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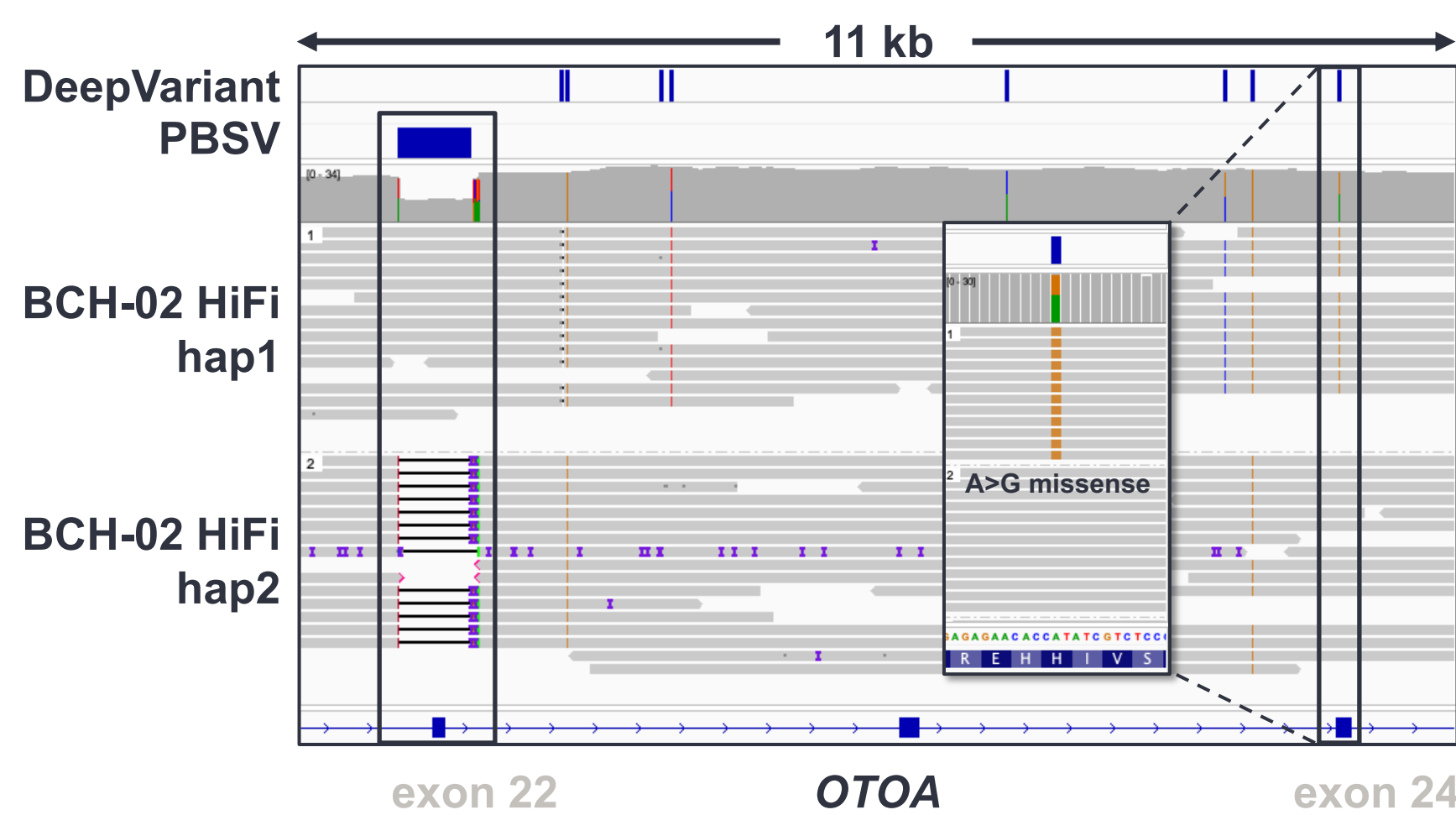
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Background

