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Mitochondrial Aspartate/Glutamate Carrier SLC25A12 and Autism Spectrum Disorder: a Meta-Analysis

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Abstract Mitochondrial dysfunction has been reported to be involved in the pathophysiology of autism spectrum disorder (ASD). Studies investigating the possible association between ASD and polymorphism in SLC25A12, which encodes the mitochondrial aspartate/glutamate carrier, have yielded inconsistent results. We conducted a systematic review and meta-analysis of such studies to elucidate if and which SLC25A12 single nucleotide polymorphisms (SNPs) are associated with ASD. We searched PubMed, Ovid, Web of Science, and ERIC databases through September 20th, 2014. Odds ratios (ORs) were aggregated using random effect models. Sensitivity analyses were conducted based on study design (family-based or case-control). Fifteen out of 79 non-duplicate records were retained for qualitative synthesis. We pooled 10 datasets from 9 studies with 2001 families, 735 individuals with ASD and 632 typically developing (TD) individuals for the meta-analysis of rs2292813, as well as 11 datasets from 10 studies with 2016 families, 852 individuals with ASD and 1058 TD individuals for the meta-analysis of rs2056202. We found a statistically significant association between ASD and variant in rs2292813 (OR=1.190, 95 % CI 1.052–1.346, P=0.006) as well as in rs2056202 (OR=1.206, 95 % CI 1.035–1.405, P=0.016). Sensitivity analyses including only studies with family-based design demonstrated significant association between ASD and polymorphism in rs2292813 (OR=1.216, 95 % CI 1.075–1.376, P=0.002) and rs2056202 (OR=1.267, 95 % CI 1.041–1.542, P=0.018). In contrast, sensitivity analyses including case-control design studies only failed to find a significant association. Further research on the role of SLC25A12 and ASD may pave the way for potential innovative therapeutic interventions.

Keywords Asperger · FBAT · Genetics · NAA · Pervasive developmental disorder · TDT

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by impairments in social communication and interaction, as well as repetitive and restricted behavior [1, 2]. Currently, the disorder is estimated to affect about 1 % of the general population [3]. ASD is recognized as a heritable condition [4–6], and genetics is reported to play an important role in its etiology [7].

Research on the genetics of ASD has taken several approaches, including genome-wide association studies (GWAS). Although the GWAS approach is suited to comprehensively investigate the genetic background of human diseases, it faces the problem that common disease-common variant model does not yield large effect size to reach statistical

threshold in GWAS study [8]. In this context, an alternative approach that investigates *a priori* a specific gene, based on its biological function, may also be fruitful. To date, a number of genes have been investigated for their possible role in the etiology of ASD in the light of pathophysiological hypotheses on this disorder [9].

Mitochondrial dysfunction has been demonstrated to be associated with neuropsychiatric conditions [10, 11]. In the case of ASD, the hypothesis of an involvement of mitochondrial dysfunction has been supported by findings of significantly higher prevalence of mitochondrial diseases in individuals with ASD compared to typically developing (TD) individuals [12]. In addition, it has been reported that individuals with ASD showed atypical serum metabolites associated with mitochondrial function, such as lactate and pyruvate [12–15]. Furthermore, neuroimaging studies have consistently shown atypical levels of metabolites associated with mitochondrial function, including N-actylaspartate (NAA) and lactate [16–19]. Finally, an increasing number of genetic studies in ASD have focused on mitochondrial DNA [14, 20] and on genes that are associated with mitochondrial function [21], such as SLC25A12, which encodes the brain mitochondrial aspartate/glutamate carrier (AGC) [22–24]. However, so far, studies investigating a possible association between single nucleotide polymorphisms (SNPs) in SLC25A12 and ASD have yielded inconsistent results (e.g., [25–27]).

To clarify a possible role of SLC25A12 in ASD, we conducted a systematic review and meta-analysis of studies that investigated the association between SNPs in SLC25A12 and ASD.

Methods and Materials

Methods for this meta-analysis have been developed according to recommendations from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement [28].

