano and Rossi 2013). Oral contraceptives are also contraindicated in women  
28 with heterozygous *PROS1* mutations, even in the absence of family history of thrombotic

1 events (van Vlijmen et al. 2016).

2

3 While the ACMG list has become the default gene list used to determine which  
4 incidental/secondary findings should be returned to participants undergoing WES/WGS, it is  
5 not necessarily a comprehensive representation of all such genes. Additionally, a number of  
6 studies have demonstrated that many patients and research participants do want to learn about  
7 incidental or secondary findings that are not medically actionable—such as genetic risk for  
8 developing adult-onset neurodegenerative conditions such as Alzheimer’s disease or  
9 Parkinson’s disease. Qualitative research on this subject has suggested many individuals find  
10 this information actionable in other (non-medical) ways and express that they would live their  
11 lives differently if they knew they were at increased risk of developing such a condition or  
12 would prepare for developing the disease (Clift et al. 2015; Yushak et al. 2016). Our study  
13 identified 21 participants with one or two copies of the *APOE* e4 allele, which significantly  
14 increases lifetime risk for developing Alzheimer’s disease (Corder et al. 1993; Bertram et al.  
15 2010). This information was reported back to participants who specifically requested their  
16 *APOE* status. Similarly, we identified three heterozygous carriers of *GBA* mutations, and  
17 while *GBA* carriers will not develop Gaucher disease—an autosomal recessive lysosomal  
18 storage disease—they are at increased risk for developing Parkinson’s disease (Tayebi et al.  
19 2003; Halperin et al. 2006; Alcalay et al. 2014). We reported this information back to  
20 participants who opted to learn all medically relevant findings. For the *GBA* mutation carriers  
21 in our study as well as the 54 carriers of mutations in other genes implicated in recessive  
22 disease, this information can also alert families to potential reproductive risk and lead to  
23 carrier testing for their partners or adult children.

24

### 25 *Manual Variant Curation*

26 In addition to limiting incidental findings to variants within a specific gene list, researchers  
27 attempting to look for WGS/WES secondary findings have also attempted to mitigate the  
28 curation workload by forgoing variant curation altogether. Several previous studies assessing

1 WGS/WES secondary findings have either completely or primarily relied on a combination of  
2 in-silico predictors and variant databases such as Clinvar  
3 (<https://www.ncbi.nlm.nih.gov/clinvar>) and HGMD ([www.hgmd.cf.ac.uk/ac/index.php](http://www.hgmd.cf.ac.uk/ac/index.php)) to  
4 classify variants rather than employing manual curation (Gonzalez-Garay et al. 2013; Tabor  
5 et al. 2014; Gambin et al. 2015; Dewey et al. 2016). This approach may be limiting and error-  
6 prone. Testing well-known, previously classified missense mutations with the commonly used  
7 in-silico predictors SIFT and Polyphen yields accuracy ranging from 62-78% (Masica and  
8 Karchin 2016). Splice site predictors are only slightly more accurate (Vreeswijk et al. 2009;  
9 Houdayer et al. 2012). Although improving, the majority of variants in Clinvar have not  
10 undergone expert review and classifications are often based on incomplete or outdated  
11 evidence and/or were classified without applying stringent criteria. Similarly, variants listed  
12 as disease mutations (DM) in the HGMD frequently do not meet criteria to be classified as  
13 likely pathogenic or pathogenic. Dewey et al found that only one-fourth of the HGMD DM  
14 variants they identified in their cohort were classified by experienced curators as likely  
15 pathogenic or pathogenic (Dewey et al. 2014). This evidence supports the necessity of manual  
16 variant curation in order to accurately classify variants, at least until such a time as a reliable  
17 publicly available database exists.

18

### 19 *Limitations*

20 Our study had several limitations. Among them, we used a minor allele frequency cutoff of  
21 0.5% when filtering variants for further curation, and this will certainly lead to the exclusion  
22 of some particularly common pathogenic and likely pathogenic variants, such as the common  
23 delta 508 *CFTR* mutation. Other filtering cutoffs also likely limited the number of disease-  
24 causing mutations identified. Our cohort size (70) is small and larger studies will be needed to  
25 determine if the rate of actionable findings identified in our cohort also applies to larger  
26 populations. Our understanding of penetrance in many disease genes is based largely on  
27 studies of families known to be affected with disease, so in the future we may learn that  
28 penetrance is lower for individuals without family histories of disease who have actionable



1 mutations described in this study.

