

# **Some bioinformatic analyses of human GDAP1 gene expression**

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## Abstract

Charcot- Marie-Tooth (CMT) represents a group of genetic disorders, which cause damage in the peripheral nervous system. It was identified and described in 1886 by Jean-Martin Charcot, Pierre Marie and Howard Henry Tooth. It is the most common inherited disorder of the peripheral nervous system, and affects approximately 1 in every 2,500 people. A severe form of CMT has been linked to mutations in the coding region of Ganglioside-induced Differentiation Associated Protein (GDAP1), a member of the glutathione transferase (GST) family which is located in the outer membrane of mitochondria. GDAP1 mutations cause axonal, demyelinating and intermediate forms of CMT. In some cases the same mutation can cause different CMT phenotypes. The overall hypothesis for this thesis was, that changes in the expression in GDAP1 may lead to these phenotypic differences.

The methodology used to investigate the expression of human GDAP1 gene was a bioinformatic approach. The results demonstrated that in normal healthy tissues, GDAP1 had ubiquitous expression, particularly in neural and endocrine tissues. This pattern of expression was different to the expression of mouse GDAP1, where expression was predominantly in nervous tissues.

GDAP1 has mainly been studied in the context of peripheral neuropathies, based on its genetic linkage with CMT disease. In this study, the expression of GDAP1 was shown to be altered in some other diseases, such as brain cancers. Five out of six microarray studies found that glioblastoma expressed lower levels of GDAP1, than normal brain tissue. This was further verified by immunohistochemistry, showing weaker staining for GDAP1 in glioblastoma glial cell samples, compared to normal brain tissue.

Limited studies have investigated the transcriptional regulation of GDAP1. A comparison between the human and mouse GDAP1 5' flanking regions for transcription factor binding was undertaken. Some differences in the transcription factor binding sites between human and mouse GDAP were found. These were further highlighted by microarray studies, focussing on some transcription factor families. In EGR family members, no impact on GDAP1 expression in mouse cells where EGR1 had been depleted was seen, whereas human GDAP1 was strongly upregulated, when WTAP (an EGR family member) was knocked down.

This project also investigated, whether genetic polymorphisms in the 5' flanking region of the GDAP1 gene can alter GDAP1 expression. The study characterised a variable poly-A region, approximately -240bp upstream of the ATG. This region had a length varying between 11 and 15 A nucleotides. These results identified a novel SNP (G/A) in position -398 and also confirmed SNPs in positions -832 and -510. Transcription factors found lying under these SNPs were altered between the wild type and variant alleles. This variation could cause changes in the expression of GDAP1.

Finally, this project aimed to determine the potential of GDAP1L1 as a compensation for GDAP1 proteins in humans. Limited support was found supporting this hypothesis. The results showed an inverse relationship between the expression of GDAP1 and GDAP1L1 in 15% of the profiles investigated.

Taken together, this study presents new insights into the human GDAP1 expression. Evidence is provided of changes in GDAP1 expression in diseases other than CMT (such as cancers), and that common polymorphisms in the 5' flanking region of GDAP1 may contribute to variation in GDAP1 expression. Also, the results show a difference in the expression patterns of human and mouse GDAP1 genes, supporting the need for further studies in human models, to support this hypothesis. Further work will be required to fully understand the implications of changes in GDAP1 expression in CMT disease.

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