# Whole exome sequencing identified mutations causing hearing loss in five consanguineous Pakistan families

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# Abstract

Background Hearing loss is the most common sensory defect that affects over 6% of the population worldwide. About 50%-60% of hearing loss patients are attributed to genetic causes. Currently more than 100 genes have been reported to cause non-syndromic hearing loss. It's possible and efficient to screen all potential disease-causing genes for hereditary hearing loss by whole exome sequencing (WES).

Methods We collected 5 consanguineous pedigrees with hearing loss from Pakistan and applied WES on selected patients for each pedigree, followed by bioinformatics analysis and Sanger validation to identify the causing genes for them.

Results Variants in 7 genes were identified and validated in these pedigrees. We identified single candidate for 3 pedigrees, which were GIPC3 (c.937T>C), LOXHD1 (c.2935G>A) and TMPRSS3 (c.941T>C). And the remaining 2 pedigrees each contained two candidates, which were TECTA (c.4045G>A) and MYO15A (c.3310G>T and c.1705G>C) for one pedigree and DFNB59 (c.494G>A) and TRIOBP (c.1952C>T) for the other pedigree. The candidates were validated in all available samples by Sanger sequencing.

Conclusion The candidate variants in hearing loss genes were validated to be co-segregated in the pedigrees, which may indicate the reasons for such patients. We also suggested that WES may be suitable strategy for hearing loss screening in clinical detection.


# Background

Hearing loss is the most common sensory defect that affects ~1/500 newborns [1] and 466 million people worldwide (https://www.who.int/pbd/deafness/estimates/en/). About 50%~60% of hearing loss patients are attributed to genetic causes [1, 2]. Hereditary hearing loss is a genetically heterogeneous disorder [3] that can be divided into syndromic hearing loss and non-syndromic hearing loss, among which non-syndromic hearing loss is the predominant type with a proportion of ~80% [4]. Currently more than 100 genes have been reported to cause non-syndromic hearing loss (https://hereditaryhearingloss.org/), and the total number of genes related to hearing loss is expected to be several hundreds.

There are mature gene panels for hearing loss detection, and the genes involved range from 4 to more than 100. However, except for several genes, such as *GJB2* [5-7] or *SLC26A4* [8-10], most causing genes contribute a little fraction for the disorder. So, we may not obtain a satisfied result by gene panel screening for many cases in clinical detection. As the whole exome sequencing (WES) technology rapidly developed and its cost becomes cheaper, it's possible and efficient to screen all potential disease-causing genes for hereditary hearing loss by WES [11, 12].

Recessive inheritance hearing loss is worth studying because such patients usually have normal parents, which makes the disorder seem to be "sudden strike", and this situation is more difficult to prevent. Consanguineous pedigree is suitable natural model to study recessive disorders [13]. In Pakistan, there are numerous consanguineous pedigrees because of their custom, which may bring more opportunity to study and recognize such disorders [14, 15].

In this study, we collected 5 consanguineous pedigrees with hearing loss from Pakistan and applied WES to identify the causing genes for them. We identified several variants in hearing loss genes that co-segregated in the pedigrees, which may indicate the reasons for such patients.

# Methods

### Participants and clinical diagnosis

In the present study we collected 5 consanguineous pedigrees containing 22 patients of hearing loss from rural areas in Pakistan. All the patients showed different degrees of hearing loss. The most likely inheritance mode for these pedigrees were autosomal recessive (Fig1). The study was approved by the ethical committee of National institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan and all participants provided written informed consent.

### DNA extraction and Whole exome sequencing

According to the manufacturer's instructions, genomic DNA was isolated from peripheral blood leukocytes of all participants using DNA QIAamp mini kit (Qiagen, Hilden, Germany). One patient from each pedigree was selected and performed WES. Exons were captured using BGI-Exome kit V4 and sequenced by BGI-seq 500 with 100bp paired-end.

