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Differential Plasma microRNA Profiles in HBeAg Positive and HBeAg Negative Children with Chronic Hepatitis B

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Abstract

Background and aim: Children with chronic hepatitis B (CHB) are at high risk of progressive liver disease. Current clinical practice predicts the risk of disease progression on the basis of persistence of HBeAg and level of hepatitis B virus (HBV) DNA; however, novel diagnostic markers are warranted. Identification of specific miRNA profiles in children chronically infected with HBV and an improved understanding of miRNAs in the pathogenesis of CHB may lead to advances in the management of children with CHB.

Patients and methods: miRNA PCR panels were employed to screen plasma levels of 739 miRNAs in pooled samples from HBeAg positive, HBeAg negative, and healthy children. The three groups' plasma miRNA profiles were compared, and aberrantly expressed miRNAs were identified. The identified miRNAs were then validated. Individual RT-PCRs were performed on plasma from 34 HBeAg positive, 26 HBeAg negative, and 60 healthy children.

Results: A panel of 16 plasma miRNAs were identified as aberrantly expressed in HBeAg positive and HBeAg negative children ($p<0.001$), Figure 1. Levels of all of the miRNAs were upregulated in HBeAg positive children compared with in HBeAg negative children. A positive correlation was furthermore found between plasma levels of the identified miRNAs and HBV DNA ($p<0.001$), Figure 2.

Figure 1

Levels of 16 identified miRNAs in plasma from HBeAg positive, HBeAg negative, and healthy children

Legend: A panel of 16 miRNAs was identified as significantly differentially expressed in plasma from 34 HBeAg positive, 26 HBeAg negative, and 60 healthy children. All 16 of the identified miRNAs were highly upregulated in plasma from HBeAg positive children compared with levels in plasma from HBeAg negative children. All miRNAs had their lowest expression in healthy children. Results are corrected for age and gender. $-\Delta C_T$: -delta cycle threshold. The bars represent geometric means of $-\Delta C_T$ values ±SEM.

