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Autoantibody and autism spectrum disorder: A systematic review

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ABSTRACT

Autism spectrum disorder (ASD) is a neurodevelopmental disorder affecting 1.7 % of US pediatric population, with a growing prevalence world-wide. Autoimmunity is one of potential pathogenic factors for ASD, which is attracting much attention and undergoing extensive investigations. For more than a decade, many groups have been studying the association between autoantibodies and ASD. Although several narrative reviews have been published on autoantibodies and ASD, no systematic review or meta-analysis has been performed. In this study, we conducted the first systematic review and evaluated available evidence for the association between ASD and major autoantibodies to identifiable antigens, together with a broader discussion of autoantibodies with no identifiable antigens. The goal is to examine studies of pediatric subjects specifically and overall, we found that children with ASD expressed trends of higher levels of antibodies reactive to folate receptor α autoantibody, anti-myelin basic protein antibodies, anti-myelin-associated glycoprotein antibodies, anti-ribosomal P protein antibodies, anti-endothelial cell antibodies, and anti-nuclear antibody, compared to healthy controls. However, the quality of evidence is low across the board because most studies were small and many did not include comparison controls. In addition, we were not able to perform a meta-analysis due to large between-study heterogeneity or lack of quantitative measures in most studies. Finally, we discussed future directions for the development of diagnostic guidelines and therapeutic targets for possible autoimmune-mediated ASD subtypes.

1. Introduction

Autism spectrum disorder (ASD) is a heterogeneous neurological and developmental disorders featured by impairments in communication, social interaction, stereotypical repetitive behaviors and restricted interests (Faras, Ateeqi Al, & Tidmarsh, 2010). The latest report from United States Centers for Disease Control and Prevention revealed the prevalence of ASD to be 1 in 59 children in the United States (Baio et al., 2018), nearly tripled from 1 in 150 children in 2000. The incidence of ASD is also increasing worldwide (Buescher, Cidav, Knapp, & Mandell, 2014; Ouellette-Kuntz et al., 2013; Wu et al., 2017). The diagnosis of this disorder was introduced in 1943 (Kanner, 1968). The pathology and etiology still remain elusive, although an interplay between genetics, neurological and environmental factors are widely accepted as plausible mechanisms of pathogenesis (Pardo, Vargas, & Zimmerman, 2009).

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In the past 15 years, increasing number of studies uncovered associations between immune system abnormalities or cytokine aberrations with ASD. Although the causality between autoimmunity and ASD has never been established, these observational studies suggest that immunological dysregulation is an important comorbidity of ASD and may play a role in its pathogenesis (Siniscalco, Schultz, Brigida, & Antonucci, 2018). Evidence includes upregulation of inflammation markers (Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2004), activated microglial cells in post-mortem brains (Morgan et al., 2010), frequent aberrations of fundamental genes involved in immune regulation, and alteration of macrophage-monocyte lineage cell responses in ASD patients (Siniscalco et al., 2018). Studies also demonstrated improvement of core symptoms both in ASD patients and in animal models by normalization of the immune system, such as treatment with Intravenous Immunoglobulin (IVIG) as shown by a recent pilot study (Melamed, Heffron, Testori, & Lipe, 2018). Further, rodent model of maternal immune activation reveals that mothers with inflammation induction were more likely to have decedents with ASD-related behaviors (Hsiao, McBride, Chow, Mazmanian, & Patterson, 2012). Studies have not identified specific causative factors, but they suggest that dysregulated innate immune responses contribute to the immune abnormalities associated with ASD (Jyonouchi, Geng, Streck, & Toruner, 2011).

Autoimmune diseases can activate abnormal immune responses which have been hypothesized to precipitate the development of ASD in subsets of patients. For example, the antigen-antibody complexes may prompt neutrophil migration and provoke an inflammatory response (AL-Ayadhi & Mostafa, 2011). The evidence of autoimmunity linked with ASD has emerged through epidemiological studies. Clinical studies show high percentage of autoimmune disorders such as type 1 diabetes and inflammatory bowel disease in ASD individuals (Kohane et al., 2012) as well as in ASD families (Comi, Zimmerman, Frye, Law, & Peeden, 1999; Mouridsen, Rich, Isager, & Nedergaard, 2007; Atladottir et al., 2009). A Danish registry (including all 689,196 children born in Denmark during 1993–2004, with 3325 children developed ASD) found that mothers with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and type 1 diabetes were more likely to bear children with ASD (Keil et al., 2010). A common genetic variant related to autoimmunity was found in both patients with RA and ASD, Human Leukocyte Antigen − antigen D Related (HLA − DR) B1*04 alleles, further supporting the hypothesis that autoimmune disease may play a pathogenic role in the development of ASD (Rom et al., 2018).

