

## **Identification of a novel mutation in *MAGT1* and progressive multifocal leucoencephalopathy in a 58-year-old man with XMEN disease**

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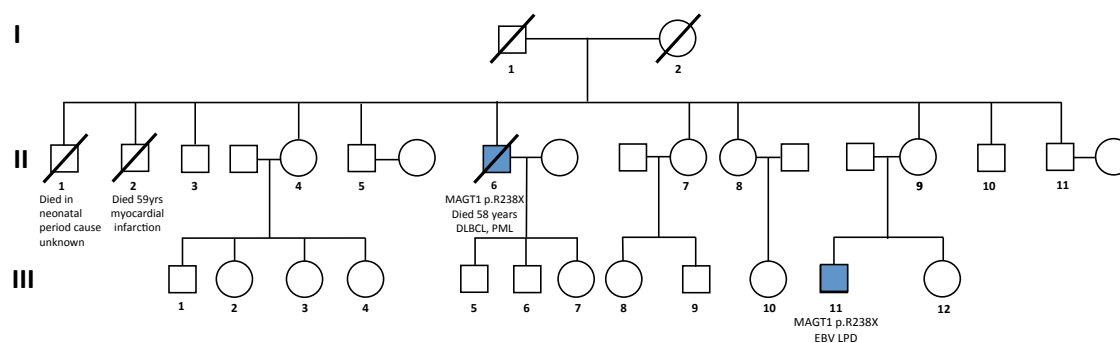
Work originated from the Department of Clinical Immunology and the Nuffield Department of Medicine, John Radcliffe Hospital, Oxford.

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## Supplementary Material

### Family pedigree

Family history revealed that 1 of the index patient's (II.6 in pedigree below) male siblings (II.1) had died of unknown causes in the neonatal period. All other family members, apart from the affected nephew (III.11), are said to be healthy with no history suggestive of primary immune deficiency or lymphoma. Genetic testing of the family is currently underway.



**Supp. Fig. 1 Family Pedigree**

### Methods

This research was approved by Institutional Review Board at the National Institute of Allergy and Infectious Diseases, NIH and the nephew provided written informed consent.

**Quantification of MagT1 RNA levels:** RNA was prepared from cryopreserved PBMCs using TRIzol (Ambion). RNA was treated with RQ1 RNase-free DNase (Promega), the DNase was inactivated at 65°C for 10 minutes, and cDNA was prepared from 0.7 ug of RNA using random hexamers with a SuperScript First-Strand Synthesis System kit (Invitrogen). Relative quantitative PCR was performed on ABI 7500 Real Time PCR System (ABI), using FastStart Universal SYBR Green Master (ROX) (Roche). Primer sequences used were MAGT1 exon1-For: 5'- TAG CCG GAG CAA AGT TTC AC - 3', MAGT1 exon1-Rev: 5'- GTC GCA AAC GAT GAG CAG -3', GAPDH-For: 5'- CCCC GGTTTCTATAAATTGAGC -3', and GAPDH-Rev: 5'- CACCTTCCCCATGGTGTCT -3'. PCR amplification was performed by denaturation at 95°C for 2 minutes, followed by 45 two-step

cycles of 95°C for 15 seconds and 60°C for 45 seconds. Dissociation curve analysis confirmed the specificity of the SYBR Green–amplified products. The mRNA level for the MAGT1 gene was normalized against that of the housekeeping gene GAPDH.

**Antigen-Specific CFSE and OX40 Assay:** Each experiment was conducted on 11 age and sex matched healthy controls, seropositive for EBV, CMV and VZV; on 2 independent occasions for the patient with XMEN disease and on 3 independent occasions for the patient with a hypomorphic mutation in *XIAP*(c.396G>T; p.G466X). Representative plots are shown.

**Antigen-Specific CFSE:** Peripheral blood mononuclear cells were isolated from whole blood using a lymphoprep gradient (Axis-Shield), and then diluted in AIMV medium (Gibco) to achieve a concentration of  $1 \times 10^6$ /ml. They were then stained with CFSE (1 $\mu$ M/ml) and after incubating for 15 minutes at 37C, quenched with 10mls of ice-cold RPMI (Sigma-Aldrich), and washed twice before plating at 200, 000 cells/200 $\mu$ l. The cells were then incubated at 37C, 5%CO<sub>2</sub> with medium alone or with 15 $\mu$ g/ml PHA (Sigma-Aldrich), or 1 $\mu$ g/well EBV lysate (Source Bioscience). After 5 days the cells were washed with PBS, and stained with anti-CD3PE-CY7 and anti-CD4-APC (BD Bioscience) for 15 minutes before analysis with flow cytometry (BD FACScanto II). After gating for CD3 positivity, T-cells with a blast-like appearance were selected using forward/side scatter. Finally, proliferating CD4 positive cells were measured by diminishing CFSE intensity compared to an unstimulated control.

**OX40 Assay:** 500 $\mu$ l aliquots of heparinised whole blood, diluted 1:1 in IMDM (Sigma-Aldrich), were prepared in sterile FACS tubes. These were then incubated with medium alone, 15 $\mu$ g/ml PHA (Sigma-Aldrich), 2 $\mu$ g/ml EBV, CMV, or VZV lysate (Source Bioscience) for 42-44 hours at 37C, 5% CO<sub>2</sub>. After incubation 100 $\mu$ l of each sample was stained with anti-CD25-FITC, anti-CD134-PE, anti-CD14-PerCP, 7-AAD, anti-CD4-APC, and anti-CD3-PE-C7 (BD Bioscience) for 15 minutes. Red cells were lysed with 1ml FACSlyse (BD Bioscience); the samples were washed twice and fixed with 1%

paraformaldehyde before being analysed using flow cytometry (BD FACScanto II). Cells were initially gated to acquire 30, 000 CD3+ events, a negative gate was then applied to exclude CD14 positive monocytes and apoptotic cells as these may aberrantly express activation markers. Dual expression of activation markers CD25 and CD134 (OX-40) was then measured amongst the CD4+ population; the gate coordinates were fixed to give 0.1% double positive events in the nil tube.