www.nature.com/jhg

ARTICLE

Actionable secondary findings in the 73 ACMG-recommended genes in 1559 Thai exomes

Wanna Chetruengchai[1,2,3] and Vorasuk Shotelersuk[1,2]✉

High-throughput DNA sequencing provides not only primary diagnosis but also makes available other genetic variants with potential health implications. the American College of Medical Genetics and Genomics (ACMG) has recommended a list of medically actionable genes since 2013 and very recently released an updated ACMG SF v3.0 list comprising 73 genes. Here, we analyzed exome data of 1559 unrelated Thai individuals to determine the frequency and spectrum of pathogenic (P) or likely pathogenic (LP) variants in the 73 genes. Based on the ACMG guidelines for the interpretation of sequence variants, 68 different P/LP variants in 26 genes associated with 18 diseases inherited in an autosomal-dominant manner of 186 individuals (11.9%; 186/1559) were identified. Of these, 22 P/LP variants in 15 genes associated with 13 diseases of 85 individuals (5.5%; 85/1559) were also reported as P/LP in the ClinVar archive. The majority harbored variants in genes related to cardiovascular diseases (4.7%; 74/1559), followed by cancer phenotypes (0.5%; 8/1559). None of the individuals in our cohort harbored biallelic variants in genes responsible for diseases inherited in an autosomal recessive manner. The results would serve as a basis for precision medicine practice at individual and population levels.

Journal of Human Genetics; https://doi.org/10.1038/s10038-021-00982-2

INTRODUCTION

High-throughput DNA sequencing including genome sequencing (GS) and exome sequencing (ES) has increasingly been used in clinical medicine to guide patient care, mainly owing to the improved efficiency of analyzing and interpreting genomic data and its less-expensive cost [1]. Not only giving the molecular diagnosis for the presenting symptoms, GS/ES would also offer genetic variants in other genes. The variants in disease-causing genes unrelated to the patient's primary diagnosis but have the potential medical value are defined as secondary findings (SFs). SFs may help prevent a disease from occurring and guide the management if the disease develops.

In 2013, the American College of Medical Genetics and Genomics (ACMG) released recommendations and encouraged the evaluation for reporting of pathogenic (P) or likely pathogenic (LP) variants in 56 medically actionable genes of 24 different diseases [2]. In 2017, ACMG SF v2.0 has revised the list to 59 actionable genes of 27 various diseases [3]. Using this SF v2.0, studies of 196 Koreans [4], 1116 Hong Kong Chineses [5], 280 Lebaneses [6], 161 Taiwaneses [7], 954 East Asians [8], and 1005 Qatari people [9] demonstrated frequencies of SFs ranging from 1 to 9% [10].

Very recently, the list of actionable genes was updated in 2021 to include a total of 73 genes for 35 phenotypes [11]. Evidence-based guidelines were also recommended by ACMG, the Association for Molecular Pathology (AMP), and the College of American Pathologists (CAP) in 2015 for the assessment of evidence to standardize the interpretation of genetic variants [12]. The ACMG Secondary Findings Maintenance Working Group (SFWG) also updated the policy statement on the SF gene list in 2021 [13]. An increase of 14 genes from ACMG SF v2.0 list to 73 genes in the SF v3.0 list would likely increase the frequency of SFs. Of the 14 newly added genes, three genes are related to cancer (*PALB2, MAX, TMEM127*), four to cardiovascular (*CASQ2, TRDN, FLNC, TTN*), two to inborn errors of metabolism (*BTD, GAA*), and four to miscellaneous phenotypes (*HFE, ACVRL1, ENG, HNF1A, RPE65*).

Here, we analyzed exomes of 1559 unrelated Thais to determine the frequency and spectrum of SFs in the newly updated SF v3.0 list of ACMG 73 medically actionable genes, which could be used as a basis to improve the Thai population's healthcare.

MATERIAL AND METHODS

Study samples

Exomes of 1565 unrelated Thai individuals who were parents of patients with various rare diseases were recruited. Informed consent was obtained from each individual. The study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University.