Autoantibodies have been detected in patients with behavioral disorders, such as autism, Tourette's syndrome (Singer et al., 2008), attention deficit hyperactivity disorder (Peterson et al., 2000), and obsessive-compulsive disorders (Ohtsuka & Suzuki, 2000). Previous studies have reported a wide spectrum of autoantibodies associated with ASD (Kobeissy, 2015). These antibodies include those reactive to endothelial cells

(Connolly et al., 1999), serotonin receptor (Todd, Hickok, Anderson, & Cohen, 1988), anti-virus antibody (Singh, Lin, & Yang, 1998), folate receptor α autoantibody (FRAA) (Ramaekers et al., 2005; Cabanlit, Wills, Goines, Ashwood, & Van de Water, 2007), anti-neuro autoantibodies such as myelin binding protein (MBP) (Singh, Warren, Odell, Warren, & Cole, 1993), antibodies against neuronal and glial proteins (Kirkman et al., 2008), and cerebellar peptides in correlation with food allergy (A. Vojdani et al., 2013). Some systemic autoantibodies were also detected in ASD patients (Montiel & Zavala, 2002). Several groups have identified the presence of IgG class antibodies reactive with fetal brain proteins in the serum of mothers with ASD children (2012, Braunschweig et al., 2008; Croen et al., 2008; Singer et al., 2008). By extracting the IgG antibodies from mothers of ASD children and injecting the antibodies into gestational monkeys or mice, investigators found that their offspring displayed behavioral and nervous system abnormalities (Dalton et al., 2003).

These studies raise the following questions. First, are any autoantibodies highly correlated with ASD and could be of diagnostic value? Second, what may be their roles in ASD pathogenesis? Finally, whether these antibodies can be used to guide clinical treatment of ASD, especially in the subset of patients who may have an immune-mediated etiology? Besides, the debate on which autoantibodies may be involved in ASD pathogenesis, if any, has not reached a consensus. The pathological threshold of autoantibody levels and ASD subtypes defined by these antibodies are also inconclusive.

For example, in the study of folate receptor α autoantibody in ASD patients, there is a promising clinical trial that supplements folinic acid (a reduced folate derivative which is more metabolically available) in FRAA-positive ASD children which showed apparent improvement in verbal communication and behavioral modification (Frye, Sequeira, Quadros, James, & Rossignol, 2013). The target antigen in this case is the folate receptor alpha involved in folate transport to the fetus during development and to the brain in neonates. Further proof supporting the role of folate receptor antibodies in the pathology of developmental deficits has been demonstrated in a rat model of exposure to rat folate receptor antibodies (Desai, Sequeira, & Quadros, 2017). However, high quality, large scale longitudinal studies and randomized trials are warranted before any conclusions are drawn.

Two systematic reviews and meta-analysis have specifically focused on autoimmunity and ASD, and quantitatively evaluated the association between them. Wu. et, al reported a statistically significant association between family history of autoimmune disease including hypothyroidism, type 1 diabetes, rheumatoid arthritis, as well as psoriasis and a higher risk of ASD (Wu et al., 2015). Chen et al. showed that mothers who developed gestational autoimmune disease had a 30% higher probability to have ASD off-springs (Chen et al., 2016). To our knowledge, despite several narrative review papers have been published on the topic of autoantibodies and ASD (Edmiston, Ashwood, & Van de Water, 2017; Meltzer & Van de Water, 2017), no systematic review or meta-analysis has been performed examining auto-immune factors and autoantibodies specifically in individuals with ASD. To further our understanding of the association between autoantibodies and the development of ASD, we conducted the first comprehensive and systematic review of available population studies and attempted a meta-analysis, if feasible, to examine a panel of autoantibodies in ASD pediatric patients as compared to the controls based on human observational studies selected by pre-defined criteria.

2. Method

2.1. Inclusion and exclusion criteria

Studies measuring and comparing levels of autoantibodies in subjects with ASD and non-ASD healthy or neurotypical controls (HC) were included. The eligibility criteria include (a). the study targeted ASD patients; (b). case-control or cohort studies; (c). the diagnosis of ASD was made in accordance with DSM-III, IV or V standards; (d). pediatric patient group; (e). antibody level comparison was conducted in greater than or equal to two independent studies; and (f). reporting or providing data for autoantibodies identified in ASD patients, with the concentrations reported using a mean and standard deviation, or sample size and percentage positivity. The exclusion criteria included (a). measurement of antibodies in any specimen other than peripheral blood or cerebrospinal fluid; (b). the methodology to measure antibody levels is any technique other than immunofluorescence (IF), enzyme-linked immunosorbent assay (ELISA) or immunoblotting; (c). measurement of protein levels other than autoantibodies; (d). study of autoimmune disease in ASD patients or family members without measuring autoantibody levels or positive percentage; (e). study of maternal antibodies; and (f). evaluation of antibody gene expression profiles.

2.2. Search strategy

A literature search of PUBMED, EMBASE, PMC, Google Scholar, and PsycINFO was conducted up to May 2018. The following types of articles were excluded: anecdotal case reports of single patients, analysis of clinical database or registries, review articles, commentaries, mechanism of action research articles, meta-analysis or systematic reviews, and study designs that satisfy the inclusion criteria but do not involve behavioral assessment. We also included unpublished, recently completed studies in the above categories for which authors granted the right to include in the present study.

Studies in ASD were searched with the terms “autism”, “children” as medical subject headings (MeSH). The term “autism” and “children” was used as free text terms. These terms were combined using the set operator AND with articles searched with the following terms: “autoantibody” and “autoimmune”, both as MeSH terms and as free text items. Terms of “rat”, “mouse”, or “review” were excluded during search, all as MeSH terms and as free text items. We did not impose language restrictions. Titles and abstracts of the papers identified by this initial search strategy were evaluated by two independent reviewers. We obtained potentially relevant papers and conducted subsequent full text screen. We also conducted a recursive search of the literature based on the bibliographies of the relevant full text articles. Two independent reviewers conducted full text screen using pre-designed eligibility criteria. Any disagreement between investigators was resolved by consensus.