- It is estimated that 60% of pediatric hearing loss cases have genetic cause.
- There are 124 known non-syndromic hearing loss genes and ~400 syndromic forms of hearing loss.
- Gene panels and short-read NGS are standard of care for pediatric hearing loss.
- Traditional short read whole exome sequencing (srWES) explains ~40% of cases.
- Copy number variations (CNVs) in the STRC region are the most common cause of mild-moderate hearing loss in children.
- PacBio HiFi reads (99.9% accuracy, 15-20 kb) enable comprehensive variant detection in human genomes, extending to repetitive regions of the genome not accessible with short-read WGS (srWGS) or WES (srWES).
- HiFi reads match or surpass srWGS for single nucleotide variant and small indel (<50 bp) detection while also improving detection of structural variants (SVs, ≥50 bp), with recall far exceeding that of srWGS.
- Here we apply HiFi-WGS to 10 probands with unexplained hearing loss who had previously undergone srWES and srWGS with a negative result.

Results

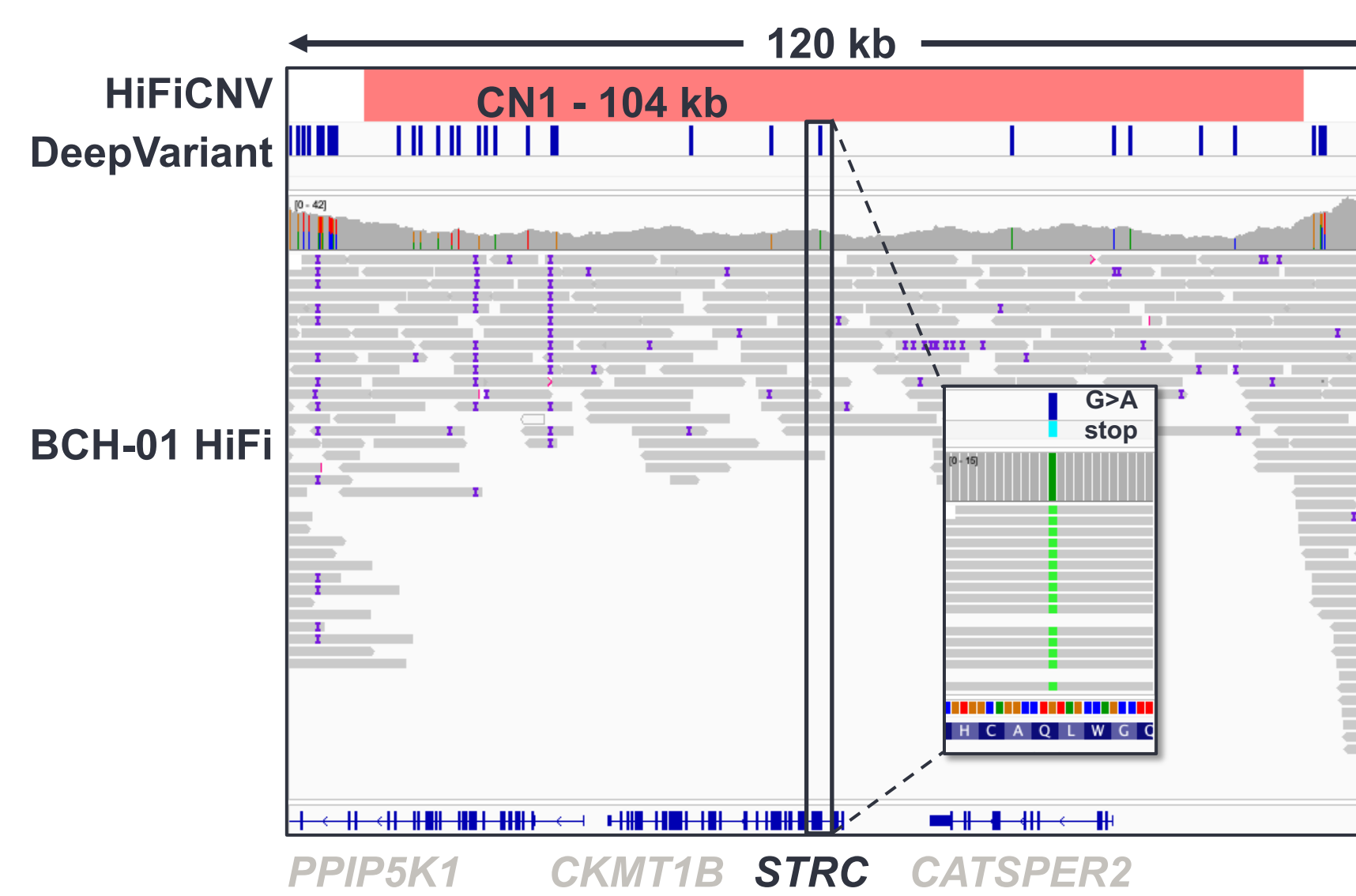
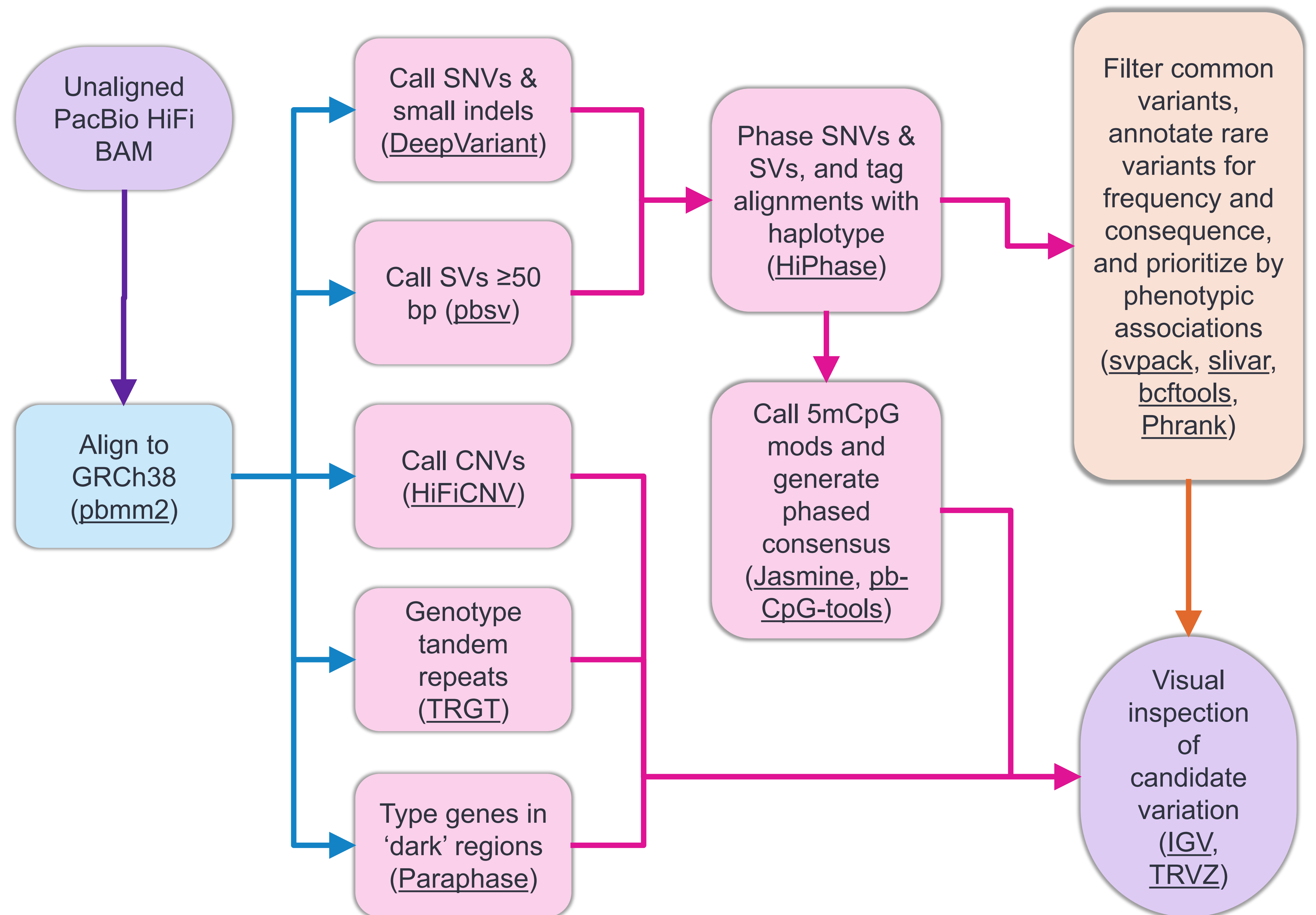
- We identified a median of 4,505,589 SNVs, 981,037 small indels, and 22,682 SVs per sample.
- Variants of phenotypic interest were identified in 7 cases, with 3 cases explained: 1) a compound heterozygous 769 bp deletion and A>G missense variant in *OTOA*, 2) a compound heterozygous ~104 kb deletion and G>A stop-gain variant in *STRC*, and 3) a copy number neutral 403 kb inversion interrupting *MITF*.