Sequencing and variant calling

Genomic DNA was extracted from peripheral blood leukocytes. The DNA samples were prepared as an Illumina sequencing library enriched by either TruSeq® Exome Kit (Illumina, San Diego, CA) or SureSelect Human All Exons, reagents (Agilent Inc,® Santa Clara, CA) according to the

[1]Excellence Center for Genomics and Precision Medicine, King Chulalongkorn Memorial Hospital, the Thai Red Cross Society, Bangkok, Thailand. [2]Center of Excellence for Medical Genomics, Medical Genomics Cluster, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. [3]Interdisciplinary Program of Biomedical Sciences, Graduate School, Chulalongkorn University, Bangkok, Thailand. ✉email: vorasuk.s@chula.ac.th

Received: 22 July 2021 Revised: 26 September 2021 Accepted: 27 September 2021
Published online: 08 October 2021

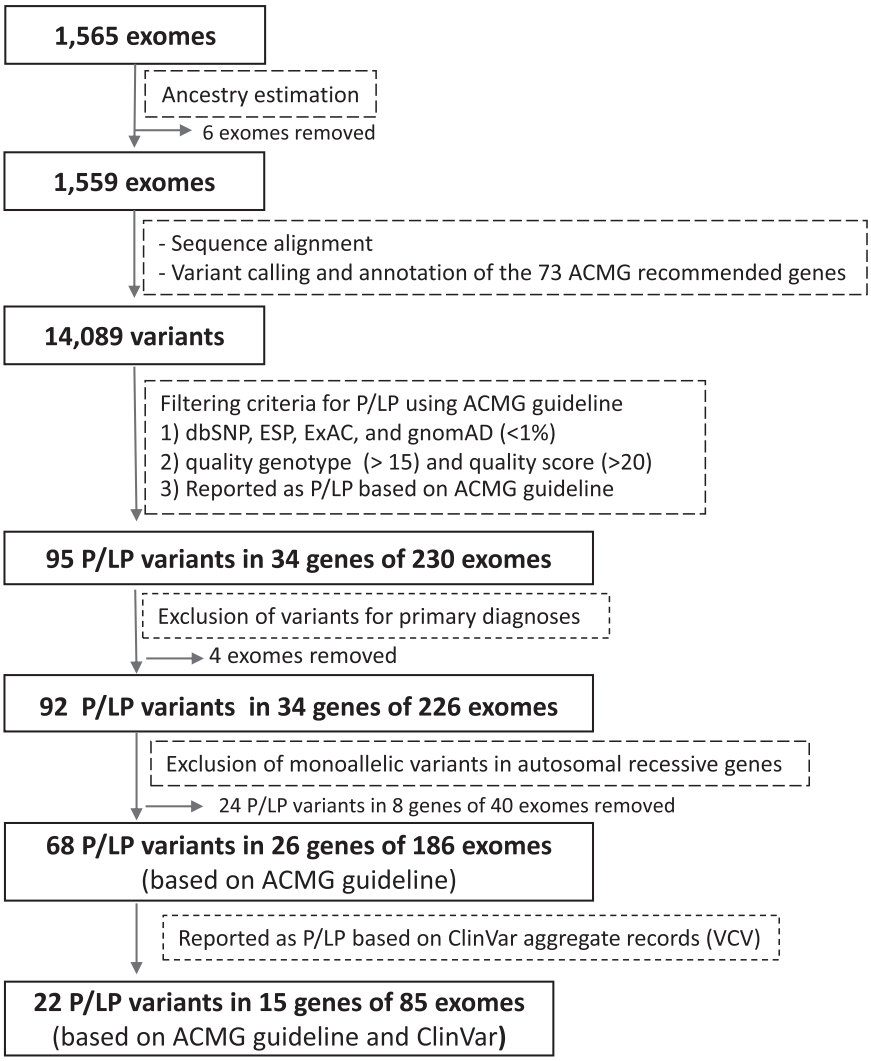

Fig. 1 Schematic workflow of the secondary findings (SFs)

manufacturer's standard protocol. Sequencing was performed on the NextSeq 500 or Hiseq 4000 Systems.

Reads files (FASTQ) were generated from the sequencing platform. Reads were aligned to the human reference genome (GRCh37) using the Burrows-Wheeler Aligner package v 0.7.15 [14]. Variant calling was performed using the Genome Analysis Tool Kit (GATK Best Practice V3.7, Broad Institute), which is called by HaplotypeCaller [15].

