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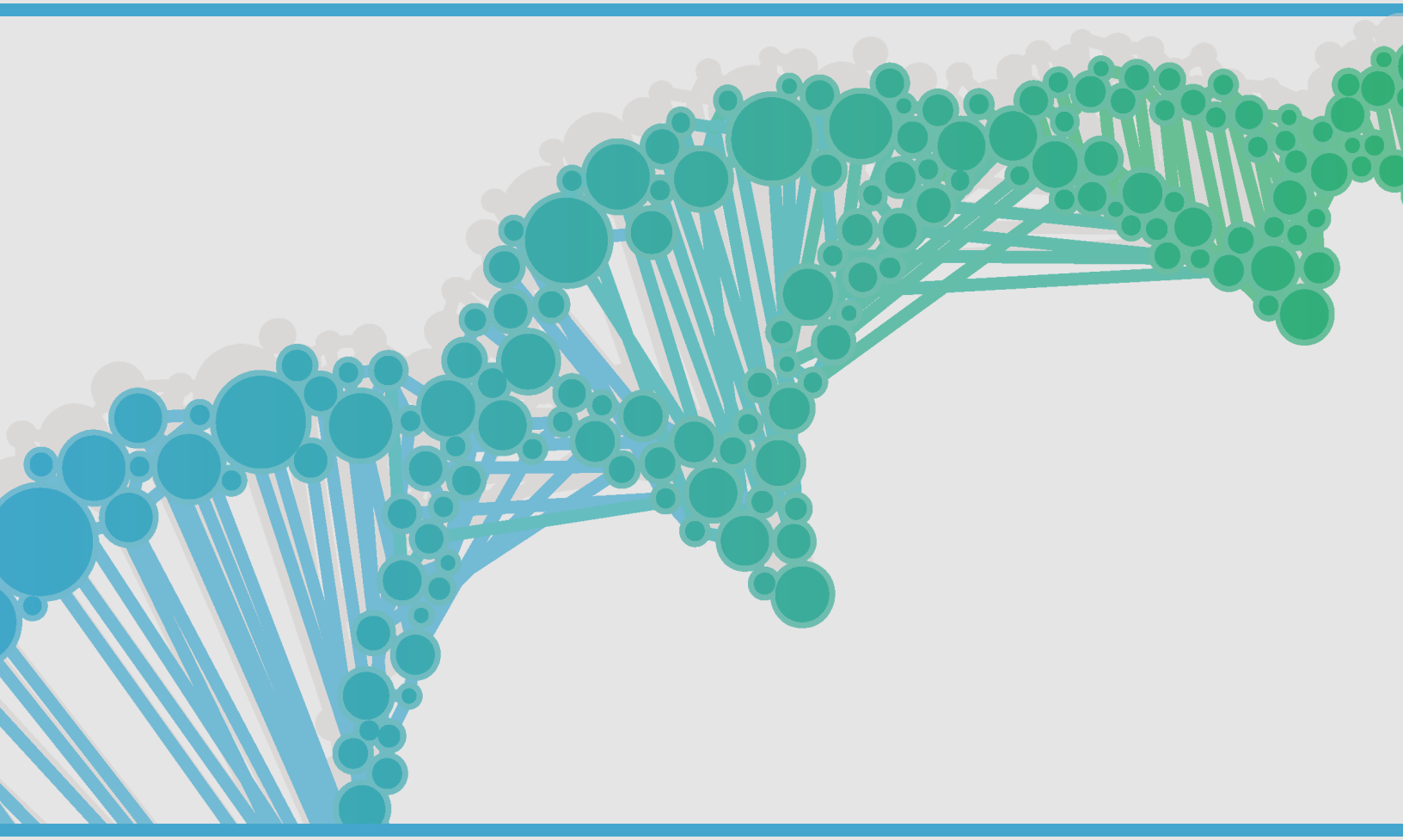

CASE STUDY

Trio analysis in NxClinical – Heritable retinal dystrophies in the Costa Rican population

NxClinical software facilitates trio and familial analysis with rich concurrent visualization of trio samples down to the single base level as well as numerous filters for compound events and inheritance based filtering.

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Trio analysis in N_xClinical – Heritable retinal dystrophies in the Costa Rican population