### Bioinformatics analysis

Low-quality reads were removed by SOAPnuke [16], then the reads were mapped to the human genome reference (UCSCGRCh37/hg19) by Burrows–Wheeler Aligner (BWA-MEM, version 0.7.10) [17]. Variants were called using the Genome Analysis Tool Kit (GATK, version 3.3) [18]. Variant Effect Predictor (VEP) [19] was used to annotate and classify all the variants. After that all the variants were filtered based on their frequency in public databases (e.g., 1000 genome project, exome sequencing project and ExAC) and our in-house databases, and the variants with MAF<0.005 were retained. Then homozygous variants and compound heterozygous variants were selected because the most likely inheritance mode for these pedigrees were autosomal recessive. Finally, we applied several variants prediction tools including SIFT [20] (http://provean.jcvi.org/), PolyPhen2 [21] (http://genetics.bwh.harvard.edu/pph2/), MutationTaster [22] (http://www.mutationtaster.org/) and CADD [23] to predict the functional impact of candidate variants [24].

### Sanger validation

DNA from all available samples in the five pedigrees was Sanger sequenced to validate the variants and confirm their co-segregation in pedigree. Forward and reverse primers were designed by Primer3. After PCR amplification, the purified product was sequenced on ABI 3730XL DNA Analyzer.

## Results

### Clinical features

All the patients showed different degrees of hearing loss. In the first pedigree (HL1), all patients showed severe deafness, and one patient (III1) was selected to perform WES. In the second pedigree (HL2), all patients showed congenital profound deafness and mute, and one patient (IV1) was selected to perform WES. In the third pedigree (HL3), all patients showed moderate deafness, their hearing loss started after seizures, and one patient (V1) was selected to perform WES. In the fourth pedigree (HL4), all patients showed congenital profound deafness, and one patient (IV1) was selected to perform WES. In the fifth pedigree (HL5), all patients showed moderate deafness, and one patient (III5) was selected to perform WES.

### Genetic analysis

WES was applied on the selected patients. An average depth of target region was 146X with a coverage of 99.85%, and the coverage of target region that sequenced at least 10 times (depth >= 10 X) was 98.20% (Table 1). For each individual, more than ten thousand variants that may influence protein were identified. After the frequency filtration (MAF<0.005), about 15~32 exon variants were retained. Further inheritance model filtration kept 1~6 candidates left (Table 1). All the rare variants detected in exon region for the pedigrees were listed in supplementary table.

We identified a stop-codon lost homozygous variant *GIPC3*: c.937T>C from the patient in HL1 pedigree, and the variant prediction tools provided a benign prediction. For the patient from HL2 pedigree, we identified 4 variants in 2 genes. However, one gene was reported to cause autosomal dominant hearing loss so we first analyzed the other one. And then a homozygous variant *LOXHD1*: c.2935G>A was regarded as candidate for this pedigree. The variant prediction tools provided a damaging prediction. For the patient from HL3 pedigree, 6 variants in 5 genes were identified at first, further analysis indicated that only 2 genes may cause autosomal recessive deafness. So, a homozygous variant *TECTA*: c.4045G>A and two compound heterozygous variants c.3310G>T and c.1705G>C in *MYO15A* were regarded as candidates. The variant prediction tools provided benign prediction for the first two variants and damaging prediction for the last variant. For the patient from HL4 pedigree, 3 variants in 3 genes were identified, and two of them may cause autosomal recessive deafness. The two homozygous variants were *DFNB59*: c.494G>A and *TRIOBP*:c.1952C>T. The variant prediction tools provided damaging prediction for the first variant and benign prediction for the last variant. For the patient from HL5 pedigree, only one homozygous candidate was identified, *TMPRSS3*: c.941T>C. And the variant prediction tools provided a damaging prediction. All these variants were not reported to cause hearing loss before. The detailed information was listed in Table 2.

In summary, we identified one most likely causing variant for HL1, HL2 and HL5 pedigrees and two most likely causing candidates for HL3 and HL4 pedigrees.

### Sanger validation

To validate co-segregation in pedigree, we applied Sanger sequencing on all available samples. In total, 6 samples (II1~2 and III1~4) were sequenced for HL1 pedigree, 6 samples (III1~2 and IV1~4) were sequenced for HL2 pedigree, 4 samples (IV1 and V1~3) were sequenced for HL3 pedigree, and 21 samples (I1, II1~11 and III1~9) were sequenced for HL5 pedigree. For HL4 pedigree, the samples collected at the first time were degraded and we failed to collect additional samples. All the variants selected to perform Sanger sequencing were co-segregated in pedigree except for HL4 (Fig 2).