2

3

#### 4 *Conclusions*

5 We demonstrate that exome sequencing of participants in a longitudinal wellness study  
6 reveals important medical information in a considerable fraction of the population. The  
7 exome results from our cohort include several medically actionable variants in genes not  
8 included in the ACMG list, which suggests a need for a more comprehensive list of genes to  
9 guide the return of incidental findings. Indeed, an expanded list of genes for the return of  
10 incidental findings would present challenges, as a larger list would require more resources to  
11 accurately curate and classify variants and could lead to costly follow-up. More research is  
12 needed to better understand how common medically actionable variants are, which other  
13 types of results patients find utility in, and to better understand the costs and benefits of  
14 returning more extensive secondary findings to patients undergoing exome or genome  
15 sequencing. Nonetheless, our study provides a general approach for how to use genome  
16 sequencing, interpretation and reporting of medically relevant variants for the population at  
17 large.

18

19

#### 20 **Methods**

21

##### 22 *Recruitment and Study Population*

23 Participants were enrolled as part of Stanford's iPOP (Integrated Personal Omics Profiling)  
24 research study (IRB 23602), which entails longitudinal multi-omics profiling of a cohort of  
25 unrelated adult volunteers enriched for pre-diabetics. The iPOP study has been described  
26 previously (Chen et al. 2012; The Integrative Human Microbiome Project 2014). All research  
27 participants received genetic counseling by a medical geneticist or genetic counselor prior to  
28 enrollment and signed a consent form approved by the Stanford University Institutional

1 Review Board. Participants were able to opt in or out of receiving incidental findings, and if  
2 they opted in, were also given the option of selecting whether they wanted only actionable  
3 results or all results with medical relevance.

4

#### 5 *Whole Exome Sequencing*

6 Whole exome sequencing was performed on 70 individuals. Briefly, DNA was isolated from  
7 blood using Gentra Puregene Kits (Qiagen) according to manufacturer's protocol. Exome  
8 sequencing was performed at Personalis—a CLIA- and CAP-accredited facility—using the  
9 ACE Clinical Exome Test which covers exomes in a more comprehensive fashion  
10 (Patwardhan et al. 2015) and additional genomic regions of interest. Variants were called  
11 using an updated version of the HugerSeq pipeline (Lam et al. 2012) which used GATK3.3 or  
12 higher (McKenna et al. 2010).

13

#### 14 *Variant Filtering and Analysis*

15 The overall workflow is depicted in Figure 1. Two types of genomic results were assessed—  
16 rare variants in known Mendelian disease genes and variants with pharmacogenetic  
17 annotations in the PharmGKB database (<https://www.pharmgkb.org>). Rare variants were  
18 filtered according to the steps depicted in Figure 2. Initially variants were filtered based on  
19 confidence metrics including Phred scores (minimum 20) and read depth (minimum 10).  
20 Variants were also excluded if they had a minor allele frequency higher than 0.5% in the 1000  
21 Genomes database ([www.internationalgenome.org/1000-genomes-browsers](http://www.internationalgenome.org/1000-genomes-browsers)) or Exome  
22 Aggregation Consortium (ExAC) database ([www.exac.broadinstitute.org](http://www.exac.broadinstitute.org)). We then removed  
23 variants that did not appear in one the 3,651 genes in the OMIM database categorized as a  
24 gene associated with Mendelian disease (downloaded January 2016), or on the list of 59  
25 genes in which the ACMG recommends reporting incidental findings (Green et al. 2013;  
26 Kalia et al. 2017). Additional filtering was performed by 1) the removal of variants for which  
27 manual examination of the aligned reads indicated a likely sequencing error; 2) the removal  
28 of variants expected to cause serious, highly penetrant disease at a young age and for which

1 the participant did not have the associated phenotype (variants were only removed when the  
2 patient had a genotype that would be expected to cause disease were the variant pathogenic—  
3 i.e. homozygous for a recessive disease or heterozygous for a dominant disease); our  
4 experience revealed that these were usually artifacts; 3) when the curators determined there  
5 was insufficient evidence that the gene in which the variant resided was associated with  
6 disease; and 4) when the minor allele frequency of the variant in the 1000 Genomes or ExAC  
7 database was above 0.5% in a subpopulation but had initially passed filtering because the  
8 overall population minor allele frequency was below that cutoff. The remaining rare variants  
9 then underwent manual curation and classification by a trained genetic counselor according to  
10 ACMG criteria for the classification of sequence variants: 1) variants of a type likely to cause  
11 loss of gene function (insertions and deletions, nonsense, splice), 2) variants with an exact  
12 match in the Human Genome Mutation Database (HGMD), and 3) coding or canonical splice-  
13 site variants in one of the 59 genes in which ACMG recommends reporting incidental  
14 findings (Richards et al. 2015; Kalia et al. 2017).