Selection Criteria

Study Type

Studies were included if they:

1) were peer-reviewed in order to ensure high levels of methodological adequacy and to avoid the inevitable bias caused by dependence on investigators agreeing to provide data from unpublished studies, as suggested by the Cochrane Group [29].

2) adopted either a "family-based association" or "case-control" design.

Population

Individuals, of any age and with no restriction of gender, diagnosed with ASD, including Asperger's syndrome, or pervasive developmental disorders according to standardized tools.

Outcome

Association between SNPs in SLC25A12 and ASD. We did not select *a priori* any specific SNPs. However, in order to provide a robust estimate of effects, consistently with other recent meta-analyses (e.g. [9]), we performed meta-analyses only if four or more datasets were available for each single SNP.

Search Methods for Identification of Studies

Electronic searches were conducted by the two authors independently in the following databases, available via the University of Tokyo Medical Library and the New York University (NYU) Medical Library: PubMed, Ovid databases (Ovid MEDLINE®, EMBASE Classic + EMBASE, PsycINFO), Web of knowledge databases (including Web of Science, Biological Abstracts, BIOSIS, Current Contents Connect, Data Citation Index, Derwet Innovations Index, FSTA, INspec, MEDLINE, and SciELO), and ERIC. The last search was performed on September 20th, 2014. Supplement 1 reports the search terms and syntax for each database.

Identification and Selection of Studies

In stage 1, the two authors screened title and abstracts of all non-duplicated papers and agreed on a final list of references to assess. The full-text version of the articles passing stage 1 was assessed for eligibility by the two authors, independently. Discrepancies were resolved with consensus. Reference lists of the retained papers were also scanned to determine if any relevant studies had been missed during the database searches. Data from multiple reports of the same study were linked together. Where required, the corresponding author was contacted to inquire on study eligibility.

Data Extraction

The two authors independently extracted the following data: 1) names of the first authors, 2) year of publication, 3) study design (i.e., family-based association or case-control), 4) number of participants, 5) ethnicity of participants, 6) diagnostic criteria, and 7) transmitted or non-transmitted risk allele counts from heterozygous parents to individuals with ASD for family-based studies and risk and non-risk allele count or risk allele frequency in case-control studies. When the study did not report sufficient data to calculate effect size, we

contacted corresponding authors via e-mail to request additional information. If the contact with the author was not successful, we could not include the study in the meta-analysis.

Statistical Analysis

We adopted a previously reported method to integrate results from family-based design and case-control design [30]. Odds ratio (OR) was used as an effect size [9, 31]. Specifically, we calculated logarithm of ORs and standard error from each retained study using number of transmitted and non-transmitted risk alleles in family-based studies and count or frequency of risk allele in case-control studies. For one family-based study, where number of transmitted and non-transmitted risk alleles were not available, we calculated OR using chi-square and number of families [32]. In one study reporting result of transmission disequilibrium test (TDT) and family-based association tests (FBAT) [33], we chose TDT with the aim to decrease between-study heterogeneity of included studies, because the large majority of included studies reported results based on TDT design [25, 26, 34]. We calculated standardized error of logarithm of OR using p value for the study by Carayol et al. [35] in order to include it into the meta-analysis.

We aggregated effect sizes using Comprehensive Meta-Analysis software (Biostat, Inc., Englewood, New Jersey, USA), following the procedure described in a recent meta-analysis of SNPs of oxytocin receptor gene in individuals with ASD [9].

We used a random effect model to account for heterogeneity in results of included studies. Statistical threshold for significance was set at $P<0.025$ (=0.05/2, corrected for multiple comparisons).

Publication Bias Assessment

We examined publication bias quantitatively according to the Egger's liner regression method [36]. Significance for publication bias was set at $P<0.10$ [36].

Between-Study Heterogeneity Assessment

Given the considerable between-study heterogeneity in participants, such as ethnicity, and study design (i.e., family-based design or case-control design), we employed I^2 score to evaluate between-study heterogeneity [37, 38]. Although there is no definite score for cutoff, the following categorization has been suggested for interpretation: "0 to 40 %: might not be important", "30 to 60 %: may represent moderate heterogeneity", "50 to 90 %: may represent substantial heterogeneity", and "75 to 100 %: considerable heterogeneity" [29].