2.3. Data extraction

One reviewer extracted individual autoantibody data from each study (sample size, mean, standard deviation and percentages of samples positive for the autoantibody in the serum or CSF of ASD and control groups) into a pre-piloted Microsoft Excel spreadsheet (XP professional edition; Microsoft Corp, Redmond, WA, USA). A second reviewer cross-checked each data variable extracted against the original publication. Disagreement was resolved via discussion with a third extractor. In addition, the following information was extracted wherever available: age of participants, gender of participants, immune methodology and definition of positive result. When necessary, we contacted the authors to obtain original data via e-mail.

3. Results

A total of 420 studies were identified from the initial search, after excluding duplicates. Titles and abstracts were scanned resulting in identification of 51 articles to be assessed for eligibility. A total of 32 studies were excluded due to the following reasons: analysis of antibodies against unknown antigens or studies that did not perform statistical analysis (11 studies); the antibody analyzed that was not assessed in at least one other studies (7 studies) or maternal autoantibodies (2 studies); review papers of autoantibodies (3 studies); study that assessed autoantibodies in non-pediatric population (1 study); non-autoantibodies (5 studies), and methodology to measure antibody levels that is not a technique rather than immunoassay such as IF, ELISA and immunoblotting (1 study). We excluded one additional study which was published in Russian language, by Klushnik et al. (2015), for which we were not able to accurately assess the antibody detecting methods used by the authors. 1 other unpublished study, with only abstract online, was also excluded (Montiel & Zavala, 2002). Finally, 19 studies met inclusion criteria for systematic review and meta-analysis. We attempted to perform meta-analysis on data for 6 different autoantibodies that were each assessed by at least two independent studies, including anti-myelin basic protein (MBP) antibody, anti-myelin-associated glycoprotein (MAG) antibody, anti-ribosomal P protein antibody, anti-endothelial cell antibody (AECA), folate receptor α autoantibody (FRAA), and antinuclear antibody (ANA). However, due to large between-study heterogeneity and lack of quantitative comparisons, a meta-analysis was ultimately deemed unfeasible. Six autoantibodies were excluded in the initial attempt for meta-analysis because of either having only been assessed by

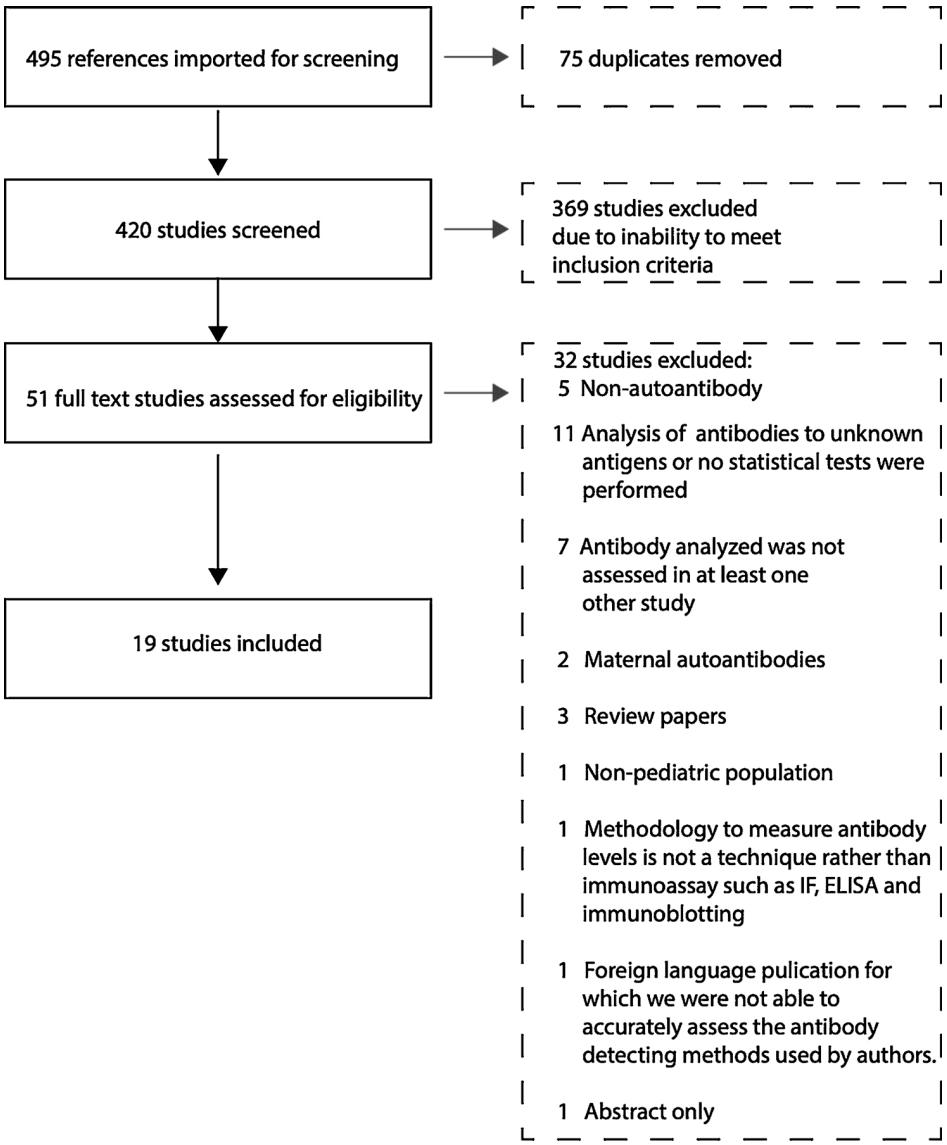

Fig. 1. Flow chart for study selection.

