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Review

Are retinoids potential therapeutic agents in disorders of social cognition including autism?

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ABSTRACT

Increasing evidence suggests that the nonapeptide, oxytocin (OT), helps shape social and affiliative behaviors not only in lower mammals but also in humans. Recently, an essential mediator of brain OT release has been discovered, ADP-ribosyl cyclase and/or CD38. We have subsequently shown that polymorphisms across the CD38 gene are associated with autism spectrum disorders (ASD). Notably, CD38 expression in lymphoblastoid cells (LBC) is reduced in cell lines derived from ASD subjects compared to parental cell lines. Intriguingly, a correlation was observed between CD38 expression and measures of social function in ASD. Finally, we have shown that all-*trans* retinoic acid (ATRA), a known inducer of CD38 transcription, can rescue low CD38 expressing LBC lines derived from ASD subjects and restore normal levels of transcription of this ectoenzyme providing 'proof of principle' in a peripheral model that retinoids are potential therapeutic agents in ASD.

1. Oxytocin

Classically, the nonapeptide oxytocin (OT) has been viewed as a hypothalamic neuropeptide that is released into the general circulation from the neural lobe of the pituitary, inducing uterine contractions during parturition and milk ejection during lactation. OT is derived from a pre-prohormone precursor that is synthesized in the hypothalamus and stored in vesicles at the posterior pituitary for storage and subsequent release into the bloodstream (see [1] for comprehensive review of the oxytocin receptor system).

2. OT and social behavior

Beyond the long-known peripheral effects of OT, a wealth of animal studies have elaborated the role of OT, or their analogues such as isotocin and vasotocin [2], in molding social behavior from fish to mammals [3]. In the past few years the role of OT has also been examined in our own species, and similar to what has been learned from animal studies, it appears that this nonapeptides also influence social behaviors in humans [4,5]. Indeed, OT has been suggested as the 'great facilitator of life' in a recent review [6].

In humans, intranasal administration of OT has been shown to increase trust [7], facilitate mind-reading [8], enhance human memory for social identity [9], increase positive communication between couples [10], increase gaze to the eye region [11] and increase generosity [12]. Intriguingly, OT plasma levels have been linked to individual patterns of maternal-fetal attachment [13] and salivary OT levels were associated with bonding to own parents and inversely related to psychological distress, particularly depressive symptoms [14]. Social anxiety symptom severity, adjusted for age and gender in a healthy group of subjects, was associated with higher plasma oxytocin levels [15]. Imaging studies reinforce the role of OT in influencing human social behavior with evidence demonstrating that OT modulates the amygdala and other brain regions [16].

3. Oxytocin receptor gene (*OXTR*)

The OT receptor gene is present in single copy in the haploid human genome and was mapped to the gene locus 3p25–3p26.2 [1]. The gene spans 17 kb and contains 3 introns and 4 exons. Exons 1 and 2 correspond to the 5′ non-coding region. Exons 3 and 4 encode the amino acids of the OT receptor. Intron 3, which is the largest at

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12 kb, separates the coding region immediately after the putative transmembrane domain 6. Exon 4 contains the sequence encoding the seventh transmembrane domain, the COOH terminus, and the entire 3'-non-coding region, including the polyadenylation signals. The OT receptor protein is a typical member of the rhodopsin-type (class I) G protein class of receptors (GPCR) family [1]. The seven transmembrane-helices are most highly conserved among the GPCR family members. Conserved residues among the GPCRs may be involved in a common mechanism for activation and signal transduction to the G protein. The OT receptor signals via the Gq/11 and Gi class of guanosine triphosphate (GTP) binding proteins that stimulate, together with $G_{\beta x}$, the phospholipase C-β isoforms [17]. The selectivity of the OT receptor for G-proteins appears to be more complex than the selectivity of the vasopressin receptors. Coexpression of different combinations of intracellular domains demonstrates that all intracellular loops may contribute to the selectivity of the OT receptor to some extent. In addition to the intracellular domains, a highly conserved region of the C-terminal domain is involved in the selectivity of coupling to Gq/11 but not Gi.

4. Oxytocin and autism spectrum disorders (ASD)

Autistic disorders (AD) are a group of disorders characterized by the three core deficits: qualitative impairment in social interaction and communication, and restricted repetitive and stereotyped patterns of behavior, interests, and activities [18]. The three disorders autism, Asperger syndrome (AS) and pervasive developmental disorder-not otherwise specified (PDD-NOS) are currently thought of by most researchers as a continuum of the same disorder with varying degrees of severity, associated intellectual functioning and medical conditions. Recent studies estimated the prevalence of AD to around 0.5–1% [19]. Genetic factors play an important role in ASD [20].

