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Detection and confirmation of deafness-causing copy number variations in the *STRC* gene by massively parallel sequencing and comparative genomic hybridization

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Abstract

Objective—Copy number variations (CNVs), a major cause of genetic hearing loss, most frequently involve the *STRC* gene, located on chr15q15.3 and causally related to autosomal recessive non-syndromic hearing loss (ARNSHL) at the DFNB16 locus. The interpretation of *STRC* sequence data can be challenging due to the existence of a virtually identical pseudogene, *pSTRC* that promotes complex genomic rearrangements in this genomic region. Targeted genomic enrichment with massively parallel sequencing (TGE+MPS) has emerged as the preferred method by which to provide comprehensive genetic testing for hearing loss. We aimed to identify CNVs in the *STRC* region using established and validated bioinformatics methods.

Methods—We used TGE+MPS to identify the genetic cause of hearing loss. CNV results were confirmed with customized array comparative genomic hybridization (array CGH).

Results—Three probands with progressive mild-to-moderate hearing loss were found among 40 subjects with ARNSHL to segregate homozygous *STRC* deletions and gene to pseudogene conversion. Array CGH showed that the deletions/conversions span multiple genes outside of the exons captured by TGE+MPS.

Conclusion—These data further validate the necessity to integrate the detection of both simple variant changes and complex genomic rearrangements in the clinical diagnosis of genetic hearing loss.

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Declaration of conflicting interests

All authors have declared no conflicts of interest.

Keywords

Hearing loss; copy number variations; STRC; massively parallel sequencing; array CHG

Introduction

Complex genomic alterations are an important cause of disease. While attention is most frequently focused on single nucleotide variations (SNVs), copy number variations (CNVs), traditionally considered the alteration through deletion, insertion and/or duplication of more than ~1 kbp, are implicated in many different diseases and neurodevelopmental disorders[1, 2]. Included in this list is sensorineural hearing loss (SNHL), a disease phenotype in which CNVs play a major role. Using comprehensive genetic testing platforms, CNVs have been identified in about 20% of all deafness-causing genes and can be found in about 15% of patients who undergo genetic testing[3].

STRC is the deafness-causing gene most frequently associated with CNV events, with the CNV event most often leading to complete deletion of this gene[3]. These deletions have an estimated prevalence of 1% in deaf populations[4]. Defining the extent of a *STRC* CNV is challenging because the genomic region includes four genes, three of which have highly homologous pseudogenes. For example, the pseudo-*STRC* gene (*pSTRC*) has 98% homology to the functional *STRC* gene. Accurately assessing the rearrangement size in this region takes on added importance because when the neighboring *CATSPER2* gene is included in the CNV and is also deleted, the phenotype becomes complex[5]. In addition to hearing loss, males have infertility secondary to altered sperm motility and therefore carry the diagnosis of deafness infertility syndrome (DIS)[6, 7]. Female fertility is not affected.

In this study, we compare the results of TGE+MPS and high-resolution array CGH to characterize CNVs involving the *STRC* gene in three Japanese families segregating deafness at the DFNB16 locus.

Subjects and Methods

Families screened in this study were identified as part of a comprehensive genetic screen of 194 Japanese subjects (114 females) from unrelated and non-consanguineous families identified in 33 otolaryngology clinics in 28 prefectures across Japan. The complete details of this cohort have been previously reported [8]. For each proband, informed consent was obtained to participate in this study, which was approved by the human subjects ethical committee associated with each clinic. Clinical information and blood samples were obtained for each proband and for all consenting affected and unaffected relatives.

Targeted Genomic Enrichment and Massively Parallel Sequencing

We sequenced all exons of all known non-syndromic hearing loss genes and non-syndromic hearing loss mimic genes as described previously. TGE and library preparation were completed using a modified solution-phase protocol based on the Agilent SureSelect target enrichment system. Prior to library preparation, genomic DNA quality was assessed by gel electrophoresis and Nanodrop 1000 spectrophotometer (Thermo Scientific, Inc) (260/280

ration = 1.8–2.2) and quantitated using a Qubit 2.0 fluorometer (Life Technologies, CA, USA). Three μg of gDNA was randomly fragmented to an average size of 250 bp by sonication with a Covaris acoustic solubilizer (Covaris Inc., Woburn, MA, USA), fragment ends were repaired, A-tails were added and sequencing adaptors were ligated prior to the first amplification. Solid phase reverse immobilization purification was performed between each enzymatic reaction. Hybridization and capture with RNA baits was followed by a second amplification before pooling for sequencing. In all cases, the smallest number of amplifications possible was used: typically eight cycles for the pre-hybridization PCR (range 8–10 cycles using NEB Phusion HF Master Mix) and 14 cycles for the post-hybridization PCR (range 12–16 cycles using Agilent Herculase II Fusion DNA Polymerase). All samples were barcoded and multiplexed before sequencing. We performed sequencing using the Illumina HiSeq system in pools of 48 samples per lane using 100-bp paired-end reads.

