Next-generation sequencing identifies rare pathogenic and novel candidate variants in a cohort of Chinese patients with syndromic or non-syndromic hearing loss

Abstract:

- **Background**: Some disorders like Hearing Loss (HL) caused by mutations other than the hotspot mutations in the common disorder genes, which could be difficult, and need a lot of time to be discovered by the traditional ways of DNA analysis.
- The **problem**: Hotspot mutations in specific DNA regions are not necessarily the only deafness-causing genes, so more efficient technologies for another regions analysis is needed
- **Method**: In this paper, we analyzed clinical and molecular data from 21 Chinese deaf families who did not have hotspot mutations in the common deafness genes GJB2, SLC26A4, GJB3, and MT-RNR1.
- The kind of **algorithm** we are interested in is Targeted Next Generation Sequencing (TGS) of 127 known deafness genes, which have been applied to 12 families, while for the remaining nine families, whole-exome sequencing (WES) or trio-WES was used.
- **Results:** GJB2, CDH23, EDNRB, MYO15A, OTOA, OTOF, TBC1D24, SALL1, TMC1, TWNK, USH1C, and USH1G were found to have potential pathogenic mutations in a total of 12 deafness genes in 13 probands, with eight of the detected mutations being novel. Also, one proband with heterozygous deletion of chromosome 4p16.3-4p15.32 had a copy number variant (CNV).
- As a result, the overall diagnostic rate for our deafness patients using NGS was 66.67% (14/21).
- **Conclusions:** These observations expand the mutation scope of deafness-causing genes and encourage the use of NGS detection technologies in Chinese deaf populations for routine molecular diagnosis.

Introduction:

- Hearing loss affects 1 in 300-1000 infants, besides nearly half of the patients with HL have a genetic cause that has been identified.
- HL can be the result of non-syndromic (70%) or syndromic (30%).
- Around 110 genes and 150 loci have been found to be associated with HL.
- GJB2 (121,011), SLC26A4 (605,646), mtDNA 12SrRNA (561,000), and GJB3 (603,324) are the most commonly detected genes in Chinese deaf populations, accounting for 30-50% of cases.
- In the remaining cases, Deafness is caused by rare mutations in known deafness genes or unknown etiologies.
- In this paper, we enrolled 21 Chinese patients with either syndromic or nonsyndromic HL who were previously evaluated also had no hotspot mutations in the common deafness genes GJB2, SLC26A4, MT-RNR1, or GJB3.
- Next-generation sequencing (NGS) technologies, including targeted NGS (TGS) and whole-exome sequencing (WES), were performed on the probands of each family to identify rare pathogenic mutations.
- The results of this study highlight the genetic heterogeneity of HL and the importance of using a next-generation sequencing (NGS) method in patients with complicated clinical phenotype.

Related work:

- In 2019, Genomic DNA was extracted from peripheral blood samples of all subjects. At least 0.5μg of genomic DNA from the probands were sheared by Covaris S2 (Covaris, MA, USA). AMPure beads (Beckman Coulter, CA, USA) were used for purification recycling, Followed by enrichment and PCR, And then Ion PI Chip v3 and a BSE4000 sequencing machine were used for high-throughput sequencing.
- Finally, the sequences were compared to the human reference genome (hg19, NCBI release GRCh37) using the TMAP Alignment Program, and variants were analyzed using Torrent Variant Caller (Xia et al., 2015).
- In this study, Five novel variants were found in three genes. Among them, MYO15A encodes myosin, OTOF encodes otoferlin, and RDX encodes radixin. When these genes are mutated, they may cause protein length or function changes, which cause hearing loss.

Methodology:

- Blood samples were obtained from the family members and genomic DNA was extracted from the whole blood.
- Prescreening of hotspot mutations in GJB2 (c.35delG, c.176_191del16, c.235delC, c.299-300delAT), SLC26A4 (c.919-2A>G, c.1174A>T, c.1226G>A, c.1229C>T, c.1707+5G>A, c.1975G>C, c.2027T>A, c.2168A>G), MTRNR1 (m.1494C>T, m.1555A>G), and GJB3 (c.538C>T) was performed in all probands by microarray (Capital Bío, China; Xiang et al., 2019).
- The blood samples collected from the probands of the 21 deaf families, 12 were analyzed by TGS of 127 deafness-causing genes, six were analyzed by single proband WES, and the remaining three were analyzed by trio-WES.
- DNA sequencing was performed with a **HiSeq2000 sequencer (Illumina)**. Raw data generated by NGS were filtered to obtain high-quality clean reads and was further aligned to the NCBI Human Reference Genome (hg19/GRCh37) using the Burrows-Wheeler Aligner (BWA). SAM tools and GATK were applied for the annotation of BAM files.

Result:

- The results identified some novels and previously reported mutations in rare deafness-causing genes.
- These findings highlight the importance of combining detailed clinical evaluation with next-generation sequencing in the genetic diagnosis of non-syndromic hearing loss and syndromic hearing loss patients.
- The availability of precise molecular data in the very early stage of the disease may contribute to better monitoring of the disease itself and may help to improve the management of individual treatment strategies, Such as cochlear implantation and early speech therapy.
