CASE STUDY

Comprehensive Genomic Analysis of Complementary Microarray and Next Generation Sequencing Data for Accurate Clinical Diagnostics

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AT A GLANCE

In this case study, you will learn:

- how Greenwood Genetics brings together NGS and Array results in routine genetic testing for improved diagnostic yield
- how the Cartagenia Bench platform allows for a seamless integration of copy number and molecular variants
- how this approach was used to solve a diagnostic case of Brittle Cornea

Introduction

Recently, next generation sequencing (NGS) has proven its diagnostic utility for a growing range of clinical applications. The intent being to replace multitude primary molecular diagnostic tools, such as Sanger Sequencing, qPCR, MLPA and array-based copy number analysis (aCNA), with a single methodology. However, extraction of copy number variations from NGS data has been challenging. Here we present a case study demonstrating the successful application of both NGS and aCNA as complementary methods for accurate clinical diagnostics using the Cartagenia Bench Lab software suite as the integrated data analysis and interpretation platform.

Case

A 2-month old male infant was referred with keratoglobus, blue sclera and abnormal red reflex in his eyes (Figure 1). The family history for these clinical findings and other related disorders was negative. The initial diagnosis pointed to brittle cornea syndrome (autosomal recessive) or osteogenesis imperfect (autosomal dominant) used to identify candidate genes responsible for the patient's phenotype.

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Figure 1: The figure depicts the proband's habitus as well as a close up of the keratoglobus and blue sclera.

Methods & Results

Initially, the Cytogenetics Laboratory at the Greenwood Genetics Center (GGC) performed aCNA using the Affymetrix Cytoscan HD platform. The microarray data were analyzed using Cartagenia's Bench Lab platform. Of the nine CNV regions identified, six were chromosomal deletions. Alignment of these regions with OMIM genes revealed a copy number loss of a 114.1 kb segment on chromosome 16. This deletion encompasses the entire ZNF469 locus which is known to have regulatory and structural function in the assembly of collagen fibers and has been associated with Brittle Cornea syndrome type 1.

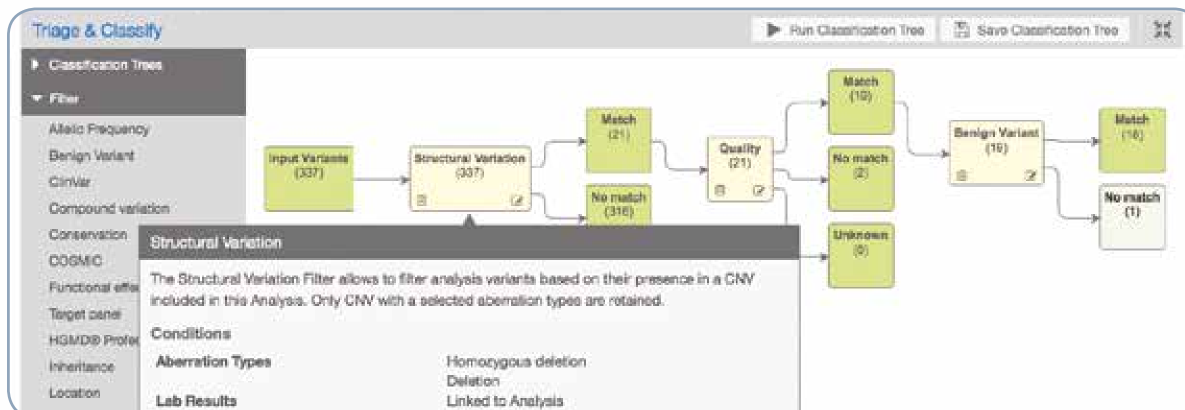

Figure 2: Filter strategy for NGS data based on aCNA results.

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Benign Variant

The Benign Variant filter filters a list of variants based on their presence in a controlled set of benign variants or reference set. Select and configure the sets you want to include in the filtering. Combinations of multiple sets can be applied such that a variant goes to the MATCH output when the variant is found in all, any, or a minimal number of sets.