Ancestry estimation

Our samples were mixed with the samples from the 1000 Genomes Project by PLINK 1.90 beta 6.9 and visualize principal component analysis (PCA) in R package. Samples that did not cluster together were removed.

Variant annotation and interpretation

To annotate genetic variants, we used ANNOVAR, which uses data from different databases including RefGene (20190929 version), dbSNP150 (avsnp150; 20170929), ljb26_all (20140925 version), and Clinvar (20210120 version) [16] ANNOVAR also provided allele frequencies across global populations from respective data sets including the ESP (esp6500siv2_all; 20141222 version), ExAC project (exac03;20151129 version), 1000 Genomes project (1000g2015aug_all;20150824 version), and gnomAD (20190323 version). The variant interpretation was limited to the 73 actionable genes [11]. We included only variants that have allele frequencies <1%, quality genotype (DP) > 15, quality score >20, and were reported as P/LP by VarSome's ACMG classification [17]. Then, we removed variants present in both the participants and their children which were responsible for the children's presenting symptoms and excluded monoallelic variants in autosomal recessive genes. Finally, we described variants that were also reported as P/LP in Clinvar aggregate records (VCV) (see Fig. 1 for the schematic workflow).

RESULTS

Of the 1565 exomes, six were removed because they did not cluster with the majority. The final cohort is clustered with the East Asian population (Fig. 2) and consists of 1559 samples with 769 males and 790 females. ES allowed us to detect 14,089 variants in the 73 selected genes. Using the filtering criteria for pathogenicity based on ACMG guidelines, 95 P/LP variants in 34 genes of 230 individuals were identified (Supplementary Table S1). Four individuals harbored variants in the genes responsible for the presenting symptoms in their children and were excluded. These include a *RET* missense variant, c.1438 G > A (p.Glu480Lys), leading to multiple endocrine neoplasia type 2 and three variants in *ATP7B*, c.3316 G > A (p.Val1106Ile), c.3426 G > C (p.Gln1142His) and c.1708-1 G > C, causing Wilson disease in their affected children (individuals with the red fonts in Supplementary Table S1).

According to the ACMG recommendations, the second finding of recessive diseases should only be returned when the individual carries biallelic mutations in the same gene. Forty individuals were identified to carry monoallelic P/LP variants in eight autosomal recessive disorders and were excluded (Supplementary Table S1).

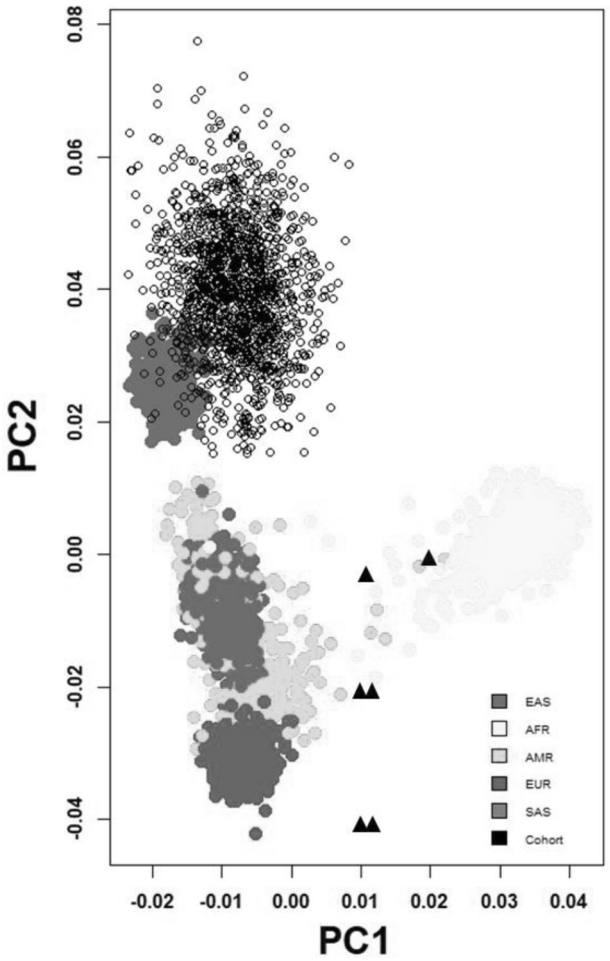

Fig. 2 A Principal component analysis was performed by comparing our 1565 exome data with 1000 Genome Project. 1559 exomes were clustered together while the six exomes represented by triangles were excluded from further analyses. *EUR* European, *EAS* East Asian, *AMR* admixed American, *SAS* South Asian, *AFR* African

