Medicine

UNIVERSITY OF Southampton

Primary immunodeficiency caused by a novel compound heterozygote mutation in *MTHFD1*

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Introduction

Primary immunodeficiency diseases (PID) are a heterogeneous class of diseases where the patient presents with insufficient immune function to reliably combat infections, specifically caused by endogenous factors, such as genetics, as opposed to exogenous factors, such as HIV. PID will usually lead to chronic or repeat infections with pathogenic or opportunistic organisms throughout life, and in many cases can be fatal without effective treatment [1].

Genetic studies, facilitated by emergent technologies such as next-generation sequencing can allow us to identify the molecular aetiology, and hopefully tailor treatment thanks to this information. Whole-exome sequencing, where only the protein-coding regions of the genome (*ca.* 1%) are sequenced has enjoyed particular success since 2009 in providing a cost-efficient means of obtaining these molecular diagnoses [2].

Aims

We aimed to determine the molecular aetiology in a family (Fig. 1) where two brothers were diagnosed with severe combined immunodeficiency disease (AKA 'boy in the bubble' disease). Laboratory tests have also shown that the brothers suffer from a severe anaemia, corrected by folinic acid treatment.

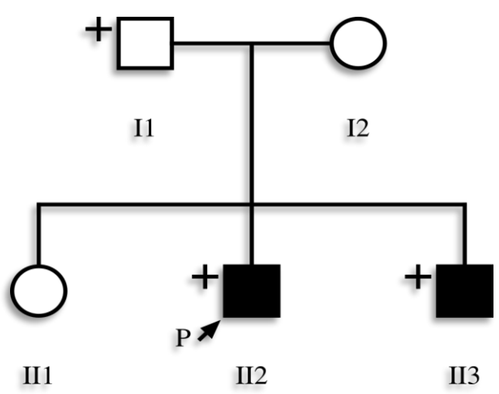

Fig. 1 | Pedigree showing affectation with disease. Squares represent males, circles females. Black fill – affected, '+' – whole-exome sequenced, P – proband.

Methods

We applied whole-exome sequencing to DNA samples from the father and two sons (Fig. 1), filtering for rare genetic variants that were present in both affected sons, and not in the unaffected father. An overview of the analysis workflow for the whole-exome sequencing data is shown (Fig. 2).

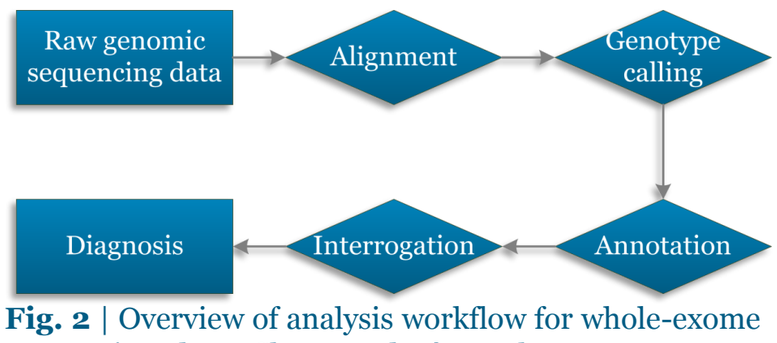

Fig. 2 | Overview of analysis workflow for whole-exome sequencing data. Short-reads from the sequencer are aligned to the reference human genome, variable position are 'called', assigning a genotype which is then annotated with biological information. Interrogation of these data will hopefully lead to a molecular diagnosis.

Results

A novel single nucleotide polymorphism (SNP) in *MTHFD1* was identified, resulting in a Leu-51-Pro substitution in the encoded protein C-1-tetrahydrofolate synthase, cytoplasmic (C1-THF synthase). This SNP was inherited from the mother.

Leu-51 is highly conserved evolutionarily across species, and is expected to be deleterious by *in silico* metrics. The residue is within 10 Å of the substrate binding site (Fig. 3) and the substitution of a proline is expected to significantly disturb the encompassing α-helix [3,4].

In addition to this SNP, further interrogation led to the identification of a paternally inherited novel *MTHFD1* exon 13 deletion. The deletion was identified by a reduction of the depth of sequence coverage (approximately proportional to the allelic dosage present in the genomic DNA, Fig. 4), and validated by Sanger sequencing. We have shown that it leads to nonsense-mediated decay of the mRNA, and is thus not translated.