Copy number analysis on MPS data

We completed read mapping with BWA and re-alignment with GATK, using the indexed BAM file to call variants and generate a pileup using samtools, retaining only the position and depth information. CNV calling was completed using a custom R script, a previously published tool[9]. This method normalizes read-depth data by sample batch using a sliding-window approach. We used the 2011-07-01 version of the tool with default settings. All CNV calls were curated through manual inspection.

Array-CGH

We designed a custom aCGH platform using the Agilent SureDesign website to select for inclusion of 86 known deaf genes, with probes covering these genomic regions at 150–200 bp intervals on the Agilent 8x60K platform (Agilent Technologies, Santa Clara, CA). aCGH was completed using the same gDNA samples as for TGE+MPS. 10ul of gDNA solution (0.5ug of DNA) was fragmented, labeled with cyanine-3 (reference DNA) or cyanine-5 (subjects), and hybridized. Scanning of the array was completed following the manufacturer's recommended protocols; scanned aCGH data were analyzed using CytoGenomics software v3.0.6.6 (Agilent Technologies).

Results

Case Details

Case 1: Patient # 167—This 12-year-old male was identified with hearing loss at a school check-up. His brother was also having hearing loss. Newborn hearing screening data were not available so the exact age-of-onset was not known. He did not have any complaints of vertigo or tinnitus. Both parents had normal hearing (Figure 1).

Case 2: Patient # 260—This 12-year-old male was first noted to have hearing loss at a school check up. Although he was diagnosed with mild hearing loss, he was not aware of any difficulty hearing. His brother (patient #261) had a similar mild sloping hearing loss (Figure 1).

Case 3: Patient # 4590—This 26-year-old female noticed hearing loss in early childhood. Serial audiological assessments showed a slow deterioration in hearing. She had no episodes of vertigo however she did complain of tinnitus. Her elder brother (patient #4591) had more severely progressive hearing loss (Figure 1).

CNVs in the *STRC* genomic region

Two probands were homozygous for a deletion of *STRC* (prevalence amongst deaf subjects in this study, 7.5%). We also identified one subject who was heterozygous for a *STRC* gene deletion and one with a heterozygous gene duplication of *STRC*.

CNV analysis derived from TGE+MPS identified a homozygous deletion encompassing *STRC* and *CATSPER2* genes in two probands (#167 and #4590) and a biallelic partial *STRC* gene to pseudogene conversion in one proband (#260) (Figure 2).

As shown in Figure 3, array CGH confirmed a homozygous deletion of *STRC* through *CATSPER2* in Case 1 and 3. Segregation analysis confirmed a heterozygous deletion in the parental samples of Case 3, with no obvious changes in the *pSTRC* region. Case 2 also carried a homozygous deletion of the *STRC* to *CATSPER2* region, with at least two or more copies of the *pSTRC* region predicted (indicated by blue dots). Parental samples of Case 2 showed only heterozygous deletion of the *STRC* to *CATSPER2* region.

DISCUSSION

We identified three patients with ARNSHL caused by CNVs in *STRC* in a Japanese hearing loss population for an estimated prevalence of 7.5% (3 /40 ARNSHL probands). This figure is similar to that reported by Vona et al. who found six of 94 SNHL subjects (6.4%) with homozygous or compound heterozygous *STRC* deletions as a cause of hearing loss using whole-genome aCGH[10]. The carrier frequency of *STRC* deletions has been estimated at 1.1 to 2.5% [4, 11]. We analyzed only a limited number of 40 ARNSHL samples, thus further study is necessary to clarify the prevalence of CNV in the *STRC*.

CNVs are found in 13% of the human genome and may be underestimated as a cause of SNHL to difficulties and limitations in their detection.[13] Although several methods including multiplex ligation-dependent probe amplification (MLPA), SNP arrays, and aCGH can be used to identify CNVs, these analyses are not routinely included in genetic testing for SNHL. Fortunately, with the implementation of TGE+MPS in the comprehensive evaluation of SNHL, detection of CNVs can be integrated into the bioinformatics analysis. Furthermore, targeted genomic regions can be expanded with additional probes to improve CNV identification when the gene locations or positions in which CNVs commonly occurred were well established.

