Validation of Candidate Disease-Causing Variants in Children with Primary Immunodeficiency

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Introduction

Primary immunodeficiencies are caused by hereditary or genetic defects, which affect the immune system resulting in an increased susceptibility to infection in children.

Here we look at two particular genes, IL7R and PIK3CD.

IL7R or IL7R alpha are both names for the unique alpha chain of the interleukin 7 receptor, which is necessary for T cell development and survival and proliferation of naïve and mature T cells. IL7R mutations cause an autosomal recessive form of severe combined immunodeficiency (SCID).

PIK3CD encodes the p110δ subunit of phosphatidylinositol-3-OH kinase (PI(3)K). PI(3)K plays an important role in the growth and maturation of B and T cells (Lucas et al., 2014). So far, only heterozygous gain of function mutations have been known to cause a combined immunodeficiency called APDS (activated PI(3)Kinase delta syndrome) which increases susceptibility to infections and progressive lung disease.

Results

Fig 1: PIK3CD disease-causing variant. (A) Integrated Genomic Viewer (IGV) shot of the PIK3CD homozygous frameshift deletion found in the whole exome sequencing (WES) results. (B) Sanger sequencing results for the PIK3CD homozygous frameshift deletion in patient DNA and control DNA. The blue box shows the deleted region and the red box shows the remaining nucleotides from the deleted region.

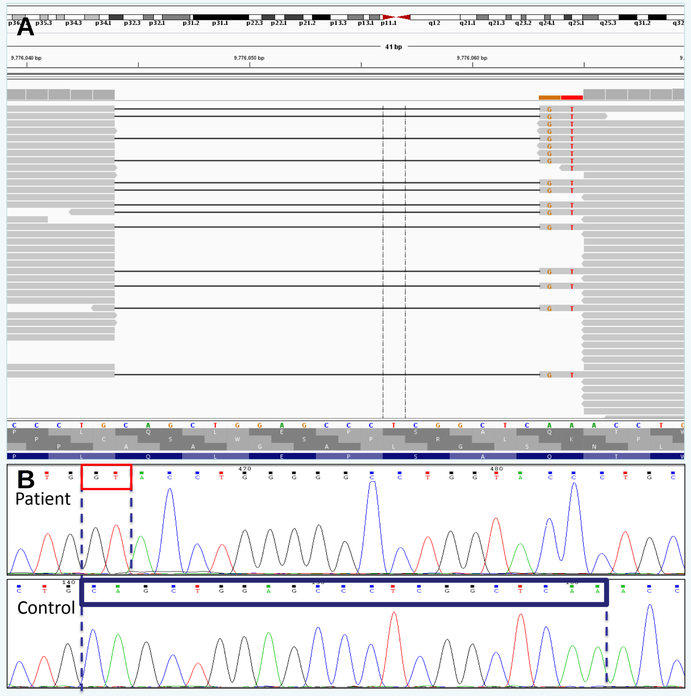
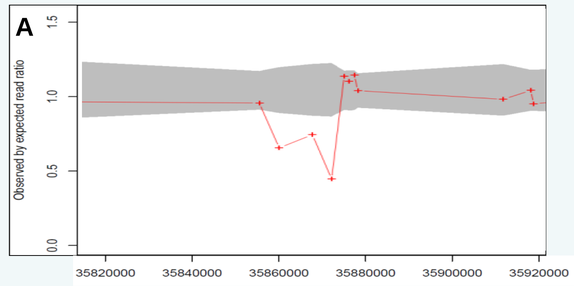
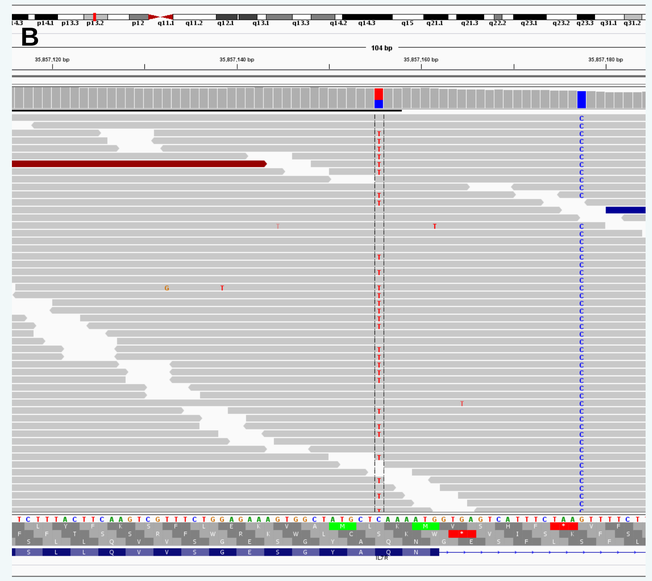
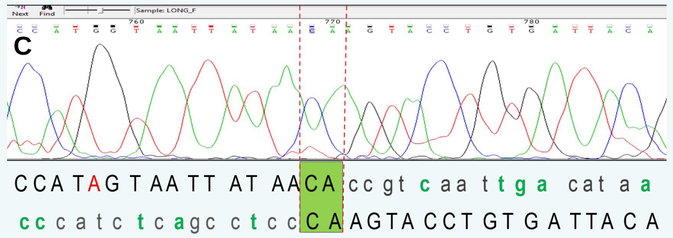
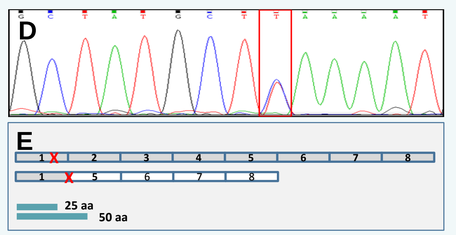


