LETTER TO THE EDITOR

Identification of clinically actionable secondary genetic variants from whole-genome sequencing in a large-scale Chinese population

To the Editor:
Clinical DNA sequencing is increasingly being chosen as a diagnostic test for Mendelian disorders in genomic medicine. Besides the primary findings, clinically actionable secondary genetic variants could be detected in the DNA sequencing. The genetic variants from genes proposed by American College of Medical Genetics and Genomics (ACMG) should be reported to clinician as secondary findings if the annotation suggested pathogenic or likely pathogenic. With the increasing application of DNA sequencing in the clinic, the ACMG updated the SF v3.0 list to 73 genes in 2021. Ethnic disparities exist in allele frequency of pathogenic variants. From the NHLBI Exome Sequencing Project (ESP), 0.7% and 0.5% of adults of European and African ancestry, respectively, were expected to have highly actionable penetrant pathogenic variants. Approximately 7% of 196 Korean individuals exhibited pathogenic variants, and at least one pathogenic variant was reported in 21% of 2049 Japanese individuals. The carrier frequency of secondary findings was highly variable among populations, but the prevalence of pathogenic or likely pathogenic variants (P/LP) in Chinese population remains unclear.

We analysed 4480 individuals’ whole-genome sequencing data from Westlake BioBank for Chinese pilot project (WBBC) to evaluate the prevalence of pathogenic genetic variants in the Chinese population for the 73 genes recommended by ACMG, and further investigated the ethnic differences among worldwide populations. A total of 9373 variants were found in the coding region, splicing site, intron and UTR in the WBBC samples, with 97.3% of these being missense and synonymous variants (Table S1). Following the variant classification standard (Figure 1 and Supporting Information), we identified 295 P/LP variants (99 pathogenic and 196 likely pathogenic variants, Table S2), accounting for 3.15% of the variants. For autosomal dominant inheritance (AD), the ratio of the P/LP variants was highest for TNNT2 (24.14%), LDLR (21.65%) and SCN5A (14.69%) genes (Table S3). The highest ratio of the P/LP variants was shown by MUTYH (24.07%), ATP7B (23.93%) and GAA (12.93%) for the autosomal recessive inheritance (AR). Additionally, 20% (3/15) of the variants were P/LP variants in GLA (X-linked inheritance) gene.

