Finding Causative Mutation Candidates in Rare Disease Studies using NextGENe's Variant Comparison Tool

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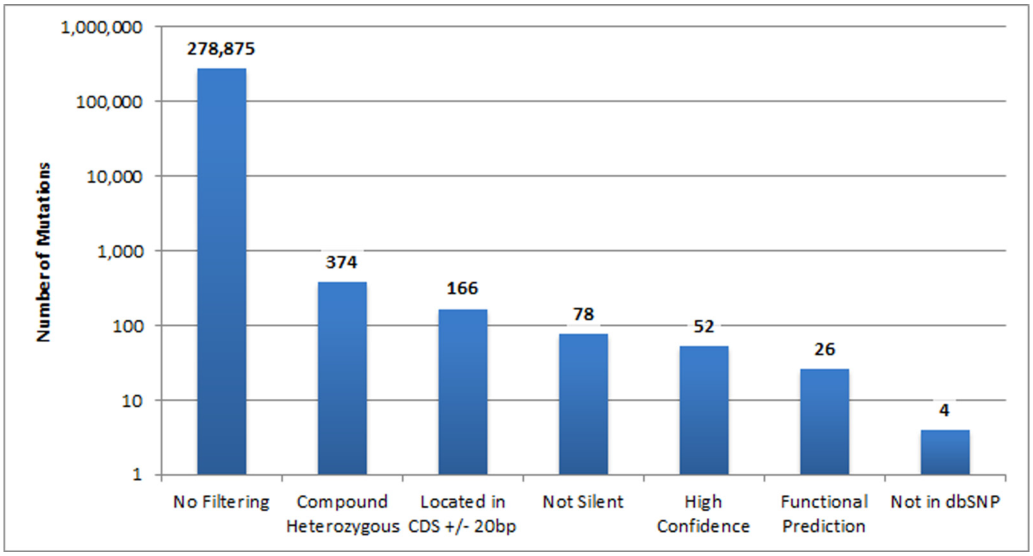


Figure 1: Number of Mutations Left After Each Filtering Step

Introduction

As the cost of sequencing continues to decline, large sequencing projects are no longer limited to large labs with dedicated bioinformaticians. Increased sequencing output requires tools that can handle more advanced applications, while remaining easy to use for biologists. In collaboration with researchers at the NIH, SoftGenetics has developed a biologist and geneticist-friendly tool designed to speed up and simplify the discovery of causative mutations. Sequencing the exomes of a small family or several individuals with similar phenotypes can result in a list of hundreds of thousands of possible mutations. NextGENe's Variant Comparison tool makes it easy to narrow down this list based on a number of criteria:

- Expected genotype - including control samples and inheritance patterns
- Annotated gene information - exons, coding sequences, splice sites, etc
- Database information – dbSNP, COSMIC
- Functional Prediction - dbNSFP (including 1000 genomes frequency and PhyloP)
- Type of mutation – Indels or Substitutions (Silent, Missense, or Nonsense)
- Inclusive and Exclusive region of interest (ROI) filtering
- Mutation confidence score filtering