Based on the ACMG guidelines for the interpretation of sequence variants, this resulted in a set of 68 different P/LP variants in 26 genes associated with 18 diseases inherited in an autosomal-dominant manner of 186 individuals (11.9%; 186/1559). Of these, 22 unique P/LP variants in 15 genes of 85 individuals (5.5%; 85/1559) were also reported as P/LP in the ClinVar archive. The 15 genes are associated with 13 autosomal-dominant diseases, which can be classified into three specific phenotype categories: cancer, cardiovascular, and miscellaneous phenotypes (Table 1).

SFs associated with oncogenic diseases were found in eight individuals representing 9.4% (8/85) of the individuals with SFs and 0.5% (8/1559) of our cohort. The most prevalent variants were c.802-2 A > G (rs587782455) in *PTEN* associated with PTEN hamartoma tumor syndromes.

Variants in genes related to cardiovascular diseases were detected in 74 individuals, representing 87% (74/85) of the patients with identified SFs and 4.7% (74/1559) of our cohort. The most prevalent variant in this phenotypic category is c. 1019 C > T (p.Thr340Met; rs34833812) in *TGFBR2* responsible for Loeys-Dietz syndrome 2 found in 19 individuals. For cardiomyopathies, c.1000 G > A (p.Glu334Lys; rs573916965) in *MYBPC3* found in 16 individuals is the most common one. Regarding arrhythmic phenotype, the most prevalent variant is c.3507 C > A (p.Tyr1169Ter; rs148894066) in *DSP* causing arrhythmogenic right ventricular cardiomyopathy (ARVC) observed in 9 individuals (0.6%; 9/1559). Notably, 10 individuals harbored c.1056 C > A (p.Cys352Ter; rs13306515) in *LDLR*-associated with familial hypercholesterolemia (FH) and cardiovascular disease.

DISCUSSION

GS/ES offers SFs with the potential to improve healthcare. The extent of their benefits relates to their frequency, which depends on the number of analyzed genes and varies by ethnic background. The most widely used list of medically actionable genes is recommended by ACMG, which increases from 56 genes in 2013, and 59 genes in 2017 to 73 genes in 2021. This would likely increase the frequency of SFs and the benefits of performing GS/ES.

Of the identified SFs in our Thai cohort, genes associated with cardiovascular phenotype were the most frequently mutated (4.7%; 74/1559). In all, 2.2% of our cohort (35/1559) risk for cardiomyopathy including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy, and arrhythmogenic cardiomyopathy. HCM has been reported to be the most prevalent genetic cardiac disease with prevalences estimated at 1 in 500 to 1 in 200 individuals [18]. If all the P/LP variants identified in our cohort lead to diseases, the prevalence of HCM in our Thai population would be 1 in 92 (17/1559). Notably, P/LP in *TGFBR2* were found in 19 individuals, leading to the prevalence of hereditary connective tissue disorders including Loeys-Dietz syndrome 2, and familial thoracic aortic aneurysms in our Thai population of 1 in 83 (19/1559). If aortopathy is identified as presymptomatic, an available effective intervention such as a prophylactic aortic surgery could save lives [19].

FH is an autosomal dominant disease, with a significantly increased cardiovascular risk. FH is a common genetic disease with an incidence of 0.2–0.5% worldwide [20]. Our finding of 0.9% or 1 in 104 individuals (15/1559) is consistent with the high frequencies found in Southeast Asian countries including 1.4% in Malaysian, and ~1% in Filipino and Vietnamese [21].

