Proteomic profile in congenital microcephaly

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Abstract

Why? Autosomal recessive primary microcephaly consists of a group of disorders characterized by microcephaly and intellectual disability. This study is essential to complement previous findings of MCPH as it helps clarify the role of different genes and proteins involved in the underlying pathophysiology of MCPH. To date, 27 different mutations have been identified.

What? This study defines a number of changes in gene expression occurring in MCPH. This helps deepen our understanding of the effect of MCPH mutations on gene expression. This study also shows the functions of proteins that increase, are unaffected, or become dysfunctional due to MCPH. We identified a marked reduction about 30 proteins with vital roles in several processes including cell cytoskeleton dynamics, cell cycle progression, ciliary functions, and apoptosis.

How? We used Cdk5rap2 (Hartwig's anemia mice) which a model that closely represent MCPH3. Gel electrophoresis was utilized in order to separate brain proteins. Fixation and protein identification was then done in order to detect changes in the level of the tested proteins.

Introduction

Autosomal recessive primary microcephaly (MCPH) is a disorder of neurodevelopment. It is characterized by mental dysfunction that is nonprogressive and microcephaly at birth. MCPH subtype 3 (MCPH3) is caused by variants of cyclin-dependent kinase 5 regulatory subunit-associated protein 2 gene (CDK5RAP2). Defects arise due to depletion of neural progenitors when this gene is impaired. Cdk5rap2 mutant mice (an/an) that were used are microcephalic. Proteomic testing will help uncover more information about MCPH path mechanism.