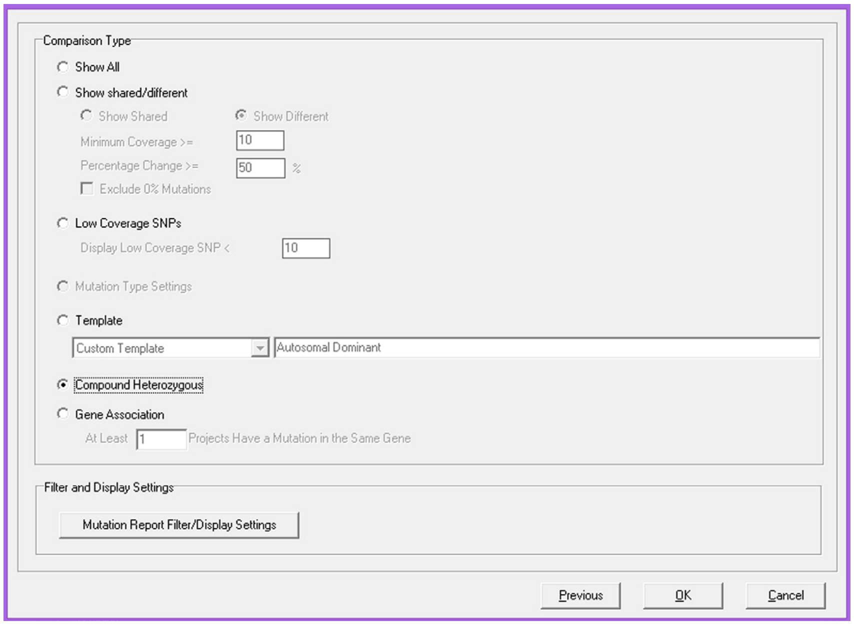
Procedure

After aligning reads in NextGENe to take advantage of its superior indel detection or importing pre-aligned reads from BAM format, the projects are loaded into the Variant Comparison tool. There are seven options for comparing the samples (figure 2):

1. Show all mutations
2. Show shared or different mutations
   a. Minimum coverage (both samples) and minimum difference can be specified
   b. "Ignore 0% Mutations" will always treat positions with no mutant alleles as different even if they are in the expected range. For example, a position with no mutant alleles in one sample and 15% mutant alleles in another will be treated as different even if the range is set to 30%.
3. Show mutations with low coverage in at least one sample

Procedure (cont.)

4. Manually specify expected mutation types
   Homozygous, Heterozygous, Negative, Undetermined, Present, Negative, or some combination
5. Specify relationship (Father, Mother, Son, Daughter) and phenotype (Affected or Unaffected) for each sample and select an inheritance template to automatically adjust expected mutation types
6. Perform compound heterozygous filtering
   a. Requires sequence data for at least one affected offspring
   b. Additional samples can be used for more specific filtering
7. Gene Association- projects share variants in the same gene, but not necessarily the same variant



Comparison Type

○ Show All

○ Show shared/different

○ Show Shared ● Show Different

Minimum Coverage >= 10

Percentage Change >= 50 %

□ Exclude 0% Mutations

○ Low Coverage SNPs

Display Low Coverage SNP < 10

○ Mutation Type Settings

○ Template

Custom Template | Autosomal Dominant

● Compound Heterozygous

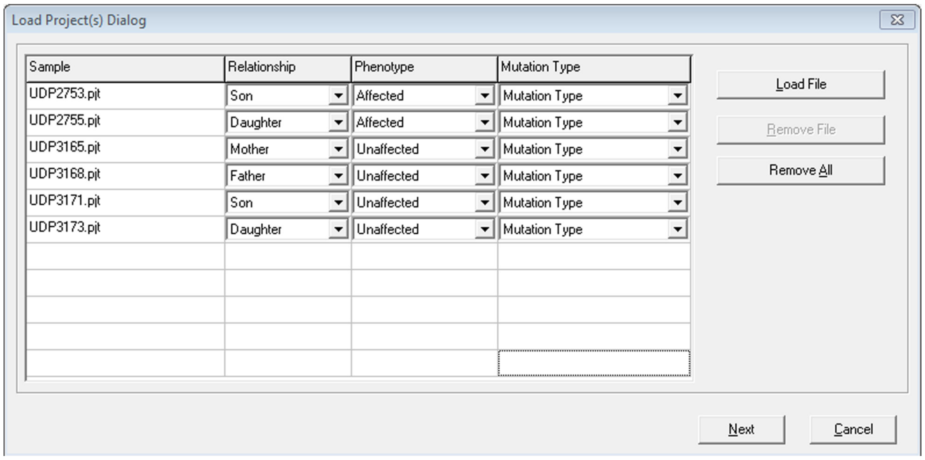
○ Gene Association

At Least 1 Projects Have a Mutation in the Same Gene

Filter and Display Settings

Figure 2: Mutation Comparison Selection

Functional prediction, conservation, and 1000 genomes information can be imported from the dbNSFP database using the Track Manager tool. The tool includes a link to the website where the database can be downloaded. More information about the scores can be found in the dbNSFP papers [2,3] which are linked in the "References" dialog found in the NextGENe "Help" menu. The same track manager tool can import the COSMIC database [1] and custom information, such as VCF files.



