Stress variables and methylation of the DDR1 gene in early intervention psychotic patients

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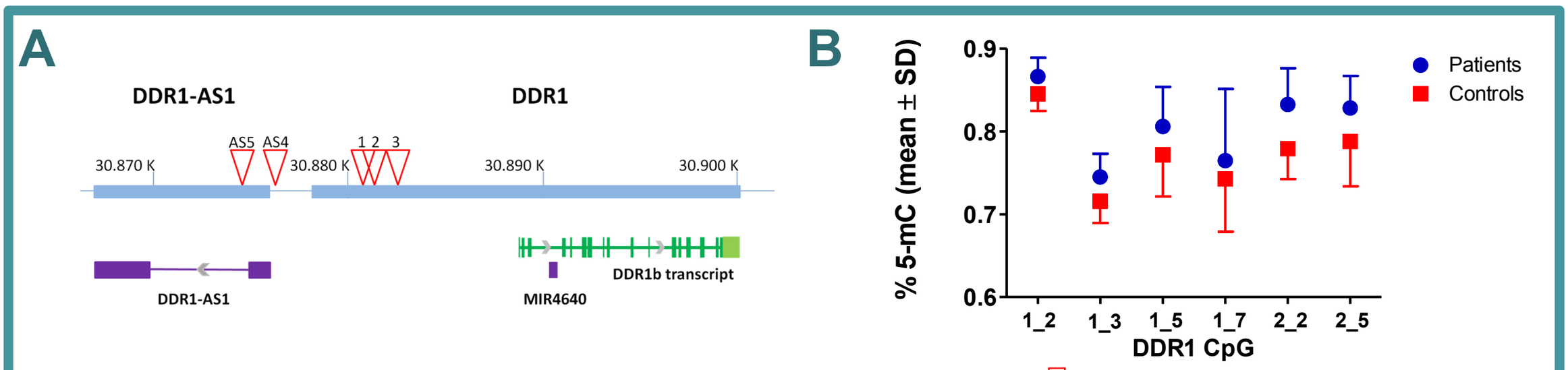

A. Schematic drawing of DDR1 locus (chr. 6) showing the 5 methylated regions analyzed (▽) and referred as AS4, AS5, 1, 2 and 3.
B. Plot graph showing methylation levels (5-mC) of the CpG that were different comparing patients and controls (see Table 1 for lineal regression analysis).

Introduction

We previously observed an association between DDR1 genetic variants and schizophrenia[1]. Also we have reported that DDR1 is a myelin protein[2] and an increased expression of DDR1 isoform c in postmortem brains from schizophrenic patients[3]. DNA methylation at specific CpG sites is an important regulator of myelin genes[4]. Compelling evidence shows that life event stressing factors are able to modify gene methylation patterns[5].

Aim

Here we aimed to show that life event stressing factors modify the pattern of DDR1 methylation in early intervention psychotic patients (PP).

Methods

We selected 60 patients attended at the Early intervention Program with a schizophrenia spectrum disorder diagnostic according to DSMV criteria, and 40 controls matched by sex and age. Stress variables were measured using Childhood Trauma Questionnaire (CTQ), Holmes–Rahe's Social Reajustment Rating Scale (HR-SRRS) and Perceived Stress Scale (PSS). Neutrophil to lymphocyte rate (NLR) was used as inflammatory marker and awakening saliva cortisol as stress biomarker. Severity of depressive and psychotic symptoms were measured by Calgary Depression Scale and PANSS respectively. Each dose of antipsychotic was transformed into chlorpromazine equivalent. Levels of methylation at 5 regions (45 CpG islands) were measured in peripheral blood DNA using Massarray EpiTYPER technology^{R} (Agena Biosciences).
After a bivariate analysis between dependent (methylation) and independent variables (all the rest) was conducted to explore associations between variables, we carried out a linear regression analysis using CpG island as dependent variable and sex, participant group (patient or control), CTQ, HR-SRRS, PSS, cortisol and NLR as independent variables.

Results

PPs (sex ratio 1:1) had a mean age of 24.7±5.4 years and controls (sex ratio 1:1) 24.6±5.4 years. Age, psychotic symptoms, depression and chlorpromazine equivalents associations with methylation did not pass the significance filter for multiple testing. Table 1 shows the linear regression analysis carried out to measure which variables associate with the methylation level at each CpG DDR1 island.

Founding Source
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Methylation at CpGs 1_2, 1_3, 1_5, 1_7, 2_2 and 2_5 is statistically different between patients and controls (Figure 1 B). Stress variables show an indirect association (negative *β* values) with methylation levels. Conversely, the inflammation NLR parameter showed a direct relationship with CpG methylation levels.

Table 2. Linear regression analysis using DDR1 methylation CpG islands as dependent variables and sex, diagnostic group, stress test scores, stress and plasma inflammatory biomarkers as independent variables.

DDR1 CpG[a]	Variable[b]	Variable statistics		Model	
		β	P	Adj R^2	P
CpG_1_2	Diagnostic	0.452	0.001	0.160	0.013
CpG_1_3	Diagnostic	0.429	1.7×10^{-4}	0.460	1.2×10^{-7}
	PSS	-0.211	0.034		
	NLR	0.341	0.001		
CpG_1_5	Diagnostic	0.235	0.049	0.355	1.6×10^{-5}
	NLR	0.479	4.1×10^{-5}		
CpG_1_7	Diagnostic	0.289	0.031	0.189	0.006
	PSS	-0.298	0.015		
	NLR	0.353	0.005		
CpG_2_2	Diagnostic	0.462	4.0×10^{-4}	0.284	2.5×10^{-4}
	PSS	-0.205	0.072		
	NLR	0.212	0.068		
CpG_2_5	Diagnostic	0.394	0.004	0.178	0.008
	NLR	0.241	0.053		

[a]Log transformed variables.
[b]Independent variables included into the equation were: Sex, diagnostic group, CTQ total score, HR-SRRS total score, PSS total score, saliva awakening cortisol and NLR.
Only variables (dependent and independent) showing statistical significance into the equation are shown.
CTQ: Childhood Trauma Questionnaire; HR-SRRS: Holmes and Rahe's Social Readjustment Rating Scale: NLR: Neutrophyl/lymphocyte ratio; PSS: Perceived Stress Scale.

Conclusions:

We show for the first time that DDR1 CpG methylation levels are increased in PPs compared to controls. In some regions the methylation levels are in part explained by stress and inflammation biomarkers but in opposite directions. Our results suggest that DDR1 methylation levels could be used as a PPs biomarker, but further studies are needed to confirm the present results.

References

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