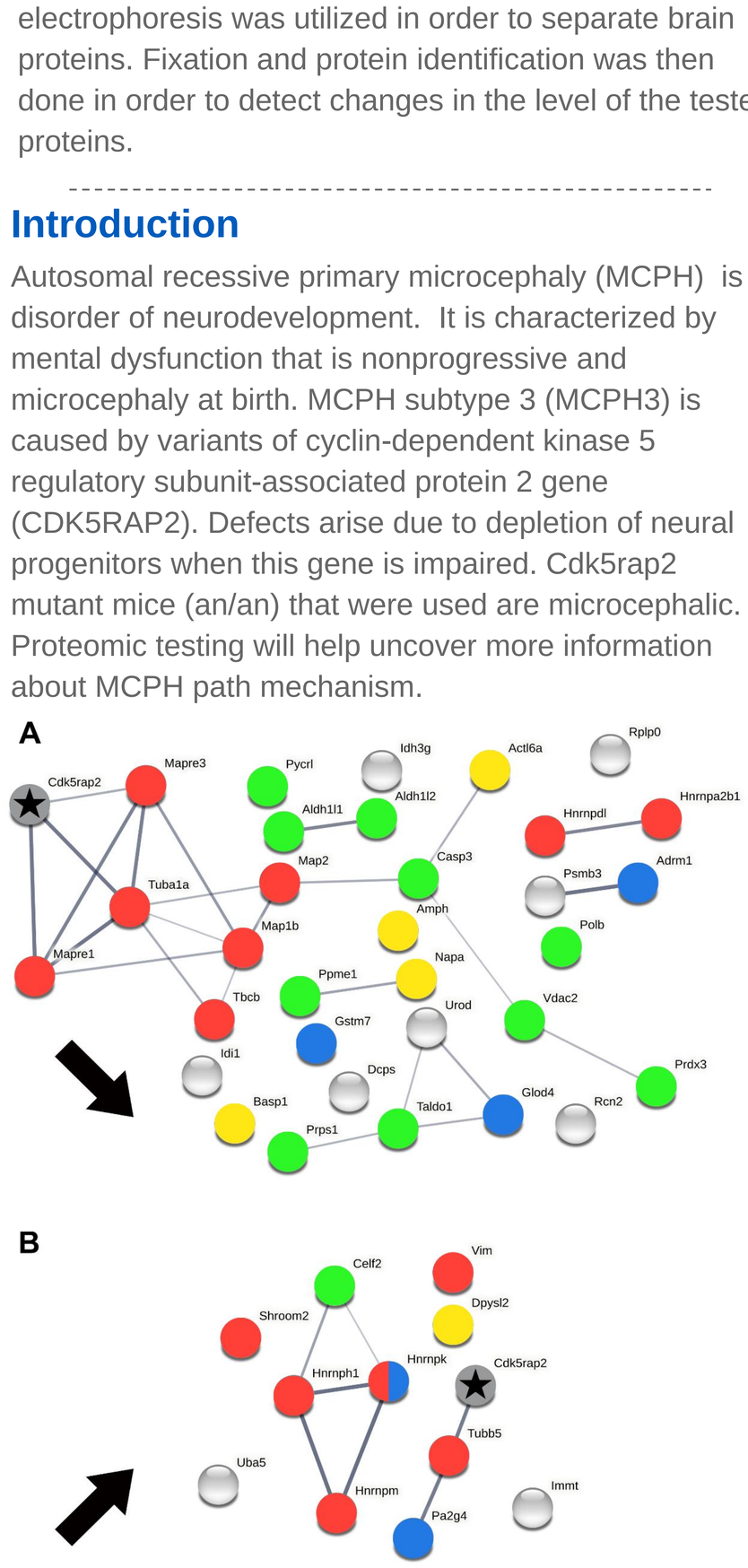


Figure.1 Protein-protein interactions red (cytoskeleton proteins), blue (cell cycle and cilia), green (apoptosis) yellow (synapse function/vesicular transport).

Methods

Mice: Cdk5rap2 mutant was used as a model for MCPH3, which carry an inversion of Cdk5rap2 exon and are therefore microcephalic.

Tissue Collection: issue collection was collected at postnatal day 0 (P0). The cerebral cortices of the mice were then pooled and snap-frozen using liquid nitrogen (-80 °C). The tissue was then lyophilized, homogenized, and then put in lysis buffer. Extraction and measuring protein concentration was then done according to the protocol of the BioRad DC Protein Assay kit.

Gel Electrophoresis: large-gel two-dimensional gel electrophoresis (2-DE) was performed to separate proteins. They were then 2-DE gels were visualized by a trained observer on a light box. The intensities of the proteins were then measured using densitometric measurements.

Protein identification: Protein spots were excised the 2-DE and trypsin was added to perform in-gel digestion. Proteins were considered identified when at least three peptides were distinguished.

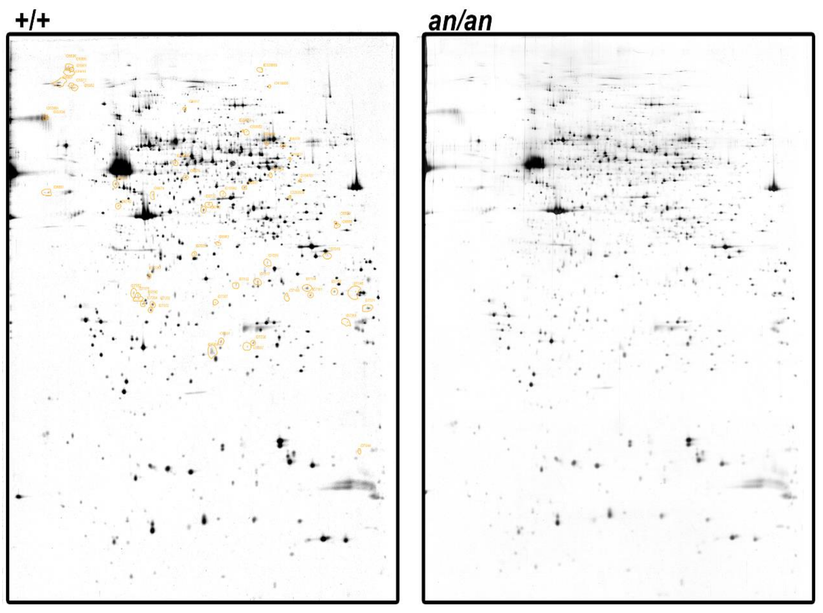
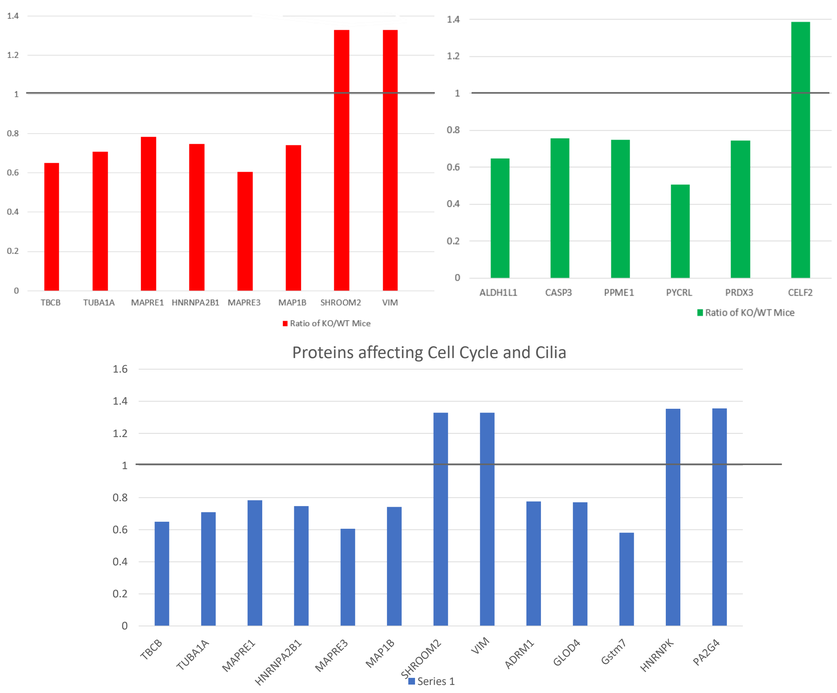


