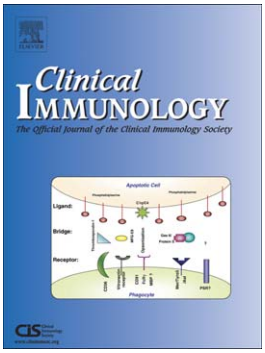

A novel *FOXP3* mutation causing fetal akinesia and recurrent male miscarriages

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A novel *FOXP3* mutation causing fetal akinesia and recurrent male miscarriages

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To the editor,

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare primary immunodeficiency (PID) due to mutations in the *FOXP3* gene which is located at Xp11.23 [1]. *FOXP3* encodes the 431-amino acid transcriptional regulator protein forkhead box p3 (Foxp3) that is critical for the development and maintenance of CD3+CD4+ CD25+ Foxp3+ regulatory T cells (Tregs) [2]. Mutations in the Treg master transcription factor, Foxp3, effects Treg development and function which culminates in multiple autoimmune conditions forming the clinical IPEX syndrome [3]. IPEX patients usually present in infancy with the clinical triad of enteropathy, autoimmune endocrinopathies and dermatitis, although many other autoimmune phenomenon have been reported [4].

Pregnancy presents an immunological challenge to the human body with maternal and fetal exposure to polymorphic allogeneic foreign antigens from tissues. Fetal tissue has immunogenic potential for the mother and requires rapid development of feto-maternal tolerance. Acquisition of this tolerance during pregnancy was thought to be solely reliant on maternal tolerance of an allogeneic fetus. Evidence now shows that both mother and fetus must develop tolerance to allogeneic antigens for successful gestation [5]. Tregs play a central role in this process and studies of fetal Tregs show that they possess potent lymphocyte suppressor activity [6].

Carneiro-Sampaio and colleagues describe two families with fetal-onset IPEX and proband males presenting with symptoms of classical IPEX syndrome [7]. Most IPEX cases previously reported have uneventful gestations and are born at birth without incident, but a detailed family history may reveal miscarriages in previous generations [4].We present a case of a mother with a novel *FOXP3* mutation and recurrent male miscarriages with no male

fetus surviving the beyond the second gestational trimester. With the lack of a proband with clinical symptoms of IPEX this case demonstrates the benefits of rapid next generation sequencing (NGS) technologies for analysis of PID genes in the diagnosis of challenging clinical presentations.

A 41yr old female was referred for assessment following multiple male miscarriages. The patient has a background of atopic dermatitis, asthma, allergic rhinoconjuctivitis and irritable bowel syndrome; although she had not undergone endoscopic or histological evaluation. There was no previous family history to suggest PID. Blood tests showed normal full blood counts, lymphocyte subset populations; CD3+ CD4+ $CD25^{high}$ $CD127^{low}$ Treg percentage was 3.6% of total CD3+ CD4+ T cells. IgE was raised at 291 iu/ml. Viral IgG serology was detected to CMV, Parvovirus, Rubella and was negative for HIV. She was blood group O+ with no anti-erythrocyte antibodies detected. Thyroid function tests were normal and there was no history of diabetes. No organ specific autoantibodies were detected. The patient's first pregnancy aged 34yrs resulted in the death of a male fetus at 18 weeks due to hydrops fetalis. A limited post-mortem did not identifying any obvious gross abnormalities. Aged 35yrs, the patient gave birth to a healthy female at full term. Aged 37yrs, a 3^{rd} pregnancy resulted in a male with fatal hydrops fetalis at 20 weeks. Fetal histology showed CD3+CD4+ cellular infiltrate within the kidneys and liver. Skeletal muscle was almost completely replaced by fibrosis tissue and macrophages. A 4^{th} pregnancy aged 39yr resulted in a male fetus with fetal akinesia, progressive hydrops and termination at 18 weeks. Fetal histology showed near absent skeletal muscle with reactive CD3+ T cell infiltrating the pancreas, myocardium, portal tracts, oesophagus and choroid plexus (Figure 1). PCR on histological samples for CMV, Parvovirus B19 and Toxoplasma was negative. Fetal DNA screening was performed by next generation sequencing (NGS) with library preparation using a Sure Select XT kit with sequencing via Illumina MiSeq, with confirmation by Sanger

Sequencing. The mutation *FOXP3* c.1009C>T p.(Arg337Ter) was identified in fetal tissue of the 3rd and 4th miscarriages. Carrier status of p. Arg337Ter, with random X inactivation, was confirmed in the mother. Samples were unavailable from the 1st miscarriage, but the similarities are suggestive that the *FOXP3* mutation was also present. The patient's daughter remains well and given her age genetic analysis has not yet been undertaken.

The miscarriage timing in of the miscarriages in the second trimester is possibly due to the immunological events occurring at that gestational stage. The second trimester is the first time that the fetus is significantly challenged by its own endogenous immune system with the requirement for robust self-tolerance. This immunological hurdle is usually met by an increase of CD4+ CD25+ Foxp3+ fetal Tregs in peripheral fetal lymphoid organs to approximately 15-20% of total CD4+ cells, the highest Treg proportion of CD4+ T cells ever during life [8]. In this case we believe that compromise in fetal Treg development due to *FOXP3*c.1009C>T p.(Arg337Ter) caused lethal *in utero* reactive T cell infiltration of multiple organs leading to fetal kinesia and progressive hydrops. The mutation c.1009C>T is located in exon 10 of *FOXP3* (Genbank accession number NM_014009.3 and results in termination of the Foxp3 protein translation at p.(Arg337) with loss of the 94-amino acids that form the forkhead domain and C-terminus. The arginine residue at amino acid position 337 of Foxp3 is highly conserved and involved in binding to DNA [9] and the majority of *FOXP3* mutations that cause IPEX syndrome are located within the forkhead domain, supporting that this region of the protein is critical to its function [4].

Recently, recurrent male miscarriages with no surviving fetus and a similar clinical and histological picture have been reported [10]. However, fetal akinesia and has not been described in cases of IPEX syndrome and is usually associated with defects in the neuromuscular pathway which complicated the differential diagnosis in our case. Given this atypical *in utero* presentation, the approach of a NGS gene panel allowed multiple genes to

be sequenced in a short time for a molecular diagnosis to be quickly achieved. This can then offer the opportunity for antenatal testing in subsequent pregnancies. This case furthers demonstrates the uses of genomic medicine in patients and families affected by PID.

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Figure 1. (A) Fetal pancreatic tissue with dense predominantly CD3+CD4+ lymphoid inflammatory. (B) Fetal myocardial tissue with lymphocytic inflammatory infiltration and scattered macrophages (x200 magnification).

- IPEX syndrome is recognised to manifest clinically *in utero.*
- IPEX syndrome should be considered in cases of recurrent male miscarriage with fetal akinesia.
- The mutation *FOXP3* c.1009C>T p.(Arg337Ter) causes severe *in utero* IPEX syndrome.
- Next generation sequencing of gene panels can aid diagnosis in atypical presentations of primary immunodeficiency.