FIGURE 1 Scheme of pathogenic/likely pathogenic (P/LP) variants analysis pipeline. These variants were extracted from 4480 Chinese individuals in the WBBC project cohort. A total of 167120 variants were annotated by the ANNOVAR, ClinVar and HGMD. The database HGMD Professional classified the pathogenic variants into disease-causing or likely disease-causing mutation (DM or DM?)
| Gene      | Transcript | cDNA     | Protein   | ID       | WBBC   | EAS | EUR | gnomAD   | P/LP | Inheritance | Diseases                                  |
|-----------|------------|----------|-----------|----------|--------|-----|-----|----------|------|--------------|-------------------------------------------|
| APC       | NM_000383.6| c.5912C>G| p.Ser1971Cys | rs754691867 | 0.0012 | 0   | 0   | 0.000065 | LP   | AD           | Familial adenomatous polyposis            |
| APOB      | NM_000384.3| c.10579C>T| p.Arg3527Trp | rs144467873 | 0.0011 | 0.001| 0   | 0.000065 | LP   | AD           | Familial hypercholesterolemia            |
| ATP7B     | NM_000503.4| c.2333G>T| p.Arg778Leu | rs28942074  | 0.0018 | 0   | 0   | 0.000097 | P    | AR           | Wilson's disease                          |
| ATP7B     | NM_000503.4| c.3316G>A | p.Val1106Ile | rs541208827 | 0.0018 | 0.002| 0   | 0.0002   | LP   | AR           | Wilson's disease                          |
| ATP7B     | NM_000503.4| c.3443T>C | p.Ile1148Thr | rs60431989  | 0.0013 | 0   | 0   | 0.00032  | P    | AR           | Wilson's disease                          |
| BRC2      | NM_000509.3| c.7088A>G | p.Tyr2363Cys | rs80358939  | 0.0009 | 0   | 0   | 0        | LP   | AD           | Hereditary breast and ovarian cancer      |
| BTD       | NM_000600.4| c.1330G>C | p.Asp444His  | rs13078881  | 0.0006 | 0   | 0.0427| 0.0286   | LP   | AR           | Biotinidase deficiency                   |
| DSG2      | NM_001943.5| c.1592T>G| p.His531Cys  | rs200485060 | 0.0016 | 0   | 0   | 0.000065 | LP   | AD           | Arrhythmogenic right ventricular cardiomyopathy |
| GAA       | NM_000152.5| c.2132C>G| p.Asp711Arg  | rs759292700 | 0.0018 | 0   | 0   | 0.000032 | LP   | AR           | Pompe disease                            |
| GAA       | NM_000152.5| c.32-13T>G| .           | rs386834236  | 0.0003 | 0   | 0.007| 0.003    | P    | AR           | Pompe disease                            |
| GLA       | NM_000169.3| c.1067G>A | p.Arg356Gln  | rs869312163 | 0.0015 | 0   | 0   | 0        | LP   | XL           | Fabry disease                            |
| GLA       | NM_000169.3| c.640-801G>A | .         | rs199473684  | 0.0010 | 0   | 0   | 0.00046  | P    | XL           | Fabry disease                            |
| LDLR      | NM_000527.5| c.1765G>A | p.Asp589Asn  | rs201971888 | 0.0015 | 0.003| 0   | 0.00032  | LP   | AD           | Familial hypercholesterolemia            |
| LDLR      | NM_000527.5| c.344G>A  | p.Arg115His  | rs201102461 | 0.0017 | 0.001| 0   | 0.0001   | LP   | AD           | Familial hypercholesterolemia            |
| LDLR      | NM_000527.5| c.769C>T  | p.Arg257Trp  | rs200990725 | 0.0015 | 0.003| 0   | 0.00065  | LP   | AD           | Familial hypercholesterolemia            |
| MSH2      | NM_000251.2| c.14C>A   | p.Pro589Gln  | rs56170584  | 0.0025 | 0   | 0   | 0        | LP   | AD           | Lynch syndrome                           |
| MSH2      | NM_000251.2| c.2516A>G | p.His839Arg  | rs63750277  | 0.0012 | 0   | 0   | 0.00065  | LP   | AD           | Lynch syndrome                           |
| MUTYH     | NM_001048171.1| c.1145G>A | p.Gly382Asp  | rs36053993  | 0.0002 | 0   | 0.0089| 0.0032   | P    | AR           | MUTYH-associated polyposis               |
| MYBPC3    | NM_000256.3| c.2504G>T | p.Arg835Leu  | rs527305885 | 0.0013 | 0.002| 0   | 0.00065  | LP   | AD           | Hypertrophic cardiomyopathy              |
| MYH7      | NM_000257.4| c.1322G>T | p.Thr441Met  | rs121913653 | 0.0011 | 0   | 0   | 0.0002   | LP   | AD           | Hypertrophic cardiomyopathy              |
| RYR1      | NM_000540.2| c.11518G>A | p.Val3840Ile | rs140616359 | 0.0010 | 0.001| 0   | 0.00065  | LP   | AD           | Malignant hyperthermia                   |
| SCN5A     | NM_198056.2| c.3539C>T | p.Ala1180Val | rs4130765   | 0.0033 | 0.001| 0   | 0.0002   | LP   | AD           | Long QT syndrome 3                       |

