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The importance of mitochondrial DNA damage and repair in the occurrence of insulin resistance in nonalcoholic fatty liver disease

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is one of the most common disorders affecting the liver. An important issue in the development of NAFLD is insulin resistance (IR), which is related to the fatty liver through the excessive accumulation of free fatty acids, chronic hepatitis, and increased oxidative stress in the liver cells. Recent literature shows the link between NAFLD and increased production of reactive oxygen species (ROS), which make the liver particularly vulnerable to oxidative stress due to a high number of mitochondria present in hepatocytes. Therefore, it can lead to an oxidative damage to the mitochondrial DNA (mtDNA), and a base excision repair (BER) is mainly responsible for repairing this type of damage.

Considering the information presented above, **the aim of the project is to determine the molecular basis of IR-related processes in NAFLD in the context of mtDNA damage accumulation and/or BER pathway impairment as well as degradation of damaged mtDNA.**

MATERIALS & METHODS

In the current academic year, an analysis of the mitochondrial DNA copy number (mtDNA CN) was conducted in patients with NAFLD as well as in the control group. Additionally, the results were compared with the expression levels of genes associated with BER. The study group consisted of 99 individuals, while the control group comprised 43 individuals. Previously isolated DNA underwent real-time PCR analysis using the TaqMan™ Universal PCR Master Mix (Applied Biosystems™). Measurement values were averaged for each sample. Statistical analysis was performed using SigmaPlot14 and GraphPad Prism 8. The comparison of mtDNA CN between patients and controls was conducted using the Mann-Whitney U test due to the non-normal distribution of the obtained results. The correlation between mtDNA CN and expression analysis was assessed using linear regression. Results were considered statistically significant at $p < 0.05$.

CONLUSION

- There is a correlation between the copy number and the expression level of genes associated with BER.
- The elevated copy number could result from a higher amount of damaged DNA.
- The expression increases when there is a greater demand for repair within the cell, indicating more genetic damage OR, the expression is increased due to impairment and damage in repair pathways, resulting in more DNA damage.

ACKNOWLEDGMENT

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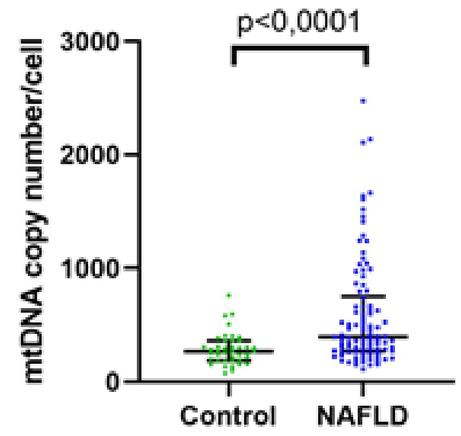
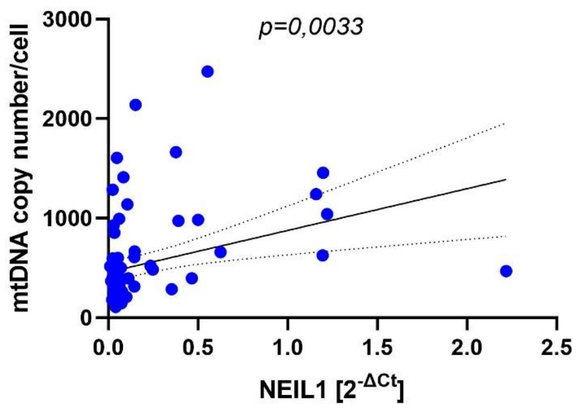
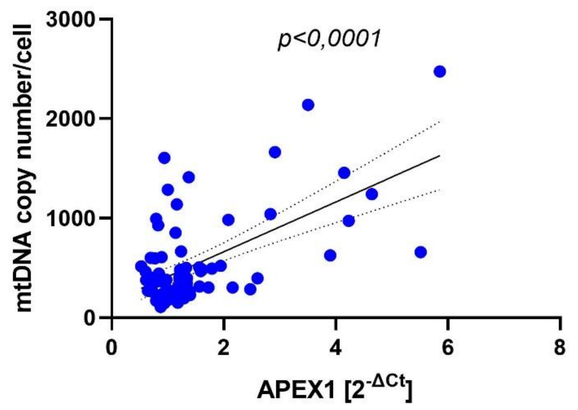
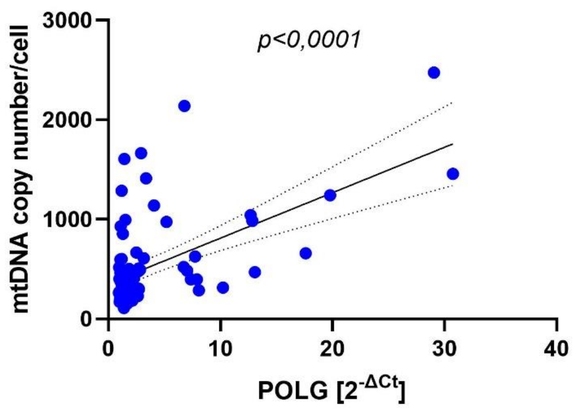
RESULTS