## Discussion

In this study, we identified several variants in genes reported to cause hearing loss that co-segregated in the pedigree. For HL1 pedigree, the variant in *GIPC3* gene may cause the disorder. *GIPC3* encodes a 312-residue protein that contains three predicted low complexity regions and a central conserved PDZ domain [25]. The PDZ domain of *GIPC3* is required for the survival of spiral ganglion and hair cells in the mouse ears. This gene was reported to cause autosomal recessive deafness 15, non-syndromic genetic deafness and audiogenic seizures. Currently 11 pathogenic variants were reported in this gene from clinvar.

For HL2 pedigree, the variant in *LOXHD1* gene was the likely causative one. *LOXHD1* encodes a highly conserved stereociliary protein consisting of 15 polycystin-1/lipoxygenase/alpha-toxin (PLAT) domains, which facilitates proteins interacting with the plasma membrane [26]. *Loxhd1* in mice plays a crucial role in maintaining normal function of cochlear hair cells [27]. It was reported to cause disorders including autosomal recessive deafness 77. Currently 28 pathogenic variants were reported in this gene from clinvar.

For HL3 pedigree, a homozygous variant in *TECTA* and two compound heterozygous variants in *MYO15A* were the likely candidates. *TECTA* encodes a protein containing 2,155 amino acids that is one of the major non-collagenous glycoproteins of the tectorial membrane, a non-cellular matrix overlying the cochlear neuroepithelium that lies over stereocilia of the hair cells and is critical for the mechanical amplification and transmission of sound [28, 29]. This gene was reported to cause autosomal recessive deafness 21, and 40 pathogenic variants were reported in this gene from clinvar. The protein encoded by *MYO15A* is a member of the unconventional myosin super-family and plays an indispensable role in the graded elongation of stereocilia and actin organization in hair cells of the inner ear, which are essential for normal hearing function [30]. *MYO15A* was reported to cause autosomal recessive deafness 3, and 112 pathogenic variants were reported

in this gene from clinvar. Considering the transmission of variants in the pedigree (this is a consanguineous pedigree), we thought the homozygous variant in *TECTA* was more likely to cause the disorder in this pedigree.

For HL4 pedigree, homozygous variants were detected in both *DFNB59* and *TRIOBP* genes. *DFNB59* encodes a protein contains 352 amino acids which plays a crucial role in auditory nerve signaling transmission [31]. It was reported to cause autosomal recessive deafness 59, and 9 pathogenic variants were reported in this gene from clinvar. *TRIOBP* encodes a protein contains 652 amino acids which plays a role in regulation of adherens junctions as well as reorganization of the actin cytoskeleton [32]. Actually, little is known about the exact function of *TRIOBP*, and multiple roles of this gene raised the issue why pathogenic variants in this gene do not lead to other pathologies than isolated hearing loss. This gene was reported to cause autosomal recessive deafness 28, and 26 pathogenic variants were reported in this gene from clinvar. The variant in *TRIOBP* was annotated as likely benign in deafness variation database. So, we thought the variant in *DFNB59* was more likely to be responsible for the disorder in this pedigree.

For HL5 pedigree, the variant in *TMPRSS3* may cause the disorder. The protein encoded by this gene contains a serine protease domain, a transmembrane domain, an LDL receptor-like domain, and a scavenger receptor cysteine-rich domain. It plays an important role in activating the ENaC sodium channel, which is regulated by serine protease activity [33], and maintains a low $Na^+$ concentration in the endolymph of the inner ear [34]. *TMPRSS3* was reported to cause autosomal recessive deafness 8, and 23 pathogenic variants were reported in this gene from clinvar.