Using a customized aCGH pipeline, we confirm the validity of this approach for the detection of *STRC*-associated CNVs. There was high concordance between the MPS CNV analysis and the aCGH results provided more data throughout the *STRC* genomic region. This result is important as CNVs in *STRC* are a common cause of mild-to-moderate ARNSHL[3, 14, 15]. In summary, we propose that genomic alterations including CNVs should

be considered as a major cause of SNHL and that genetic testing must integrate SNV and CNV identification.

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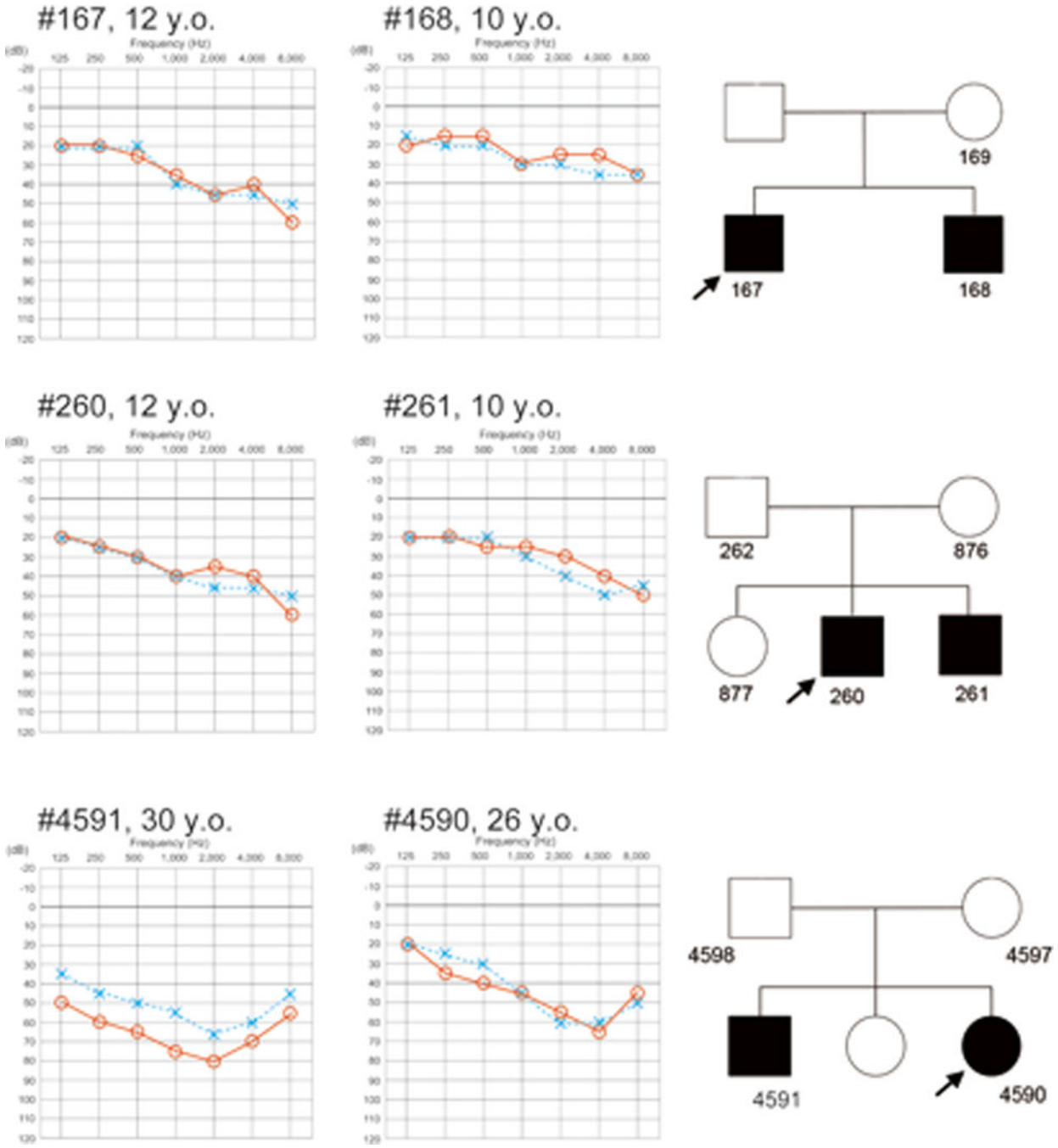

Figure 1.

Audiograms of the proband and siblings with homozygous *STRC* deletions. Audiograms show mild-to-moderate hearing loss and mild age-associated progression of hearing loss. Each number in the pedigree is the assigned patient number.