less than two studies, or if assessed two or more times, inadequate information was provided in one or more papers. The majority of anti-neuronal antibodies were not included in the initial attempt for meta-analysis due to lack of the same statistical comparison in other studies (14 studies). Details and rationales for study selection and exclusion are summarized in Fig. 1.

Table 1 presents characteristics of included studies. Baseline characteristics of participants, sample sizes, antibody detection methods, and the identities of autoantibodies were listed. Table 2 includes autoantibody concentrations in ASD and control groups, detection thresholds for determining positivity, as well as percentages of subjects who were deemed positive. In the following section, we present detailed results from included autoantibodies, along with statistical considerations. Overall, we found that studies consistently report ASD children displayed higher levels of FRAA, anti-MAG antibody, anti-MBP antibody, anti-ribosomal P antibody, AECA, and ANA, compared to HCs. However, the quality of evidence is low based on the following grounds: (1) most studies suffered from small sample sizes (Table 1), (2) many did not include the same comparison antibody levels in cases to controls (Table 1), and (3) many lacked information regarding the raw autoantibody concentrations in ASD vs control groups, as well as threshold for determining positivity (Table 2). Unfortunately, we were not able to perform a meta-analysis due to large between-study heterogeneity, small numbers of studies with quantitative comparisons, and lack of the control groups in many studies.

Table 1
Summary characteristics of included studies in the present systematic review.

Article	Study Design	Antibody	No. of ASD			No. of HCs			Mean age of subjects	Location	Method of detection
			Total	Male	Female	Total	Male	Female			
Mostafa and AL-Ayadhi, 2011	CC	Anti-ribosomal P	70	55	15	48	37	11	8.1	serum	ELISA
AL-Ayadhi, 2005	CC	MBP	65	61	4	65	NA	NA	8.2	serum	ELISA
Mostafa and Al-Ayadhi, 2013	CC	MBP	42	32	10	42	33	9	8.12	serum	ELISA
		MAG	42	32	10	42	33	9	8.12	serum	EIA (enzyme immunoassay technique)
Mostafa et al., 2014	CC	ANA	100	74	26	100	74	26	8	serum	IF (Immunofluorescence)
Al-Ayadhi and Mostafa, 2014	CC	ANA	60	49	11	60	50	10	7	serum	ELISA
Bashir and Al-Ayadhi, 2015	CC	AECA	55	47	8	25	21	4	7.98	serum	ELISA
Mostafa, El-Khashab, & AL-Ayadhi, 2015	CC	MBP	80	66	14	80	66	14	7.4	serum	ELISA
Connolly et al., 1999	CC	AECA	11	7	4	71	NA	NA		serum	Immunoassay (IHC)
		ANA	11	7	4	71	NA	NA	4.7	serum	Immunoassay (IFA)
Frye et al., 2012	CC	FRA	93	84	9	6	NA	NA	7.25	serum	Assay for blocking & binding FRAAs
		FRA	93	84	9	NA	NA	NA	7.25	plasma	Assay of FR autoantibodies of the blocking type
Frye et al., 2016	CO	FRA	87	70	17	12	NA	NA	6.83	serum	Assay for blocking & binding FRAAs
Montiel and Zavala, 2002	CC	ANA	35	NA	NA	54	NA	NA	NA	serum	IF
Mostafa et al., 2016	CC	Anti-ribosomal P	60	NA	NA	60	NA	NA		serum	ELISA
Mostafa et al., 2008	CC	MAG	32	25	7	32	25	7	4	serum	EIA
Ramaekers et al., 2007	CC	FRA	25	18	7	25	14	11	6.88	serum CSF	Assay of FR autoantibodies of the blocking type
Singh, Lin, Newell, & Nelson, 2002	CC	MBP	125	NA	NA	92	NA	NA	4–10	serum	Immunoblotting
Singh et al., 1993	CC	MBP	33	NA	NA	50	NA	NA		serum	Western immunoblotting
Singh and Rivas, 2004	CC	ANA	60	NA	NA	55	NA	NA	6.7	serum	IF + EIA
Vojdani et al., 2002	CC	MBP	40	23	17	40	NA	NA	6.4	serum	ELISA
Total			1610			1299					

Note: CC- case control study; CO - cohort study.

Table 2
Summary of the findings of antibody concentrations for ASD vs. controls.