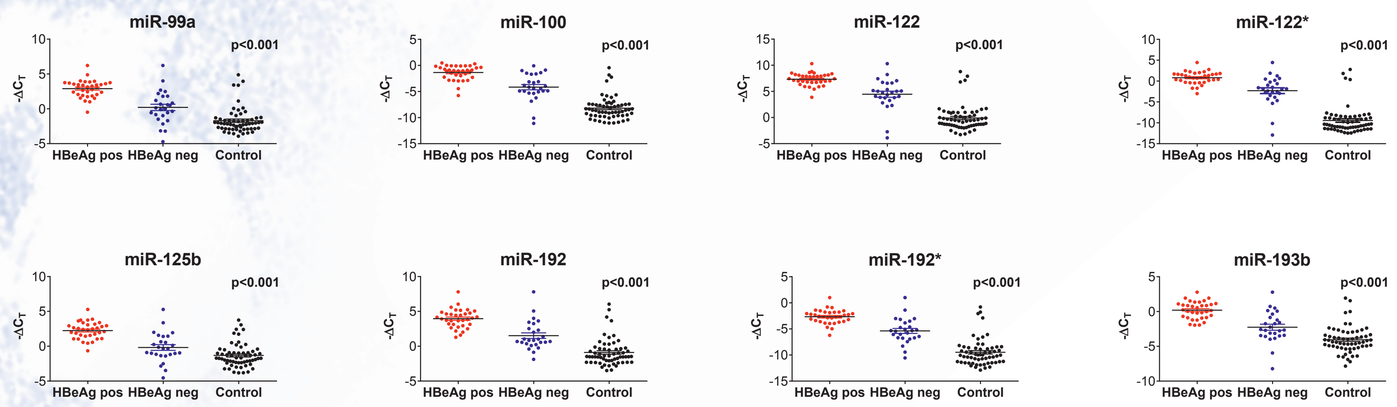


Table 1

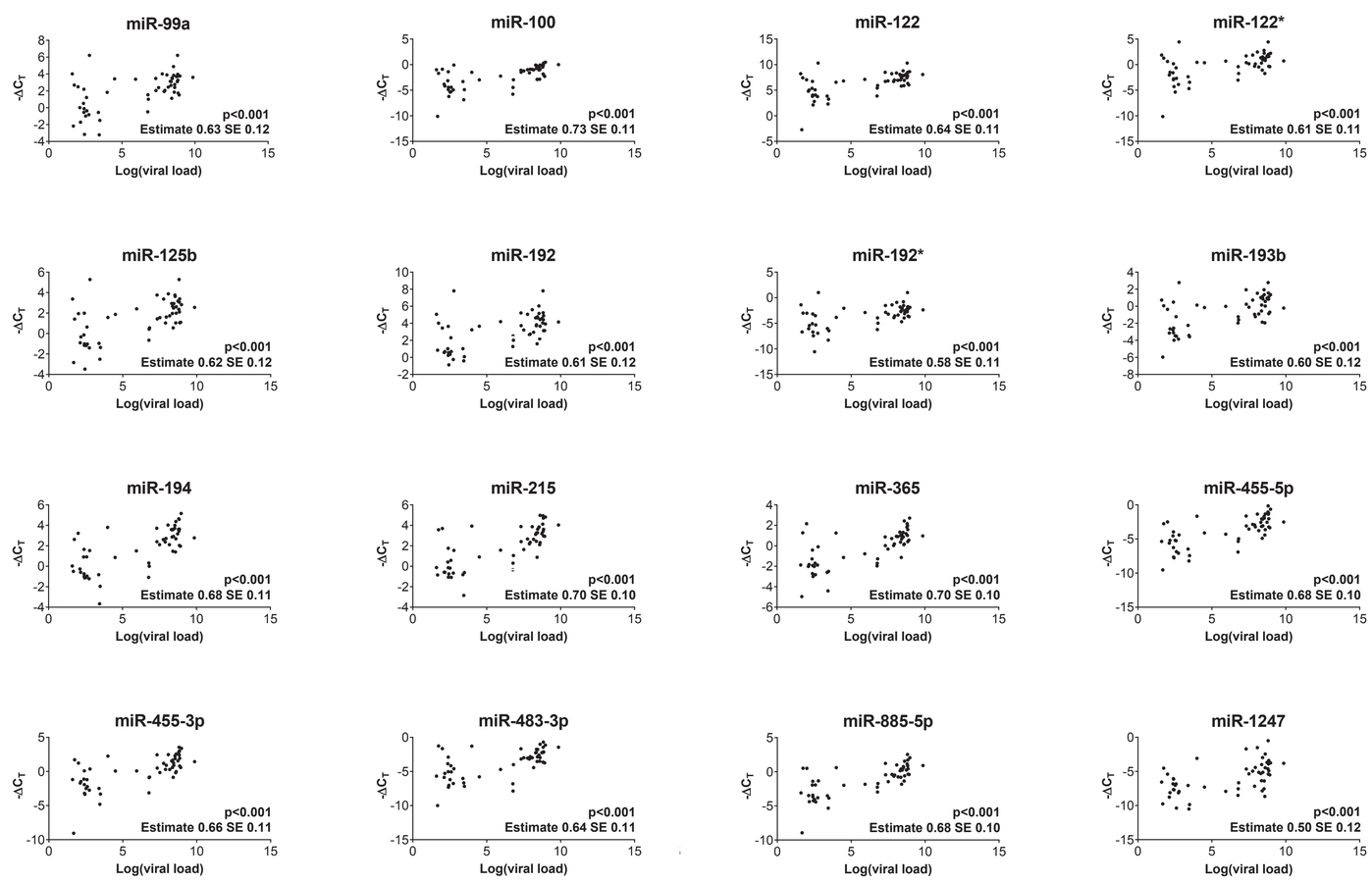
Characteristics of children with chronic hepatitis B and of healthy controls

	HBeAg pos	HBeAg neg	Healthy controls
No of patients	**34**	**26**	**60**
Male	14 (41%)	12 (46%)	33 (55%)
Female	20 (59%)	14 (54%)	27 (45%)
Age (years)			
Mean	**8,8**	**12,0**	**7,1**
SD	3,7	3,4	3,7
ALT (U/l ref. value 5-45)			
Mean	**46,5**	**25,6**	**14,3**
SD	27,6	12,6	5,3
HBV DNA (IU/ml)			
Mean	**5,1E+08**	**8,5E+02**	**NA**
SD	1,2E+09	2,0E+03	
Genotypes			
A	**2** (6%)	**2** (8%)	
B	**11** (32%)	**2** (8%)	
C	**5** (15%)	**2** (8%)	
D	**12** (35%)	**7** (27%)	
E	**3** (9%)	**2** (8%)	
F	**1** (3%)	**0** (0%)	
NA	**0** (0%)	**11** (42%)	**60** (100%)

Figure 2

Correlation between circulating miRNAs and HBV DNA

Legend: The relationship between circulating miRNAs and viral load was investigated and a strong positive correlation was found between plasma levels of all 16 miRNAs and HBV DNA. Results are corrected for age, gender, and ALT.



Conclusion

We are the first to investigate the plasma miRNA profile of children with CHB. Our data indicates the existence of a relationship between abundance of circulating miRNAs and risk of disease progression. Certain miRNAs may be applied as predictive markers for risk of disease progression in children with CHB. Further studies are warranted to advance understanding of miRNAs in the pathogenesis of CHB, hopefully leading to the identification of future therapeutic targets.