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The Regulation of the Incorrect Splicing of *ISCU* in Hereditary Myopathy with Lactic Acidosis

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Akademisk avhandling

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Title
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Abstract
Patients suffering from hereditary myopathy with lactic acidosis (HML) can be found in the northern Swedish counties of Ångermanland and Västerbotten. HML is a rare autosomal recessive disease where patients display a low tolerance to exercise at an early age. Exercise can trigger symptoms such as palpitations, tachycardia, muscle cramps and dyspnoea. Extensive exercise or strict diets can result in myoglobinuria and life-threatening levels of lactic acid. The disease is caused by a nonsense G > C mutation (c.418 + 328G < C) in the last intron of the iron-sulphur (FeS) cluster assembly gene (ISCU), resulting in nonsense-mediated decay (NMD) of the transcript due to incorrect splicing. The ISCU protein is involved in the assembly of FeS clusters, which are essential cofactors for a wide range of proteins. Patient muscles display decreased levels of several FeS cluster proteins: mitochondrial aconitase in the tricarboxylic acid (TCA) cycle and Complex I, II (succinate dehydrogenase [SDH]) and III in the electron transport chain (ETC). The incorrect splicing of ISCU occurs to the highest extent in HML patient skeletal muscle, restricting the loss of ISCU protein to muscles, thereby preventing a more severe phenotype.

We found that the incorrect splicing occurs to the highest extent in slow-fibre muscle, which may be caused by the serine/arginine-rich splicing factor (SRSF3) as it is expressed at higher levels in slow-fibre muscle compared to other muscles, and since it is able to activate the incorrect splicing of ISCU. Following muscle, there is a gradual decrease of the incorrect splicing in heart, brain, liver and kidney, which is negatively correlated with the levels of the splicing inhibitor polypyrimidine-tract binding protein 1 (PTBP1). Overexpression of PTBP1 in HML patient myoblasts resulted in a drastic decrease in the incorrect splicing, while a PTBP1 knockdown had the opposite effect. Our results suggest that PTBP1 acts as a dominant inhibitor of the incorrect splicing and is likely the main cause for the tissue-specific splicing of ISCU in HML. We also identified RBM39 and MBNL1 as activators of the incorrect splicing of ISCU, which, together with the low levels of PTBP1, could explain the high levels of incorrect splicing in muscle.

Since almost 95% of all human gene transcripts are alternatively spliced, it is not surprising that a wide range of diseases are caused by mutations that affect splicing. Further knowledge of the function of splicing, such as tissue-specific splicing, can provide vital information for the development of therapies for diseases caused by splicing.

Keywords
HML, ISCU, Splicing, SRSF3, PTBP1, RBM39, MBNL1

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