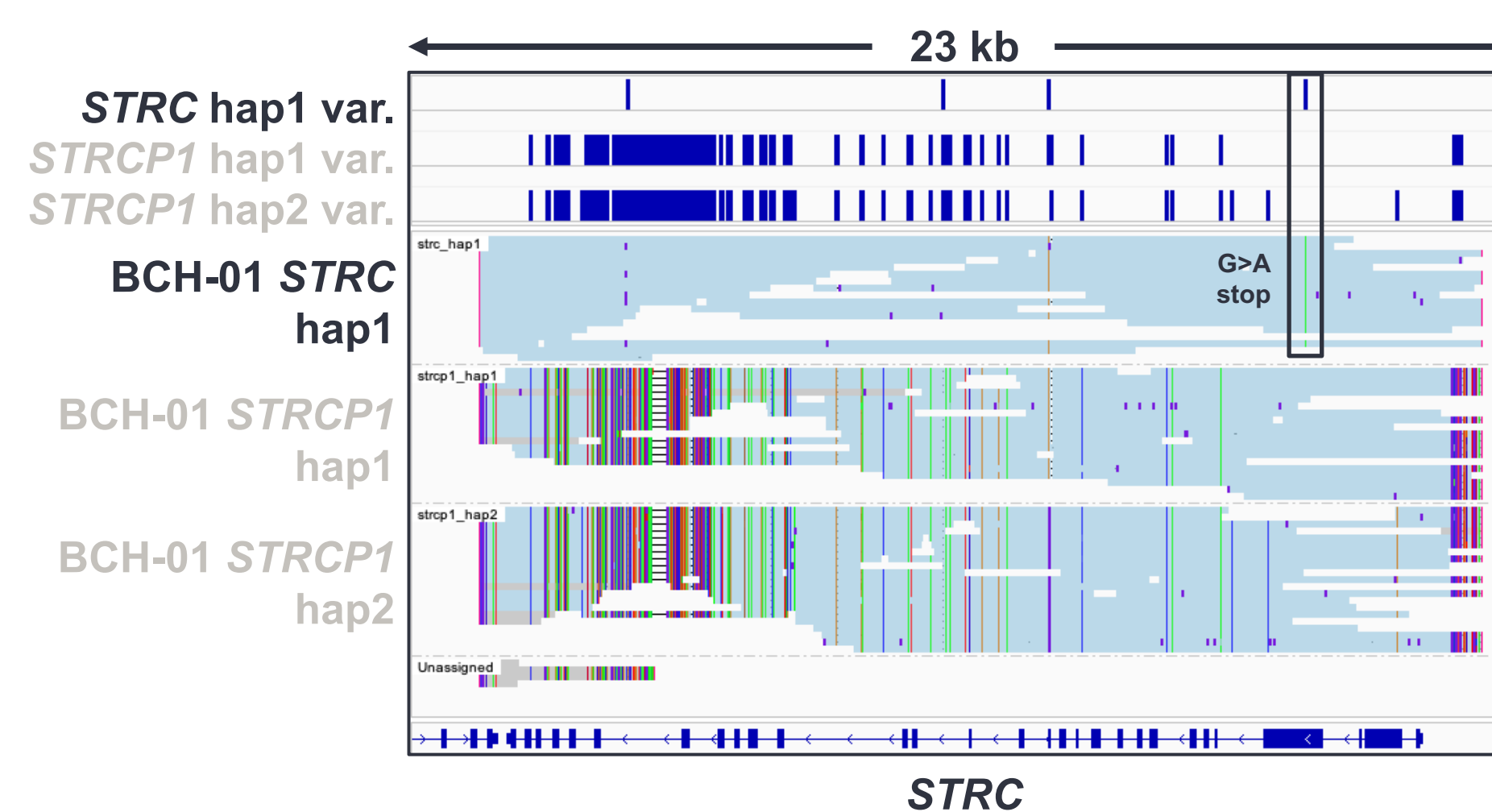
Identification of compound heterozygous pathogenic variants in *OTOA*. In haplotype 1, structural variant caller pbsv detected a 769 bp deletion at chr16:21,735,945 covering exon 22 of *OTOA*. In haplotype 2, DeepVariant identifies chr16:21,744,915 A>G (absent in gnomAD and ClinVar; VUS in LOVD), a missense variant in an exon that is challenging to map with short reads. These variants are shown to be *in trans*.