Study	Autoantibody	Antibody concentration				Percentage of subjects positive for antibody expression		
		ASD		HC				
		Mean/median	SD/IQR	Mean/median	SD/IQR	Standard for determining positivity	ASD (%)	HC (%)
Mostafa and AL-Ayadhi, 2011	Anti-ribosomal P	NA	NA	NA	NA	unclear	44.3	NA
AL-Ayadhi, 2005	MBP	NA	NA	NA	NA	unclear	54	9
Mostafa and Al-Ayadhi, 2013	MBP	227 pg/mL	133 (IQR)	97 pg/mL	63 (IQR)	unclear	57.10	NA
	MAG	2468.9 BTU	644.4 BTU	1285.9 BTU	435.1 BTU	unclear	66.70	NA
Mostafa et al., 2014	ANA	NA	NA	NA	NA	titer ≥1:80	25	4
Al-Ayadhi and Mostafa, 2014	ANA	2.9	2.6 (IQR)	1.7	1 (IQR)	unclear	46.70	NA
Bashir and Al-Ayadhi, 2015	AECA	306.4 pg/mL	45.6 pg/mL	209.6 pg/mL	24.6 pg/mL	Best cutoff value (serum AECAs) 254 pg/mL, with the area under the curve being 0.64	NA	NA
Mostafa et al., 2015	MBP	NA	NA	NA	NA	Patients were considered to have increased serum anti-MBP if their levels were above the 95th percentile of the control values (242 ng/mL)	75	NA
Connolly et al., 1999	AECA	NA	NA	NA	NA	Positive: staining scored 2+ or 3+	50	0 HC, 9.5 NNI
	ANA	NA	NA	NA	NA	ANA titer of ≥1:80 as abnormal	27 (3/11)	3 (2/71)
Frye et al., 2012	FRA	NA	NA	NA	NA	Negative < 0.5, low 0.5–2, Medium 2–10, High > 10, expressed as pmoles of IgG Ab/mL of serum	75.30	17 + 17 (low positive)
Frye et al., 2016	FRA (blocking)	NA	NA	NA	NA	Blocking FRAAs are expressed as pmoles of folic acid blocked from binding to FRα per ml of serum	15	Not tested
	FRA (binding)	NA	NA	NA	NA	binding FRAAs are expressed as pmoles of IgG antibody per ml of serum.	46.10	Not tested
Montiel et al., 2002	ANA	NA	NA	NA	NA	unclear	37	NA
Mostafa et al., 2016	Anti-ribosomal P	NA	NA	NA	NA	unclear	58.30	5
Mostafa et al., 2008	MAG	2100 BTU	1995 (IQR)	1138 BTU	87.5 (IQR)	unclear	62.50	3.25
Ramaekers et al., 2007	FRA (blocking)	1.05 pmols/mL	Range: 0.1–4.19pol/mL	NA	NA	unclear	76	0
Singh et al., 2002	MBP	NA	NA	NA	NA	unclear	56	0
Singh et al., 1993	MBP	NA	NA	NA	NA	unclear	58	14
Singh and Rivas, 2004	ANA	NA	NA	NA	NA	unclear	5−7	6−8
Vojdani et al., 2002	MBP	NA	NA	NA	NA	unclear	67	15: IgG & IgA, 18: IgM

3.1. Folate receptor α autoantibody (FRAA)

Frye's group conducted a series of studies on the association between FRAA and autism. They reported that FRAA was likely to be associated with ASD syndromes through multiple publications (Frye et al., 2012, 2013). They studied the serum of 93 ASD subjects and found that 70 (75.3%) have FRAAs, out of which 56 (60.0%) were positive for blocking FRAAs and 41 (44%) were positive for binding FRAA (Frye et al., 2012). ASD children who are positive for binding FRAA were found to have higher levels of serum B12 than those negative for binding FRAA. On the other hand, children who are positive for blocking FRAA were found to have relatively better redox metabolism and inflammation markers than children who are negative for blocking FRAA. However, although Frye's studies used a linear model to address the association between antibody and autism symptoms, it did not provide information regarding FRAAs' levels in HCs. Ramaekers et al. is the only FRAA study that included HCs, and they found that 19 out of 25 sera of ASD patients had positive FRAAs (76%) while no antibody was detected in control children ($p < 0.0001$) (Ramaekers, Blau, Sequeira, Nassogne, & Quadros, 2007).

3.2. Anti-MAG and anti-MBP antibodies

Higher levels of anti-MAG and anti-MBP antibodies were detected in ASD patients compared to controls, raising the hypothesis of neuroinflammation as these antibodies have been shown to react against neuronal tissue. ASD children were found to have high proportion (57.58%) of anti-MBP antibody comparing to HCs (9.09%) (p value < 0.001) (Singh et al., 1993). The anti-MAG antibody was found positive in 62.5% of ASD children and only 3.25% in HCs (p value < 0.001) (Mostafa, El-Sayed, & El-Aziz, 2008).

3.3. Anti-ribosomal P protein antibody

Anti-ribosomal P protein antibody specifically targets the highly conserved phosphoproteins at the large ribosomal subunit (60 s) (Gerli & Caponi, 2009). Mostafa et al. found that 58.3% ASD children had increased level of serum anti-ribosomal P antibodies, while it was only detected in 5.0% HCs (p-value < 0.001) (Mostafa, Bjorklund, Urbina, & Al-Ayadhi, 2016). The same group has described anti-ribosomal P antibodies in ASD patients in a prior publication, but HC was not reported (Mostafa & AL-Ayadhi, 2011). The antibody is known to react against multiple tissues, including neuronal tissue.