Abbreviations: EAS, the allele frequency of East Asian in the 1000 Genome Project; EUR, the allele frequency of European in the 1000 Genome Project; gnomAD, gnomAD_hg19_r211; Mode of inheritance, AD (autosomal dominant), AR (autosomal recessive) and XL (X-linked); WBBC, the allele frequency of Chinese in the Westlake BioBank for Chinese.
At the population level, approximately 17.37% (778/4480) of Chinese individuals carried at least one reported P/LP variant, whereas 4.2% (186/4480) of individuals had the pathogenic (P) variants. Because the 4480 samples also included individuals with Parkinson’s disease (PD), we estimated a population frequency of 16.6% for P/LP variants in the PD patients and 18% in relatively healthy individuals. The proportion of P/LP carriers showed no significant differences between the PD patients and relatively healthy individuals \( (p = .297) \). Excluding the autosomal recessive condition carriers, the prevalence of P/LP variants was 10.9% (488/4480) compared to 1.4% (62/4480) for pathogenic variants in the WBBC cohort. For the autosomal dominant cardiovascular and cancer diseases, we found that 7.32% and 2.67% of the individuals carried P/LP variants in 31 cardiovascular and 27 cancer genes, respectively. A closer look at the single gene, MUTYH (3.15%, AR), ATP7B (2.86%, AR), SCN5A (1.96%, AD), LDLR (1.72%, AD) and GAA (1.03%, AR) showed a relatively high population frequency of the P/LP variants in the Chinese population (Table S3).

Our study observed significant ethnic differences in allele frequency of likely pathogenic or pathogenic variants between Chinese and European populations (Table 1 and Figure 2). We found that 24 P/LP variants from 15 genes exhibited relatively remarkable ethnic differences (Table 1). The minor allele frequencies of variants p.Pro5Gln (MSH2, Figure 2A), c.850-2A>G (MUTYH, Figure 2B) and p.Ala1180Val (SCN5A, Figure 2D) in the WBBC were relatively higher than in non-East Asian populations (Supporting Information). Contrastingly, p.Gly382Asp (MUTYH, Figure 2C), c.-32-13T>G (GAA, Figure 2E) and p.Asp444His (BTD, Figure 2F) showed a significantly high allele frequency in European population. We found an unusual difference in the pathogenic variant p.Asp444His in the BTD gene where the allele frequency exceeded 2% in South Asian, European and Admixed American populations (MAF_SAS = 0.035, MAF_EUR = 0.043 and MAF_AMR = 0.019). However, this variant was very rarely detected in the East Asian population (MAF_WBBC = 0.0006 and MAF_EAS = 0). In fact, the prevalence of biotinidase deficiency in East Asian (1/15 000 in Japanese and 1/620 400 in Chinese\(^8\)) was lower than other ethnic groups (e.g., 1/9000 in Brazil\(^9\); please refer to the Supporting Information for more details). To access the full list of the variants, we provided a user-friendly
website to search for the annotation and frequency of variants in Chinese and other populations (https://wbcc.westlake.edu.cn/).

Considering the ethnic discrepancies in incidence of diseases, the recommendation list should include highly penetrant phenotypes and genes in the East Asian population. Citrin deficiency, an inherited autosomal recessive metabolic disease, was initially reported and found mostly in individuals of East Asian ancestry. We found four heterozygous pathogenic variants of SLC25A13, c.550C>T (p.Arg184*), c.615+5G>A, c.852_855del and c.1180+1G>A in 1.5% (66/4480) in the individuals from WBBC. The c.852_855del variant in SLC25A13 gene was the most common variants among East Asians (MAF_WBBC = 0.006 and MAF_EAS = 0.004) but rarely detected in other populations.

In conclusion, we found that approximately 17.37% (778/4480) of Chinese individuals carried at least one reported P/LP variant in the 73 genes recommended by ACMG, and 295 P/LP genetic variants were detected in our WBBC pilot cohort. We observed ethnic differences in allele frequency of P/LP variants between Chinese and European populations, 24 P/LP variants from 15 genes exhibited relatively remarkable ethnic differences (such as rs13078881 on BTD for biotinidase deficiency). We also suggested that high-penetrance genes (e.g., SLC25A13 gene for citrin deficiency) in the East Asians should be included in the recommendation list. Prevention and early intervention could reduce the risk of potentially severe consequences of genetic disorders for the undiagnosed carriers; therefore, secondary findings should be incorporated in clinical DNA sequencing reports appropriately.