15

16 Participants had varying degrees of personal and family medical history available for the  
17 curators to take into consideration when classifying variants. For some participants this  
18 information was limited to a medical history intake form and basic medical records; for others  
19 much more extensive medical history and/or a three-generation pedigree were available.

20 Additional variants were curated when they were identified in genes associated with a  
21 potentially Mendelian disease in the participant's family history.

22

23 Participants in whom medically significant likely pathogenic or pathogenic variants were  
24 identified were encouraged to discuss the results with their physician and, when necessary,  
25 referred to a genetics clinic for follow up. Participants were given the option at the time of  
26 consent of selecting whether they would like to receive genomic results, and if so, whether  
27 they would prefer actionable results only or all medically relevant results identified.

28 Actionable results were defined as likely pathogenic or pathogenic variants in genes

1 associated with diseases that are moderately to highly penetrant, the identification of which  
2 was likely to result in altered medical care in the form of treatment, screening, or preventative  
3 measures. Additionally, non-actionable findings with medical relevance were returned to  
4 participants who opted to receive them during the consent process. These results included  
5 likely pathogenic and pathogenic heterozygous variants in genes implicated in recessive  
6 diseases, as well as likely pathogenic and pathogenic variants in genes associated with  
7 diseases such as Parkinson's disease or Alzheimer's disease, for which limited or no highly  
8 effective treatment or preventative measures are available. All pathogenic and likely  
9 pathogenic variants were reviewed by two genetic counselors and a medical geneticist.  
10 Results were then reported back to participants by a genetic counselor in accordance with  
11 their stated preferences.

12

13 Participants' genotypes were also examined for common SNPs with pharmacogenetic  
14 annotations that reached a level 1A classification in the PharmGKB database ([pharmgkb.org](http://pharmgkb.org)).  
15 Level 1A variants represent those with the highest level of validation.

16

17

## 18 **Data Access**

19 Data from participants who consented to make their sequences completely public is available  
20 at <http://ihmpdccc.org/resources/osdf.php>. Data will also be submitted to dbGAP prior to  
21 publication.

22

23

## 24 **Acknowledgments**

25 Our work was supported by grants from the NIH Common Fund Human Microbiome  
26 Project (HMP) (1U54DE02378901) (M.P.S. and T.M.) and American Diabetes  
27 Association (grants 1-14-TS-28 and 1-11-CT-35) (T.M.). M.R.S and H.L.R. are supported by  
28 grants from the Swiss National Science Foundation (SNSF):

1 P300PA\_161005, P2GEP3\_151825, M.R.S.; P300PA\_164703, H.L.R.). J.D. is funded by the  
2 Mobilize Center (grant NIH U54 EB020405). This work was also supported by a gift from the  
3 Forbes Family Fund. The authors would like to thank the Stanford Genetics Bioinformatics  
4 Service Center for computational and informatics support, as well as the volunteers who  
5 participated in our study

6

7

#### 8 **Disclosure Declarations**

9 M.P.S. is a founder and member of the science advisory board of  
10 Personalis, SensOmics and Qbio and a science advisory board member of Genapsys. L.M.S.  
11 is a founder and member of the science advisory board of Sophia Genetics and Levitas