Background

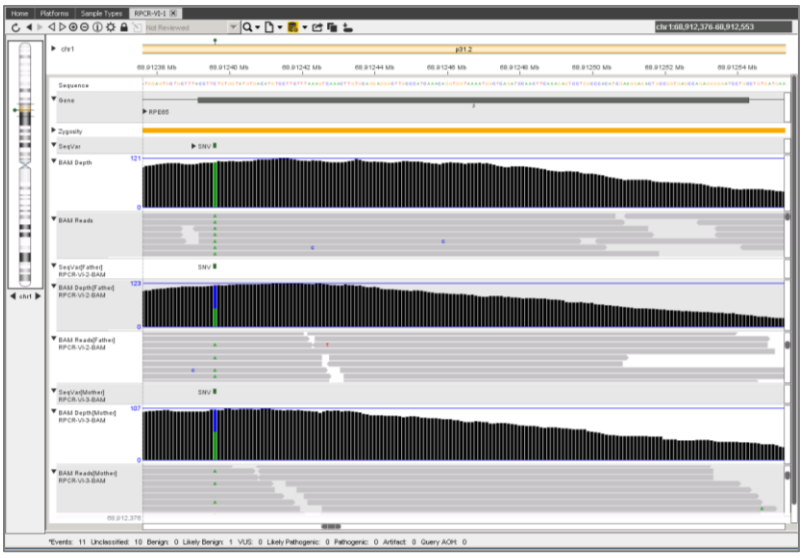
The Costa Rican population has distant relatedness; as a result, a high incidence of autozygosity and founder effects are predicted to have resulted in rare diseases. The population has an unusually high prevalence of inherited retinal dystrophies (RDs) which is a significant cause of childhood vision loss in Costa Rica. IRDs have considerable phenotypic and genotypic heterogeneity with over 200 disease-causing genes identified, most of which are autosomal recessive. Dr. Daynna Wolff, Director, Clinical Cytogenetics and Genomics, Medical University of South Carolina used N_xClinical 4.0 for integrative analysis of copy number, sequence variants, and autozygosity mapping to study 31 affected children from 23 Costa Rican families. Autozygosity-directed mutation assessment using familial and trio analysis revealed biallelic mutations in the RPE65 gene in 87% of affected individuals with four apparent founder mutations.

Quick Glance

N_xClinical software facilitates trio and familial analysis with rich concurrent visualization of trio samples down to the single base level as well as numerous filters for compound events and inheritance based filtering.

Benefits:

- Integrated analysis and visualization of copy number, sequence variants, and AOH
- Compound event filtering quickly identifies causative variants which could otherwise be missed when using multiple software platforms for analysis



Trio Analysis

Whole-exome sequencing (WES) data of trios were analyzed in N_xClinical for all exons in *RPE65*. Various tracks (e.g. BAM Depth, BAM Reads, and SeqVar) for the trios are displayed concurrently in the N_xClinical interface facilitating analysis and interpretation. A set of trio samples is presented in the figures. The gold bar under the chromosome ideogram at the top indicates AOH for the proband. Beneath that, each bar in the BAM Depth track depicts a single base.

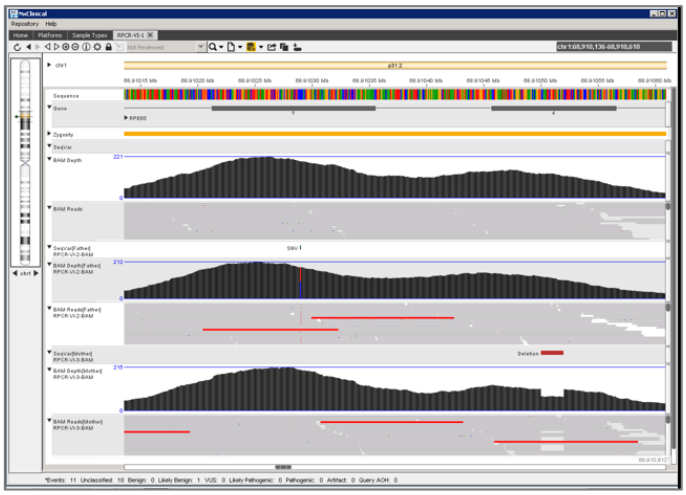
The figure on the right is zoomed in on an SNV on exon three. The proband has a homozygous A mutation, as indicated by a green bar in the BAM Depth track. Each parent is heterozygous for the A mutation resulting in a 50/50 blue and green coloring at that location, with the mutant A allele represented in blue and the reference C allele, in green. The second figure shows the region containing exons 4 and 5 with an additional heterozygous

SNV in the father and a 20bp deletion in the mother. Note that the read depth shows a dip in the plot indicating a deletion in this area in the mother's BAM Depth track.

Results

Identification of founder mutations

The study unveiled a high degree of autozygosity in the genomes of affected probands, with an average of ~4% homozygosity in each genome (range 2.4 to 9.3%). By comparing these regions to a BED file with known IRD genes, an interesting stretch of homozygosity was found on 1p31.3, coincident with the region containing the *RPE65* gene. Four recurrent RPE65 mutations were identified consistent with founder mutations.



Trio analysis identifies compound heterozygous mutations

Analysis of trios in N_xClinical lead to discovery of compound heterozygote mutations in parents, easily visible via BAM reads in the N_xClinical browser. Only a few samples did not have *RPE65* mutations but displayed other aberrations in genes associated with IRDs, including a hemizygous deletion on the X chromosome.

Conclusions

Dr. Wolff found the numerous filtering and visualization tools in N_xClinical to be extremely useful for trio and familial analysis. The ability to see read pile-ups shows the extent of coverage for the exons and provides further evidence of deletions of certain areas, as indicated by a dip in the Read Depth for such regions. The fine resolution of the interface allows visualization down to the single base level, indicating the base ascertained at each location in the genome. Filters to show inheritance pattern and compound events pinpoint the areas to review to find causative mutations.

N_xClinical allowed integrative analysis of copy number, areas of homozygosity and sequence variants resulting in a comprehensive genetic analysis of this unique Costa Rican population. The analysis identified causative mutations for retinal dystrophy in *RPE65*, which may respond to a new gene therapy that has demonstrated improved vision in clinical trials.

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