Functional studies of YBX3 variants associated with nemaline myopathy

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INTRODUCTION & OBJECTIVES

Two *YBX3* variants, p.S34R and p.R129W, were found by exome sequencing in a Finnish patient with an unusual form of nemaline myopathy (NM). The healthy parents carry one variant each, the affected child being compound heterozygous. The father and the child also carry seemingly recessive *TPM3* and *RYR1* variants (Fig. 1).

YBX3 is a transcription factor, shuttling in and out of the nucleus. Both variants are located at highly conserved positions in the DNA-binding domain of YBX3. The Ser34 location is thought to affect the formation of nuclear YBX3 complexes and binding to single-stranded DNA. The Arg129 location is situated close to an implicated regulatory region of nuclear/cytoplasmic trafficking of YBX3 in myogenic cells.

The aim of the study is to functionally characterize YBX3 and assess the pathogenicity of the variants.

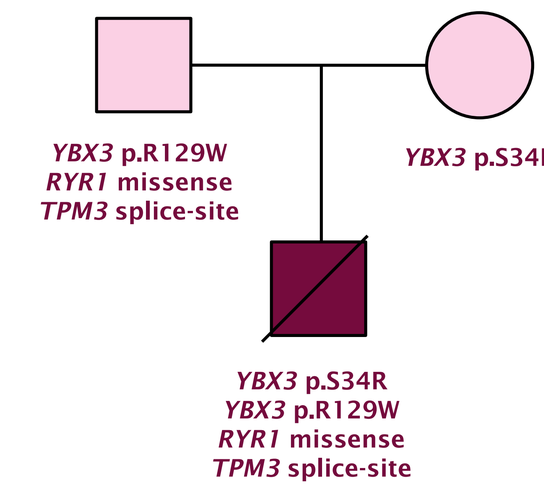

Figure 1. Pedigree of the family

METHODS

Immunofluorescence microscopy (IF)

HeLa cells were transfected with N-terminally DYK-tagged *YBX3* wild-type and the variants p.S34R and p.R129W in the pcDNA3.1 vector to assess the intracellular localization of the protein.

CellInsight High Content Analysis

Cells were seeded on a 24-well plate, transfected, imaged and analyzed using the Thermo Scientific CellInsight High Content imaging system and the HCS Studio Cell Analysis Software (Fig. 2).

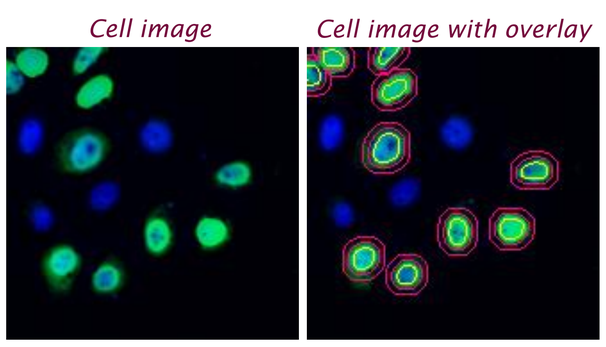

Figure 2. Demo of used CellInsight analysis principle showing the region of interest (ROI) boundaries around the nucleus in yellow and the cytoplasm in red.

I-TASSER protein structure prediction

The YBX3 wild-type and variant amino acid sequences were run by the I-TASSER V5.0 server ("Zhang-server") to predict the possible protein structure changes caused by the variants.

RESULTS

Immunofluorescence microscopy

Immunofluorescence microscopy revealed a tendency for *YBX3* p.S34R/p.R129W co-transfected HeLa cells to accumulate the protein in the nuclei, whilst cells transfected with the wild-type plasmid had a more varied distribution of the protein throughout the nucleus and the cytosol (Fig. 3).

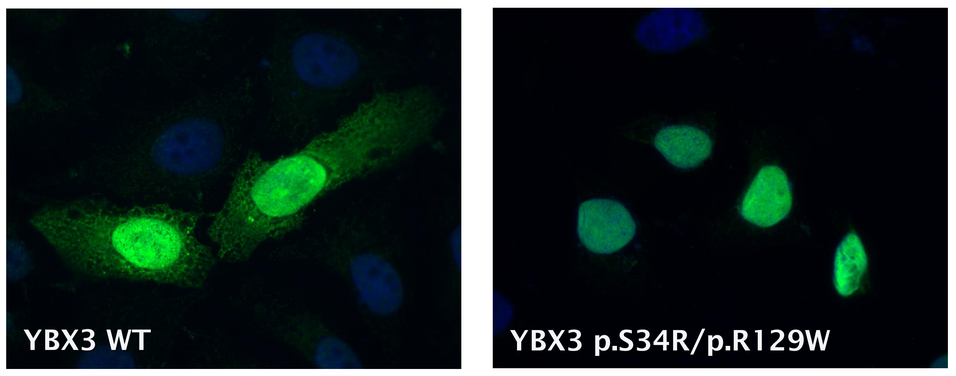

Figure 3. Example of HeLa cells transfected with pcDNA3.1.-(k)-DYK/*YBX3* wild-type and the pcDNA3.1.-(k)-DYK/*YBX3* p.S34R and p.R129W plasmids respectively. In the wild-type cells, the protein is seen in both the nucleus and the cytosol, while it remains within the nuclei in the p.S34R and p.R129W co-transfected cells.

