

# CpG methylation status changes within the protein tyrosine phosphatase non-receptor type 22 gene promoters in children and adolescents of Greek origin with Type 1 Diabetes

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## Introduction

Protein tyrosine phosphatase non-receptor type 22 (PTPN22) is a well-established genetic locus of type 1 diabetes (T1D). The aim of this study is to compare the methylation level of PTPN22 between children and adolescents of Greek origin with T1D and healthy controls.

## Methods

Twenty T1DM participants and 20 age-/gender-matched healthy youngsters were enrolled.

- DNA was extracted from white blood cells
- Genomic DNA(800ng) was then treated with sodium bisulphate using the EZ DNA Methylation-Gold Kit.
- Treatment with sodium bisulfite converts unmethylated cytosines into uracils, whereas methylated cytosines remain unchanged under the same conditions.
- DNA was then amplified by PCR reaction was performed in a total volume of 50 µl targeting a specific sequence of the gene promoters
- Amplicons were analyzed by electrophoresis (1% agarose gel stained with ethidium bromide) and visualized by ultraviolet trans-illumination.
- PCR products were purified and then sequenced with Next Generation Sequencing- Illumina, in order to identify DNA methylation changes
- Comparisons between groups were performed with students t-test or its non- parametric analogue, Mann Whitney U test, as appropriate.

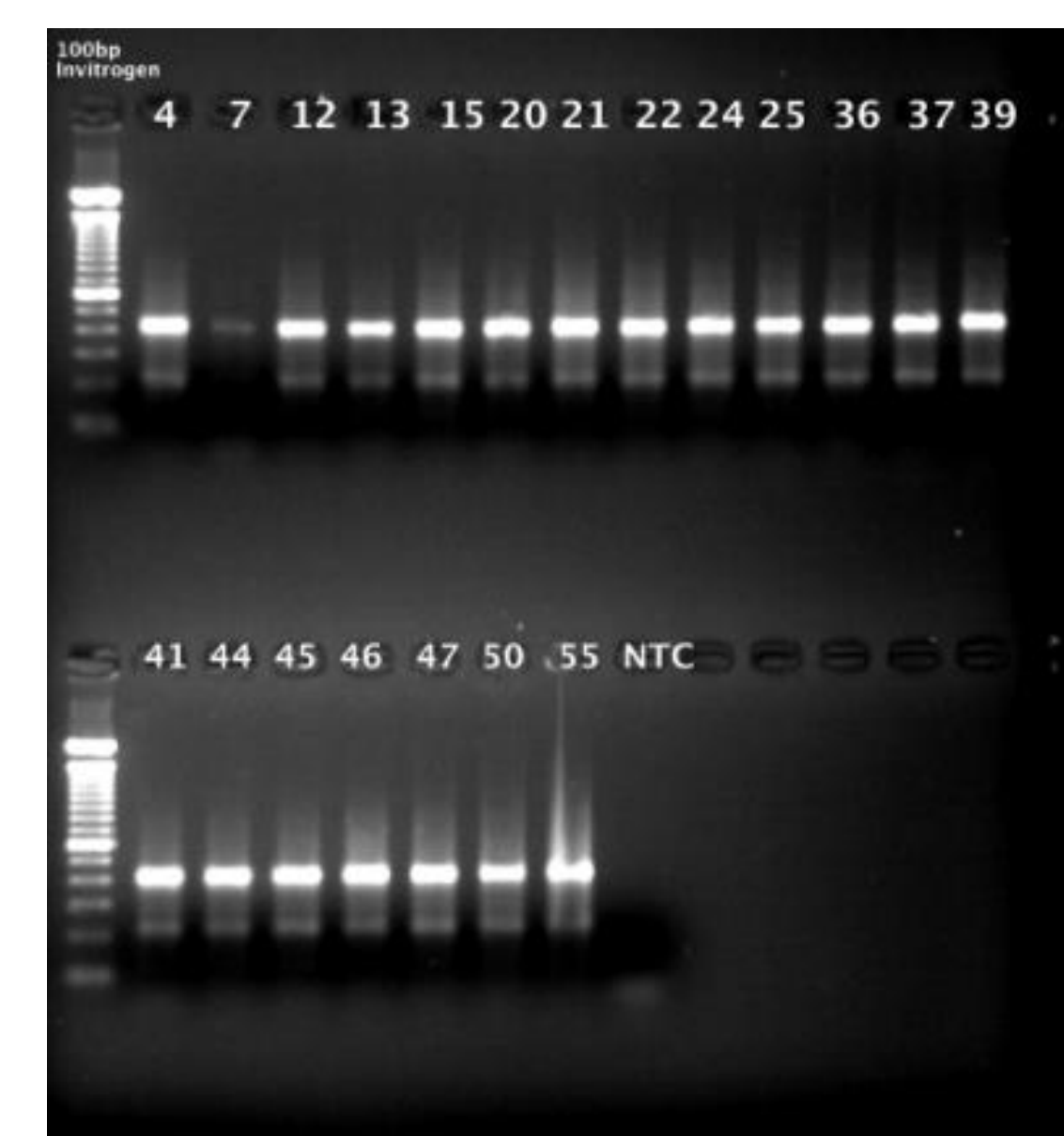
**Figure 1. PTPN22 forward and reverse primers**

	Forward primer	Reverse primer
<b>PTP N22</b>	5'TCGTCGGCAGCGTCA GATGTGTATAAGAGACAG TTTTGGTTTATGTTGTAG AGT3'	5'GTCTCGTGGGCTCGGAGAGAT GTGTATATAAGAGAAGACAGTTAC ATATATAAATAATAAAATAAAAT3'

## Results

Methylation profile at 4 CpGs of the PTPN22 promoter, was analyzed. The results showed the methylation level was statistically significant lower at position 1-4826 (p 0.026) in patients compared to controls. Furthermore, there was a tendency for statistically significant hypomethylation at position 3-5018 in patients than in control group.

**Figure 2. PTPN22 PCR products of mDNA in T1D patients**



**Table 1. Hypomethylated CpGs of the PTPN-22 promoters in patients and controls**

4 CpGs in PTPN22 gene	DNA methylation (%)		
	T1D (n=20)	Controls (n=20)	p
	Overall mean methylation percentage		
Mean methylation	0.4 ± 0.1	0.46 ± 0.14	0.179
Range	0-1	0-1	
CpG sites			
<b>1-4826</b>	<b>0.35 ± 0.17</b>	<b>0.52 ± 0.28</b>	<b>0.026</b>
2-4950	0.45 ± 0.22	0.51 ± 0.29	0.64
<b>3-5018</b>	<b>0.24 ± 0.09</b>	<b>0.30 ± 0.13</b>	<b>0.065</b>
4-5046	0.56 ± 0.37	0.50 ± 0.34	0.659

## Conclusions

To our knowledge, this is the first time to detect hypomethylation of PTPN22 gene in patients with T1D.

## References

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2. Wang Z et al. Beyond Genetics: What Causes Type 1 Diabetes. *Clinic Rev Allerg Immunol* DOI 10.1007/s12016-016-8592-1

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