Sensitivity Analysis

In order to test if findings were influenced by study design, we conducted a sensitivity analysis including only family-based design and another one focusing only on case-control design. Statistical threshold for significance was set for $P=0.0125$ (=0.05/4, case-control and family-based designs for two SNPs).

One-Study Removed Sensitivity Analysis

To challenge the robustness of the results, we conducted "one-study removed" sensitivity analysis. Using this method, we evaluate whether results were influenced by only one single study [39, 40]. According to this approach, the more the sensitivity analysis preserves significance, the more the result is replicable. We applied the "one-study removed" procedure to the sensitivity analysis including only studies with family-based design. We did not apply this procedure to the sensitivity analysis including only case-control studies, since this did not yield significant results (see "Results" section). We set a statistical threshold at $P<0.025$.

Results

Figure 1 shows the PRISMA studies selection flowchart. Supplemental Table 1 reports studies excluded from the qualitative synthesis, with reasons.

Our search identified a total of 15 studies for qualitative synthesis and potentially relevant for the meta-analysis (Table 1) [25–27, 32–35, 41–48]. Twenty-three types of SNP (rs11757, rs12692976, rs17499593, rs17581284, rs1878583, rs2056202, rs2271758, rs2292813, rs35678, rs3749004, rs3765166, rs3770445, rs3821095, rs4307059, rs6433317, rs6716901, rs6724337, rs6758704, rs7573003, rs7586207, rs925881, rs908670, and rs970948) were investigated once or more in the identified 15 studies. Among these 23 SNPs, only rs2292813 and rs2056202 were investigated in four or more datasets. Eleven studies, with a total of 12 datasets (either family-based or case-control datasets; the study by Blasi et al. [25] provided both types of datasets) focused on rs2292813 [25–27, 32–35, 41, 44, 46, 47]. Two datasets from two studies [34, 46] were excluded given lack of complete data necessary to carry out the analysis (We sent an e-mail to corresponding authors to gather such data on 22nd Sep 2014 and waited for their response for one month. If the authors did not respond to our e-mail, we did not include the study). Therefore, 9 studies were used for the meta-analysis of rs2292813.

Twelve studies with a total of 14 datasets investigated rs2056202 [25–27, 32–34, 41–44, 46, 47] (the studies by Blasi et al. [25] and Correia et al. [42] both provided family-based as well as case-control datasets). Two datasets from two studies