In all, 0.5% (8/1559) of our cohort are in the cancer phenotype category. Six P/LP variants were found in *PTEN* associated with PTEN hamartoma tumor syndromes. These syndromes include Cowden syndrome (CS), Lhermitte–Duclos disease (LD), Bannayan–Riley–Ruvalcaba syndrome (BRRS), and possibly Proteus syndrome (PS). Variants in *BRCA1, BRCA2*, and *PALB2* for breast and/or ovarian cancer have a prevalence of 0.13% (2/1559) or 1 in 780, similar to the previous estimation of 0.12–0.25% in other ethnics [22].

All SFs identified in our Thai cohort are associated with autosomal dominant diseases. Actually, 24 monoallelic P/LP variants in eight genes, *ATP7B, BTD, CASQ2, GAA, HFE, MUTYH, RPE65*, and *TRDN*, are responsible for autosomal recessive diseases were found in 40 individuals representing 2.6% of our cohort. In all, 17 out of these 24 P/LP variants in eight genes of 30 individuals were also reported as P/LP in the ClinVar archive (Supplementary Table S1). Variants in *ATP7B* responsible for Wilson disease were found in 16 individuals (1% of our cohort). This frequency is lower than those in Hong Kong Chinese (1.3%), Lebanese (1.4%), Korean (2%), Taiwanese (3.1%), and French (3.2%) [5–7, 23, 24]. Two individuals (0.1%) of our cohort carried P/LP variants in *MUTYH,* which increases the lifetime risk of colorectal cancer. It is much lower than 1–2% of monoallelic found in the Caucasian population [25]. As the ACMG recommends to return SFs of recessive diseases only when the individuals harbor biallelic P/LP variants and all in our cohort carried monoallelic variants, these SFs, therefore, are not included as actionable in our study. Nonetheless, these variants may still be relevant to the individuals if the other allele is actually present but unidentified by the technique or when they plan to have children.

Table 1. Disease phenotypes with the 22 pathogenic/likely pathogenic variants as secondary findings in 15 genes of 85 unrelated Thai individuals

Phenotype	Disease	Gene	Individuals	dbSNP ID	Genomic position	RefSeq mRNA	Nucleotide change	Amino acid change	ACMG classification	Evidence	Clinvar classification
Cancer (8)	Hereditary breast and ovarian cancer (2)	*BRCA1*	1	rs80357251	chr17: 41244913	NM_007300.4	c.2635 G > T	p.Glu879Ter	P	PVS1, PP5,PM2, PP3	P
		PALB2	1	rs747148023	chr16: 23641062	NM_024675.4	c.2411_2412del	p.Ser804CysfsTer10	P	PVS1, PP5,PM2	P/LP
	PTEN hamartoma tumor syndrome(6)	*PTEN*	6	rs587782455	chr10: 89720649	NM_000314.8	c.802-2 A > G	NA	P	PVS1, PP5,PM2, PP3	P
Cardiovascular (74)	Aortopathies (19)	*TGFBR2*	19	rs34833812	chr3: 30713619	NM_003242.6	c.1019 C > T	p.Thr340Met	LP	PM1,PP2, PP3,PP5, BS2	CIP;B(3), LB (5), LP (1), VUS(2)
	Arrhythmogenic right ventricular cardiomyopathy(10)	*DSP*	9	rs148894066	chr60: 7579930	NM_004415.4	c.3507 C > A	p.Tyr1169Ter	P	PVS1, PM2, PP3,PP5	LP
		PKP2	1	NA	chr12: 32994140	NM_004572.4	c.1511-1 G > C	NA	P	PVS1, PM2, PP3,PP5	P
	Catecholaminergic polymorphic ventricular tachycardia(1)	*RYR2*	1	rs794728707	chr1: 237540658	NM_001035.3	c.499 A > G	p.Lys167Glu	LP	PM1, PM2,PP3, PP5	LP
	Dilated cardiomyopathy(8)	*TNNT2*	6	rs397516484	chr1: 201328372	NM_001276345.2	c.863 G > C	p.Arg288Pro	LP	PM2, PM5, PP2,PP3	CIP;LP(4),P (1), VUS(1)
		TTN	1	rs886038916	chr2: 179418821	NM_001256850.1	c.84094 C > T	p.Arg28032Ter	P	PVS1, PP5, PM2,PP3	P/LP
			1	rs1057522831	chr2: 179415988	NM_001256850.1	c.86348-1 G > A	NA	P	PVS1, PM2, PP3,PP5	LP
	Familial hypercholesterolemia (15)	*LDLR*	1	rs112029328	chr19: 11213463	NM_000527.5	c.313 + 1 G > A	NA	P	PVS1, PM2, PP3	CIP; LB(1), LP (2), P(17)
			1	rs201102461	chr19: 11215926	NM_000527.5	c.344 G > A	p.Arg115His	LP	PM1, PM2,PP2	CIP;LB(1),LP (2), P(1),VUS (3)
			3	rs730882109	chr19: 11227576	NM_000527.5	c.1747C > T	p.His583Tyr	P	PM1, PM2, PM5,PP2, PP3	CIP;LB(1),LP (5), P(7),VUS (1)
			10	rs13306515	chr19: 11221443	NM_000527.5	c.1056 C > A	p.Cys352Ter	P	PVS1, PM2,PP5, PP3	P/LP
	Hypertrophic cardiomyopathy(17)	*MYBPC3*	16	rs573916965	chr11: 47364637	NM_000256.3	c.1000 G > A	p.Glu334Lys	LP	PM2,PP2, PP3, PP5	CIP;B(2),LP(1), P(2),VUS(6)
		MYH7	1	rs397516142	chr14: 23894566	NM_000257.4	c.2348 G > A	p.Arg783His	LP	PM1, PM2, PM5,PP2, BP4	CIP;LP(3),VUS (3)
	Long QT syndrome types 1 and 2 (2)	*KCNQ1*	1	rs199472776	chr11: 2608860	NM_000218.3	c.1189 C > T	p.Arg397Trp	LP	PM2, PM5, PP2, PP3	CIP;B(1),LP (3), VUS(9)
			1	rs199472737	chr11: 2594172	NM_000218.3	c.877 C > T	p.Arg293Cys	LP	PM2,PP2, PP3, PP5	CIP;LP(1),VUS (5)