Load Project(s) Dialog

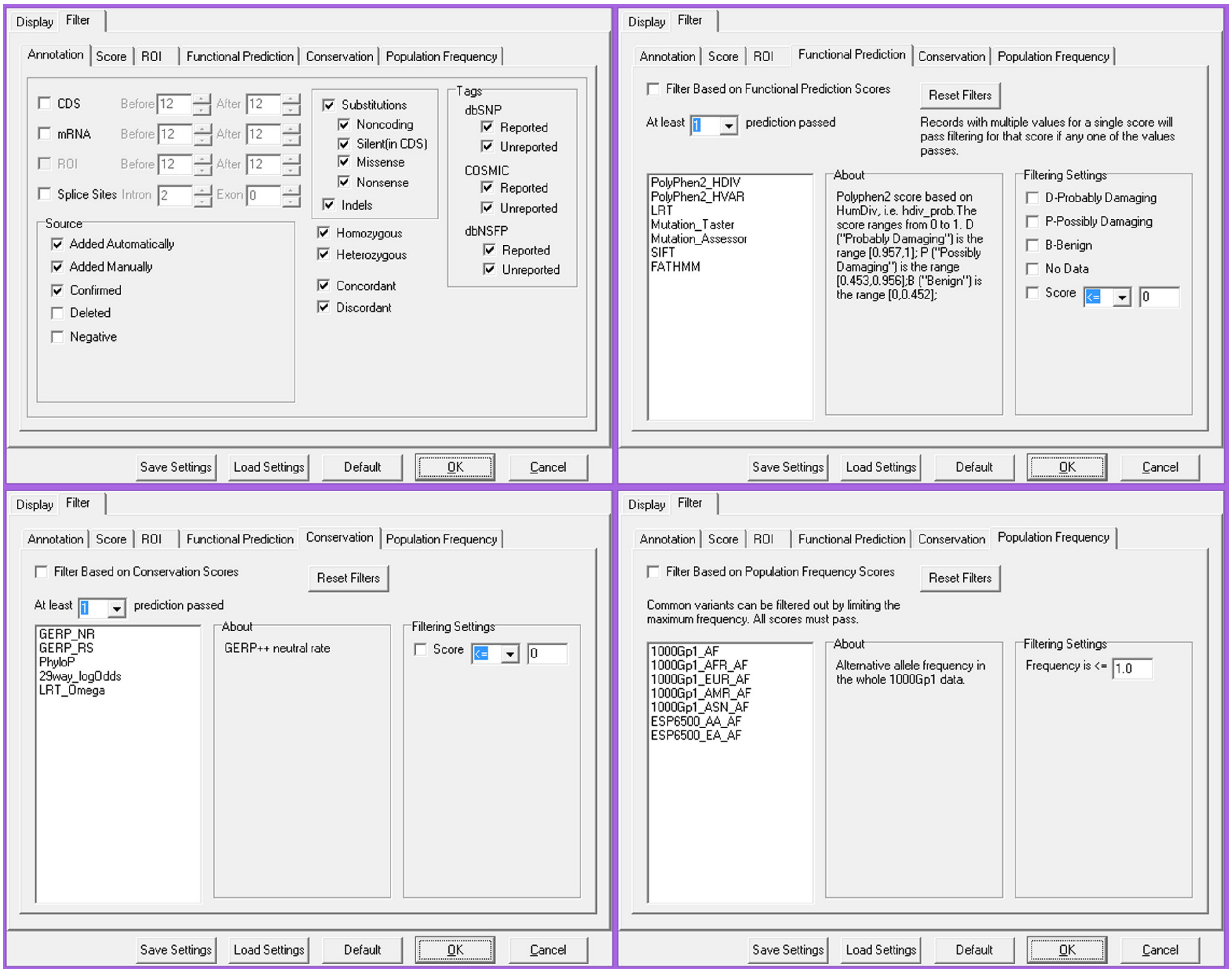
Sample	Relationship	Phenotype	Mutation Type
UDP2753.pjt	Son	Affected	Mutation Type
UDP2755.pjt	Daughter	Affected	Mutation Type
UDP3165.pjt	Mother	Unaffected	Mutation Type
UDP3168.pjt	Father	Unaffected	Mutation Type
UDP3171.pjt	Son	Unaffected	Mutation Type
UDP3173.pjt	Daughter	Unaffected	Mutation Type

