Validation of whole exome sequencing results in patients with inborn errors of immunity

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1. Aims

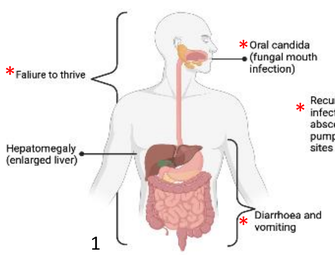
- To validate the mutations found in individuals in exon 6 of their *DOCK2* and *IL2RG* gene using Polymerase chain reaction (PCR) and Sanger sequencing (SS).
- To validate the segregation of the mutation of *IL2RG* in the TRIM family. So how the reported clinical phenotype links with the mutation in patient TRIM027.
- To investigate whether the reported clinical phenotype links with the mutation found in patient F98407.
- To explain the effects of the *DOCK2* and *IL2RG* mutations on both patients.

2. Introduction

- Next Generation Sequencing and SS are biochemical techniques we used to sequence patients with a clinical phenotype to find potential mutations in their genome to come up with a molecular diagnosis.
- Discovery of a mutation might help clinicians come up with a treatment plan.
- Therefore, genetic counselling can be offered to families, so they better understand the risks of future pregnancies.
- Identifying a mutation allows screening of other siblings at an earlier age so there can be early interventions e.g., stem cells transplant to alleviate symptoms or even cure the disease.

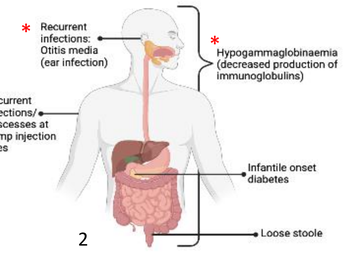
The clinical phenotypes of patients F98407 and TRIM027:

Patient: F98407
DOCK2



* Symptoms have been seen in 5 patients with *DOCK2*[3].

Patient: TRIM027
IL2RG



* Symptoms have been seen in patients with a mutation in *IL2RG* gene[4,5].

DOCK2:

- Expressed in hematopoietic cells and in peripheral blood leukocytes[6].
- Remodels the actin skeleton which is important for lymphocyte migration due to chemokine signalling[6].

IL2RG:

- The protein encoded by *IL2RG* plays a vital role in the signalling components of interleukin receptors which regulate the growth of B cells and T cells[7].
- *IL2RG* plays an important role in regulating the immune system[7].

3. Methods

Design primers → PCR and Gel electrophoresis of *IL2RG* + *DOCK2* amplicons → DNA gel extraction of *DOCK2* band → Sanger Sequencing

4. Results

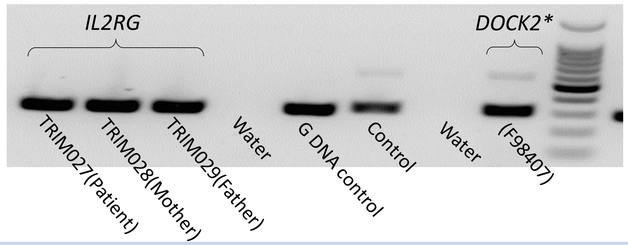


Figure 1: The PCR products of patients, family and control DNA are represented as dark bands on the agarose gel. The size of these bands was determined by the molecular weight ladder on the far right.