Table 1 continued

Phenotype	Disease	Gene	Individuals	dbSNP ID	Genomic position	RefSeq mRNA	Nucleotide change	Amino acid change	ACMG classification	Evidence	Clinvar classification
	Long QT syndrome 3; Brugada syndrome (2)	*SCN5A*	1	rs199473561	chr3: 38655260	NM_001160161.2	c.677 C > T	p.Ala226Val	LP	PM1, PM2,PP2, PP3,PP5	CIP;B(1),LB(4), LP(1),VUS(5)
			1	rs199473603	chr3: 38603958	NM_001160161.2	c.3749 C > T	p.Thr1250Met	LP	PM1, PM2,PP2, PP3,PP5	CIP;B(1),LP(2), VUS(11)
Miscellaneous (3)	Malignant hyperthermia (3)	*RYR1*	1	rs200069592	chr19: 38951020	NM_000540.3	c.2366 G > A	p.Arg789Gln	LP	PM1, PM2, PP2, PP3	CIP;LB(10,LP (1),VUS(2)
			2	rs193922748	chr19: 38931469	NM_000540.3	c.130 C > T	p.Arg44Cys	LP	PM2,PP2, PP3,PP5	CIP,LP(1),P(1), VUS(8)

This study was the first to provide the prevalence of SFs from ES in a Thai population. Most are related to cardiovascular and cancer phenotypes. Early surveillance is critical to decreasing the risk of developing diseases and sudden death. The information would serve as a basis for precision medicine practice for the Thai population.