## References

- Alcalay RN, Dinur T, Quinn T, Sakanaka K, Levy O, Waters C, Fahn S, Dorovski T, Chung WK, Pauciulo M et al. 2014. Comparison of Parkinson risk in Ashkenazi Jewish patients with Gaucher disease and GBA heterozygotes. *JAMA neurology* **71**: 752-757.
- Amendola LM, Dorschner MO, Robertson PD, Salama JS, Hart R, Shirts BH, Murray ML, Tokita MJ, Gallego CJ, Kim DS et al. 2015. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome research* **25**: 305-315.
- Bacon S, Kyithar MP, Rizvi SR, Donnelly E, McCarthy A, Burke M, Colclough K, Ellard S, Byrne MM. 2016. Successful maintenance on sulphonylurea therapy and low diabetes complication rates in a HNF1A-MODY cohort. *Diabetic medicine : a journal of the British Diabetic Association* **33**: 976-984.
- Banno F, Kita T, Fernandez JA, Yanamoto H, Tashima Y, Kokame K, Griffin JH, Miyata T. 2015. Exacerbated venous thromboembolism in mice carrying a protein S K196E mutation. *Blood* **126**: 2247-2253.
- Bazalova Z, Rypackova B, Broz J, Brunerova L, Polak J, Rusavy Z, Treslova L, Andel M. 2010. Three novel mutations in MODY and its phenotype in three different Czech families. *Diabetes research and clinical practice* **88**: 132-138.
- Benn DE, Croxson MS, Tucker K, Bambach CP, Richardson AL, Delbridge L, Pullan PT, Hammond J, Marsh DJ, Robinson BG. 2003. Novel succinate dehydrogenase subunit B (SDHB) mutations in familial pheochromocytomas and paragangliomas, but an absence of somatic SDHB mutations in sporadic pheochromocytomas. *Oncogene* **22**: 1358-1364.
- Bertram L, Lill CM, Tanzi RE. 2010. The genetics of Alzheimer disease: back to the future. *Neuron* **68**: 270-281.
- Boursi B, Sella T, Liberman E, Shapira S, David M, Kazanov D, Arber N, Kraus S. 2013. The APC p.I1307K polymorphism is a significant risk factor for CRC in average risk Ashkenazi Jews. *European journal of cancer (Oxford, England : 1990)* **49**: 3680-3685.
- Burke W, Antommara AH, Bennett R, Botkin J, Clayton EW, Henderson GE, Holm IA, Jarvik GP, Khoury MJ, Knoppers BM et al. 2013. Recommendations for returning genomic incidental findings? We need to talk! *Genetics in medicine : official journal of the American College of Medical Genetics* **15**: 854-859.
- Calabria AC, Li C, Gallagher PR, Stanley CA, De Leon DD. 2012. GLP-1 receptor antagonist exendin-(9-39) elevates fasting blood glucose levels in congenital hyperinsulinism owing to inactivating mutations in the ATP-sensitive K<sup>+</sup> channel. *Diabetes* **61**: 2585-2591.
- CHEK2 Breast Cancer Case-Control Consortium. 2004. CHEK2\*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. *Am J Hum Genet* **74**: 1175-1182.
- Chen R, Mias GI, Li-Pook-Than J, Jiang L, Lam HY, Chen R, Miriami E, Karczewski KJ, Hariharan M, Dewey FE et al. 2012. Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell* **148**: 1293-1307.
- Clift KE, Halverson CM, Fiksdal AS, Kumbamu A, Sharp RR, McCormick JB. 2015. Patients' views on incidental findings from clinical exome sequencing. *Applied & translational genomics* **4**: 38-43.
- Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science (New York, NY)* **261**: 921-923.
- Costa A, Bescos M, Velho G, Chevre J, Vidal J, Sesimalo G, Bellanne-Chantelot C, Froguel P, Casamitjana R, Rivera-Fillat F et al. 2000. Genetic and clinical characterisation of

