



# *all-trans* Retinoic Acid upregulates reduced CD38 transcription in lymphoblastoid cell lines from patients with Autism Spectrum Disorder

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## Introduction

Evidence suggests that Oxytocin may contribute vulnerability to Autism Spectrum Disorders (ASD). Recently, a new player in the Oxytocin story was revealed by the CD38 knockout mouse (1). These animals fail to sufficiently secrete Oxytocin from hypothalamic neurons and present with deficits in social behavior that could be restored by Oxytocin administration.

Two association studies strengthen the notion that CD38 is a factor in risk for ASD (2,3), and we reported that CD38 expression was reduced in lymphoblast cell (LBC) lines from ASD subjects (2).

In the recent work, the hypothesis was tested whether reduced levels of CD38 correlate with behavioral phenotypes and IQ in ASD subjects as determined by the evaluation of caretaker questionnaires and clinical data. Since the CD38 gene is induced by *all-trans* Retinoic Acid (ATRA; vitamin A), we explored whether ATRA would 'rescue' ASD LBC lines and restore normal levels of CD38 expression, and whether the functional (4) polymorphism rs6449182 influences transcriptional activity.

## Methods

**Participants** - EBV-transformed cell lines were derived from 42 probands (30 male, 12 female) and their healthy parents (80 LBC lines). Subjects were diagnosed with ASD by two trained clinicians, and clinical data was collected (ADI-R, ADOS-G, VABS, WISC-II).

**Treatment** - Cell lines were grown under suitable culture conditions and incubated for 48 hours with ATRA at a final concentration of 0.1  $\mu$ M dissolved in ethanol, and the equivalent volume of ethanol was added to the control flasks.

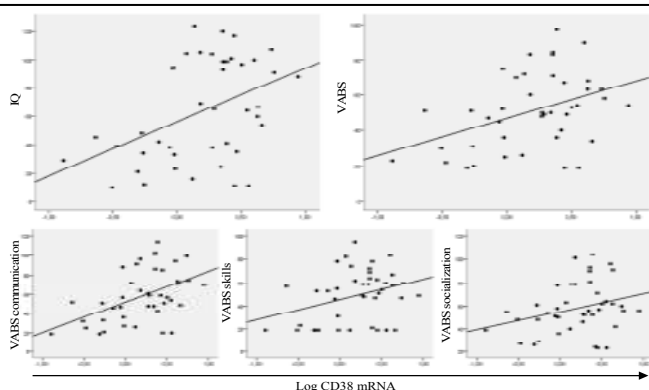
**Quantitative PCR** - All cell lines were harvested followed by RNA-extraction and quantitative RT-PCR using SYBR green ROX mix and specific primers. CD38 expression was normalized to  $\beta$ -actin.

**Genotyping** - The SNP rs6449182 was chosen via the dbSNP-homepage and genotyped using the SNaPshot Method™ (Applied BioSystems) and analyzed on an ABI PRISM 310 automated sequencer.

**Statistics** - SPSS 17 (Windows) or STATA software suite was used for all statistical analyses.

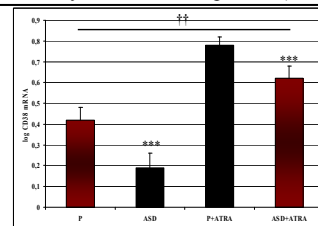
## Results

•Cell lines were chosen mostly as in our previous work, and an additional 38 LBC lines were added. We replicate our initial findings that CD38 mRNA is significantly reduced in ASD LBCs ( $p < 0.001$ , STATA linear regression).

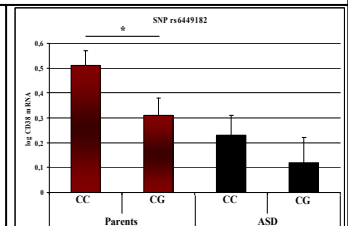


**Figure 1: Pearson Correlations for log CD38 mRNA with IQ and VABS scores.** CD38 expression levels significantly correlate with IQ ( $r = 0.43$ ,  $p = 0.004$ ) and VABS overall scores ( $r = 0.431$ ,  $p = 0.008$ ) and subscores (VABS communication:  $r = 0.487$ ,  $p = 0.001$ ; VABS skills:  $r = 0.329$ ,  $p = 0.034$ ) except for VABS socialization score ( $r = 0.294$ ,  $p = 0.059$ ).

•We did not find a correlation for CD38 mRNA with age in neither LBCs of probands (age range: 3-17 years;  $r = 0.104$ ,  $p = 0.051$ ) nor parents (age range: 31-64 years;  $r = 0.057$ ,  $p = 0.61$ ).



**Figure 2: CD38 antigen level.** Prolonged exposure to 0.1  $\mu$ M ATRA elevates CD38 antigen levels of ASD LBCs above that of untreated parental LBCs *in vitro* ( $\dagger\dagger$  ind. samples t-test,  $p = 0.002$ ). CD38 mRNA is significantly reduced in untreated as well as ATRA-treated conditions compared to parental LBCs ( $***$  ind. samples t-test,  $p < 0.001$ ).



**Figure 3: Log CD38 mRNA levels blotted over rs6449182.** A functional polymorphism (rs6449182) located within the CpG-island spanning the 5'-end of the first CD38-gene intron including the RARE is significantly associated with reduced basal mRNA levels in the parental group (\* ind. samples t-test,  $p = 0.024$ ). The G allele is accompanied by reduced ATRA sensitivity (data not shown).

## Discussion

•Replication of our initial findings suggest that reduced levels of CD38 reflect a generalized defect in ASD, which do not correlate with the age of the blood donors.

•The hypothesis drawn from the CD38 KO mouse such that ASD patients with low CD38 levels should present with lower functioning and social skills was confirmed by the significant correlation for IQ and VABS scores with antigen mRNA.

•The treatment of LBCs with nanomolar concentrations of ATRA shows that the reduced CD38 level observed in ASD can be restored to normal values and provides a 'proof-of-principle' for pharmacological applications with the low-toxicity retinoid.

•The finding that the SNP rs6449182 is significantly associated with basal CD38 mRNA levels in parental lines emphasizes the need for further evaluation of this and other polymorphisms in ASD.

## References

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