REFERENCES

1. Kamolvisit W, Phowthongkum P, Boonsimma P, Kuptanon C, Rojnueangnit K, Wattanasirichaigoon D, et al. Rapid exome sequencing as the first-tier investigation for diagnosis of acutely and severely ill children and adults in Thailand. Clin Genet. 2021;100:100–105.
2. Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med 2013;15:565–74.
3. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, et al. 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. Genet Med 2017;19:249–55.
4. Jang MA, Lee SH, Kim N, Ki CS. Frequency and spectrum of actionable pathogenic secondary findings in 196 Korean exomes. Genet Med 2015;17:1007–11.
5. Yu MHC, Mak CCY, Fung JLF, Lee M, Tsang MHY, Chau JFT, et al. Actionable secondary findings in 1116 Hong Kong Chinese based on exome sequencing data. J Hum Genet. 2020;66:637–641.
6. Jalkh N, Mehawej C, Chouery E. Actionable exomic secondary findings in 280 Lebanese participants. Front Genet 2020;11:208.
7. Kuo CW, Hwu WL, Chien YH, Hsu C, Hung MZ, Lin IL, et al. Frequency and spectrum of actionable pathogenic secondary findings in Taiwanese exomes. Mol Genet Genom Med 2020;8:e1455.
8. Tang CS, Dattani S, So MT, Cherny SS, Tam PKH, Sham PC, et al. Actionable secondary findings from whole-genome sequencing of 954 East Asians. Hum Genet 2018;137:31–7.
9. Jain A, Gandhi S, Koshy R, Scaria V. Incidental and clinically actionable genetic variants in 1005 whole exomes and genomes from Qatar. Mol Genet Genomics. 2018;293:919–29.
10. Haer-Wigman L, van der Schoot V, Feenstra I. Vulto-van Silfhout AT, Gilissen C, Brunner HG, et al. 1 in 38 individuals at risk of a dominant medically actionable disease. Eur J Hum Genet 2019;27:325–30.
11. Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23:13891–1390.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. 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. Genet Med 2015;17:405–24.
13. Miller DT, Lee K, Gordon AS, Amendola LM, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23:1391–1398.
14. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25:1754–60.
15. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20:1297–303.
16. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38:e164.
17. Kopanos C, Tsiolkas V, Kouris A, Chapple CE, Albarca Aguilera M, Meyer R, et al. VarSome: the human genomic variant search engine. Bioinformatics 2019;35:1978–80.
18. Semsarian C, Ingles J, Maron MS, Maron BJ. New perspectives on the prevalence of hypertrophic cardiomyopathy. J Am Coll Cardiol 2015;65:1249–54.
19. Aftab M, Cikach FS, Zhu Y, Idrees JJ, Rigelsky CM, Kalahasti V, et al. Loeys-Dietz syndrome: intermediate-term outcomes of medically and surgically managed patients. J Thorac Cardiovasc Surg 2019;157:439–50. e5
20. Najam O, Ray KK. Familial hypercholesterolemia: a review of the natural history, diagnosis, and management. Cardiol Ther 2015;4:25–38.
21. Huang CC, Charng MJ. Genetic diagnosis of familial hypercholesterolemia in Asia. Front Genet 2020;11:833.
22. Petrucelli N, Daly MB, Feldman GL. Hereditary breast and ovarian cancer due to mutations in BRCA1 and BRCA2. Genet Med 2010;12:245–59.
23. Collet C, Laplanche JL, Page J, Morel H, Woimant F, Poujois A. High genetic carrier frequency of Wilson's disease in France: discrepancies with clinical prevalence. BMC Med Genet 2018;19:143.

24. Park HD, Ki CS, Lee SY, Kim JW. Carrier frequency of the R778L, A874V, and N1270S mutations in the ATP7B gene in a Korean population. Clin Genet 2009;75:405–7.
25. Fabisikova K, Hamidova O, Behulova RL, Zavodna K, Priscakova P, Repiska V. Case report: the role of molecular analysis of the MUTYH gene in asymptomatic individuals. Front Genet 2020;11:590486.

ACKNOWLEDGEMENTS

We would like to thank the patients and their families for participating in this study, clinicians for collecting clinical information, and bioinformaticians for analyzing and managing the database. This work was supported by Thailand Research Fund (DPG6180001), Health Systems Research Institute, TSRI Fund (CU_FRB640001_01_30_10), and the Second Century Fund (C2F), and Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (764002-HE01).

AUTHOR CONTRIBUTIONS

W.C.: data curation, formal analysis, and writing the original draft; V.S.: conceptualization, funding acquisition, and editing the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s10038-021-00982-2.

Correspondence and requests for materials should be addressed to Vorasuk Shotelersuk.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.