NATIONAL HEALTH
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Professor Brian Fowler and Dr Ann Brown
Chairman and Honorary Treasurer
ERNDIM

Report on ERNDIM Training Grant awarded to Dr GF van der Watt.

Thank you for your kind contribution

The grant monies awarded by ERNDIM were used towards travel and accommodation costs that enabled me to visit the Sheffield Childrens Hospital Inherited Metabolic Disease Laboratory in the United Kingdom for a period of 2 weeks in February 2009.

Background:

Over the last 14 months, running the metabolic diagnostic service in Cape Town, South Africa we have diagnosed 6 new cases of glutaric aciduria type 1 (GA1) (OMIM #231670). In all these cases the diagnosis was suspected based on clinical findings and organic acid analysis and confirmed locally by mutational analysis of the glutaryl CoA dehydrogenase (GCD) gene. The recent implementation of a quality organic acid screening service within our institution has played a significant role in picking up these patients that may otherwise have gone undiagnosed. The interesting finding in all 6 cases and in another three known cases has been that all these patients are African children of Southern African ancestry and all are homozygous for the same A294T substitution, furthermore there is no history of consanguinity in any of the affected families. We believe that the carrier frequency of this allele may be high in the local population and have set about investigating this disorder in our population.

A third leg in the laboratory diagnosis of GA1 is measurement of GCD activity using radiolabeled glutaryl-CoA in cultured fibroblasts. This assay plays an essential role in investigating suspected GA1 patients as urine organic screening is not completely sensitive and mutational analysis with complete gene sequencing is expensive and cannot confirm all cases. Very few laboratories in the world perform this assay because the labeled reagent is not available commercially. Sheffield Children's Hospital's Inherited Metabolic Disease Laboratory is one of these.

Drs Jim Bonham, Simon Olpin and Nigel Manning at Sheffield Childrens all kindly agreed to allow me to visit their laboratory to learn how to synthesize the radiolabeled reagent and run the assay that they have developed for this disorder.

Training Visit:

During the time at Sheffield Childrens after attending a radiation safety course presented by Mrs Tracy Soames I accompanied Dr Olpin and learnt how to synthesize 1,5^{14}C glutaryl-CoA from 1,5^{14}C glutaric acid. The method involves boiling radioactive glutaryl anhydride under reflux – a tricky business and not for the feint hearted. After synthesizing a batch of the reagent, I then learnt to set up and run the assay on cultured fibroblasts – this involves trapping released ^{14}CO2 in fiber-glass filter discs and quantifying the captured radioactivity by beta emission. After demonstrating expected results in known normal and abnormal cell lines I then repeated the assay on 3 fibroblast cell lines from our own patients with the homozygous A294T mutation – all three patients had no detectable GCD enzyme activity.

In addition to the time spent setting up and running the GCD assay I was also fortunate enough to review many of the routine diagnostic methods employed within the unit – these included a review of the following:

- Overview of Software applications to facilitate processing and interpretation of Urine organic Acid analysis – Dr Nigel manning
- Two dimensional TLC for amino acid screening in body fluids – Dr Camilla Reed
- Fibroblast Immunofluorohistochemical staining for catalase in the screening for peroxisomal disorders – Dr Camilla Reed
- Fibroblast Fillipin staining for Niemann Pick type C disease – Dr Camilla Reed
- Very Long Chain Fatty Acid analysis by GCMS, methodology and aspects of method development – Dr Nigel Manning
- Methodologies, data management and structural organization of the dried blood spot NBS program at Sheffield Children's Hospital – Professor Rodney Pollitt and Dr Melanie Downing

Tutorials on various topics around fatty acid oxidation defects and demonstration of interpretation and validation of acylcarnitine profiles were also provided throughout the time I spent in the lab by Dr Olpin and I also attended various patient centred discussions and case presentation

Conclusion:

The visit to Sheffield Children's was a marked success and I would like to take this opportunity to thank ERNDIM and the staff at Sheffield Children's for making the trip possible. I would like to extend special thanks to Dr Jim Bonham, the laboratory head and joint head of the diagnostic directorate at Sheffield Children's for supporting and facilitating my visit and a special thanks to Dr's Simon Olpin, Nigel Manning and Camilla Reed for their friendly help, willingness to share their expertise, patience and excellent sense of humour.

The knowledge gained and techniques learnt will go a long way to enabling us to improve the diagnostic service that we provide in South Africa and we hope to set up the GCD assay here in the near future. It will certainly be put to good use and make a significant contribution towards our investigation of and research into this disorder and it's molecular basis in Southern Africa.

Thank all you once again

Kindest Regards

George vd Watt

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Simon Olpin and George vd Watt during his visit to Sheffield.