- maturity-onset diabetes of the young in Spanish families. *European journal of endocrinology* **142**: 380-386.
- De Stefano V, Rossi E. 2013. Testing for inherited thrombophilia and consequences for antithrombotic prophylaxis in patients with venous thromboembolism and their relatives. A review of the Guidelines from Scientific Societies and Working Groups. *Thrombosis and haemostasis* **110**: 697-705.
- Dewey FE, Grove ME, Pan C, Goldstein BA, Bernstein JA, Chaib H, Merker JD, Goldfeder RL, Enns GM, David SP et al. 2014. Clinical interpretation and implications of whole-genome sequencing. *Jama* **311**: 1035-1045.
- Dewey FE, Grove ME, Priest JR, Waggott D, Batra P, Miller CL, Wheeler M, Zia A, Pan C, Karzcewski KJ et al. 2015. Sequence to Medical Phenotypes: A Framework for Interpretation of Human Whole Genome DNA Sequence Data. *PLoS genetics* **11**: e1005496.
- Dewey FE, Murray MF, Overton JD, Habegger L, Leader JB, Fetterolf SN, O'Dushlaine C, Van Hout CV, Staples J, Gonzaga-Jauregui C et al. 2016. Distribution and clinical impact of functional variants in 50,726 whole-exome sequences from the DiscovEHR study. *Science (New York, NY)* **354**.
- Dorschner MO, Amendola LM, Turner EH, Robertson PD, Shirts BH, Gallego CJ, Bennett RL, Jones KL, Tokita MJ, Bennett JT et al. 2013. Actionable, pathogenic incidental findings in 1,000 participants' exomes. *American journal of human genetics* **93**: 631-640.
- Font MA, Feliubadalo L, Estivill X, Nunes V, Golomb E, Kreiss Y, Pras E, Bisceglia L, d'Adamo AP, Zelante L et al. 2001. Functional analysis of mutations in SLC7A9, and genotype-phenotype correlation in non-Type I cystinuria. *Human molecular genetics* **10**: 305-316.
- Frayling IM, Beck NE, Ilyas M, Dove-Edwin I, Goodman P, Pack K, Bell JA, Williams CB, Hodgson SV, Thomas HJ et al. 1998. The APC variants I1307K and E1317Q are associated with colorectal tumors, but not always with a family history. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 10722-10727.
- Gambin T, Jhangiani SN, Below JE, Campbell IM, Wiszniewski W, Muzny DM, Staples J, Morrison AC, Bainbridge MN, Penney S et al. 2015. Secondary findings and carrier test frequencies in a large multiethnic sample. *Genome medicine* **7**: 54.
- Gimenez-Roqueplo AP, Favier J, Rustin P, Rieubland C, Kerlan V, Plouin PF, Rotig A, Jeunemaitre X. 2002. Functional consequences of a SDHB gene mutation in an apparently sporadic pheochromocytoma. *The Journal of clinical endocrinology and metabolism* **87**: 4771-4774.
- Gonzalez-Garay ML, McGuire AL, Pereira S, Caskey CT. 2013. Personalized genomic disease risk of volunteers. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 16957-16962.
- Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, McGuire AL, Nussbaum RL, O'Daniel JM, Ormond KE et al. 2013. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genetics in medicine : official journal of the American College of Medical Genetics* **15**: 565-574.
- Halbritter J, Baum M, Hynes AM, Rice SJ, Thwaites DT, Gucev ZS, Fisher B, Spanas L, Porath JD, Braun DA et al. 2015. Fourteen monogenic genes account for 15% of nephrolithiasis/nephrocalcinosis. *Journal of the American Society of Nephrology : JASN* **26**: 543-551.
- Halperin A, Elstein D, Zimran A. 2006. Increased incidence of Parkinson disease among relatives of patients with Gaucher disease. *Blood cells, molecules & diseases* **36**: 426-428.
- Hayakawa T, Morimoto A, Nozaki Y, Kashii Y, Aihara T, Maeda K, Momoi MY. 2011. Mesenteric venous thrombosis in a child with type 2 protein S deficiency. *Journal of pediatric hematology/oncology* **33**: 141-143.
- Hayashi T, Nishioka J, Shigekiyo T, Saito S, Suzuki K. 1994. Protein S Tokushima: abnormal