ACKNOWLEDGEMENTS
This study was supported by a grant from National Natural Science Foundation of China (32061143019). We are grateful to staffs from the High-Performance Computing Center at Westlake University for technical support.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

Shu-Yang Xie, Bei-Sha Tang, Hou-Feng Zheng

1Diseases & Population (DaP) Geninfo Lab, School of Life Sciences, Westlake University, Hangzhou, Zhejiang, China
2Westlake Laboratory of Life Sciences and Biomedicine, Hangzhou, Zhejiang, China
3Institute of Basic Medical Sciences, Westlake Institute for Advanced Study, Hangzhou, Zhejiang, China
4Department of Dermatology, Hangzhou Hospital of Traditional Chinese Medicine, Hangzhou, Zhejiang, China
5Clinical Genome Center, KingMed Diagnostics, Co. Ltd., Guangzhou, Guangdong, China
6WBBC Shandong Center, Binzhou Medical University, Yantai, Shandong, China
7WBBC Jiangxi Center, Jiangxi Medical College, Shangrao, Jiangxi, China
8National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan, China
9Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, China

Correspondence
Hou-Feng Zheng, School of Life Sciences, Westlake University, Hangzhou, Zhejiang, China.
Email: zhenghoucheng@westlake.edu.cn
 Bei-Sha Tang, National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan, China.
Email: bstang7398@163.com
 Ke-Qi Liu, WBBC Jiangxi Center, Jiangxi Medical College, Shangrao, Jiangxi, China.
Email: lkq2550598@126.com

Shu-Yang Xie, WBBC Shandong Center, Binzhou Medical University, Yantai, Shandong, China.
Email: shuyangxie@aliyun.com

ORCID
Pei-Kuan Cong https://orcid.org/0000-0002-4921-5657
Saber Khederzadeh https://orcid.org/0000-0002-0115-8710
Bei-Sha Tang https://orcid.org/0000-0003-2120-1576
Hou-Feng Zheng https://orcid.org/0000-0001-5681-8598

REFERENCES
1. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 2013;15:565-574.
2. Miller DT, Lee K, Chung WK, 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;23:1381-1390.

3. Amendola LM, Dorschner MO, Robertson PD, et al. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Res*. 2015;25:305-315.

4. Jang MA, Lee SH, Kim N, et al. Frequency and spectrum of actionable pathogenic secondary findings in 196 Korean exomes. *Genet Med*. 2015;17:1007-1011.

5. Yamaguchi-Kabata Y, Yasuda J, Tanabe O, et al. Evaluation of reported pathogenic variants and their frequencies in a Japanese population based on a whole-genome reference panel of 2049 individuals. *J Hum Genet*. 2018;63:213-230.

6. Peikuan C, Weiyang B, Jinchen L, et al. Genomic analyses of 10,376 individuals in the Westlake BioBank for Chinese (WBBC) pilot project. *Nature Portfolio*. 2021. https://doi.org/10.21203/rs.3.rs-814288/v1

7. Zhu XW, Liu KQ, Wang PY, et al. Cohort profile: the Westlake BioBank for Chinese (WBBC) pilot project. *BMJ Open*. 2021;11:e045564.

8. Hong F, Huang X, Zhang Y, et al. [Screening for newborn organic aciduria in Zhejiang province: prevalence, outcome and follow-up]. *Zhejiang Da Xue Xue Bao Yi Xue Ban*. 2017;46:240-247.

9. Neto EC, Schulte J, Rubim R, et al. Newborn screening for biotinidase deficiency in Brazil: biochemical and molecular characterizations. *Braz J Med Biol Res*. 2004;37:295-299.

10. Ohura T, Kobayashi K, Tazawa Y, et al. Neonatal presentation of adult-onset type II citrullinemia. *Hum Genet*. 2001;108:87-90.

**Supporting Information**
Additional supporting information may be found in the online version of the article at the publisher’s website.