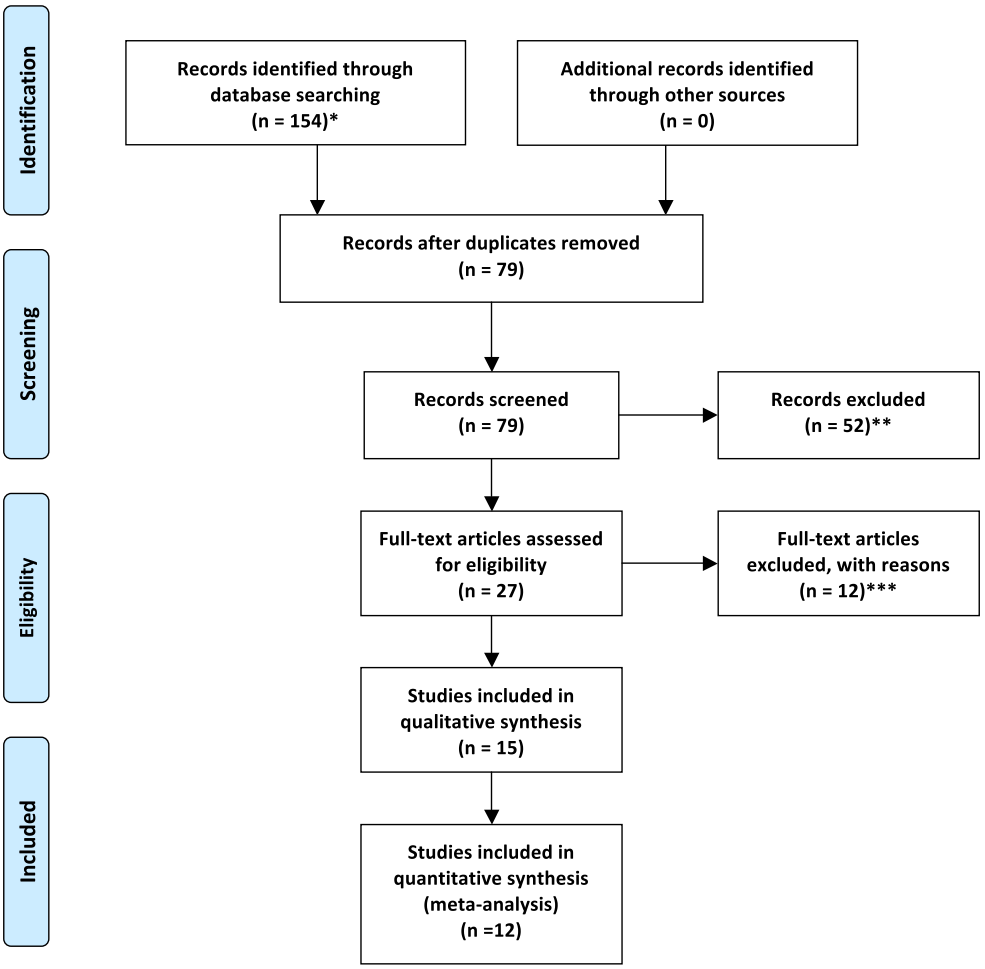

Fig. 1 The process of systematic screening of studies. A comprehensive literature search firstly identified 154 studies from databases. After removing duplicates, there were 79 independent studies for further screening. Among them, 52 studies were excluded after reading abstract, which left 27 studies for full-text screening. Twelve studies were excluded from the qualitative synthesis as a result of full-text screening. Among the remaining 15 studies, three studies were not included in the meta-analysis because they did not provide sufficient data to calculate effect sizes. Therefore, 12 studies were identified to be eligible for meta-analysis

* Results for each database are reported in Supplement1

** n=22: reviews, n=16: clearly not pertinent, n=10: meeting abstracts, n=4; patent registration

*** References of excluded studies are reported, with reasons for exclusion, in Supplementary Table 1

[46, 47] could not be retained for the meta-analysis since it was not possible to obtain additional necessary information from the authors. As for the study by Correia et al. that reported results of both family-based design and case-control design, we extracted the results from case-control dataset only, given lack of necessary data from the family-based design dataset [42]. Therefore, ten studies were used for the meta-analysis of rs2056202.

In total, 12 studies were used for the meta-analyses.

Meta-Analysis of Datasets on rs2292813

Nine studies, including seven family-based design datasets with a total of 2001 families [25–27, 32, 33, 35, 47] and three case-control design datasets recruiting 735 individuals with ASD and 632 individuals with TD [25, 41, 44], were eligible to the meta-analysis (Table 2). A random effect model meta-analysis demonstrated a significant association between rs2292813 and ASD ("G" allele increasing the risk for ASD, OR=1.190, 95 % CI 1.052–1.346, P=0.006). Between-study heterogeneity was small (I^2=13.9), and there was no significant publication bias (P=0.427) (Fig. 2a).

A sensitivity analysis focusing only on datasets with family-based design confirmed the significant association between rs2292813 and ASD ("G" allele increasing the risk for ASD, OR=1.216, 95 % CI 1.075–1.376, P=0.002), with negligible between-study heterogeneity (I^2=12.6) and no significant publication bias (P=0.178) (Table 2 and Fig. 2b). On the other hand, a sensitivity analysis focusing only on case-control design datasets showed no significant association between "G" allele in rs2292813 and ASD (OR=0.899, 95 % CI 0.594–1.360, P=0.615) (Table 2 and Fig. 2c).