A number of molecular genetic findings support the involvement of oxytocin neurotransmission in some of the social deficits and symptomatology of ASD. Firstly, significant association between OXTR SNPs [21–26] and risk for autism has been provisionally shown (but also see [27,28]) and, notably, was mediated by socialization skills in our study [23]. Additionally, SNPs associated with ASD are also associated with amygdala volume [29], depression/anxiety [29], negative emotionality [30], attention deficit [31], adult attachment styles [32,33] (but see[34]), affectivity, emotional loneliness and IQ [35], empathy and stress reactivity [36], observed parenting [37], with sexual phenotypes [38] and in our own studies with prosocial behavior [39]. Interestingly, the AVPR1a receptor of the closely related nonapeptide vasopressin has also been associated with ASD [40–42]. This provisional role of OT in ASD, is further supported by two linkage studies [43,44], clinical evidence by several groups demonstrating clinical improvement in ASD following OT administration [45–51] (also see [52,53]) and studies showing that ASD is also associated with alterations in OT plasma levels [54].

5. Dendritic release of oxytocin

An interesting feature of OT action in the brain is that although OT has profound effects on social bonding that are exerted at sites that richly express oxytocin receptors, such brain regions are innervated by few, if any, oxytocin-containing projections [55]. How then does OT exert its effect in limbic and other 'socially sensitive' brain regions in the absence of apparent direct OT containing neurons? Oxytocin synthesis takes place in hypothalamic nuclei, including the supraoptic nucleus (SON) and paraventricular nucleus (PVN). Specifically, magnocellular neurons in these two nuclei contain most of the OT and AVP in the CNS and are

characterized by dendritic release, which is regulated independently of secretion into the blood. Indeed, dendrites are apparently the major source of peptides released in the brain [55]. Importantly dendritic release does not parallel axonal release and both processes are regulated independently. Although release of OT and AVP from axons is linked to electrical activity resulting from Ca2+ entry through voltage-gated ion channels following depolarization of the terminals by invading action potentials [56], OT and AVP themselves [57], can elicit dendritic peptide release without increasing electrical activity. In OT neurons, OT itself mobilizes Ca2+ from thapsigargin-sensitive intracellular stores [58]. Activation of peptide receptors on the dendrites or soma elevates intracellular Ca2+ concentrations and triggers exocytosis of large dense-core vesicles (LDCVs), and once dendritic peptide release is triggered, because of the peptide feedback, dendritic release can be self-sustaining and, therefore, long-lasting.

6. CD38

A seminal paper by Higashida and his group led to the discovery that OT release in the brain is mediated by ADP-ribosyl cyclase and/or CD38 [59]. They used CD38 gene knockout mice (Cd38−/−), and discovered that CD38-dependent cyclic ADP ribose (cADPR)- and NAADP-sensitive intracellular Ca2+ mobilization plays a key role in OT release from soma and axon terminals of hypothalamic neurons, with marked effects on social behavior. In particular, maternal behavior was dependent on OT, and social amnesia in males was evident in the absence of this hormone. Activation of CD38 with its substrate ligand NAD+ results in hydrolysis of NAD+ and cyclic ADP-ribose (cADPR) to ADP-ribose, or in cyclization of NAD+ to cADPR. In addition to production of cADPR, the enzyme can use NADP+ as a substrate and catalyze the exchange of its nicotinamide group with nicotinic acid to produce NAADP+. cADPR mobilizes Ca2+ from ryanodine-sensitive intracellular Ca2+ stores in the endoplasmic reticulum and NAADP liberates it from other pools located in lysosomes or secretory granules. The two molecules act as second messengers independent of inositol 1,4,5-trisphosphate (IP3) [60,61]. To summarize, CD38 is a multifunctional molecule (ecto-enzyme) combining enzymatic and receptor properties and playing a key role in various physiological processes in the tissues (proliferation, differentiation, migration, adhesion, and secretion). In the brain, CD38 is found in neurons and glial cells, shows intracellular or plasma membrane location, and is enriched in neuronal perikarya and dendrites [62,63]. CD38 is critical for OT but not AVP release.