Figure.2. Overview of brain proteins altered in *an/an* mice. A representative 2-DE protein pattern from +/+ and *an/an* brains

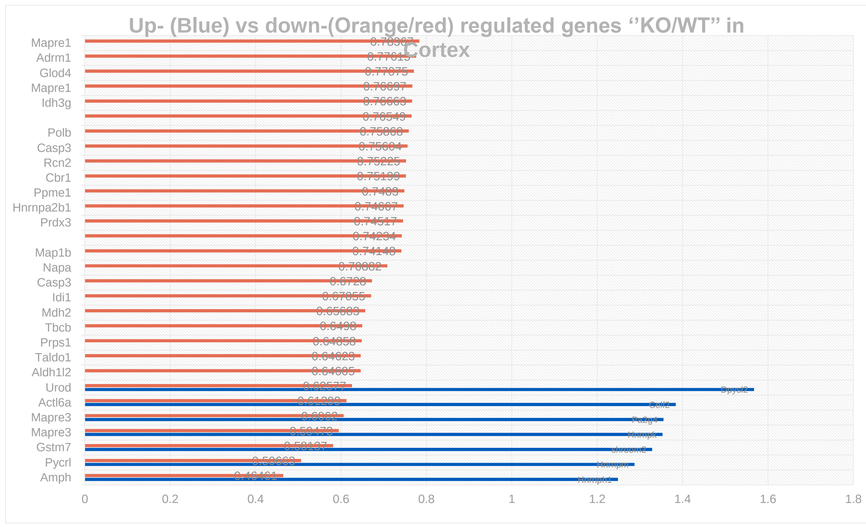
Results:

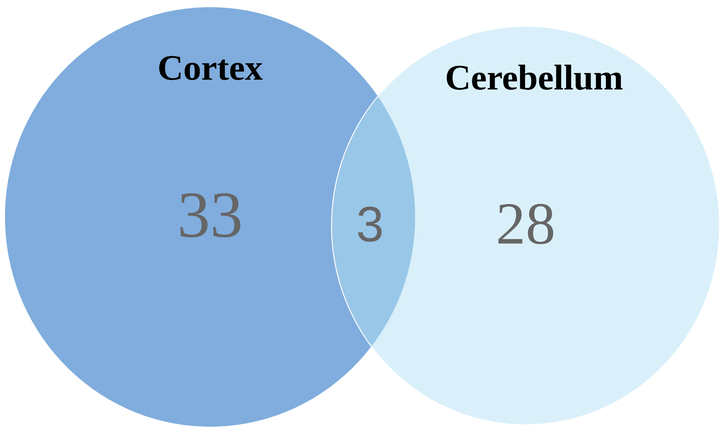
- These graphs show the change in gene expression of the protiens affecting the cytoskeleton (red), apoptosis (green), and cilia and cell cycle (blue).



Results

- Figure 4. shows the up and down regulated genes in the cortices of *an/an* mice
- Figure 5. shows the number of affected protiens in the cerebellum and cortex and the common proteins in both of them.





Conclusion

Our study showed a increase in the proteins controlling apoptosis. in addition, there was a marked decrease in the cytoskeletal proteins and proteins affecting cilia and cell cycle. Therefore, we can conclude that MCPH might have a direct effect on the aforementioned cell function. These findings, accompanied by more research, can help clarify our understanding of the disease process underlying MCPH.

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