Methods

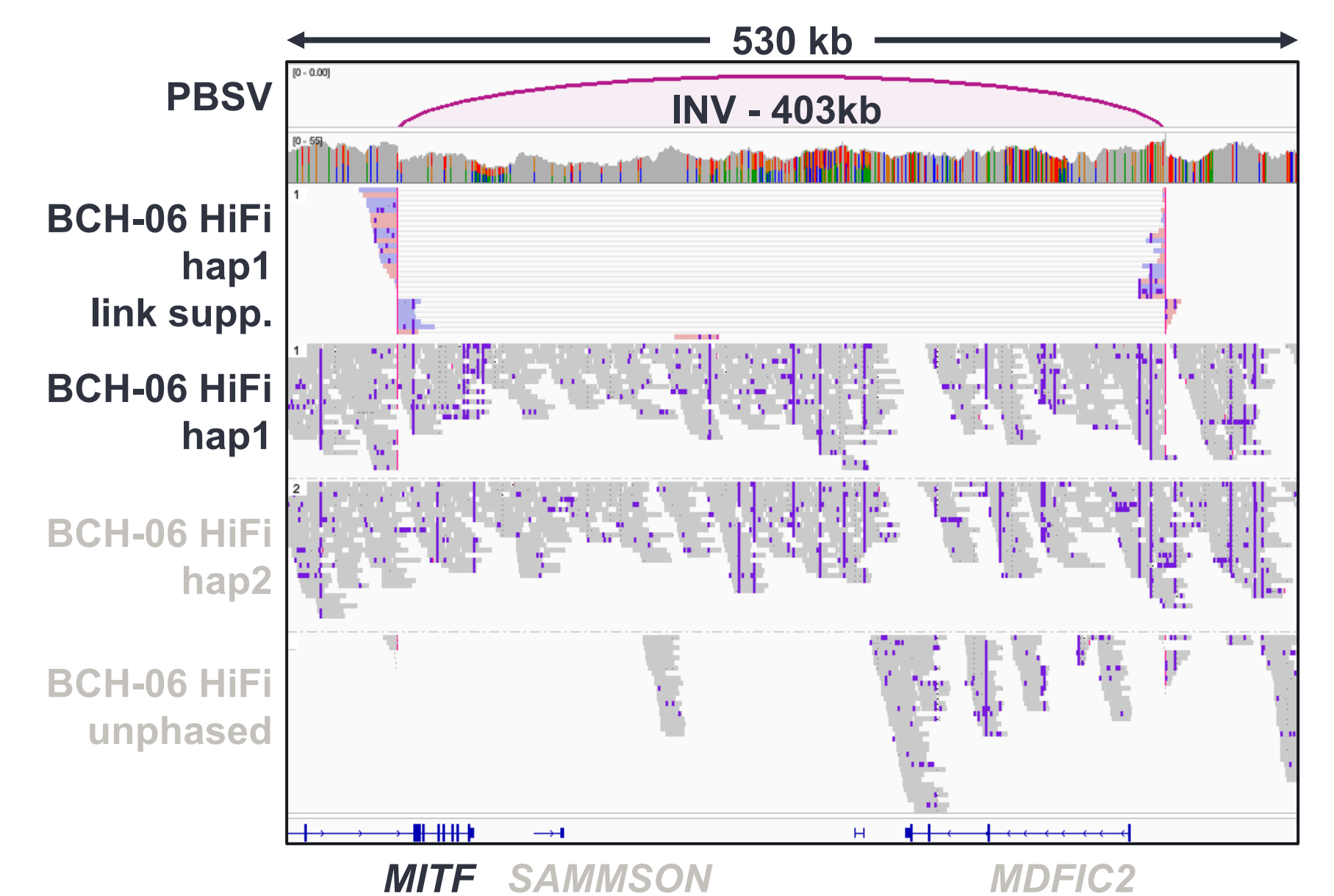
- BCH clinicians provided blood derived DNA from 10 probands with sensorineural hearing loss (1 unilateral, 9 bilateral) that was unexplained by srWES and srWGS.
- 12–15 kb insert SMRTbell libraries were prepared and sequenced to 24– to 32– fold coverage on the Sequel II system.
- Sequence data was processed by an automated workflow described below.



Identification of compound heterozygous pathogenic variants in *STRC*. Depth-based CNV caller HiFiCNV detected a 104 kb deletion (approx. chr15:43,566,001–43,670,000) covering *STRC*. On the other allele, DeepVariant identifies chr15:43,616,338 G>A (absent from gnomAD, ClinVar, and LOVD), resulting in a stop gain in an exon that is challenging to map with short reads due to a highly similar paralog.



Visualization of *STRC* & *STRCP1* haplotypes identified by Paraphase. Paraphase (*P18.025.A*) is a HiFi-based informatics method that can accurately genotype variants in gene/paralog pairs. Reads from both *STRC* and pseudogene *STRCP1* are aligned to *STRC* in the reference and grouped by haplotype. Paraphase identifies only one *STRC* haplotype, with roughly half of the expected coverage (CN1), containing a G>A stop gain at chr15:43,616,338.



Identification of inversion interrupting *MITF*. In haplotype 1, structural variant caller pbsv detected a 403 kb inversion (chr3:69,927,910–70,331,155) interrupting *MITF*. Haplotype 1 is shown twice, once with primary and supplementary linked to demonstrate that reads align to opposite strands, indicating an inversion.

Conclusion

HiFi-WGS increases the ability to explain rare disease cases by allowing for the detection of a broad range of variants, especially in regions that are difficult to map with srWGS.



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Conflict of interests: WJR, CN, JMH, XC, CF, EML, CL, CS, and MAE are employees and shareholders of PacBio.