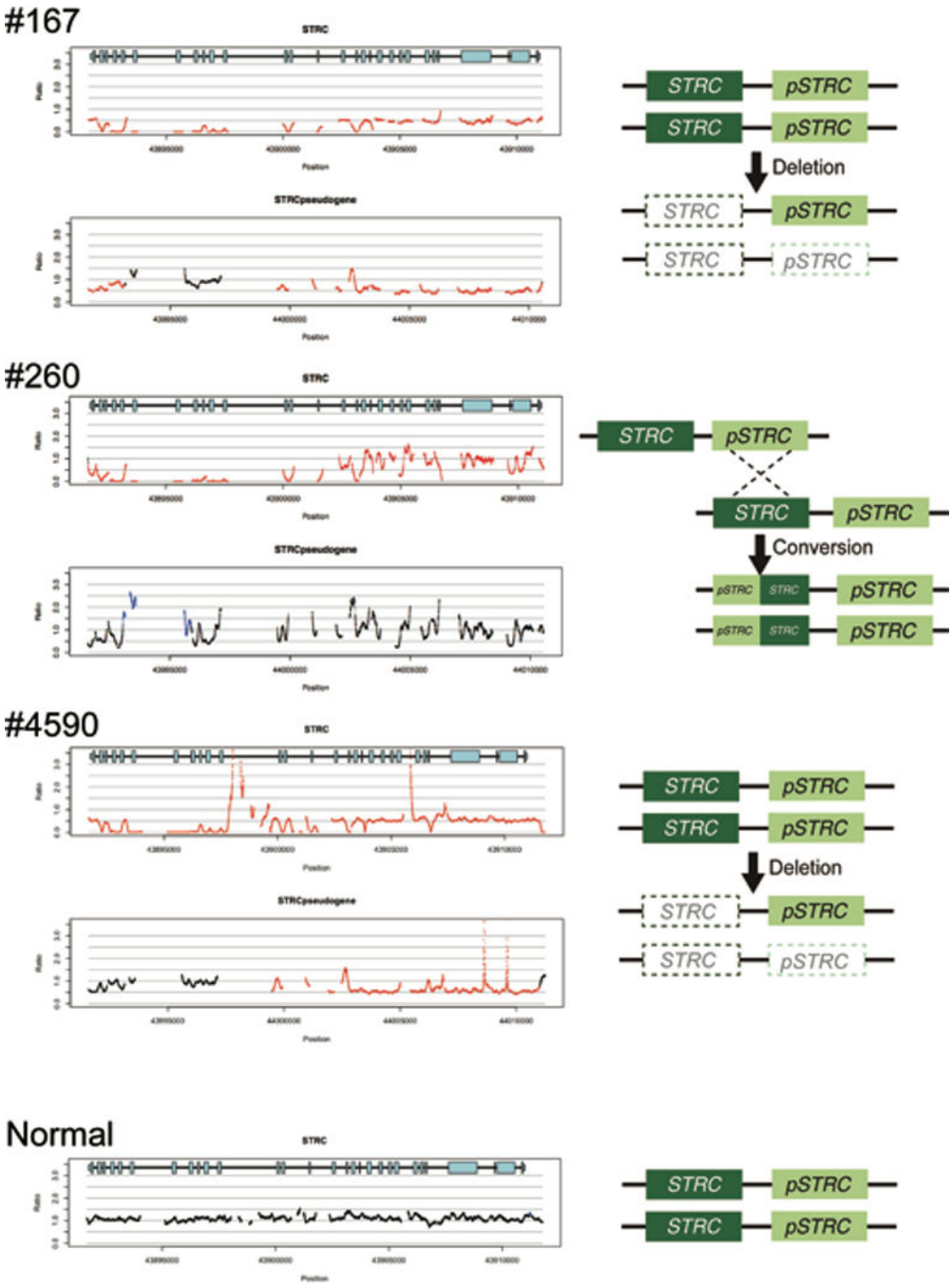

Figure 2.

CNV identification by a CNV-calling script implemented with TGE+MPS. The horizontal axis indicates chromosomal position, and the vertical axis indicates the number of gene copies shown as a ratio; 1.0 indicates the normal 2 copies (black lines), and 0.5 and 0.0 indicates 1 copy (heterozygous deletion) or 0 copy (homozygous deletion), respectively indicated by red lines. Likewise, a figure above 1.0 shows copy gain (heterozygous or homozygous gene duplication) as indicated by blue lines.

