

Abstract

Definitive diagnosis of inherited metabolic disorders commonly depends on the measurement of enzyme activity (which is often complicated) and/or molecular genetic testing. Yet even the standard mutation analysis can bring false negative results in the case of gross chromosomal rearrangements or incorrect regulation of gene expression due to the mutations in regulatory regions. In the present study I focused on characterization of complex mutations affecting the gene encoding ornithine transcarbamylase (OTC) followed by studies of regulatory regions of *OTC* and *GBA* (the gene encoding β -glucocerebrosidase).

In the first study we identified 14 novel mutations including three large deletions in a cohort of 37 patients with OTC deficiency (OTCD). Subsequently we evaluated clinical significance of all these mutations. We also found a heterozygote carrying a hypomorphic mutation and manifesting OTCD most likely due to unfavorable X-inactivation which was observed independently in three different peripheral tissues.

In order to evaluate the clinical significance of a promoter variation c.-366A>G found in a family with mild OTCD we identified three alternative transcription start sites (TSSs) of human *OTC* and delimited the promoter. We also found a distal enhancer and performed functional analysis of both regulatory regions. Our results indicate that tissue specific expression of *OTC* in the liver depends on the promoter-enhancer interaction. The variation c.-366A>G decreased the promoter-enhancer transcriptional activity by 50%.

A detailed characterization of human *OTC* promoter revealed two positive *cis*-acting regulatory elements corresponding to HNF-4 α binding sites. Both sites, similarly as a third HNF-4 α recognition motif found in the proximal promoter, are located within 35 bases upstream of the TSSs. Since the *OTC* promoter lacks general core promoter elements such as TATA-box or initiators on standard positions, our results strongly suggest an important role of HNF-4 α in the control of *OTC* transcription in human.

A similar approach as in the *OTC* gene studies was used in studies of an upstream promoter of *GBA*. We identified three alternative TSSs and performed function analysis of the alternative promoter. Its transcriptional activity was lower than that of the normal promoter while expression profiles across multiple tissues were comparable. We hypothesized that phenotypic differences in patients with the same genotype may be caused by variable expression of mutant *GBA*; however, our hypothesis was not confirmed experimentally in a group of twenty Gaucher patients.

In conclusion, our findings extend the possibilities of molecular genetic testing for OTCD and Gaucher disease.

Keywords: Ornithine transcarbamylase, ornithine transcarbamylase deficiency, acid β -glucocerebrosidase, Gaucher disease, gene expression, regulation of transcription, promoter, enhancer.