We calculated the density of reported pathogenic variants in these genes and they were 11.7/kb, 4.2/kb, 6.2/kb, 10.6/kb, 8.5/kb, 3.7/kb and 16.8/kb for *GIPC3*, *LOXHD1*, *TECTA*, *MYO15A*, *DFNB59*, *TRIOBP* and *TMPRSS3*, respectively. The density may indicate the degree of understanding or focusing for different genes. And the genes with low density such as *LOXHD1* and *TRIOBP* may have potential research value.

The majority of causing genes we identified for these pedigrees were not common hearing loss genes. If we applied common hearing loss gene panel to screen these patients, we would obtain negative results and the causing gene/variant for the patients would be missed. So, WES may be better strategy than panel sequencing for hearing loss screening even in clinical detection.

# Conclusion

In conclusion, we applied WES on five consanguineous pedigrees (one patient per pedigree) with hearing loss from Pakistan, followed by Sanger sequencing for all available samples among the pedigrees to identify the causing genes for them. Several variants in hearing loss genes were validated to be co-segregated in the pedigrees, which may indicate the reasons for such patients. Moreover, we suggested that WES may be suitable strategy for hearing loss screening in clinical detection.

# Declarations

### Ethics approval and consent to participate

This study was approved by the ethical committee of National institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan and all participants provided written informed consent.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The data that support the findings of this study have been deposited in the CNSA (https://db.cngb.org/cnsa/) of CNGBdb with accession code CNP0000508-sub010963.

### Competing interests

The authors declare that they have no competing interests.


### Funding

No funding was obtained for this study.


### Authors' contributions

YZ analyzed and interpreted the WES data and write the manuscript. MT analyzed and interpreted the patient data and modified the manuscript. SH analyzed and interpreted the WES data. UA performed Sanger sequencing. JZ designed the project and guided the analysis of WES data. SB designed the project, guided the analysis of patient data and helped the manuscript. All authors read and approved the final manuscript.


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# Tables

**Table 1. Sequencing and variants data**

| Pedigrees | HL1 | HL2 | HL3 | HL4 | HL5 |
|---|---|---|---|---|---|
| Samples applied WES | III1 | IV1 | V1 | IV1 | III5 |
| Sequencing depth (X) | 136.98 | 136.47 | 142.36 | 143.74 | 170.79 |
| Coverage (%) | 99.75 | 99.88 | 99.9 | 99.83 | 99.9 |
| 10X coverage (%) | 98.11 | 98.21 | 98.21 | 97.84 | 98.65 |
| Exon variants with MAF<0.005 | 23 | 22 | 32 | 15 | 19 |
| Variants followed recessive model | 1 | 4 | 6 | 3 | 1 |
| Variants applied Sanger validation | 1 | 1 | 3 | 0 | 1 |

**Table 2. Detailed information for candidate variants**

| Pedigree | Variant | RS_number | Gene | Strand | DNA change | AA change | Type | SIFT | PolyPhen2 | MutationTaster | CADD | ACMG classify |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HL1 | 19-3590186-T-C | rs1466835034 | GIPC3 | + | c.937T>C | p.*313Gluext*98 | homozygous | NA | NA | polymorphism | Benign | VUS |
| HL2 | 18-44063569-C-T | rs774836161 | LOXHD1 | - | c.6136G>A | p.Glu2046Lys | homozygous | Damaging | Damaging | disease_causing | Damaging | VUS |
| HL3 | 11-121016765-G-A | rs141024429 | TECTA | + | c.4045G>A | p.Ala1349Thr | homozygous | Tolerated | Benign | disease_causing | Benign | VUS |
| HL3 | 17-18025424-G-T | rs919809633 | MYO15A | - | c.3310G>T | p.Gly1104Cys | compound heterozygous | Damaging | Benign | polymorphism | Benign | VUS |
| HL3 | 17-18069800-G-C | rs535441567 | MYO15A | - | c.9913G>C | p.Glu3305Gln | compound heterozygous | Damaging | Damaging | disease_causing | Damaging | VUS |
| HL4 | 2-179320814-G-A | NA | DFNB59 | - | c.494G>A | p.Ser165Asn | homozygous | Damaging | Damaging | disease_causing | Damaging | VUS |
| HL4 | 22-38120515-C-T | rs760246167 | TRIOBP | + | c.1952C>T | p.Ser651Phe | homozygous | Damaging | Benign | polymorphism | Benign | VUS |
| HL5 | 21-43795850-A-G | NA | TMPRSS3 | - | c.941T>C | p.Leu314Pro | homozygous | Damaging | Damaging | disease_causing | Damaging | VUS |