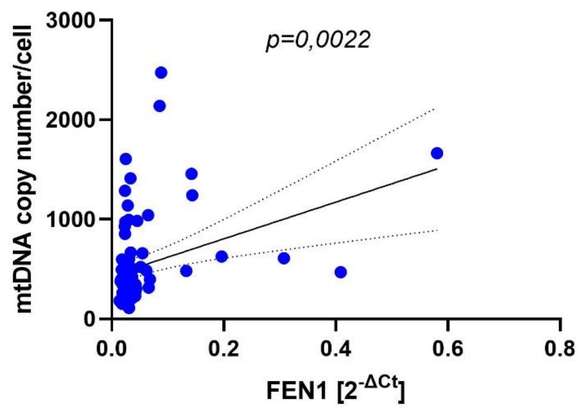
- mtDNA copy number is elevated in patients with NAFLD
- The higher mtDNA copy numer in the cell, the higher mRNA level of the examined genes, i.e. NEIL1, APEX1, POLG, FEN1, PARP1, XRCC1, LIG1, and LIG3

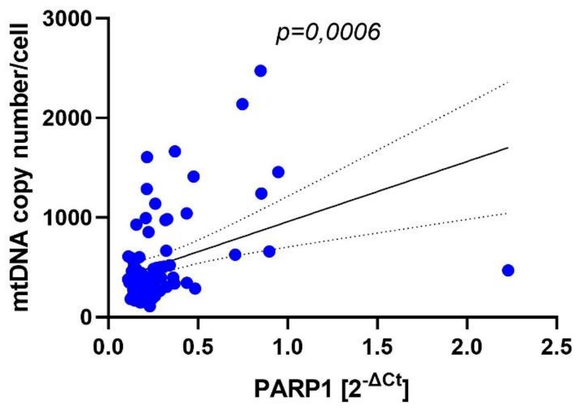


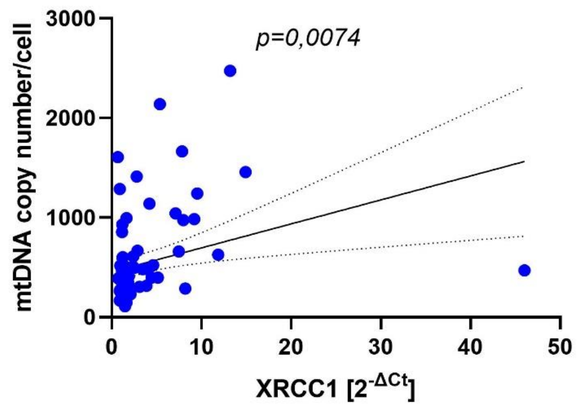


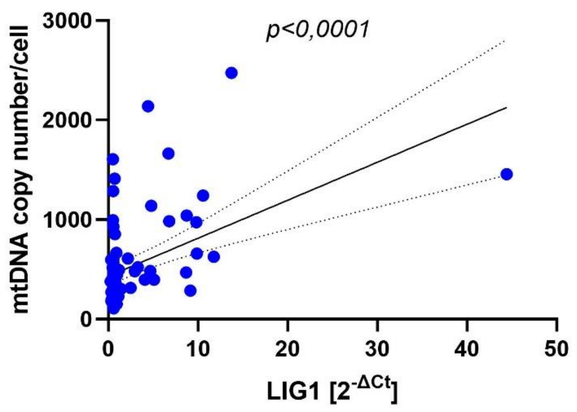












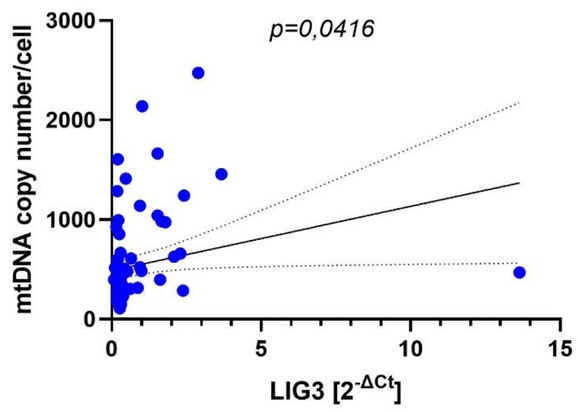


Figure 1. A) The number of copies of mitochondrial DNA in the blood of patients with NAFLD and controls, depicted using the $2^{-\Delta Ct}$ method. B) The number of copies of mitochondrial DNA in patients with NAFLD based on the expression level of the studied gene. The results presented as scatter dot plots, horizontal lines represent medians and whiskers denote 95% confidence interval.