- molecule with a substitution of Glu for Lys-155 in the second epidermal growth factor-like domain of protein S. *Blood* **83**: 683-690.
- Holtzman NA. 2013. ACMG recommendations on incidental findings are flawed scientifically and ethically. *Genetics in medicine : official journal of the American College of Medical Genetics* **15**: 750-751.
- Houdayer C, Caux-Moncoutier V, Krieger S, Barrois M, Bonnet F, Bourdon V, Bronner M, Buisson M, Coulet F, Gaildrat P et al. 2012. Guidelines for splicing analysis in molecular diagnosis derived from a set of 327 combined in silico/in vitro studies on BRCA1 and BRCA2 variants. *Human mutation* **33**: 1228-1238.
- Huang SS, Liu Y, Jing ZC, Wang XJ, Mao YM. 2016. Common genetic risk factors of venous thromboembolism in Western and Asian populations. *Genetics and molecular research : GMR* **15**: 15017644.
- Ikejiri M, Wada H, Sakamoto Y, Ito N, Nishioka J, Nakatani K, Tsuji A, Yamada N, Nakamura M, Ito M et al. 2010. The association of protein S Tokushima-K196E with a risk of deep vein thrombosis. *International journal of hematology* **92**: 302-305.
- Jang MA, Lee SH, Kim N, Ki CS. 2015. Frequency and spectrum of actionable pathogenic secondary findings in 196 Korean exomes. *Genetics in medicine : official journal of the American College of Medical Genetics* **17**: 1007-1011.
- Johnston JJ, Rubinstein WS, Facio FM, Ng D, Singh LN, Teer JK, Mullikin JC, Biesecker LG. 2012. Secondary variants in individuals undergoing exome sequencing: screening of 572 individuals identifies high-penetrance mutations in cancer-susceptibility genes. *American journal of human genetics* **91**: 97-108.
- Jurgens J, Ling H, Hetrick K, Pugh E, Schiettecatte F, Doheny K, Hamosh A, Avramopoulos D, Valle D, Sobreira N. 2015. Assessment of incidental findings in 232 whole-exome sequences from the Baylor-Hopkins Center for Mendelian Genomics. *Genetics in medicine : official journal of the American College of Medical Genetics* **17**: 782-788.
- Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, Herman GE, Hufnagel SB, Klein TE, Korf BR et al. 2017. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genetics in medicine : official journal of the American College of Medical Genetics* **19**: 249-255.
- Kim E, Rath EM, Tsang VH, Duff AP, Robinson BG, Church WB, Benn DE, Dwight T, Clifton-Bligh RJ. 2015. Structural and functional consequences of succinate dehydrogenase subunit B mutations. *Endocrine-related cancer* **22**: 387-397.
- Kimura N, Takekoshi K, Horii A, Morimoto R, Imai T, Oki Y, Saito T, Midorikawa S, Arao T, Sugisawa C et al. 2014. Clinicopathological study of SDHB mutation-related pheochromocytoma and sympathetic paraganglioma. *Endocrine-related cancer* **21**: L13-16.
- Kimura R, Sakata T, Kokubo Y, Okamoto A, Okayama A, Tomoike H, Miyata T. 2006. Plasma protein S activity correlates with protein S genotype but is not sensitive to identify K196E mutant carriers. *Journal of thrombosis and haemostasis : JTH* **4**: 2010-2013.
- Komine K, Shimodaira H, Takao M, Soeda H, Zhang X, Takahashi M, Ishioka C. 2015. Functional Complementation Assay for 47 MUTYH Variants in a MutY-Disrupted Escherichia coli Strain. *Human mutation* **36**: 704-711.
- Laken SJ, Petersen GM, Gruber SB, Oddoux C, Ostrer H, Giardiello FM, Hamilton SR, Hampel H, Markowitz A, Klimstra D et al. 1997. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. *Nature genetics* **17**: 79-83.
- Lam HY, Pan C, Clark MJ, Lacroute P, Chen R, Haraksingh R, O'Huallachain M, Gerstein MB, Kidd JM, Bustamante CD et al. 2012. Detecting and annotating genetic variations using the HugerSeq pipeline. *Nature biotechnology* **30**: 226-229.
- Lawrence L, Sincan M, Markello T, Adams DR, Gill F, Godfrey R, Golas G, Groden C, Landis D, Nehrebecky M et al. 2014. The implications of familial incidental findings from exome sequencing: the NIH Undiagnosed Diseases Program experience. *Genetics in medicine : official journal of the American College of Medical Genetics*



16: 741-750.