# Figures

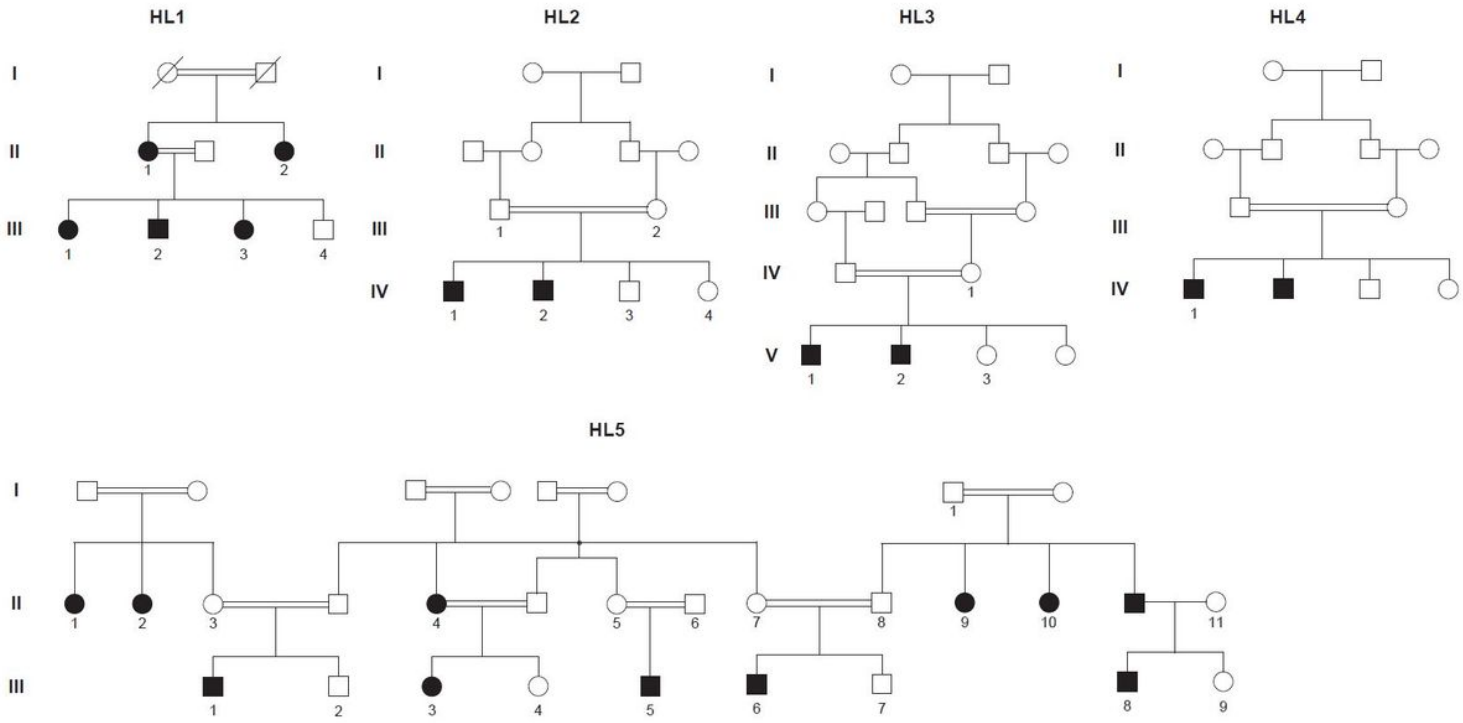


**Figure 1**

Pedigree figures of the five consanguineous families. The squares and the circles represent males and females, respectively. The black-filled symbols indicate patients with hearing loss and a diagonal line symbol indicates a deceased family member. The samples with numbers indicate that their DNA are available.