A homozygous deletion in the *STRC* region and a heterozygous deletion in the *pseudo-STRC* gene (*pSTR*C) is seen in patients #167 and #4590. A homozygous deletion of the last 11 exons in *STRC* (left side in the gene diagram) and homozygous duplication of the corresponding region of *pSTRC* is seen in patient #260. The hypothesized mechanisms for these events are shown to the right.

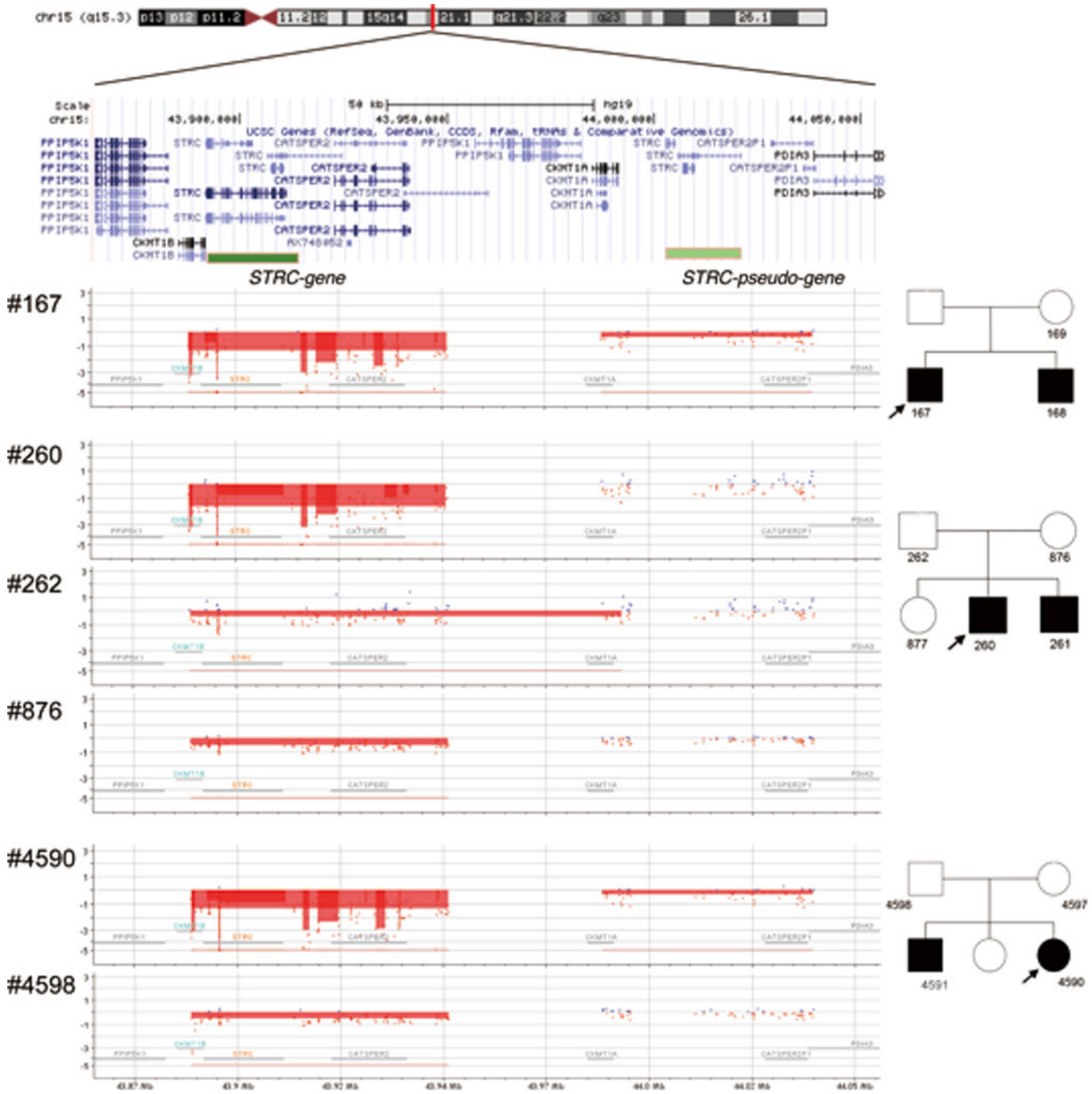

Figure 3.

Results of array CGH for patients and parent(s). The upper part of the figure shows the genomic location across multiple genes. The results show deletion of both copies of *STRC* and hemizygous deletion of *pSTRC* in patients #167 and #4590 by CytoGnomics software, with parental sample #4598 showing hemizygous deletion of *STRC* but no changes in the *pSTRC* region. For the family of the proband #260, deletions in only *STRC* to *CATSPER2* region were observed.