CellInsight High Content Analysis

The CellInsight High Content Analysis findings are consistent with the IF results, suggesting that the results are true. YBX3 should shuttle between the cytoplasm and the nucleus, and a wider variablilty in the wild-type cells is seen. In the p.S34R/p.R129W co-transfected cells the variability is less extensive and the protein tends to gravitate towards the nucleus (Fig. 4)

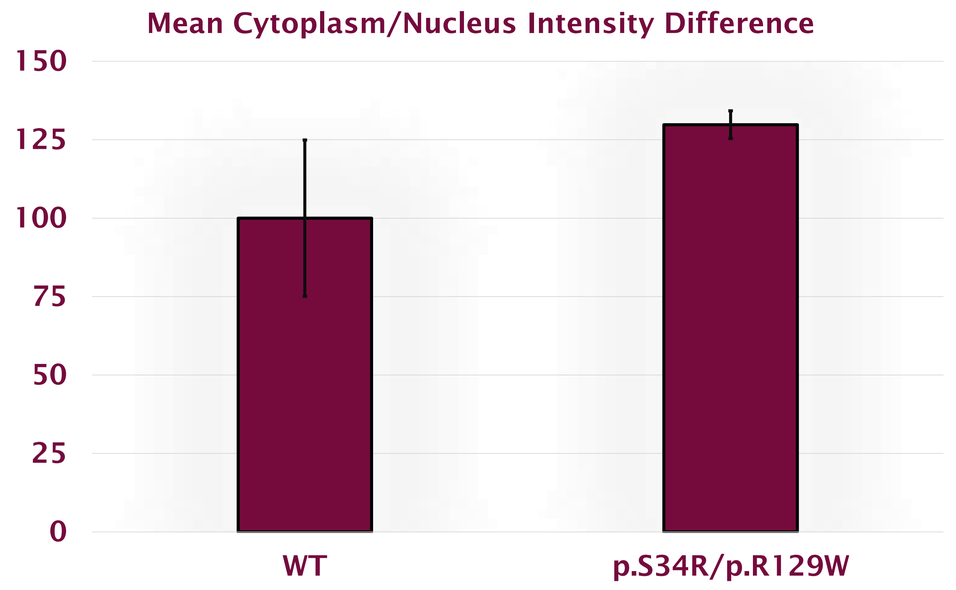

Figure 4. Average of nuclei to cytoplasm intensity differences for cells transfected with pcDNA3.1.-(k)-DYK/YBX3 wild-type and the pcDNA3.1.-(k)-DYK/*YBX3* p.S34R and p.R129W plasmids respectively, normalized to wild-type and with the background removed. *YBX3* p.S34R/p.R129W co-transfected cells have the tendency to express the protein at higher levels in the nuclei than in the cytoplasm. The variability is larger in the wild-type, because under normal conditions, the protein shuttles between the nucleus and the cytoplasm.

I-TASSER protein structure prediction

I-TASSER predicted significant changes to the protein structure as a result of the variants. The structures with the highest C-scores are presented below (Fig. 5). The wild-type model includes a protruding tail-like sheet structure, while both variants are predicted to create more globular tertiary forms.

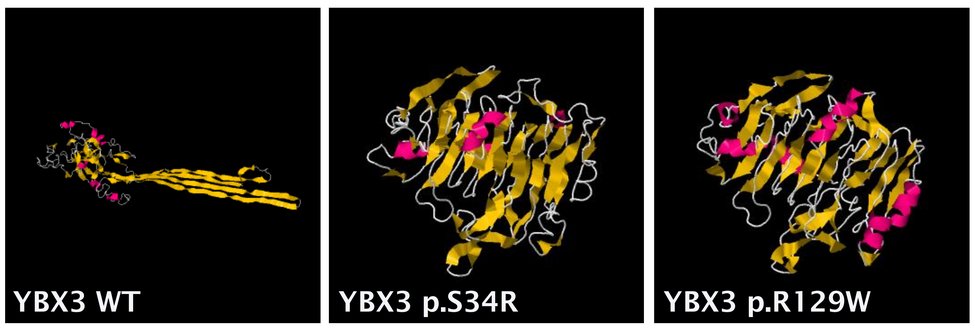

Figure 5. I-TASSER generated structural models of YBX3 wild-type (C-score -1.11) and variants p.S34R (C-score -1.04) and p.R129W (C-score -1.44) respectively.

DISCUSSION

YBX3 has not previously been recorded as an NM-causing gene. Functional and structural characterization, if any, of the human YBX3 has been limited.

Our studies show, that both of the variants studied are likely to affect the protein structure significantly. This could hinder the forming of important bonds and the shuttling of the protein between the nucleus and the cytoplasm.

While the two other variants in *TPM3* and *RYR1* carried by the patient have been interpreted as recessive, the *YBX3* variants p.S34R and p.R129W could be disease-causing when inherited together, without the balancing effect of a normal allele.

Further studies on protein function will be conducted, including repeat experiments using the methods described here.

CONCLUSIONS

- The variants studied seem to affect the structure and function of YBX3. This might lead to the inability of the protein to shuttle between the cytosol and the nucleus.
- *YBX3* can be considered a potential new NM-causing gene – the assessment is still ongoing, but the preliminary results indicate an effect on protein function.
- The putative effect of the two *YBX3* variants on the expression of *RYR1* and *TPM3* is to be assessed.

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