- Li D, Morales A, Gonzalez-Quintana J, Norton N, Siegfried JD, Hofmeyer M, Hershberger RE. 2010. Identification of novel mutations in RBM20 in patients with dilated cardiomyopathy. *Clinical and translational science* **3**: 90-97.
- Liang J, Lin C, Hu F, Wang F, Zhu L, Yao X, Wang Y, Zhao Y. 2013. APC polymorphisms and the risk of colorectal neoplasia: a HuGE review and meta-analysis. *American journal of epidemiology* **177**: 1169-1179.
- Manolio TA, Chisholm RL, Ozenberger B, Roden DM, Williams MS, Wilson R, Bick D, Bottinger EP, Brilliant MH, Eng C et al. 2013. Implementing genomic medicine in the clinic: the future is here. *Genetics in medicine : official journal of the American College of Medical Genetics* **15**: 258-267.
- Masica DL, Karchin R. 2016. Towards Increasing the Clinical Relevance of In Silico Methods to Predict Pathogenic Missense Variants. *PLoS computational biology* **12**: e1004725.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M et al. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome research* **20**: 1297-1303.
- Meijers-Heijboer H, van den Ouweland A, Klijn J, Wasielewski M, de Snoo A, Oldenburg R, Hollestelle A, Houben M, Crepin E, van Veghel-Plandsoen M et al. 2002. Low-penetrance susceptibility to breast cancer due to CHEK2(\*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. *Nature genetics* **31**: 55-59.
- Miyaki M, Iijima T, Yamaguchi T, Hishima T, Tamura K, Utsunomiya J, Mori T. 2005. Germline mutations of the MYH gene in Japanese patients with multiple colorectal adenomas. *Mutation research* **578**: 430-433.
- Miyata T, Sato Y, Ishikawa J, Okada H, Takeshita S, Sakata T, Kokame K, Kimura R, Honda S, Kawasaki T et al. 2009. Prevalence of genetic mutations in protein S, protein C and antithrombin genes in Japanese patients with deep vein thrombosis. *Thrombosis research* **124**: 14-18.
- National Comprehensive Cancer Network. 2016. National Comprehensive Cancer Network. Colorectal Cancer Screening (Version 2.2016).
- National Comprehensive Cancer Network. 2017. National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Breast and Ovarian (Version 2.2017).
- National Diabetes Statistics Report. 2014. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. Centers for Disease Control and Prevention. U.S. Department of Health and Human Services, Atlanta, GA.
- Neki R, Fujita T, Kokame K, Nakanishi I, Waguri M, Imayoshi Y, Suehara N, Ikeda T, Miyata T. 2011. Genetic analysis of patients with deep vein thrombosis during pregnancy and postpartum. *International journal of hematology* **94**: 150-155.
- Olschwang S, Blanche H, de Moncuit C, Thomas G. 2007. Similar colorectal cancer risk in patients with monoallelic and biallelic mutations in the MYH gene identified in a population with adenomatous polyposis. *Genetic testing* **11**: 315-320.
- Patwardhan A, Harris J, Leng N, Bartha G, Church DM, Luo S, Haudenschild C, Pratt M, Zook J, Salit M et al. 2015. Achieving high-sensitivity for clinical applications using augmented exome sequencing. *Genome medicine* **7**: 71.
- Pern F, Bogdanova N, Schurmann P, Lin M, Ay A, Langer F, Hillemanns P, Christiansen H, Park-Simon TW, Dork T. 2012. Mutation analysis of BRCA1, BRCA2, PALB2 and BRD7 in a hospital-based series of German patients with triple-negative breast cancer. *PloS one* **7**: e47993.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E et al. 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine : official journal of the American College of Medical Genetics* **17**: 405-424.
- Ruggieri V, Pin E, Russo MT, Barone F, Degani P, Sanchez M, Quaia M, Minoprio A, Turco

- E, Mazzei F et al. 2013. Loss of MUTYH function in human cells leads to accumulation of oxidative damage and genetic instability. *Oncogene* **32**: 4500-4508.
- Saxena N, Maio N, Crooks DR, Ricketts CJ, Yang Y, Wei MH, Fan TW, Lane AN, Sourbier C, Singh A et al. 2016. SDHB-Deficient Cancers: The Role of Mutations That Impair Iron Sulfur Cluster Delivery. *Journal of the National Cancer Institute* **108**.
- Shepherd M, Shields B, Ellard S, Rubio-Cabezas O, Hattersley AT. 2009. A genetic diagnosis of HNF1A diabetes alters treatment and improves glycaemic control in the majority of insulin-treated patients. *Diabetic medicine : a journal of the British Diabetic Association* **26**: 437-441.
- Shih HA, Couch FJ, Nathanson KL, Blackwood MA, Rebbeck TR, Armstrong KA, Calzone K, Stopfer J, Seal S, Stratton MR et al. 2002. BRCA1 and BRCA2 mutation frequency in women evaluated in a breast cancer risk evaluation clinic. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **20**: 994-999.
- Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, Ganapathy K, Bhatti T, Stanley CA, Ganguly A. 2013. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. *The Journal of clinical endocrinology and metabolism* **98**: E355-363.
- Stern HS, Viertelhausen S, Hunter AG, O'Rourke K, Cappelli M, Perras H, Serfas K, Blumenthall A, Dewar D, Baumann E et al. 2001. APC I1307K increases risk of transition from polyp to colorectal carcinoma in Ashkenazi Jews. *Gastroenterology* **120**: 392-400.
- Tabor HK, Auer PL, Jamal SM, Chong JX, Yu JH, Gordon AS, Graubert TA, O'Donnell CJ, Rich SS, Nickerson DA et al. 2014. Pathogenic variants for Mendelian and complex traits in exomes of 6,517 European and African Americans: implications for the return of incidental results. *American journal of human genetics* **95**: 183-193.
- Tayebi N, Walker J, Stubblefield B, Orvisky E, LaMarca ME, Wong K, Rosenbaum H, Schiffmann R, Bembi B, Sidransky E. 2003. Gaucher disease with parkinsonian manifestations: does glucocerebrosidase deficiency contribute to a vulnerability to parkinsonism? *Molecular genetics and metabolism* **79**: 104-109.
- The Integrative Human Microbiome Project. 2014. The Integrative Human Microbiome Project: dynamic analysis of microbiome-host omics profiles during periods of human health and disease. *Cell Host Microbe* **16**: 276-289.
- Thusberg J, Olatubosun A, Vihinen M. 2011. Performance of mutation pathogenicity prediction methods on missense variants. *Human mutation* **32**: 358-368.
- Tonooka N, Tomura H, Takahashi Y, Onigata K, Kikuchi N, Horikawa Y, Mori M, Takeda J. 2002. High frequency of mutations in the HNF-1alpha gene in non-obese patients with diabetes of youth in Japanese and identification of a case of digenic inheritance. *Diabetologia* **45**: 1709-1712.
- Turkovic L, Gurrin LC, Bahlo M, Dite GS, Southey MC, Hopper JL. 2010. Comparing the frequency of common genetic variants and haplotypes between carriers and non-carriers of BRCA1 and BRCA2 deleterious mutations in Australian women diagnosed with breast cancer before 40 years of age. *BMC cancer* **10**: 466.
- Vail PJ, Morris B, van Kan A, Burdett BC, Moyes K, Theisen A, Kerr ID, Wenstrup RJ, Egginton JM. 2015. Comparison of locus-specific databases for BRCA1 and BRCA2 variants reveals disparity in variant classification within and among databases. *Journal of community genetics* **6**: 351-359.
- van Vlijmen EF, Wiewel-Verschueren S, Monster TB, Meijer K. 2016. Combined oral contraceptives, thrombophilia and the risk of venous thromboembolism: a systematic review and meta-analysis. *Journal of thrombosis and haemostasis : JTH* **14**: 1393-1403.
- Vaxillaire M, Rouard M, Yamagata K, Oda N, Kaisaki PJ, Boriraj VV, Chevre JC, Boccio V, Cox RD, Lathrop GM et al. 1997. Identification of nine novel mutations in the hepatocyte nuclear factor 1 alpha gene associated with maturity-onset diabetes of the young (MODY3). *Human molecular genetics* **6**: 583-586.

