IDENTIFICATION OF VARIANTS ASSOCIATED WITH THE PRIMARY IMMUNODEFICIENCY MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASE (MSMD) IN SOUTH AFRICA

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Background
Mendelian Susceptibility to Mycobacterial Disease (MSMD) is a group of rare inborn errors of immunity characterized by a predisposition to clinical disease caused by weakly pathogenic mycobacteria such as environmental mycobacteria and BCG vaccines. MSMD designation does not account for all the clinical features seen in patients, as affected individuals are prone to salmonellosis, candidiasis and tuberculosis (TB), and more rarely to infections with other intra-macrophagic bacteria, fungi, parasites, and in some cases, viruses. To date, a total of nine MSMD-causing genes have been identified. Of these, seven are autosomal (*IFNGR1, IFNGR2, STAT1, IL12B, IL12RB1, ISG15*, and *IRF8*) and two are X-linked (*NEMO* and *CYBB*). It has been well established that IFN-γ mediated immunity is essential for the control of mycobacterial infections and perhaps other intra-macrophage pathogens. To date, the disruption of IFN-γ immunity has been reported in all genetic etiologies of MSMD. The prevalence of MSMD is currently unknown in South Africa and is almost certainly under-detected due to the massive TB/HIV epidemic. In this study, our aim was to identify the genetic etiology of five patients with suspected MSMD using a whole exome sequencing approach. These mutations are being functionally validated using in house phenotypic and functional profiling.

Methods
A total of five suspected MSMD patients were recruited for the study. All patients were under the age of ten years on first enrolment in the study (range: 3 months – 10 years). Blood was drawn from each of the index cases and DNA extracted. Whole exome sequencing (WES) was performed on the Ion Torrent PGM at the Central Analytical Facility at Stellenbosch University and the data was processed using an in-house bioinformatics pipeline, TAPER™. All candidate variants were validated using Sanger sequencing.

Results
Plausible disease-causing mutations were identified in all five patients with suspected MSMD. Variants were identified in *IL12B* (*c. 320 A>G*), *IL12RB2* (*c. 1874 C>T; c. 1258 G>A*), *IL12RB1* (*c. 139 C>T*), *IFNGR2* (*c. 708 A>T*) and *IFNGR1* (*c. 864 C>G*). Each of these variants were identified in genes known to be associated with MSMD but must be functionally verified to determine the role in IFN-γ mediated immunity and subsequently the role in MSMD. Functional profiling involves examination of lymphocyte and monocyte expression of IFN-γ and IL-12 receptors, as well as *in vitro* IL-12 induction of IFN-γ and IFN-γ induction of IL-12.

Conclusion
Investigations of MSMD patients have indicated that human IFN-γ mediated immunity is essential for the control of mycobacterial infections. This study has indicated that in South Africa MSMD is usually associated with TB rather than unusual mycobacteria. An improved understanding of the molecular mechanisms required to contain infection with mycobacteria is essential for future approaches toward containment of TB, especially in the era of rapidly increasing drug resistance. More extensive screening of patients with recurrent TB infection is recommended. The identification of novel variants associated with disease has the potential to greatly improve our understanding of the pathobiology of a disease.