A HISTOLOGICAL ANALYSIS OF GLYCOGEN CONTENT IN HEPATOCYTES OF TREFOIL FACTOR FAMILY 2 AND TREFOIL FACTOR FAMILY 3 KNOCK-OUT MICE

Edi Rođak[1], Kristina Ivić[2], Tatjana Belovari[1], Ivana Lovrić[1], Nikola Bijelić[1], Mirela Baus Lončar[3]

[1]Department of Histology and Embryology, Faculty of Medicine, University of Osijek, J. Huttlera 4, 31000 Osijek, Croatia
[2]Clinical Hospital Centre Osijek, J. Huttlera 4, 31 000 Osijek, Croatia
[3]Department of Molecular Medicine, Institute Ruđer Bošković, Bijenička 54, 10000 Zagreb, Croatia

INTRODUCTION

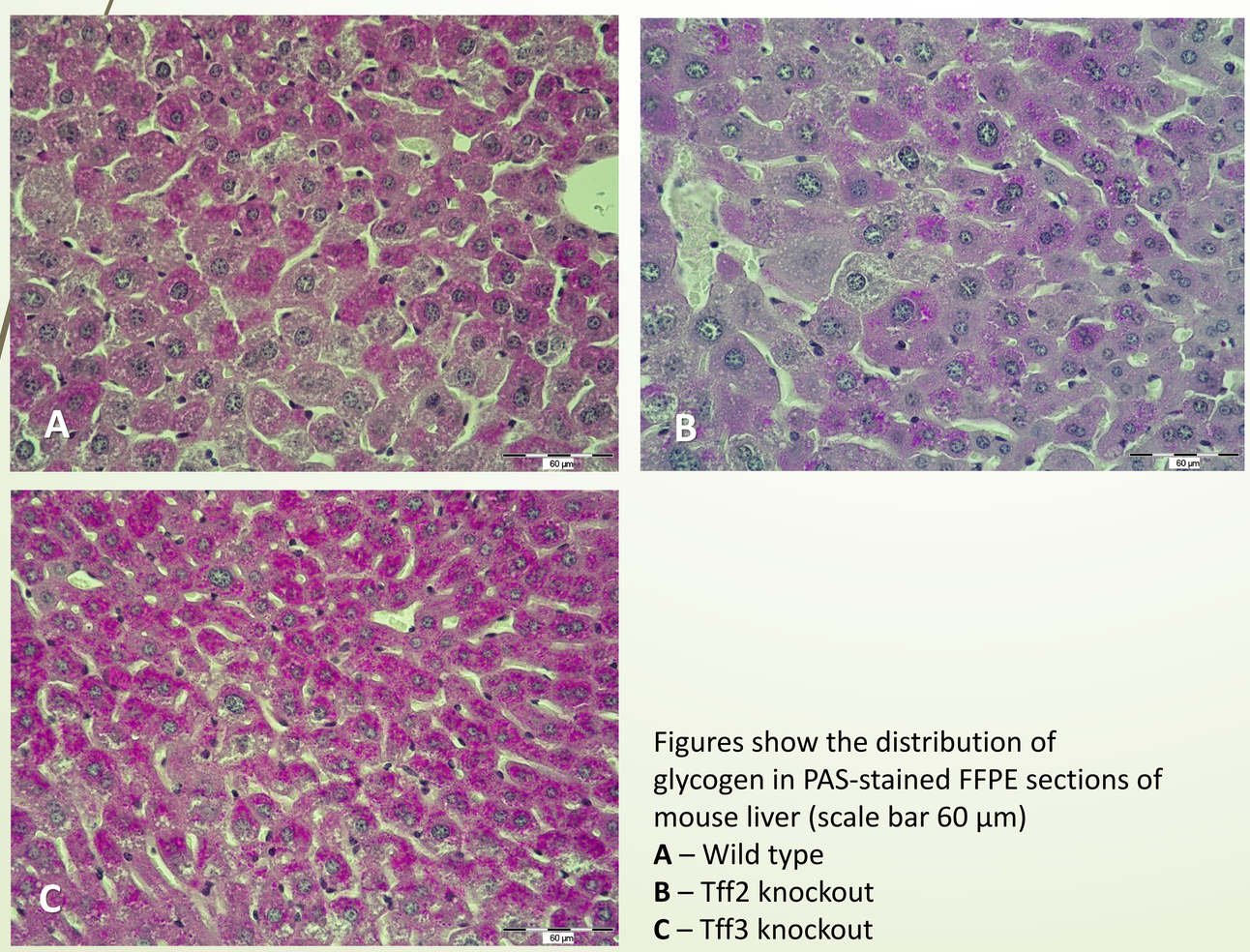
Trefoil factor family (Tff) peptide 2 and Tff peptide 3 are small peptides mostly present in the gastrointestinal mucosa and related to mucosal protection and restitution. Their mRNA can also be found in liver, lungs, brain, salivary glands, thyroid gland and many other organs. Tff3 is included in hepatic glucose metabolism and has an effect on metabolic pathways of gluconeogenesis and glycolysis. Furthermore, both Tff2 and Tff3 peptide expression positively stimulate beta-cell proliferation in the pancreatic islets.

MATERIALS AND METHODS

Tff2 and Tff3 deficient mice including appropriate wild type mice of mixed background (Sv129/C57Bl6) (N=6 per genotype) were kept on standard diet until 6 month old. Glycogen distribution was monitored in formalin-fixed and paraffin-embedded tissue sections stained using PAS method (6 µm). Areas of strongest glycogen staining were chosen for analysis and glycogen-positive cells were counted within regions of 100 cells. Number of glycogen positive cells was noted down and using an arbitrary semi-quantitative scale, the signal was classified as weak (0-35 positive cells), medium (36-70 positive cells) and strong (71 or more positive cells). Collected data was further analyzed using Mann-Whitney U test.

RESULTS

Tff3 deficient mice had the strongest accumulation of glycogen, which was statistically significant compared to wild-type mice ($p=0.005$, Mann-Whitney U test). Liver glycogen distribution in Tff2 deficient mice was heterogeneous and overall signal did not differ statistically from that of wild-type mice ($p=0.5$).



Figures show the distribution of glycogen in PAS-stained FFPE sections of mouse liver (scale bar 60 µm)
A – Wild type
B – Tff2 knockout
C – Tff3 knockout

Table 1.
Observed glycogen signal intensity using arbitrary semi-quantitative scale (in 6 animals per genotype)
(+ = weak, ++ = medium, +++ = strong)

Group	Glycogen signal intensity		
	+	++	+++
Wild type	0	5	1
Tff2 K.O.	1	2	3
Tff3 K.O.	0	0	6

Table 2.
Results of Mann-Whitney U test

Compared groups		p value
Wild type	Tff2 K.O.	0.523
Tff2 K.O.	Tff3 K.O.	0.058
Wild type	Tff3 K.O.	0.005

CONCLUSION

Our results support the notion that Tff3 peptide is included in the hepatic glucose and glycogen metabolism. Tff3 knockout animals had more glycogen positive cells and those cells had more glycogen accumulated in them when compared to wild type and Tff2 knockout animals. In conclusion, further metabolism-oriented studies are needed to elucidate the exact metabolic role of Tff3 peptide, and it's effect on physiological and pathological processes in liver.

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