- Vreeswijk MP, Kraan JN, van der Klift HM, Vink GR, Cornelisse CJ, Wijnen JT, Bakker E, van Asperen CJ, Devilee P. 2009. Intronic variants in BRCA1 and BRCA2 that affect RNA splicing can be reliably selected by splice-site prediction programs. *Human mutation* **30**: 107-114.
- Whirl-Carrillo M, McDonagh EM, Hebert JM, Gong L, Sangkuhl K, Thorn CF, Altman RB, Klein TE. 2012. Pharmacogenomics knowledge for personalized medicine. *Clinical pharmacology and therapeutics* **92**: 414-417.
- Win AK, Dowty JG, Cleary SP, Kim H, Buchanan DD, Young JP, Clendenning M, Rosty C, MacInnis RJ, Giles GG et al. 2014. Risk of colorectal cancer for carriers of mutations in MUTYH, with and without a family history of cancer. *Gastroenterology* **146**: 1208-1211.e1201-1205.
- Woodage T, King SM, Wacholder S, Hartge P, Struwing JP, McAdams M, Laken SJ, Tucker MA, Brody LC. 1998. The APC11307K allele and cancer risk in a community-based study of Ashkenazi Jews. *Nature genetics* **20**: 62-65.
- Xiang HP, Geng XP, Ge WW, Li H. 2011. Meta-analysis of CHEK2 1100delC variant and colorectal cancer susceptibility. *European journal of cancer (Oxford, England : 1990)* **47**: 2546-2551.
- Xue Y, Chen Y, Ayub Q, Huang N, Ball EV, Mort M, Phillips AD, Shaw K, Stenson PD, Cooper DN et al. 2012. Deleterious- and disease-allele prevalence in healthy individuals: insights from current predictions, mutation databases, and population-scale resequencing. *American journal of human genetics* **91**: 1022-1032.
- Yamada S, Tomura H, Nishigori H, Sho K, Mabe H, Iwatani N, Takumi T, Kito Y, Moriya N, Muroya K et al. 1999. Identification of mutations in the hepatocyte nuclear factor-1alpha gene in Japanese subjects with early-onset NIDDM and functional analysis of the mutant proteins. *Diabetes* **48**: 645-648.
- Yushak ML, Han G, Bouberhan S, Epstein L, DiGiovanna MP, Mougalian SS, Sanft TB, Abu-Khalaf MM, Chung GG, Stein SM et al. 2016. Patient preferences regarding incidental genomic findings discovered during tumor profiling. *Cancer* **122**: 1588-1597.