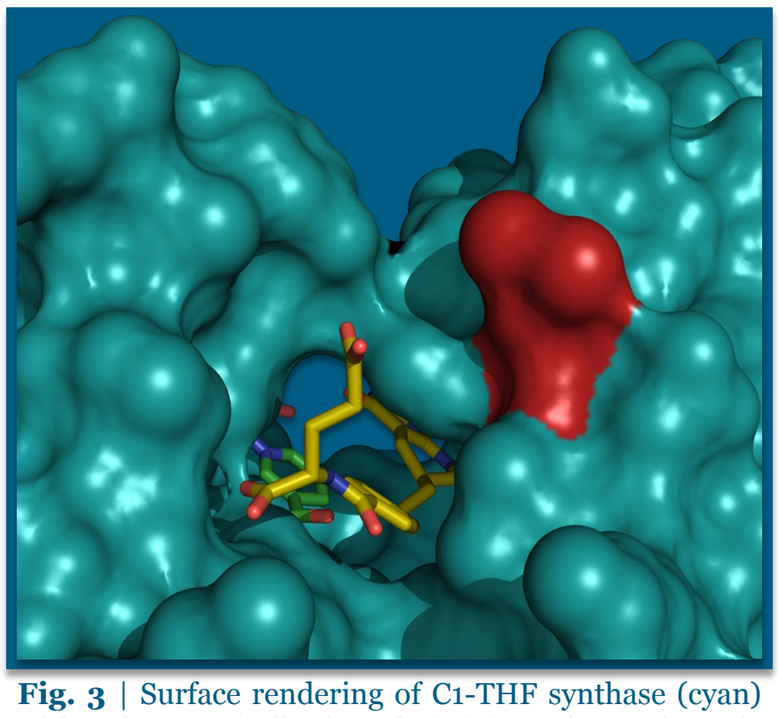

Fig. 3 | Surface rendering of C1-THF synthase (cyan) with substrate (yellow) and $NADP^+$ cofactor (green). Leu-51 is highlighted in red. PDB ID: 1DIA [3].

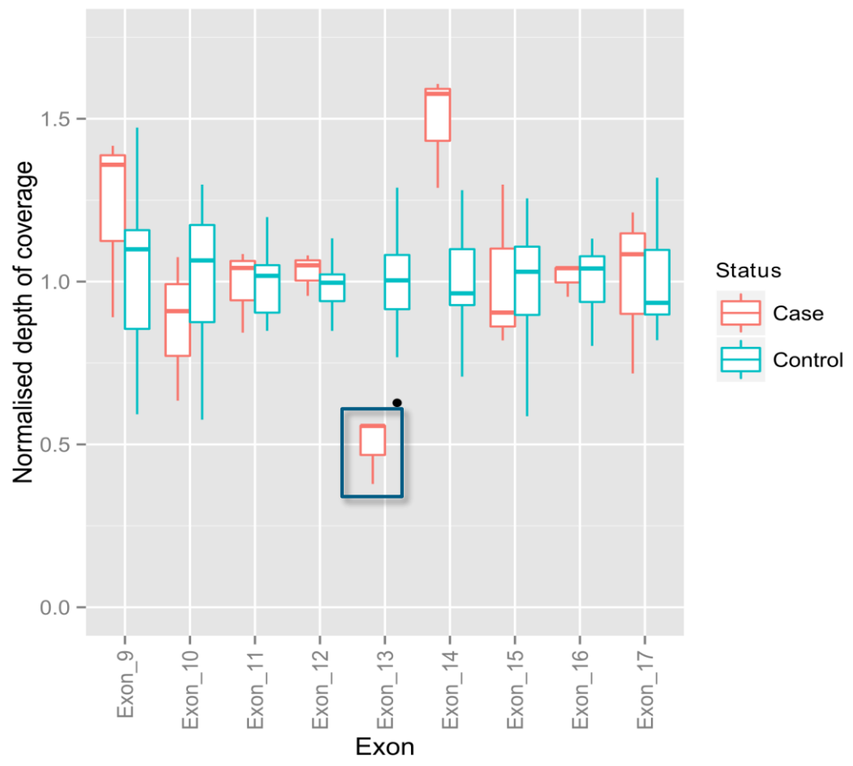

Fig. 4 | Depth of coverage for exons in *MTHFD1* in 13 control samples and the three cases, indicating an exon 13 deletion (in blue box) in the three whole-exome sequenced family members (p=0.00019 for depth between cases and controls).

As the affected brothers have inherited a different hypo-functional allele of *MTHFD1* from both of their parents (and so are compound heterozygotes for disease alleles), their cells have hypofunctional C1-THF synthase enzyme, and as such cannot efficiently metabolise folate.

Conclusions

We have identified a novel compound heterozygote in *MTHFD1* through whole-exome sequencing analysis. A previous single case study identified additional compound mutations in this gene that caused a neurological syndrome with associated PID [5]. The reduced function of C1-THF synthase prevented the generation of sufficient metabolites for cellular processes, causing disease. Treatment with supplements can be guided by these results, mostly reconstituting the immune system, with the brothers no longer requiring consideration for a bone marrow transplant.

References

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Acknowledgements

We would like to thank the patients involved in this study for their participation.
Analyses were performed on Iridis 4.
Banner image created by Christoph Bock.