3.4. Anti-endothelial cell antibody

AECAs are commonly detected in autoimmune diseases, such as SLE, rheumatoid arthritis and systemic sclerosis. Vascular endothelial cell antibodies such as AECAs were found to be elevated in ASD children measured by immunoassays including immunohistochemistry ($P = 0.004$) (Connolly et al., 1999) and ELISA ($P < 0.05$) (Bashir & Al-Ayadhi, 2015), as compared to HCs who showed either none-existent or low percentage of autoantibody expression.

3.5. Antinuclear antibody (ANA)

As a well-known systematic antibody associated with many autoimmune diseases, ANA is a good "gateway" antibody candidate to reflect the presence of autoimmunity in ASD patients. According to a study by Connolly et al., (Connolly et al., 1999), increased level of ANA was founded in 3 out of 11 ASD patients, while none of 51 HCs reported abnormal high level of ANA. In another study (Mostafa, El-Sherif, & AL-Ayadhi, 2014), 25 out of 100 (25%) ASD children showed increased level of ANA, while only 4 out of 100 (4%) HCs did ($P < 0.001$). Moreover, authors reported that the prevalence of ANA increased with the severity of ASD.

4. Discussion

Autoantibodies to a variety of known antigens, CNS regions, and neuronal proteins have been detected in ASD patients (Kobeissy, 2015). We performed the first systematic review on significant autoantibodies associated with ASD, assessed by cohort or case-control studies. This systematic review aimed to investigate abnormality of serum or CSF autoantibody(ies) in ASD compared to HCs, and to analyze if ASD patients have a typical autoantibody profile. In contrast to other reviews, we focused on well-studied autoantibodies, excluding maternal antibodies and antibodies without statistical inference. Our systematic review provided some indications that ASD children were more likely to show higher levels of FRAAs, anti-MAG, anti-MBP, anti-ribosome P, AECAs, and ANA, compared to HCs. Of note, the quality and level of our overall evidence is relatively low because most included studies had small sample sizes, many did not include comparison to controls or perform statistical analysis, and many lacked quantitative information regarding the raw autoantibody concentrations in ASD vs control groups, as well as threshold for determining positivity. Unfortunately, we were not able to perform a meta-analysis to quantify the association of any antibody and ASD risk due to significant between-study heterogeneity, very small numbers of studies for the same comparison, and lack of control groups in majority of the included studies.

4.1. Potential pathogenic mechanisms of autoantibodies

Studies found that autoantibodies provoked by CNS infection can cross-react with neuronal antigens, resulting in behavior changes (Kobeissy, 2015). A hypothesis is raised relating neuron-specific antibodies to immune-mediated diseases with cognitive and

behavioral changes, exemplified by ASD, SLE, Sydenham's chorea, and Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS) (Libbey & Fujinami, 2010). Furthermore, a variety of potential pathogenic mechanisms have been proposed regarding the roles of the autoantibodies included in this systematic review, which are briefly summarized below.

4.1.1. FRAA

A "cerebral folate deficiency syndrome" caused by FRAAs on the choroid plexus has been proposed as a potential mechanism. High-affinity blocking FRAAs may impair folate transport across the blood-cerebrospinal fluid barrier by disrupting the binding of folate to the membrane-associated folate receptors.

These autoantibodies might be stimulated by folate-binding proteins in breast milk or formula (Ramaekers et al., 2005). Maternal FRAAs can also prevent the transfer of folate across the blood-placenta barrier and blood-brain barrier in infants, thus disrupt fetal brain development which leads to ASD (Quadros et al., 2018).

4.1.2. Anti-MBP and anti-MAG antibodies

Antibodies detected against proteins in the brain or the spinal cord and their supporting tissues, such as anti MBP and MAG antibodies, are hypothesized to represent autoimmune processes in the CNS (Mostafa et al., 2016). Most of autoantibodies reactive to neuronal tissue have no corresponding antigens or epitopes identified (discussed in section 4.1.5). Various causative factors including bacterial infections and milk allergen may mediate the development of the autoantibodies by disrupting the blood-brain barrier, which exposes immunocompetent cells to the circulating T-cells and antigens from the central nervous system (Vojdani et al., 2002). It is also noted that milk intake can stimulate the production of FRAAs in ASD children (Ramaekers, Sequeira, Blau, & Quadros, 2008).

4.1.3. Anti-ribosomal P protein antibody

Studies have found an association between anti-ribosomal P protein antibodies and neurokinin A, a pro-inflammatory neuropeptide mediating neurogenic inflammation including some autoimmune diseases (Veres, Rochlitzer, & Braun, 2009). This could reflect the impact of inflammation in the course of autoimmune diseases, thus suggesting that interaction between the central nervous system and the immune system may be a pathogenic culprit (Mostafa & AL-Ayadhi, 2011).

4.1.4. Autoantibodies without statistical inference (no statistical tests were performed or reported)

In the following section, we will provide a brief summary regarding some autoantibodies for which no statistical inference could be made due to inferior study designs. These are not included in the systematic review but are worth noting in a broader context. This group includes autoantibodies that were detected in ASD patients without comparison to HC groups, exemplified by anti-neuronal N-methyl-D aspartate (NMDA) antibody and anti-serotonin antibody.

