VARIABLE FUNCTIONAL PHENOTYPES OF INHERITED IL-12 RECEPTOR VARIANTS IN PATIENTS WITH MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASE

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Introduction

In high TB burden countries such as South Africa, individuals with Mendelian Susceptibility to Mycobacterial Disease (**MSMD**) are prone to developing severe, persistent, unusual or recurrent (SPUR) TB. Several genes have been associated with MSMD, all resulting in the disruption of the IL-12-IFN-γ cytokine axis, which is essential for the effective control of mycobacterial infections.

The IL-12 receptor consists of the **IL-12Rβ1** and **IL-12Rβ2** subunits and co-expression is required to generate high affinity for IL-12.

Signal transduction through IL-12R firstly induces phosphorylation of JAK2 and TYK2, which in turn activates and **phosphorylates STAT4,** forms a homodimer and translocates to the nucleus where it activates the transcription of IL-12 responsive genes, including **IFN-γ.**

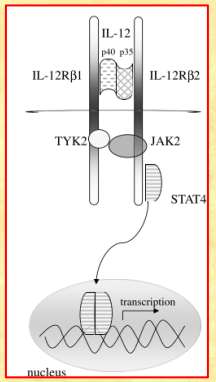

Figure 1: The IL-12 Receptor
Adapted from Haverkamp et al. 2014

Aim

The aim of this study was to assess the immunological phenotypes (relating to the IL-12-IFN-γ cytokine pathways) in four patients presenting with SPUR TB with likely disease-causing variants identified in the IL-12 receptor genes (*IL12RB1* or *IL12RB2*) and their family members using a set of in-house functional profiling assays.

Table 1: Summary of the clinical presentation and variants identified in IL-12R via WGS; N/A – age unknown

	Sex	Age	Clinical Presentation	Candidate Gene Variant Identified
P1	F	**3 y**	Recurrent and Persistent TB	***IL12RB1*** **- c.911_912del homozygous**
	F	**7 y**	Sister of P1 – Recurrent TB	***IL12RB1*** **- c.911_912del homozygous**
	M	**9 y**	Brother of P1 - Recurrent TB	***IL12RB1*** **- c.911_912del heterozygous**
	M	N/A	Father of P1 – Only one episode of pulmonary TB	***IL12RB1*** **- c.911_912del heterozygous**
P2	M	**10 y**	Severe, Unusual Disseminated TB – pericardial, rib, vertebrae and spine, cold abscess involvement	***IL12RB1*** **- c.C139T homozygous**
P3	F	**10 y**	Unusual, disseminated TB of the liver that is recurrent	***IL12RB1*** **- c.1921_1923del heterozygous & *IL12RB2* - c.G2290A heterozygous**
	F	N/A	Mother of P3 - unaffected	WGS not performed
P4	F	**19 y**	Recurrent TB - at least 5 separate confirmed episodes	***IL12RB2*** **- c.C1771T heterozygous & *IL12RB2* - c.G1259A heterozygous**
	M	**6 mo**	Son of P4 – Unusually Severe TB at 6 months old	WGS not performed

Methods

Novel, plausible disease-causing variants were identified previously in the genes coding for the IL-12 receptor in all four patients through Whole Genome Sequencing (WGS) – listed in Table 1

IL-12Rβ1 and IL-12Rβ2 expression was assessed through standard flow cytometric phenotyping. IL-12 receptor signalling was assessed by means of phospho-specific flow cytometry, which detects phosphorylated STAT4 following *in vitro* stimulation. IL-12-induced IFN-γ production was determined by Luminex following 48-hour IL-12 and BCG co-stimulation.

Results

The four patients had varying degrees of immune dysfunction, which deviated from the controls as well as from their family members. A summary of the data for γδ T cells (flow cytometry) and total PBMCs (cytokine –induced cytokine production) can be found in Table 2. A visual representation of the variable phenotypes observed in P1 and her family members are shown in Figure 2.

Table 2: Summary of the functional readouts

	% IL12RB1 (γδT cells)	% IL12RB2 (γδT cells)	IL-12 signalling (fold-change in pSTAT4 expression in γδT cells)	Induced IFN-γ production (pg/mL per 10^5 cells)
Control ranges (n=11)	22-52	5-15	1,65-2,62	300-1200 (n=4)
P1 – F	6,75	1,9	0,97	24,29
P1 - Sister	11	4,0	1,03	7,60
P1 - Brother	19,8	4,6	1,27	43,68
P1 - Father	22,3	5,6	1,74	138,65
P2 – M	8,12	8,7	1,28	37,90
P3 – F	25,5	9,6	1,72	196,19
P3 - Mother	35,9	16,3	1,11	172,86
P4 – F	43,4	3,3	2,05	1303,19
P4- Son	19,4	1,2	1,98	501,95

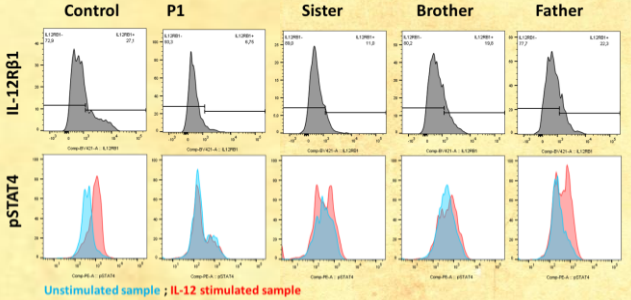

Figure 2: Variable flow-cytometric readouts of P1 and family

All individuals had an impaired phenotype compared to the controls

The individuals who have homozygous variants in the IL12R genes had more exacerbated phenotypes than heterozygous forms of the same variant.

Conclusion

The variability in the functional results for these individuals emphasizes the importance of *in vitro* assessment of genetic variants in patients and their family members to determine penetrance of particular gene variants.

References

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