Figure 3: The sample projects are loaded in the Variant Comparison Tool

In this example six sequenced exomes were examined to find potential causative mutations that follow a compound heterozygous inheritance pattern causing a form of epilepsy (figure 3). There were two parents, two affected children, and two unaffected children. For each sample mutations were called if they occurred in regions with at least 5x coverage and occurred in at least 3 reads or 20% of reads aligned at a position. If any sample had below the mutation coverage cutoff (5x) at a position, it was excluded from the final report.

Results

There were a total of 278,875 mutation calls in at least one of the six exomes. There are advantages and drawbacks to using many samples in a comparison. Using more samples allows more specific filtering, but may cause false negatives due to some samples not having adequate coverage. The compound het report lists all possible pairs of mutation calls where both are in the same gene and one is inherited from each parent. Using only compound heterozygous filtering of the initial 278,875 mutation calls reduced the list to 374 calls allowing for 497 possible pairs. There are many filters available for narrowing down the list even further (figure 4). Additional filtering was applied and the results are summarized in table 1.



☐ CDS Before 12 After 12
☐ mRNA Before 12 After 12
☐ ROI Before 12 After 12
☐ Splice Sites Intron 2 Exon 0

Source
☑ Added Automatically
☑ Added Manually
☑ Confirmed
☐ Deleted
☐ Negative

☑ Substitutions
☑ Noncoding
☑ Silent(in CDS)
☑ Missense
☑ Nonsense
☑ Indels

☑ Homozygous
☑ Heterozygous

☑ Concordant
☑ Discordant

Tags
dbSNP
☑ Reported
☑ Unreported
COSMIC
☑ Reported
☑ Unreported
dbNSFP
☑ Reported
☑ Unreported

☐ Filter Based on Functional Prediction Scores

At least 1 prediction passed

Records with multiple values for a single score will pass filtering for that score if any one of the values passes.

PolyPhen2_HDIV
PolyPhen2_HVAR
LRT
Mutation_Taster
Mutation_Assessor
SIFT
FATHMM

About
Polyphen2 score based on HumDiv, i.e. hdiv_prob.The score ranges from 0 to 1. D ("Probably Damaging") is the range [0.957,1]; P ("Possibly Damaging") is the range [0.453,0.956];B ("Benign") is the range [0,0.452];

Filtering Settings
☐ D-Probably Damaging
☐ P-Possibly Damaging
☐ B-Benign
☐ No Data
☐ Score <= 0

☐ Filter Based on Conservation Scores

At least 1 prediction passed

GERP_NR
GERP_RS
PhyloP
29way_logOdds
LRT_Omega

About
GERP++ neutral rate

Filtering Settings
☐ Score <= 0

☐ Filter Based on Population Frequency Scores

Common variants can be filtered out by limiting the maximum frequency. All scores must pass.