7. CD38 and autism spectrum disorders

The accumulating evidence discussed above, that OT plays an important role in both normal as well as dysfunctional social relationships/cognition [64,65], ipso facto targets CD38, a key mediator of OT brain release, as a potential focus of interest in normal human social behaviors as well as disorders of social cognition especially autism [66,67]. In the past year, two research groups have independently addressed the role of CD38 in autism in human subjects. Higashida and his colleagues [68] analyzed 10 single nucleotide polymorphisms (SNPs) and mutations of CD38 by resequencing DNAs mainly from a case–control study in Japan, and Caucasian cases mainly recruited to the Autism Genetic Resource Exchange (AGRE). CD38 SNPs, rs6449197 and rs3796863 showed significant associations with a subset of ASD subjects (IQ > 70; designated as high functioning autism/HFA) in 104 AGRE family trios, but not with Japanese 188 HFA subjects. Interestingly, a mutation/rare polymorphism that caused tryptophan to replace arginine at amino acid residue 140 (R140W; (rs1800561, 4693C > T)) was

found in 0.6–4.6% of the Japanese population and was associated with ASD in the smaller case–control study. The SNP was clustered in pedigrees in which the fathers and brothers of T-allele-carrier probands had ASD or ASD traits. In this cohort [68] OT plasma levels were lower in subjects with the T allele than in those without.

In our first study of CD38 [69], we examined all tagging SNPs across the CD38 gene region in 170 subjects diagnosed with ASD from 149 families (see [23] for description of the subjects). Individual SNPs and haplotypes were tested for association with ASD. Additionally, the relationship between diabetes, autism and CD38 [70], as well as the use of CD38 as a disease marker [71], suggests that it would also be worthwhile to explore CD38 expression in immune cell lines derived from ASD patients. These considerations prompted us to measure CD38 gene expression in lymphoblastoid cell lines (LBC) derived from both ASD subjects and unaffected parents. We also include in the gene expression and family-based association analysis the SNP (rs3796863), which proved significantly associated with ASD in the Munesue et al. [72] study.

8. Molecular genetic association [71]

We first examined association between CD38 tagging SNPs and DSM IV ASD. ASD subjects were evenly grouped into high and low functioning based on an IQ cutoff of 70. This subject stratification was aimed at reducing phenotypic heterogeneity in the autism sample. Significant association was observed between low functioning ASD and three – seven haplotypes (Table 1). The results shown in the Table are significant ($p < 0.05$) following permutation testing. Importantly, the SNP (and the 'C' allele) identified in the Munesue et al study [68] (rs3796863), which they found

significantly associated with ASD, is located in all except one of the significant haplotypes in our study.

9. Is CD38 expression in peripheral cells a hallmark for ASD [69,72]?

CD38 mRNA levels in LBC derived from subjects with autism and unaffected parents were also examined in our study (Fig. 1). A highly significant reduction (SPSS ANOVA-Affected status: $F = 14.72$, $p = 0.0002$, df = 1; Sex: $F = 4.680$, $p = 0.033$, df = 1; Interaction: affected x sex, $F = 2.304$, $p = 0.132$, df = 1) in CD38 expression was observed in cells from the DSM IV ASD subjects ($N = 44$) compared to "unaffected" parents ($N = 40$). Main effects are observed for diagnosis and sex. The reduction in CD38 expression is more marked in female ASD subjects than in male ASD subjects. These first results [69] have now been partially replicated in a new study from our laboratory [72]. In the new expanded study there was no effect of gender.

In our subsequent investigation [72], we have re-analyzed the EBV lines described in the first report, significantly adding to the sample with 38 new cell lines so that in the second investigation for each proband both of their parents were now included in the analysis. Cells in culture, or frozen lines were first thawed, and then cultured, and their CD38 mRNA levels measured. It was important to determine whether expression of CD38 is stable and is maintained despite repeated cycles of freezing and thawing. The new results confirm that CD38 expression in ASD patient lines is substantially lower than in those derived from the patients' parents (Fig. 2). Although these results are not a fully independent replication, we believe they nevertheless considerably strengthen our first findings that reduced CD38 transcription is a characteristic of peripheral lymphocyte cells derived from ASD subjects [69] (see Fig. 2).