Meta-Analysis of Datasets on rs2056202

Ten studies, including six family-based design datasets with 2016 families [25–27, 32–34] and five case-control design datasets recruiting 852 individuals with ASD and 1058 individuals with TD [25, 41–44], were amenable to meta-analysis

Table 1 Characteristics of studies identified for the qualitative synthesis

Study	Design	Number of families	Number of cases	Number of controls	Ethnicity	Diagnostic criteria	SNPs	Significant	Minor allele	Risk allele
Blasi et al., 2006	Family-based	261	NA	NA	Caucasian	NA	rs3749004	No	NA	A
							rs2292813	No	NA	G
							rs2056202	No	NA	G
	Case-control	NA	261	174	Caucasian	NA	rs3749004	No	G	NA
							rs2292813	No	A	NA
							rs2056202	No	A	NA
Carayol et al., 2010	Family-based	222	NA	NA	Various	ADI-R, ADOS-G	rs2292813	No	NA	G
Chien et al., 2010	Case-control	NA	465	450	Asian	DSM-IV, ADI-R	rs2292813	No	A	NA
							rs2056202	No	A	NA
Correia et al., 2006	Family-based	241	NA	NA	NA	DSM-IV, ADI-R, CARS	rs2056202	No	NA	NA
							rs11757	No	NA	NA
	Case-control	NA	NA	NA	NA	DSM-IV, ADI-R, CARS	rs2056202	No	G	NA
							rs11757	No	G	NA
Durdiaková et al., 2014	Case-control	NA	117	426	Caucasian	DSM-IV, ICD-10	rs2056202	No	A	NA
							rs3765166	No	A	NA
							rs6716901	Yes	A	NA
Kim et al., 2011	Family-based	720	NA	NA	White	ADI-R, ADOS	rs908670	No	G	NA
							rs2292813	No	A	NA
							rs2056202	No	A	NA
Lepagnol-Bestel et al., 2008	Case-control	NA	9	8	NA	NA	rs2292813	No	A	NA
							rs2056202	Yes	A	NA
Palmieri et al., 2010	Family-based	346	NA	NA	NA	DSM-IV	rs2292813	No	T	NA
							rs17499593	No	G	NA
							rs17581284	No	T	NA
							rs7586207	No	G	NA
							rs12692976	No	C	NA
							rs2271758	No	T	NA
							rs3770445	No	G	NA
							rs2056202	No	T	NA
							rs1878583	No	G	NA
							rs6724337	No	T	NA
							rs7573003	No	T	NA
Prandini et al., 2012	Family-based	227	NA	NA	Italian	DSM-IV, ADI-R, ADOS	rs4307059	Yes	NA	C
							rs35678	Yes	NA	T
Rabionet et al., 2006	Family-based	327	NA	NA	Caucasian	NA	rs6433317	No	NA	NA
							rs1878583	No	NA	NA
							rs2056202	No	NA	NA
							rs925881	No	NA	NA
							rs3821095	No	NA	NA
							rs970948	No	NA	NA
							rs6758704	No	NA	NA
							rs2292813	No	NA	NA
							rs11757	No	NA	NA
Ramoz et al., 2004	Family-based	197	NA	NA	NA	ADI-R	rs2292813	Yes	NA	G
							rs2056202	Yes	NA	G
Ramoz et al., 2008	Family-based	334	NA	NA	NA	ADI-R	rs2292813	No	NA	NA
							rs2056202	Yes	NA	G
Segurado et al., 2004	Family-based	158	NA	NA	NA	ADI-R, ADOS	rs2292813	Yes	NA	G

Table 1 (continued)

Study	Design	Number of families	Number of cases	Number of controls	Ethnicity	Diagnostic criteria	SNPs	Significant	Minor allele	Risk allele
							rs2056202	Yes	NA	G
Turunen et al., 2008	Family-based	97	NA	NA	Finnish	DSM-IV, ICD-10	rs2292813	Yes	NA	G
							rs2056202	No	NA	NA
Yan et al., 2006	Family-based	105	NA	NA	Japanese	NA	rs3769955	No	NA	NA
							rs3770448	No	NA	NA

N number, *NA* not applicable, *DSM* Diagnostic and Statistical Manual of Mental Disorders, *ADI-R* Autism Diagnostic Interview-Revised, *ADOS* Autism Diagnostic Observation Schedule, *CARS* childhood autism rating scale, *ICD* International Statistical Classification of Diseases and Related Health Problems

(Table 2). A random effect model meta-analysis showed a significant association between rs2056202 and ASD ("G" allele increasing the risk for ASD, OR=1.206, 95 % CI 1.035–1.405, P=0.016). There was moderate between-study heterogeneity (I^2=44.6), and no publication bias was observed (P=0.373) (Fig. 2d).

