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Dear Tracey

Re: Progress report

Elucidating the genetic mechanism of primary antibody deficiency in patients with *TNFRSF13B* mutations

The aims of the project
1. Characterise TACI expression on B cells from *TNFRSF13B* mutants, according to antibody phenotype (hypogammaglobulinaemic or normal).

2. Perform global gene expression analysis of memory B cells according to the presence or absence of mutant *TNFRSF13B* alleles, according to the presence or absence of antibody defects. This might identify genetic variants that result in antibody deficiency in patients with *TNFRSF13B*.

3. Assess the prevalence of somatic mutations in memory B cells from patients with germline *TNFRSF13B* mutations.

Our project is progressing well towards these aims. Assembling the cohort and genotyping to get to this point represents an enormous amount of work.

1. Patients and blood samples
The Australia and New Zealand Antibody deficiency Allele (ANZADA) cohort has recruited 302 individuals across 195 pedigrees. Genotyping for *TNFRSF13B* has been completed for every new recruit with some form of antibody deficiency. We have now identified 12 patients with mutations in this gene. Importantly, we have now recruited

first degree relatives of these probands, which has lead to identification of a total of 16 individuals with same mutation as the probands (Table). Genotyping has confirmed a wild type among another 17 healthy relatives of the *TNFRSF13B* probands.

Table. *TNFRSF13B* genotypes in ANZADA cohort

TNFRSF13B Genotype	PAD	Healthy relatives
C104R	6	10
A181E	6	5
Normal		17

TNFRSF13B expression analysed by flow cytometry

To date, most published data investigating *TNFRSF13B* expression in humans have investigated cell lines propagated from individuals with *TNFRSF13B* mutations, rather than primary cells. By contrast, we have now completed cellular analysis on fresh B cells from patients and relatives with each of the genotypes for *TNFRSF13B* expression (mRNA and protein by RT-PCR and flow cytometry, respectively).

The next stage of the project involved characterisation of the level of *TNFRSF13B* expression on memory B cells on all available Australian probands and family members, analyzed according to genotype. Our findings so far have identified a significant reduction ($P < 0.05$) in the level of *TNFRSF13B* expression on class switched memory B cell between by those individuals carrying either the A181E mutation (transmembrane domain) or C104R mutation (extracelluar domain), when compared with memory B cells from both healthy individuals and CVID patients with normal *TNFRSF13B* alleles. Significantly, there is no difference in expression levels between those with and without antibody deficiency. **This analysis has established that hypogammaglobulinaemia in patients with mutant *TNFRSF13B* is not associated with differential protein expression on the cell surface compared to those with normal immunoglobulin and mutant *TNFRSF13B*.**

We also aim was to perform global gene expression analysis of memory B cells according to the presence or absence of mutant *TNFRSF13B* alleles, and the presence or absence of antibody defects. This might identify genetic variants that result in antibody deficiency in patients with *TNFRSF13B*. The remaining experiments will be directed at this aim. Our preliminary experiments have identified appropriate donors for this phase of the project. Sample collection is underway for gene expression analysis. We will assess the prevalence of somatic mutations in memory B cells from patients with germline *TNFRSF13B* mutations. This will be achieved by sorting class-switched memory B cells will by FACS from hypogammaglobulinaemic individuals heterozygous for *TNFRSF13B*. cDNA will be isolated and analysed by next generation sequencing at our facility (JCSMR). This will permit assessment of how *TNFRSF13B* variant alleles affect abundance of expressed genes in memory B cells, and the spectrum

of somatic mutations in individuals who harbour variant *TNFRSF13B* alleles but either do or do not have antibody deficiency.

We are very grateful to the RCPA for their generous support of this exciting project.

Yours sincerely

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