The surface expression of CD38 on blood cells varies significantly throughout the life course. Normally, expression is high on cord blood cells and diminishes in cells obtained from adults [71]. Hence the age difference between ASD subjects and their parents could be a confounding factor in interpretation of our results. However, no age-related differences indexed by mRNA levels were observed in LBC lines obtained from the ASD patient group or from

Table 1
Haplotype association of CD38 SNPs with low functioning ASD subjects.

HAP	Freq.	Transmitted	Untransmitted	Chi Square	p value
rs3796863–rs1803404–rs1130169					
Likelihood ratio chi square = 12.14; df = 4; global p-value = **0.016** (**0.019**)					
CTC	0.501	85.87	64.62	4.78	**0.028**
ATT	0.201	35.87	38.81	0.939	0.332
CTT	0.194	17.13	36.38	7.47	**0.006**
rs10805347–rs3796863–rs1803404–rs1130169					
Likelihood ratio chi square = 20.69; df = 9; global p-value = **0.014** (**0.01**)					
ACTC	0.316	39.97	39.24	0.005	0.938
GCTC	0.187	42.58	20.25	9.419	**0.002**
GATT	0.186	31.53	24.23	1.101	0.294
GCTT	0.178	16.44	31.45	4.796	**0.028**
rs2286553–rs10805347–rs3796863–rs1803404–rs1130169					
Likelihood ratio chi square = 25.46; df = 11; global p-value = **0.008** (**0.019**)					
GACTC	0.317	39.99	39.72	3.25e−05	0.999
GGCTC	0.186	42.25	19.76	9.768	**0.001**
GGCTT	0.177	16.76	31.8	4.762	**0.029**
GGATT	0.173	28.97	24.45	0.493	0.482
rs3796864–rs2286553–rs10805347–rs3796863–rs1803404–rs1130169					
Likelihood ratio chi square = 41.52; df = 13; global p-value = **7.81e−05** (**0.009**)					
CGACTC	0.313	39.99	37.76	0.075	0.783
CGGCTC	0.179	41.19	18.85	9.528	**0.002**
CGGCTT	0.167	15.77	29.15	3.525	0.061
AGGATT	0.125	16.95	19	0.152	0.695
rs4516711–rs3796864–rs2286553–rs10805347–rs3796863–rs1803404–rs1130169					
Likelihood ratio chi square = 34.78; df = 12; global p-value = **0.0005** (**0.0049**)					
GCGACTC	0.297	38.99	31.84	1.006	0.315
GCGGCTC	0.180	38.16	18.97	8.064	**0.004**
GCGGCTT	0.162	14.84	27.03	3.808	0.051
CAGGATT	0.123	16.99	18	0.396	0.842

Global p-values are presented for each haplotype window followed by the p-values for the individual common haplotypes (>10%). p-values in parentheses are after permutation test. The IQ cutoff <70 was used to define the low functioning group.
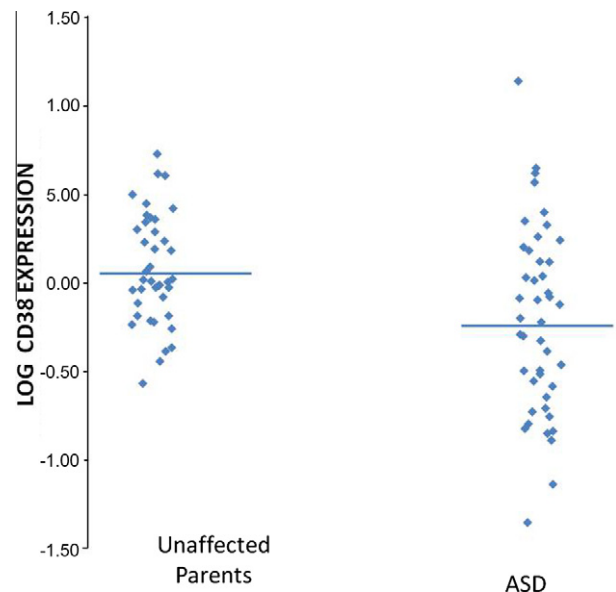