Conditions

Match **Any** of the following conditions:

Condition	Value
dbSNP	
dbSNP Allele frequency	0.01
dbSNP Allele count	200
dbSNP Validation Status	validated
dbSNP Suspicion Flag	not suspected false
1000 Genomes	
1000 Genomes Allele frequency	0.01
1000 Genomes Allele count	200
ESP6500	
ESP6500 Allele frequency	0.01
ESP6500 Allele count	200
Gleeson_Exome_reference_set	
Gleeson_Exome_reference_set Allele frequency	0.01
Gleeson_Exome_reference_set Allele count	200
ALL_Exome_reference_set	
ALL_Exome_reference_set Allele frequency	0.01
ALL_Exome_reference_set Allele count	200

Figure 3: Variants are restricted based on data quality parameters such as read depth > 20 (not shown). Benign variants are then removed by removing those present in a private knowledge database as well as those present in public database (EVS, dbSNP, 1000Genomes) with an allele frequency > 1%.

This observation was followed by NGS-based targeted resequencing of 31 genes as represented in our Connective Tissue Panel. We used a simple strategy to extract disease relevant variants (Figure 2). Benign variants were removed by alignment of the NGS data with public databases (Exome Variant Server, dbSNP and 1000Genomes) as well as our own reference data for common variants (Figure 3).

We then combined the results of from aCNA and NGS in Bench Lab by mapping the location of highconfidence, potentially payhogenic variants to the six deleted regions in this patient (Figure 4). Only one variant, a single-nucleotide deletion, was located within the 114.1 kb deletion on chromosome 16 causing a frameshift in the remaining ZNF469 allele (Figure 5). This outcome consistent with an autosomal recessive disorder of the connective tissue, brittle cornea syndrome 1, in this patient.

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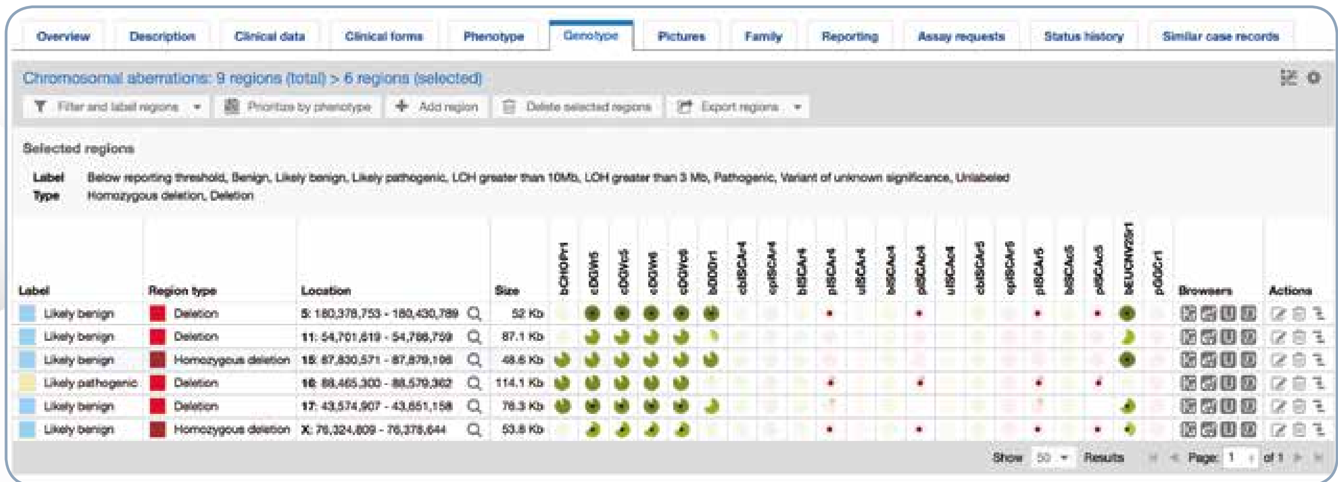

Figure 4: Integration of aCNA data to restrict the number of potentially disease-causing variants. Here, six regions showing copy number loss, based on microarray data, are used to identify candidate genes responsible for the patient's phenotype.

The above conclusions were validated by qPCR for paternal inheritance of the 114.1 kb deletion resulting in decreased genomic dosage for the exonic sequence of ZNF469 in the father and the proband. In addition, Sanger sequencing confirmed the presence of the framehift-causing, single-nucleotide deletion in ZNF469 in the mother and the proband in heterozygous and hemizygous configuration respectively.

Figure 5: Variant list of potentially disease-causing variants as the output of the filtering strategy. The approach identified a single frameshift mutation in a heterozygously deleted region. The gene is known to cause the autosomal recessive brittle cornea syndrome 1, consistent with the patient's phenotype.

Summary

In conclusion, the above example demonstrates a quick and efficient way to integrate the analysis of genomics data for accurate determination of disease-causing genomic changes using the Bench Lab data analysis and interpretation platform.

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