A sensitivity analysis focusing only on datasets with family-based design confirmed a significant association between polymorphism in rs2056202 and ASD ("G" allele increasing the risk for ASD, OR=1.267, 95 % CI 1.041–1.542, P=0.018), with substantial between-study heterogeneity (I^2=61.7) but no significant publication bias (P=0.269) (Table 2 and Fig. 2e). On the other hand, a sensitivity analysis including only case-control design datasets revealed no significant association between polymorphism in rs2056202 and ASD (OR=1.071, 95 % CI 0.854–1.343, P=0.552) (Table 2 and Fig. 2f).

One-Study Removed Sensitivity Analysis

rs2292813

Using, as mentioned, a strict threshold at P<0.025, all "one-study removed" sensitivity analyses, except one, preserved the significant association between "G" allele in rs2292813 and development of ASD. In addition, all the "one-study removed" analyses applied to the sensitivity analysis of rs2292813 with family-based design preserved the significance of the association between polymorphism in rs2292813 and ASD (Fig. 3b).

rs2056202

Six out of the 11 "one-study removed" sensitivity analyses showed a statistically significant association (P<0.025) between polymorphism in rs2056202 and ASD (Fig. 3c). In contrast, only two out of the six "one-study removed" analyses preserved the significant association between "G" allele in rs2056202 and ASD (Fig. 3d).

Discussion

The present meta-analysis showed a significant association between polymorphism of both rs2292813 and rs2056202 in SLC25A12 and ASD, with small between-study heterogeneity and without significant publication bias. Sensitivity analyses

Table 2 Results of meta-analysis and sensitivity analysis of rs2292813 and rs2056202

SNP	Number of datasets	Number of families	Number of cases/controls	Pooled OR	95 % CI	P value	I^2	Publication bias
rs2292813-G								
All datasets	10	2001	735/632	1.190	1.052–1.346	0.006	13.9	0.427
Family-based design	7	2001	NA	1.216	1.075–1.376	0.002	12.6	0.178
Case-control design	3	NA	735/632	0.899	0.594–1.360	0.615	0.0	0.196
rs2056202-G								
All datasets	11	2016	852/1058	1.206	1.035–1.405	0.016	44.6	0.373
Family-based design	6	2016	NA	1.267	1.041–1.542	0.018	61.7	0.269
Case-control design	5	NA	852/1058	1.071	0.854–1.343	0.552	1.0	0.404