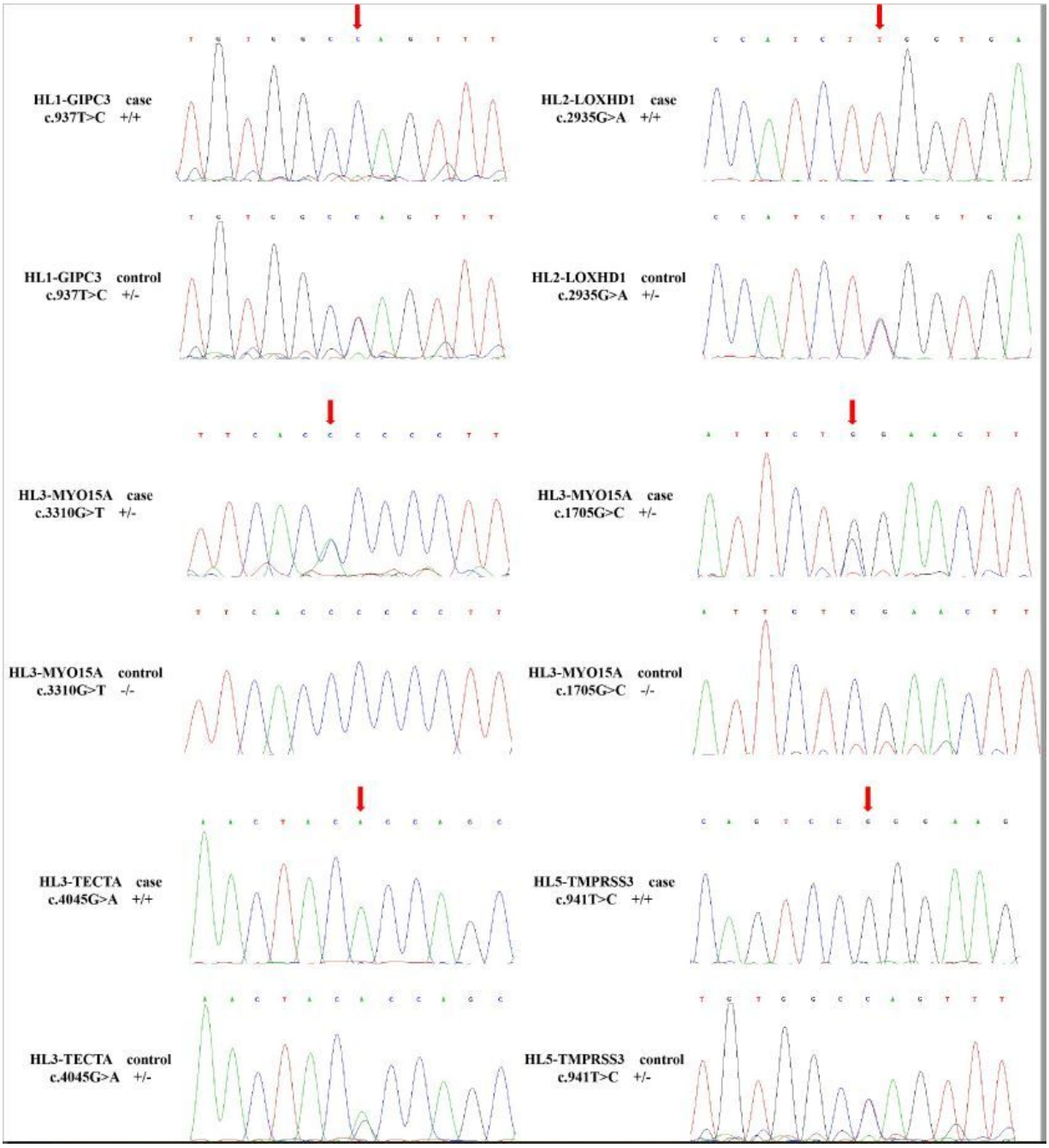


**Figure 2**

Sanger results of the candidate variants. The 6 variants are shown. Each variant contains a case and a control in the family. Sanger result of the case is on the top while Sanger result of the control is on the bottom. The red arrow indicates the variant site.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarytable.xlsx