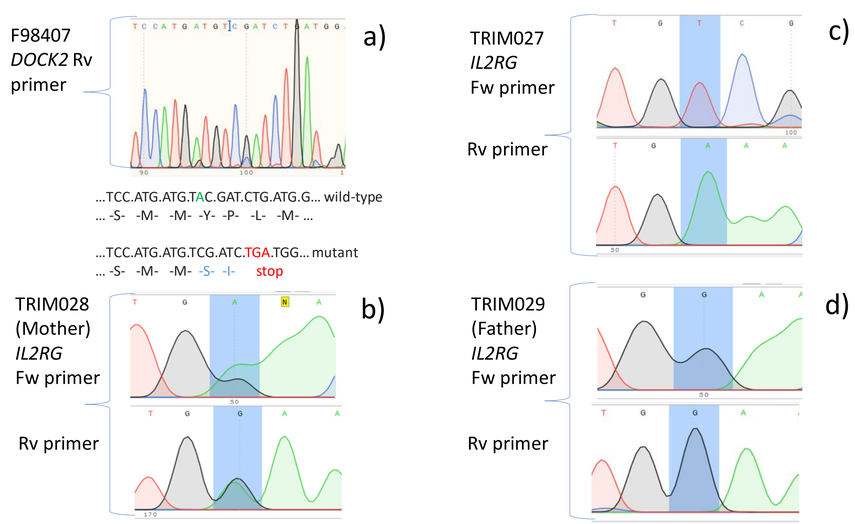


Figure 2 a-d: Each curve is assigned to a nucleotide (A,T,G,C). A) The blue line on the chromatogram shows the deleted nucleotide of *DOCK2*. The wild-type sequence represents no mutation, the mutant sequence (F98407) shows the deletion of the nucleotide and the frameshift. B-d) show the results from SS represented through the forward (Fw) and reverse primers (Rv) for the *IL2RG* family.

Patient: F98407	Patient: TRIM027	TRIM028 (Mother)	TRIM029 (Father)
Mutation: c.365 del A frameshift Homozygous for ***DOCK2*** variant	**Mutation:** c.850 G>A Missense Hemizygous for ***IL2RG*** variant	Heterozygous for ***IL2RG*** variant	Hemizygous wild-type (carries no form of variant)

5. Discussion

PCR:

- Is a biochemical technique that uses primers, enzymes and thermocycling to copy and amplify exon 6 of *DOCK2* and *IL2RG* which was then sent for SS.
- The PCR products are run by electrophoresis which separates DNA fragments based on their molecular weight and charge (Figure 1).
- A background band was observed for *DOCK2*,* so DNA gel extraction was carried out to isolate and purify *DOCK2* for SS.

Sanger Sequencing:

- SS is the secondary method which confirms the presence of the mutation. The results of SS are presented as chromatograms (Figure 2).
- **Reference sequence:** we used a reference sequence from Ensembl (genomic database) to compare it with the sequences from the patients and family members to determine abnormalities.
- **Confirmation of mutation:** searched for the affected nucleotide to see if it was substituted/deleted using the reference sequence as a guide.
- **Confirmation of the genotype**: looked at whether there was any overlap of the curves and changes in the nucleotides to determine whether the individuals are:
- **Heterozygous:** inherited two different alleles, one from each parent.
- **Hemizygous:** having only a single copy of a gene instead of two. All the genes on the single X chromosome in males are hemizygous[8].
- **Homozygous:** inherited two of the same allele, one from each parent.

6. Conclusion & Future work

- Patient F98407 has a one nucleotide deletion in exon 6 of *DOCK2*, resulting in a frameshift.
- **Effects of *DOCK2* mutation:** The Y amino acid at position 122 changed to S (Y122S) with a premature stop codon after 3 codons in the new reading frame (Y122Sfs*3).
- *DOCK2* is 1830 amino acids long, so the premature stop is quite early on, so the coded protein is not expressed and non-functional.
- Patient TRIM027 has a substitution of G>A making it a missense mutation.
- **Effects of *IL2RG* mutation:** can cause a non-functional gamma chain or it may not be expressed at all, which stops or diminishes lymphocyte development[4].
- **Future work** will include preforming a STAT5 functional test which measures STAT-5 phosphorylation to determine the functionality of the common gamma chain in *IL2RG*[9].

Acknowledgments & References

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References: 1,2. BioRender. 3.K.Dobbs et al. Inherited DOCK2 Deficiency in Patients with Early-Onset Invasive Infections. 4. Che Kang Lim IL2RG hypomorphic mutation: identification of a novel pathogenic mutation in exon 8. 5. Brahim Belaid IL2RG Chain Defect Presented as a Hyper-IgE Syndrome. 6.NIH DOCK2 dedicator of cytokinesis 2. 7. MedlinePlus IL2RG gene. 8. Charles Davis Definition of hemizygous. 9. A Arcas-Garcia. The IL-2RG R328X nonsense mutation allows partial STAT-5 phosphorylation and defines a critical region involved in the leaky-SCID phenotype.