Fig. 1. Distribution of the expression (log transformed) of the CD38 gene as depicted in a (A) scatter plot and (B) box plot. Lower expression in the ASD group is significant ($p = 0.003$). Taken from [69].
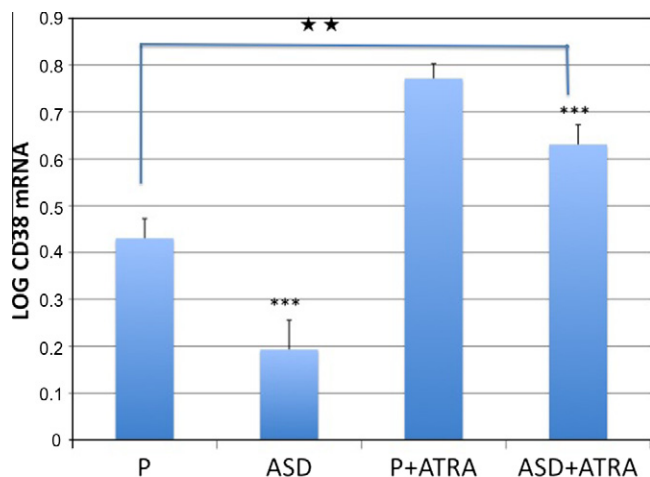
Fig. 2. The effect of 48 h 0.1 μm ATRA treatment on CD38 mRNA levels in LBC lines. ★★, independent samples t-test, $t = -3.199$, $p = 0.002$; prolonged ATRA treatment elevates reduced CD38 mRNA levels in LBC lines from ASD patients ($N = 42$) above parental (P) basal expression ($N = 78$). Also, basal and induced CD38 mRNA levels are significantly reduced in ASD cell lines compared to parental (P) cell lines (***, independent t-test; $p < 0.001$). Taken from [72].

their parents at least in the disease model currently adopted and using immortalized cell lines [69,72].

10. Retinoids

Retinoids are a class of compounds consisting of retinol (vitamin A) and its derivatives and synthetic analogs [73,74]. Natural retinoids are fundamental for many physiologic processes, such as reproduction, growth, and cellular differentiation. These effects are mediated by binding to and activating two different types of nuclear receptors, the retinoic acid (RA) receptors (RARs) and retinoid X receptors (RXRs). All-*trans*-RA (ATRA) is a high-affinity ligand for RARs only, whereas 9-cis-RA is a high-affinity ligand for both RARs and RXRs. RAR-RXR heterodimers activate the transcription of target genes in response to ATRA or 9-cis-RA, whereas RXR–RXR homodimers transactivate in response to 9-cis-RA.

11. Retinoids and CD38

all-*trans* retinoic acid (ATRA) is a potent inducer of CD38 [75] suggesting the possibility that this compound can be used to 'rescue' cells exhibiting low CD38 synthesis and hence might be a novel therapeutic strategy in treatment of autism.

In human promyelocytic leukemia HL-60 cells the expression of CD38 mRNA by RA appeared to be caused by the transcriptional stimulation of the gene, since it was blocked by an RNA synthesis inhibitor, but not by a protein synthesis inhibitor [76]. Transient transfection experiments revealed that the responsiveness to RA was conferred through a RARE (retinoic acid responsive element) consisting of two direct repeat TGACCT-like hexamer motifs with a 5-nucleotide spacer, which was located in the first intron rather than the 5-flanking region of the CD38 gene. This RARE interacted with heterodimer composed of RA receptor and retinoid X receptor in vitro. Thus, the RA-induced expression of the human CD38 gene was demonstrated to be mediated through the RARE located in the first intron.

12. In vitro rescue by retinoids of CD38 deficiency in autism [72]

We wanted to determine whether the diminished expression of CD38 in ASD could be reversed through simple treatment with ATRA. Such a demonstration would provide in vitro 'proof of principle' that retinoids could play a role in the clinical treatment of ASD.

Following 48 hours of ATRA treatment, the results indicate that the CD38 gene in the EBV lines obtained from the ASD probands conserves its ability to respond with a significant induction of CD38 mRNA (Fig. 2). The parental lines display the same ability, although to a lesser extent (paired t test $t = -13.26$ $p < 0.001$ ± ATRA). These results, demonstrating that ATRA can elevate CD38 levels in cells obtained from ASD subjects who show impaired CD38 transcription, strengthen the notion that vitamin A and related retinoids are potential therapeutic agents in the treatment of ASD. Indeed, retinoids are widely used as treatment modules in a spectrum of diseases including acne & psoriasis [77], cancer [78] and as a dietary supplement reduces child mortality between 6 months to five years in low and middle income countries [79]. We put forward the prospect that retinoids are potential therapeutic agents in autism and possibly other disorders that are characterized by dysfunctional social cognition/relationships especially where oxytocin has been suggested to play a role.