N number, *NA* not applicable, *CI* confidence interval

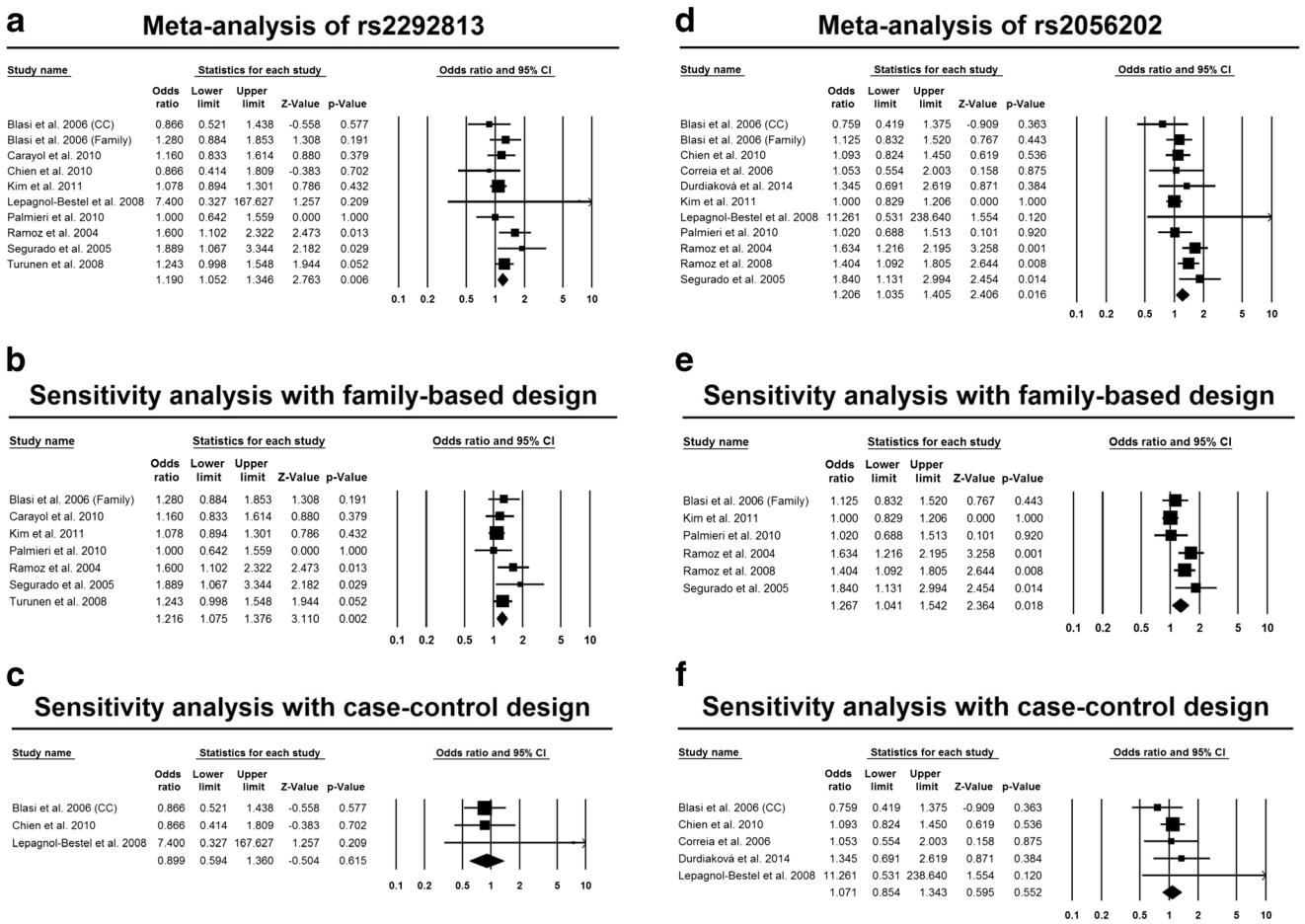

Fig. 2 Results of meta-analyses. **a** A random effect model demonstrated a significant association between polymorphism of rs2292813 and autism spectrum disorder. **b** A sensitivity analysis with studies with family-based design also demonstrated a significant association between polymorphism of rs2292813 and autism spectrum disorder. In contrast, **c** a sensitivity analysis with studies with case-control design revealed no significant association between polymorphism of rs2292813 and autism spectrum disorder. **d** A random effect model showed a statistically significant association between polymorphism in rs2056202 and autism spectrum disorder. **e** A sensitivity analysis with studies with family-based design preserved a statistically significant association between polymorphism in rs2056202 and autism spectrum disorder. On the other hand, no significant association between polymorphism in rs2056202 and autism spectrum disorder was detected in a sensitivity analysis with studies with case-control design (**f**)

focusing only on family-based design showed that both polymorphism of rs2292813 and rs2056202 in SLC25A12 were associated with ASD, with low between-study heterogeneity and without significant publication bias.

As we expected, the analyses demonstrated significance albeit small pooled OR for the association between SNPs variants in SLC25A12 and ASD. The small effect size is a possible reason why available studies have yielded inconsistent results. In addition, the small effect size might contribute to explain why SLC25A12 did not reach the threshold in existing GWAS studies of ASD [49–51]. Although effect size was small, the present result supports the possibility that SLC25A12 is involved in the etiology of ASD.

