OMICS RESEARCH SYMPOSIUM

Bioinformatics approach to analyze variants in Retinitis Pigmentosa.

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Introduction

Retinitis Pigmentosa (RP) , rare genetic disorder affecting the rods and cones present in an individual's eye that results as complete blindness (Kohei Saito 2021). It is the most occurring form of retinal dystrophy that is inherited. The disease can be inherited by three inheritance patterns namely Autosomal dominant , Autosomal recessive , and X-Linked inheritance. The prevalence of the disease is 1:3500-4000 (Bruninx 2020). A rod-cone dystrophy is the most prevalent type of RP, with night blindness as the first symptom, then results in progressive loss of peripheral vision in daylight, and eventually blindness in upcoming several decades.
Currently, mutations in 17 genes are known to induce adRP, 25 genes cause recessive RP, and 13 genes cause recessive RP. Alterations in the genes ,that induce recessive LCA (LCA is basically caused by two genes: dominant and recessive) and X-linked RP is caused by mutations in six genes (Stephen P Daiger 2007). But the causal variants are unknown for about 60% in the Retinitis Pigmentosa cases (Kohei Saito 2021).
Despite the difficulty, recent years have seen as substantial success in finding novel RP genes and screening patients for harmful mutations. This is due in part to the advancement of high-throughput mapping and sequencing technologies (Daiger, 2013).

The genomic data that had been generated for people with RP condition can reveal which SNPs (Single Nucleotide Polymorphism) are likely to cause the phenotype. To find such associations , GWAS (Genome Wide Association Studies) can reveal variants with statistical association between genome variation and disease. In near future, a more extensive study of genetic and clinical data, together that provide us better understanding of the genotype-phenotype link, may be able to offer critical information about disease progression and treatment options.

Table 1. Occurrence of Retinitis Pigmentosa and its Estimated Percentages for Retinitis Pigmentosa Types.

Category	Type	%of Total
Nonsyndromic RP	Autosomal dominant RP	20
	Autosomal recessive RP	13
	X-linked RP	8
	Isolated or unknown RP	20
	Leber congenital amaurosis	4
	Subtotal	65
Syndromic and Usher syndrome systemic RP		10
	Bardet-Biedl syndrome	5
	Other	10
	Subtotal	25
Other & unknown types of RP		10
Total		**100**

Fig(a) Abbreviation: RP, retinitis pigm

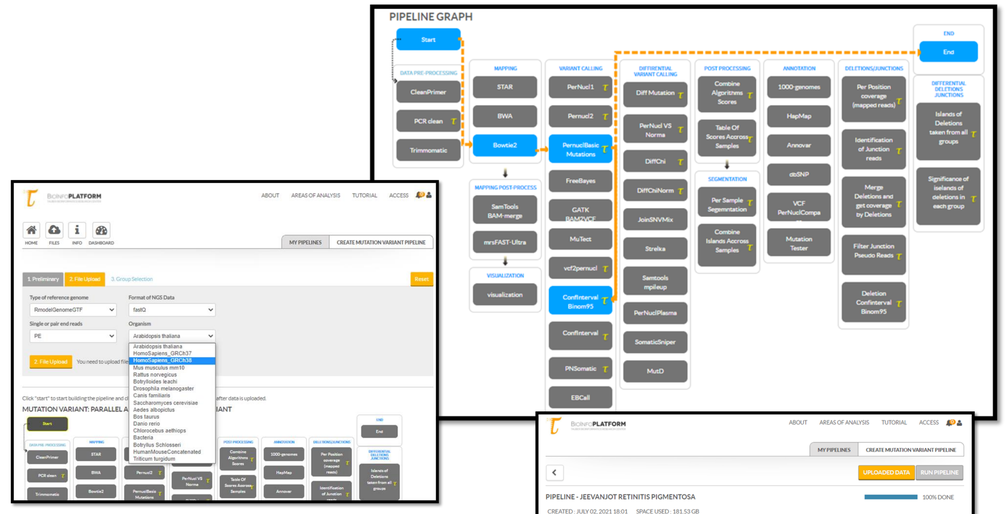
Methods

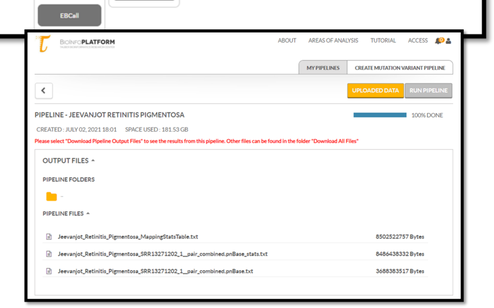
Data availability: Analysis data is collected from NCBI. The data is available as a BioProject PRJNA686229. It includes the primary data of exosome of whole sequencing of proband of Retinitis Pigmentosa.