1000Gp1_AF
1000Gp1_AFR_AF
1000Gp1_EUR_AF
1000Gp1_AMR_AF
1000Gp1_ASN_AF
ESP6500_AA_AF
ESP6500_EA_AF

About
Alternative allele frequency in the whole 1000Gp1 data.

Filtering Settings
Frequency is <= 1.0

Figure 4: Some of the filters available in the Variant Comparison Tool. Clockwise from top left: Annotation, Functional Prediction Scores, Population Frequencies, Conservation Scores

Filtering	Number of Variants	Number of Pairs of Variants
1. Compound Heterozygous	374	497
2. CDS +/- 20 bp	166	183
3. Hide Silent Mutations	78	71
4. Mutation Confidence Score >= 8	52	39
5. >=1 Functional prediction of damage or conserved normal allele (if dbNSFP information was available)	26	19
6. Hide dbSNP Mutations	4	2

Table 1: Mutation Filtering Results

At each step the new filtering was applied first (such as hiding silent mutations) and the resulting lists of mutation calls for each sample were compared for compound heterozygous inheritance. The final 2 pairs of variants included a pair in the gene known to cause this disorder. Figure 5 shows a comparison for one of those variants across all of the samples. Both of these mutations occurred at positions predicted to be conserved by PhyloP. One was predicted to be damaging according to PolyPhen-2.

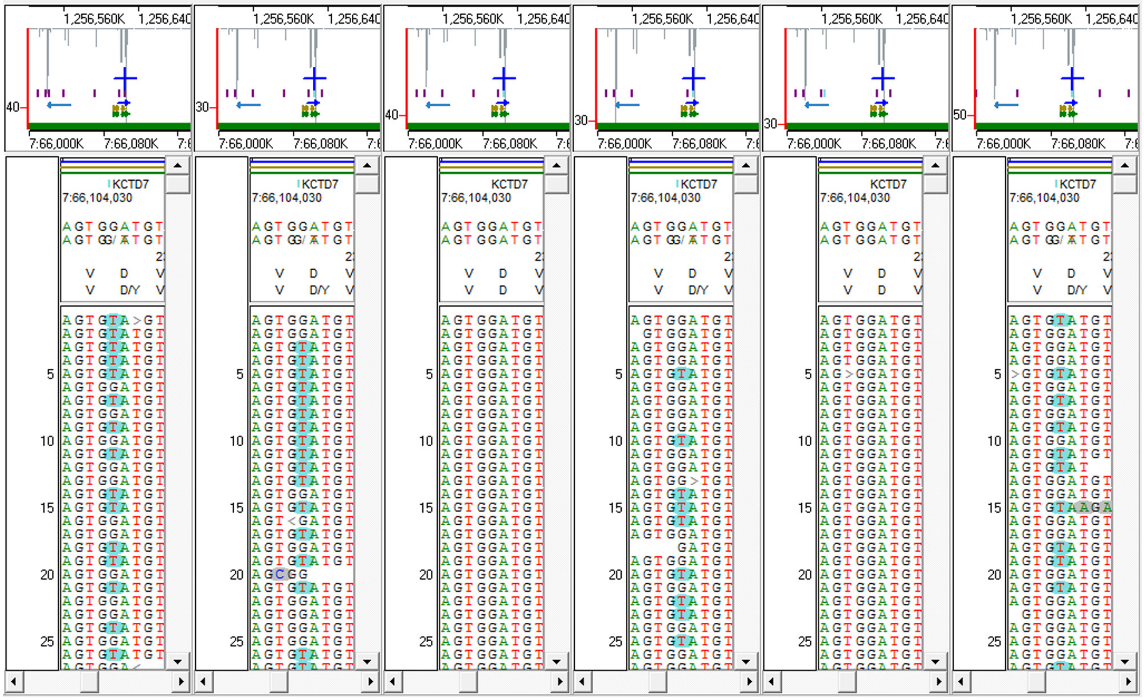
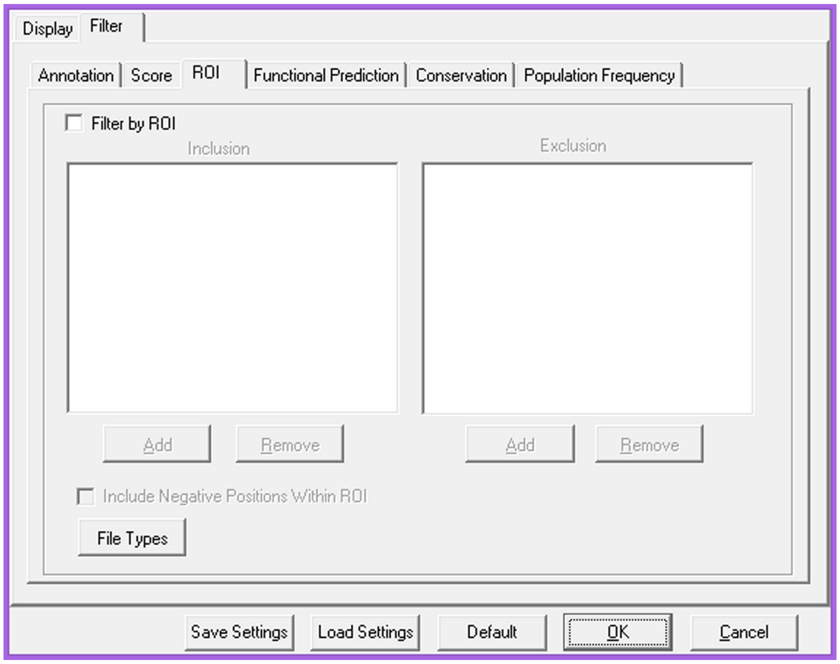