Anti-NMDA antibody: It was initially discovered in 2007 (Dalmau, Lancaster, Martinez-Hernandez, Rosenfeld, & Balice-Gordon, 2011), which is an antibody most frequently identified against surface neuronal proteins (Lee, Choi, & Kim, 2015). In SLE patients, antibodies can cross-react with double-stranded DNA, which is associated with the neurologic symptoms of these patients (DeGiorgio et al., 2001). In a mouse model, these antibodies can react with NMDA receptor (NMDAR) leading to neuronal cell death (Huerta, Kowal, DeGiorgio, Volpe, & Diamond, 2006; Kowal et al., 2006). Studies found that NMDAR plays a critical role in the synaptic activities contributing to learning and memory and the production of NMDAR antibodies generate similar effects (Dalmau et al., 2011). NMDAR antibody is associated with a special type of encephalitis characterized by a mild prodromal symptom followed by significant psychiatric symptoms, typically identified in young female patients with ovarian teratoma (Gable, Sheriff, Dalmau, Tilley, & Glaser, 2012). Early detection of the autoantibody and removal of the tumor can result in rapid improvement in this subtype of encephalitis (Dalmau et al., 2011). The current evidence of NMDAR antibodies in autism is limited to a few case reports (Scott et al., 2013; Cleland, Lieblich, Schalling, & Rahm, 2015). Its rarity may be the reason why neither large-scale case series nor cohort studies have been conducted. Furthermore, a study has found that pharmacological enhancement or suppression of NMDAR function can reduce the autistic behavior in patients. Thus, NMDAR antibody in ASD is an attractive field of research, and regulating NMDAR function may be a potentially therapeutic target for ASD treatment in the subset of patients with autoimmune etiology.

Anti-serotonin antibody: Serotonin (5-HT) is a neurotransmitter that plays an important role in the functioning of central catecholaminergic neurons, which may be disrupted in autism. Hyperserotonemia and autoantibodies reactive against brain serotonin receptors were described in ASD pediatric patients (Todd et al., 1988; Yuwiler et al., 1992). It was suggested that hyperserotonemia might stimulate autoantibody production against brain serotonin receptor, as serotonin is found to have the potential to promote mature B lymphocyte proliferation through 5-HT1A receptors (Iken, Cheng, Fargin, Goulet, & Kouassi, 1995), which lead to immune abnormalities in children with autism (Singh, Singh, & Warren, 1997). Studies have indicated that both hyperserotoninemia and autoimmunity might be modulated by a common factor, but further exploration is required (Burgess, Sweeten, McMahon, & Fujinami, 2006).

4.1.5. Autoantibodies with unknown antigens

This group is represented by antibodies against brain tissue, which are identified autoantibodies against unknown antigens in the basal ganglia, prefrontal cortex, cerebellum, cingulate gyrus (Mora et al., 2009; Singer et al., 2006; Todd et al., 1988) and the Purkinje cells (Wills et al., 2009). Antibodies against 45 and 62 kDa proteins from cerebellum were found in the plasma of ASD children by immunoblotting, with antigens extracted from rhesus macaque cerebellum (Wills et al., 2009). The presence of these autoantibodies is associated with ASD severity (Piras et al., 2014). A 52 kDa protein from the hypothalamus and thalamus of human

adult ASD patients was extracted and revealed that autoantibodies against brain tissues are detected more frequently in patients with ASD than HCs (Cabanlit et al., 2007), further supporting the hypothesis that neuroinflammation and autoimmunity play an important role in a subgroup of ASD patients.

4.2. Antibody production strongly associated with autoimmunity

Although not autoantibodies in strict terms, some antibodies are produced in response to common environmental allergens/antigens and are found more frequently in ASD children than HCs. These observations raised the possibility that the development of the antibodies and ASD are both consequences of immune dysfunction, and some hypothesized a mechanistic role of these autoantibodies in the development of ASD. The most common allergens include viral vaccines (such as MMR) and gluten.

4.2.1. Virus-induced antibody

Singh's group found that the majority of ASD patients (83%) were hyper-reactive to measles virus, as reflected by higher antibody levels to measles virus in comparison to HC ($P = 0.003$) (Singh & Jensen, 2003; Singh, 2009). In the serum of patients with ASD, along with dramatic increase of MMR antibodies, autoantibodies to brain tissue such as MBP and neuron-axon filament protein are also elevated (Singh et al., 1998, Kozlovskaia et al., 2000). This raised the hypothesis that virus-induced autoimmunity may be a cause of ASD (Cohly & Panja, 2005). A potential mechanism is that following virus infection, antigen-presenting cells can be stimulated and trigger Th1 lymphocyte activation, which would produce interferon-γ leading to altered cell permeability in the blood-brain barrier (BBB) (Neumann, Cavalie, Jenne, & Wekerle, 1995). Consequently, Th1 lymphocytes could permeate BBB and recognize anti-brain autoantibodies. The antibodies or antigen-antibody compound could impair oligodendrocyte to result in aberrant myelin sheath production and interrupting neural pathway transduction, thus contributing to neurologic and behavior abnormalities.