There is a longstanding notion that vitamin A plays a role in psychiatric illness [80] likely based on the profound effects of retinoids on brain development and processes such as long-term potentiation (LTP) and mood regulation [81]. Interestingly, during early development mice pass through a narrow post-natal window where they are very sensitive to brief exposure to retinoic acids. Such exposures have significant long-term behavioral effects [82], Interestingly, retinoid-induced abnormalities appear to be due to damage to the limbic system, a brain area that plays a crucial role in a number of behavioral pathologies. Furthermore, these post-natal effects of experimental retinoid manipulations incur minimal anatomical changes (in comparison to manipulations during the prenatal period) whereas behavioral effects are quite profound. Intriguingly, the cortex of retinoic acid deprived rats is characterized by beta-amyloid accumulation and other changes that parallel those in Alzheimer' disease [83]. Additionally, normal memory loss in aging rats can be alleviated by vitamin A therapy [84]. In the adult hippocampus, retinoids are essential for the maintenance of synaptic plasticity including LTP and neurogenesis [85], The hippocampus is a brain region dependent upon neural plasticity for its function in learning and memory, Altogether, this brief summary of a very extensive literature on retinoids and the brain firstly provides a perspective for interpreting our investigation [72] that CD38 transcription is correlated with cognitive function in ASD and secondly, that retinoids are potential therapeutic agents in autism.

13. CD38 genotype and ATRA response

Cell lines were genotyped for the rs6449182 SNP, which leads to a C→G variation. This SNP is located in intron 1 of the regulatory region of human CD38, proximal to RARE. The presence of the allele G is reported as being paralleled by increased binding of the transcription factor E2A [86]. Furthermore, the G allele marks an increased risk in CLL patients of transformation into Richter's syndrome [87].

The results from our second study [72], indicate that the presence of the G allele is paralleled by (i) reduced transcriptional levels of CD38 mRNA, a characteristic shared by ASD with those of the parental lines although in LBC lines obtained from ASD probands the difference does not attain statistical significance. Furthermore, (ii) the G allele is accompanied by reduced sensitivity to ATRA treatment (+ ATRA treatment in parental lines CC = 0.82 ± 0.04; CG = 0.69 ± 0.04 $p = 0.04$).

14. Link between CD38 expression and clinical characteristics in the ASD sample

Our results showing that CD38 expression is reduced in ASD prompted us to examine whether its expression levels might also reflect phenotypical characteristics of ASD further enhancing the value of this ectoenzyme as a potential biomarker. We looked at social functioning measures that were available for these probands since such deficits are a core clinical characteristic of autism. The results obtained clearly show a significant correlation between transcriptional levels of CD38 mRNA and IQ and Vineland Adaptive Behavioral Scores (VABS) scores [88], except for VABS socialization. Nonetheless, the correlation with the VABS total scores does prove significant ($r = 0.431$, $p = 0.008$, $N = 42$).

CD38 mediates oxytocin brain release [59] and importantly, oxytocin itself enhances social learning and memory in the limbic system [89]. If retinoids modulate CD38 transcription in the brain, which in turn mediates oxytocin release, then the relationship we have shown [72] between cognitive function and CD38 mRNA levels in LBC cells may be reflecting common state characteristics of OT-CD38-RA pathways in different tissues. Indeed, various studies have employed LBC lines to model brain dysfunctions in autism [90–92] and other neuropsychiatric disorders [93–97].

Finally, it needs to be noted that the potential use of retinoids in any therapeutic intervention must be tempered with the evidence that too high exposure to retinoids is as harmful as too little.

15. Biomarkers for autism spectrum disorders

Despite the considerable part that heredity plays in the etiology of ASD the identification of which specific genes, and how many genes, contribute to ASD remains a challenge. For example, a recent GWAS study [98] genotyped 1558 families and only a single marker attained genome-wide significance. Exploratory analysis of phenotype subtypes yielded some promising candidate genes, which did not survive correction for multiple testing. To date, only rare de novo mutations are validated genetic risk factors for ASD and most other findings from GWAS and association studies need to be considered provisional. However, such rare variants, although they might offer important clues to the underlying pathophysiology and brain biochemistry in autism, only account for a small proportion of the total genetic risk. Some chromosomal rearrangements appear causal, with the most common being maternal duplication of 15q11–q13 [99]. Rare de novo mutations of high penetrance for ASD have been identified in synaptic genes, including NLGN3, NLGN4X and SHANK3 [100–102]. Similar to other neuropsychiatric disorders copy number variations (CNV) also contribute overall risk to ASD [103] including rare deletion CNVs of SHANK3 and the surrounding 22q13.33 region found in individuals with ASD [103].

