



Medical Neurogenetics

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Letter of Intent.

To establish keratinocytes and/or melanocytes as a cell source for the enzyme assay of tyrosine hydroxylase.

1. **Objective.** To demonstrate that cultured keratinocytes and/or melanocytes provide a cell system in which the enzyme activity of tyrosine hydroxylase (TH) can be determined. Measurement of TH activity in these cells from patients suspected of having tyrosine hydroxylase deficiency can be used to confirm or exclude a diagnosis of TH deficiency.

2. **Which specific PND disease the research relates to and/or relevance to PND's** Tyrosine hydroxylase deficiency is an autosomal recessive disorder that leads to a deficiency of catecholamines in the central and peripheral nervous systems. Approximately 100 cases have been diagnosed world wide. However, it is certain that many more cases remain undiagnosed. Currently diagnosis requires a lumbar puncture followed by measurement of homovanillic acid (HVA) in cerebrospinal fluid and a finding of a low level of this metabolite. It is well known that low HVA levels are found in many patients with severe neurological disease that do not have TH deficiency (Van Der Heyden, Rotteveel, & Wevers, 2003). A final absolute diagnosis for TH deficiency cannot therefore be achieved unless pathogenic mutations are detected. From our experience only about 10% of samples that are submitted for TH genomic sequencing are found to be positive for pathogenic mutations. This demonstrates a need for a biochemical screening assay that can be used to filter candidate patients.

It has been a widely accepted dogma that an easily obtainable peripheral source of tissue is not available in which TH enzyme activity can be measured. This dogma is incorrect. There is evidence showing that keratinocytes and melanocytes possess all of the machinery

necessary to produce catecholamines including TH. We initially demonstrated that cultured human keratinocytes contain mRNA for both TH and aromatic L-amino acid decarboxylase (Chang, Mues, Pittelkow, & Hyland, 1996). The expression of TH in keratinocytes was later confirmed (Gillbro, Marles, Hibberts, & Schallreuter, 2004) and by western blot and direct enzyme assay the presence of TH protein in these cells has been demonstrated (Marles, Peters, Tobin, Hibberts, & Schallreuter, 2003; Pullar, Rizzo, & Isseroff, 2006). These cells are easily obtained (via a fairly non-invasive suction blister) and commercial, cell specific culture medium is available.

3. **Hypothesis to be tested.** Human keratinocytes and/or melanocytes can be cultured and used to assay tyrosine hydroxylase activity and to examine TH mRNA integrity. In this manner cells obtained from patients suspected of having TH deficiency can be used to provide a definitive diagnosis.

4. **Approximate amount of funding requested:** To cover 50% salary of technician and tissue culture/assay costs associated with method development and analysis of normal and TH deficient cell lines: \$40,000.

5. **Approximate timeline for research project:** 1 year.

Reference List

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