Data analysis: The analysis of data is performed by mapping on reference genome and calling variants using PerNucleotide analysis of read coverage and statistical calling of significant variants.

The analysis is performed using ***t-Bioinfo platform.***

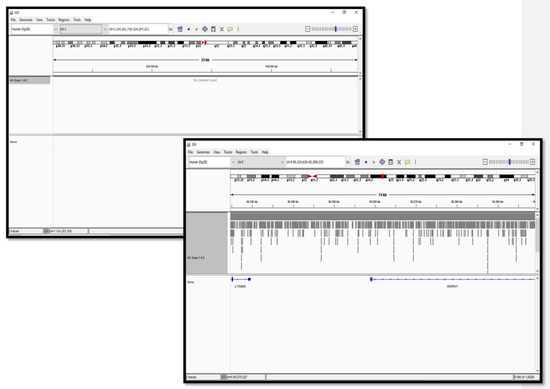
Following is the view of the pipeline graph being used in the analysis. It starts with clicking on the start button, after that few algorithms are used including Bowtie2 (Bowtie2 is a rapid alignment algorithm for seeds substrings that uses the FM-index approach to align them to the genome), PerNucl Basic Mutations (PerNucl is an algorithm for calculating all variations' per-position coverage of the reference genome), ConfIInterval Binom95 (Based on Wilks theorem for binomial distribution, MutationCallBinom95 calculates the 95 percent confidence interval for the frequency of every variation at the genomic site). Then it's the end of the graph and allow the pipeline to run.





The downloaded files are in the .txt format which is so far same as vcf file with all the data to explore. The downloaded files are to be viewed in IGV and will do comparative analysis and also look for the variants and choose top positions .

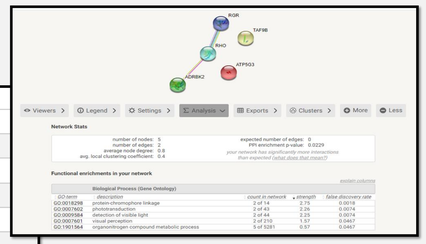
Results



For visualization, the IGV (Integrative Genomics Viewer) software had been used, a free tool for visualising vast amounts of genetic data. . For the visualization, the genome was set to be Human (hg38) , then select the resulted file to be uploaded using file from the browser option. SNPs are genetic variations that occur with a high frequency in a population. The Single Nucleotide Polymorphism Database (dbSNP) is a public-domain repository covering a wide range of common germline and somatic variants, as well as their known or expected clinical importance. This database can be loaded from the server, and archived SNPs can be seen with IGV. To view the SNPs , from the genome option uploaded the already existing datasets, chosen the All Snps 1.4.2 .

The total number of variants I got were 51,777 out of which 47,883 are single nucleotide polymorphism and 3,894 are displayed as insertions/deletions (INDELS). Amazingly , there are variants in all the chromosomes excluding the three chromosomes namely Chr 1 , Chr 16 and Y-chromosome.

All the 5 genes involved in the pathways (protein-chromophore linkage, phototransduction, detection of visible light, visual perception, organonitrogen compound metabolic process) are useful for proper vision.
Mutations in such genes can cause vision impairment.
The SNPs detected in the genes cause changes that leads to the mechanism disruption . Thus leads to Retinitis Pigmentosa.



s.no	Chr	Pos	ID	Alt
1.	2	175178203	rs1062648	A* to G
2.	3	129530997	rs759406789	G* to C
3.	10	84253016	rs769745211	G* to T
4.	22	25604363	rs751895901	C* to T
5.	X	78137889	rs781955939	A* to G

(Table 1)

[Chr – chromosome

Pos – position

Alt – alteration]

S.no	ID	FC	Gene	AA chng
1.	rs1062648	3_primer_UTR_variant	ATP5MC3	Lys --- Arg
2.	rs759406789	Missense_variant	RHO	Gly --- Ala
3.	rs769745211	Intron_variant	RGR	Gly --- Val
4.	rs751895901	Intron_variant	GRK3	Thr --- Ile
5.	rs781955939	Intron_variant	TAF9B	Lys --- Arg

(Table 2)

[FC – Functional consequences i.e when simple variants cause alterations in the product of gene and affect its phenotype.