Fig 2: IL7R disease-causing variants. (A) Graph showing copy number of the IL7R gene in patient DNA (red) and reference DNA (Grey) from WES results. (B) IGV shot of the IL7R gene showing a nonsense mutation in exon 1. (C) Sanger sequencing results for the heterozygous IL7R exon 2-4 deletion. Capital letters of the first line show the nucleotides in the sequencing results, which are from exon 1, and the simple letters are the reference sequence. Capital letters of the second line are from exon 5. (D) Sanger sequencing results for the heterozygous IL7R nonsense mutation. (E) Diagram showing the likely locations of the two variants on the IL7R alleles and the effect they have on the protein products. The variants are highly likely to be compound heterozygous as it fits the phenotype.









Methods

- The whole exome of several SCID and combined immunodeficiency patients was sequenced by deep sequencing and the results were analysed. Interesting variants were chosen for further analysis.
- The three disease-causing variants chosen were an IL7R heterozygous exon 2-4 deletion, an IL7R heterozygous exon 1 nonsense mutation and a homozygous frameshift deletion in PIK3CD.
- Sanger sequencing was carried out to confirm these variants.

Discussion

- The sanger sequencing results for the PIK3CD disease-causing variant showed a 19 base-pair homozygous deletion (Fig 1B).
- This deletion is homozygous and causes a frameshift mutaion very early on in the protein and is followed by a premature stop codon which results in the removal of the ras-binding and kinase domain of the protein, therefore the mutation is a loss-of-function mutation.
- The copy number of IL7R exons 2, 3 and 4 dropped to 0.5 in the patient DNA (Fig 2A) showing that the multi-exon deletion is heterozygous.
- The IL7R exon 2-4 deletion breakpoint is at a dinucleotide (CA) (Fig 2C). This large deletion will lead to shorter protein products.
- The IL7R exon 1 nonsense mutation, which was confirmed by Sanger sequencing (Fig 2D), causes a premature stop codon also leading to shorter protein products.
- These two IL7R disease causing variants are highly likely to be compound heterozygous as it fits the patient phenotype very well.
- Compound heterozygosity is the presence of two different mutant alleles at a particular gene locus, one on each chromosome, therefore protein products by both alleles will be affected (Fig 2E).
- Compound heterozygosity is confirmed by parental DNA analysis which could not be done due to unavailability of parental DNA.

Conclusion

Sanger sequencing results validated the candidate disease-causing variants obtained from the whole exome sequencing data.

This work shows the importance of whole exome sequencing for diagnostic purposes of rare diseases.

More work will be done on the newly discovered PIK3CD disease-causing variant.

References: Lucas, C.L., Kuehn, H.S., Zhao, F., Niemela, J.E., Deenick, E.K., Palendira, U., Avery, D.T., Moens, L., Cannons, J.L., Biancalana, M., Stoddard, J., Ouyang, W., Frucht, D.M., Rao, V.K., Atkinson, T.P., Agharahimi, A., Hussey, A.A., Folio, L.R., Olivier, K.N., Fleisher, T.A., Pittaluga, S., Holland, S.M., Cohen, J.I., Oliveira, J.B., Tangye, S.G., Schwartzberg, P.L., Lenardo, M.J. and Uzel, G. (2014) 'Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency', *Nat Immunol*, 15(1), pp. 88-97.