The current observation that CD38 expression is reduced in lymphoblastoid cells derived from ASD subjects suggests the prospect that CD38 might be an early hallmark for this disorder. As noted by Yirmiya and Charman [104] "the primary motivation for identifying the earliest signs of emerging ASDs is the desire to develop and test early or even 'preventative' interventions to lessen morbidity by changing the course of early emerging developmental perturbation, thus preventing 'secondary' neurodevelopmental disturbances." Towards evaluating the potential of CD38 as a hallmark in ASD, reduced CD38 expression needs firstly to be verified in circulating lymphocytes. Secondly, it must be stressed that CD38 transcription is a marker for other diseases. It is prognostic for HIV infected subjects [105], chronic lymphoid leukemia [106] and for diabetic patients with nephropathy [107]. Hence reduced CD38 transcription cannot be pathognomonic for

ASD but nevertheless might prove of salient clinical value in a disorder diagnosed solely using behavioral assessments reliably carried out only at the age of three [104]. Moreover, CD38 expression changes throughout the lifespan [71] and age-dependent CD38 expression in circulating lymphocytes are potential confounds in its use as a diagnostic indicator in ASD. However, the critical need in ASD is for very early (prenatal or perinatal) diagnostic tools and, hence from this perspective, CD38 mRNA levels in cord blood or amniotic fluid might be of substantial value notwithstanding subsequent age-related changes in lymphocyte CD38 expression.

One group of biomarkers for neuropsychiatric disorders that shows considerable promise is blood gene expression profiling [108–113]. Most of the studies to date have focused on human lymphocytes gene expression profiling, comparison between illness groups and normal controls, and cross-matching with human postmortem brain gene expression data. A number of studies have specifically examined gene expression patterns in ASD [90–92,114–123]. It should be noted that predating this gush of expression studies in ASD, hyperserotonemia was observed in one third of patients and platelet serotonin was suggested as a marker for this disorder [124–127].

To summarize, the difficulty to validate specific gene variants for most cases of autism likely reflects a variety of causes including gene x gene interactions, the heterogeneous nature of this disorder, and epigenetic modifications due to diverse environmental challenges. The challenge of finding specific genes contributing to ASD, except in those rare cases and their families showing Mendelian inheritance, suggests that complementary strategies to supplement more standard molecular genetic association studies would be worthwhile. Such an approach has been proposed by Le-Niculescu and his colleagues [128]. Their approach, termed convergent functional genomics (CFG), which translationally cross-matches animal model gene expression data with human genetic data and human tissue data (blood, postmortem brain), as a Bayesian strategy of cross-validating findings, reducing the false positives and false negatives inherent in each individual approach, and helping identify true candidate genes, pathways and mechanisms for neuropsychiatric disorders.

16. Conclusion

The current study adds to the growing list of potential biomarkers in ASD and moreover, uniquely observes a correlation between expression levels of CD38 in LBC derived from these subjects and social and communication skills that are core deficits in this disorder. Notably, the potential of CD38 expression as a diagnostic indicator for ASD was a hypothesis driven idea catalyzed by the seminal study of Higashida and his colleagues [59] in the CD38 knockout mouse and reinforced by two independent molecular genetic studies showing association between SNPs in the CD38 gene and ASD [68,69].

In addition to the potential of CD38 as a hallmark that may prove useful in early diagnosis of illness, the study of CD38 expression in peripheral lymphocytes has allowed us to model the potential of retinoic acids as a therapeutic agent in ASD. Indeed, we have shown that LBC derived from ASD subjects and characterized by reduced CD38 transcription can be 'rescued' by simple treatment with all-*trans* retinoic acid. We believe these results provide the first 'proof of principle' for a novel therapeutic strategy in treatment of ASD by enhancing OT secretion in the brain indirectly by ATRA induction of CD38 followed by mobilization of ryanodine-sensitive intracellular Ca2+ stores from the endoplasmic reticulum which in turn release OT.

17. Disclosure

We declare that the authors have no competing interests as defined by Molecular Medicine, or other interests that might be perceived to influence the results and discussion reported in this paper.

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