AA chng – Amino acid change]

Conclusions

The project was started to know about the variants in the retinitis pigmentosa. RP , a rare genetic disease is caused due to mutations in approx. 100 genes so far, and the variants study is of interest to conclude about the diagnosis and treatment methods. The advances in the sector of omics paved the way to many researchers to study large genomes in less time with better and better conclusion. The disease is 3-tier in which the person first suffers from nyctalopia , then to tunnel vision and ultimately the blindness. The sample for the study is taken from BioProject PRJNA686229. After running a pipeline in T-BioInfo platform , the results are being analysed and visualised using IGV. The interpretations are made on the fact that whether the varinats have functional consequence or not. In total , 5 positions I have picked for my studies and looked for their FC on dbSNP that stores information for the SNP. After comparing the variants with dbSNP all the positions under studied showed some FC including missense variant, intron variant and 3 primer UTR variant. There are no variants found in 3 chromosomes namely chr1 ,chr 16 and Y chromosome.

The concluding statement is that the above studies show 2 inheritance patterns namely arRP and X-linked. The alterations studied can lead to the alterations in the genotype that can cause phenotype changes related to age or developmental stages. All the positions I looked upto are of SNV variant type.

REFERENCES

1. Zhang Q. Retinitis Pigmentosa: Progress and Perspective. Asia Pac J Ophthalmol (Phila). 2016 Jul-Aug;5(4):265-71. doi: 10.1097/APO.0000000000000227. PMID: 27488069.
2. Daiger SP, Sullivan LS, Bowne SJ. Genes and mutations causing retinitis pigmentosa. Clin Genet. 2013 Aug;84(2):132-41. doi: 10.1111/cge.12203. Epub 2013 Jun 19. PMID: 23701314; PMCID: PMC3856531.
3. Bruninx R, Lepièce G. L'image du mois. La rétinite pigmentaire [Retinitis pigmentosa]. Rev Med Liege. 2020 Feb;75(2):73-74. French. PMID: 32030928.
4. Branham K, Matsui H, Biswas P, Guru AA, Hicks M, Suk JJ, Li H, Jakubosky D, Long T, Telenti A, Nariai N, Heckenlively JR, Frazer KA, Sieving PA, Ayyagari R. Establishing the involvement of the novel gene AGBL5 in retinitis pigmentosa by whole genome sequencing. Physiol Genomics. 2016 Dec 1;48(12):922-927. doi: 10.1152/physiolgenomics.00101.2016. Epub 2016 Oct 7. PMID: 27764769; PMCID: PMC5206392.
5. Chiang JP, Lamey T, McLaren T, Thompson JA, Montgomery H, De Roach J. Progress and prospects of next-generation sequencing testing for inherited retinal dystrophy. Expert Rev Mol Diagn. 2015;15(10):1269-75. doi: 10.1586/14737159.2015.1081057. Epub 2015 Aug 26. PMID: 26394700; PMCID: PMC4659341.
6. Riaz M, Baird PN. Genetics in Retinal Diseases. Dev Ophthalmol. 2016;55:57-62. doi: 10.1159/000431142. Epub 2015 Oct 26. PMID: 26501365.
7. Nishiguchi KM, Miya F, Mori Y, Fujita K, Akiyama M, Kamatani T, Koyanagi Y, Sato K, Takigawa T, Ueno S, Tsugita M, Kunikata H, Cisarova K, Nishino J, Murakami A, Abe T, Momozawa Y, Terasaki H, Wada Y, Sonoda KH, Rivolta C, Tsunoda T, Tsujikawa M, Ikeda Y, Nakazawa T. A hypomorphic variant in EYS detected by genome-wide association study contributes toward retinitis pigmentosa. Commun Biol. 2021 Jan 29;4(1):140. doi: 10.1038/s42003-021-01662-9. PMID: 33514863; PMCID: PMC7846782.
8. Hamel C. Retinitis pigmentosa. Orphanet J Rare Dis. 2006 Oct 11;1:40. doi: 10.1186/1750-1172-1-40. PMID: 17032466; PMCID: PMC1621055.
9. Saito, K., Gotoh, N., Kang, I. et al. A case of retinitis pigmentosa homozygous for a rare CNGA1 causal variant. Sci Rep 11, 4681 (2021). https://doi.org/10.1038/s41598-021-84098-9
10. Daiger SP, Bowne SJ, Sullivan LS. Perspective on genes and mutations causing retinitis pigmentosa. Arch Ophthalmol. 2007 Feb;125(2):151-8. doi: 10.1001/archopht.125.2.151. PMID: 17296890; PMCID: PMC2580741.

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Video Link - (154) BIOINFORMATICS APPROACH TO ANALYZE VARIANTS IN RETINITIS PIGMENTOSA - Jeevanjot Kaur - YouTube