4.2.2. Anti-gliadin antibody

Anti-gliadin antibody has been reported in celiac disease (Sanders, 2002), gluten ataxia (Hadjivassiliou et al., 2002) and in ASD children (Vojdani, Pangborn, Vojdani, & Cooper, 2016). Although it remains controversial whether autism is associated with celiac disease (ElsonIII & Smith, 2008), some studies support that in a subset of ASD patients, gluten induces immune activation (de Magistris et al., 2013), and gluten intake could stimulate autoantibody production in the brain (predominantly cerebellum), which cross-react with Purkinje cells resulting in neurological changes (Vojdani et al., 2013). In patients with gluten ataxia, the brain tissue revealed perivascular inflammation in the cerebellum, potentially leading to the degeneration of Purkinje cells. It may be caused by molecular mimicry between anti-gliadin antibodies and epitopes expressed on Purkinje cells (Hadjivassiliou et al., 2002), suggesting that immune aberrations may effectuate neurological disruption (Bushara, Goebel, Shill, Goldfarb, & Hallett, 2001).

4.3. Maternal antibodies

Our study excluded maternal antibodies so that we can focus on autoantibodies produced in ASD children, and to minimize the complexity of maternal antibody subtypes that interfere with data collection. We briefly discuss the maternal antibodies due to their significant association with ASD. Mothers with maternal autoimmune diseases are at higher risk of having ASD offspring (pooled OR 1.30) (Chen et al., 2016), and 12% of these mothers have serum autoantibodies reactive with the fetal brains (Lu & Kong, 2016). Pilot study demonstrated that injection of the serum from patients' mother into a pregnant mouse could lead to developmental deficits in its decedents (Dalton et al., 2003; Warren et al., 1990). Other studies found maternal antibodies reacted with fetal brain proteins located within 37, 39, and 73 kDa bands in ASD individuals (Braunschweig et al., 2008; Singer et al., 2008; Zimmerman et al., 2007).

4.4. Consideration of antibody detection techniques

Many techniques have been developed to assess autoantibodies. Commonly used ones include indirect immunofluorescence, ELISA and immunoblotting (and its variations such as line immunoassays). In recent years, other techniques have been developed, such as laser bead-based assays, planar arrays, and point-of-care immunoassays (Olsen, Choi, & Fritzler, 2017). In general, indirect immunofluorescence has been considered the "golden standard" for antibody detection and validation, but all techniques have their respective advantages and limitations. Some of the newer techniques, such as certain point-of-care assays, have sensitivities and specificities that surpassed the traditional methods (Olsen et al., 2017). The present systematic review selected studies that included indirect immunofluorescence, ELISA, and immunoblotting for antibody detection because these methods have been the most well-established and well-validated. Despite our adoption of a relatively narrow range of detection methodologies, we still observed high inter-study variabilities. We believe that this is partially due to the high heterogeneity that is inherent in the ASD population, and partially because of the large variation in detection protocols related to less well characterized autoantibodies such as AECA.

5. Future perspectives

More research is needed to characterize the relationships between autoantibodies and specific brain region or neuronal circuits, in order to correlate with phenotypic presentations of ASD. Future studies should also explore the basic mechanisms of autoantibodies in autoimmune encephalitis and ASD, such as molecular mimicry and regulation of immune activation versus tolerance. Furthermore, in order to establish a link between ASD and autoimmunity, future research should explore the emerging connection between HLA

haplotypes and ASD, as demonstrated by a recent study (Bennabi et al., 2018). A majority of autoimmune diseases have been associated with specific HLA class II genes. A demonstration of the association between ASD and HLA haplotypes would yield stronger support for the hypothesis that ASD, or at least subtypes of the disease, can be autoimmune in nature.

6. Conclusions

Autoimmunity has been proposed as one potential mechanism through which environmental and genetic factors can interact and contribute to the pathogenesis of ASD. Based on our systematic review, studies provided varying levels of evidence that ASD children displayed higher levels of antibodies reactive to folate receptor α, MAG, MBP, ribosome P, endothelial cell, and ANA, as compared to HCs. Given the significant between-study heterogeneity, small sample sizes in individual included studies, and lack of relevant information, the quality and level of our overall evidence is limited and future large-scale and well-designed studies are warranted to either confirm or refute the findings in previous studies.

From the clinical perspective, the key question is, what autoantibodies to test in patients with ASD, and when to test. Our systematic review suggests that there is currently insufficient evidence to develop a guideline for routine autoantibody testing and screening. In general, if there is high clinical suspicion of autoimmune-induced or related ASD (e.g. if patients have considerable disease burden of autoimmune comorbidities), patients may be tested for ANA and autoantibodies against neuronal tissues. Although there is currently no evidence between vaccine administration and ASD in general, if the ASD disease onset or progression is temporally correlated with episodes of viral infection or MMR vaccine administration, one may opt to assay for measles antibodies and anti-MMR antibodies. If an ASD patient presents with significant food allergies, he/she may warrant testing for anti-gliadin antibody. Our review calls for more high-quality clinical research on the characterization of autoantibodies in ASD patients, in order to generate high-quality evidence for guiding the development of a clinical guideline for antibody screening and testing in ASD patients.

With increasing basic and clinical research in this field and better understanding of the genetic, epigenetic and protein-level mechanisms of molecular mimicry, it is possible that in the near future, we can develop specific diagnostic panels for autoimmune encephalitis associated with ASD, identify immunomodulatory targeted therapies and establish management guidelines for autoimmune-mediated ASD subtypes, in order to optimally modify or reverse the core symptoms of the ASD patients.

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