British Association of Dermatologists Project Grant Report

John Lee

I would like to express my gratitude to the British Association of Dermatologists for the award of the project grant. This award allowed me to continue working on a research project that began during my intercalated BSc in Medical Genetics, under the supervision of Professor John McGrath (St John's Institute of Dermatology) and Professor Maddy Parsons (Randall Division of Cell & Molecular Biophysics). The focus of my project was to investigate the molecular genetic basis of inherited disorders of pigmentation.

In this project, we identified a family from the Middle East where four affected individuals presented with an unusual autosomal recessive condition causing pigmentary defects (silvery-grey hair, vitiligo spots) and progressive spastic paraparesis. Using whole-exome sequencing, we identified a homozygous intragenic deletion in a gene not previously implicated in any dermatological or neurological diseases. By gel electrophoresis and Sanger sequencing, we confirmed that this mutation indeed segregated with disease status in this family. By collaborating with researchers from all around the world, we were able to identify two further pedigrees with the same condition and found that they also harboured the same deletion mutation.

Skin biopsies were obtained from one of the patients and transmission electron microscopy revealed extensive cell death and swollen mitochondria, predominantly in melanocytes but also to a lesser extent in keratinocytes and fibroblasts. Western blotting and immunofluorescence staining in patient skin revealed significantly reduced protein expression in patient skin, indicating a loss-of-function effect of the deletion mutation.

We then used *in vitro* models in order to find out more about the effects of this loss-of-function mutation at the cellular level. Primary keratinocytes and fibroblasts were isolated from one of the patient's skin biopsy samples. We also used siRNA knockdown of the gene of interest in immortalised mouse keratinocytes, fibroblasts and melanocytes. Upon exposure to ultraviolet irradiation, knockdown and patient-derived cells exhibited increased cell death compared to controls, as quantified by cleaved caspase-3 staining and loss of cell attachment. Since the gene of interest was previously demonstrated to be involved in the extracellular signal-regulated kinase (ERK) pathway, we next investigated whether this mutation led to a loss of phosphorylated ERK (pERK) activity after stimulation with fibroblast growth factor (FGF). Whilst primary patient keratinocytes showed reduced pERK activity in response to FGF stimulation, patient fibroblasts showed no difference compared to control cells. This interesting finding suggests that the involvement of this gene in the ERK pathway is likely to be cell-type specific.

In conclusion, although interesting insights have been gained through this study, the underlying pathobiology of this rare disorder remains largely unknown. Further experiments in collaboration with colleagues at the University of Minnesota involving patient-derived induced pluripotent stem cells (iPSCs) are ongoing. Our next goal is to generate melanocytes and possibly neural cell lines from these iPSCs in order to try to further explain the pigmentary and neurological symptoms of patients. The pathomechanisms involved in this Mendelian disease may also open new avenues for research in complex polygenic versions of vitiligo and spastic paraparesis.