Figure 5: One of two causative mutations found as compared across all six projects. The projects (left to right) are the two affected children, the mother, the father, and the two unaffected children. The second unaffected child has this mutation but does not have the other mutation in the same gene.

Discussion

NextGENe's Variant Comparison tool makes it very simple to quickly sort through thousands of mutation calls in order to find a few dozen (or even fewer) candidate mutations that can be confirmed with Sanger sequencing and assessed for their phenotypic impact. NextGENe is able to import 1000 genomes frequencies and several functional scores from the dbNSFP database including PolyPhen-2, SIFT, LRT, and MutationTaster. Hiding reported (dbSNP) variations may be useful in some cases, but dbSNP does contain some clinical variants and some compound heterozygous pairs may involve a known SNP and a novel mutation. Knowledge about the disease can be used to include or exclude certain genes or regions in order to improve filtering even more (figure 6).



□ Filter by ROI

□ Include Negative Positions Within ROI

Figure 6: Multiple ROI files (BED files, VCF files, or text files specifying positions or gene names) can be loaded and used for filtering in an inclusive or exclusive manner.

Depth of coverage is very important when comparing multiple projects. Ideally all samples will have 30x or more coverage throughout the targeted regions so that confident mutation calls can be made. Missing data in any one sample may cause false negatives because that project cannot be used for filtering. In this test case all samples had more than 25x coverage at the potential causative mutation positions. Each whole-exome sequencing project consisted of 32 to 67 million aligned 100 bp paired-end Illumina reads.

References

1. Forbes, S. A., Bindal, N., Bamford, S., Cole, C., Kok, C. Y., Beare, D., ... & Futreal, P. A. (2011). COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic acids research, 39(suppl 1), D945-D950
2. Liu, X., Jian, X., & Boerwinkle, E. (2011). dbNSFP: a lightweight database of human nonsynonymous SNPs and their functional predictions. Human mutation, 32(8), 894-899.
3. Liu, X., Jian, X., & Boerwinkle, E. (2013). dbNSFP v2. 0: A Database of Human Non-synonymous SNVs and Their Functional Predictions and Annotations. Human mutation, 34(9), E2393-E2402.

Acknowledgements

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