Our results are consistent with the findings from several lines of research. Postmortem studies demonstrated atypical brain expression of AGC1 in individuals with ASD compared with TD individuals [22, 33, 44]. Although it is not possible to explore activation of AGC1 *in vivo*, results from several neuroimaging studies are in line with these postmortem studies. For instance, NAA is a metabolite that contributes to regulate osmotic pressure of neurons and becomes a precursor of N-acetylaspartylglutamate (NAAG) [52]. Magnetic resonance spectroscopy studies have recognized these as reflection of neuronal density and function [52–54]. As NAA is synthesized in neuronal mitochondria from aspartate and acetyl-coenzyme A and is coupled with glutamate to become NAAG [52, 54], NAA is reported to have a link with mitochondrial function [55]. Basic animal experiments suggested that AGC1 is associated with NAA synthesis in the brain; furthermore, evidence in human showed that individuals with AGC deficiency had significantly lower NAA levels than those with normal levels of AGC [56–59]. In line with the notion that AGC1 is atypically expressed or activated in individuals with ASD, a number of studies have reported atypical NAA and

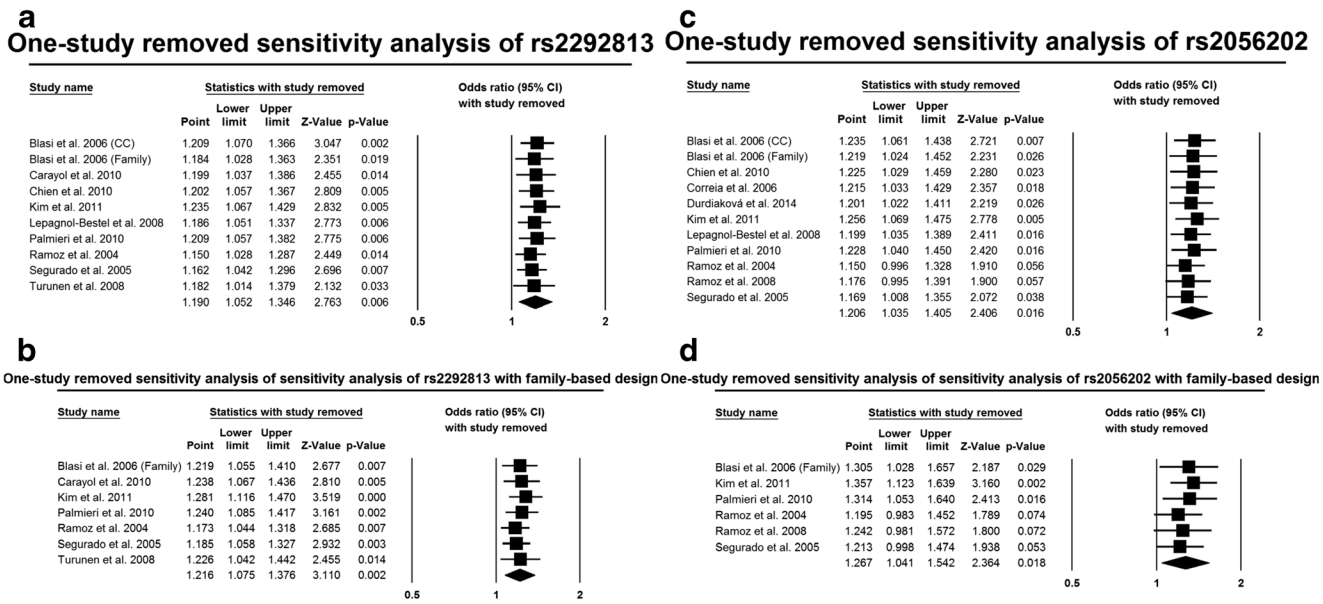

Fig. 3 Results of "one-study removed" sensitivity analysis. **a** "One-study removed" sensitivity analysis of rs2292813 showed that all the sensitivity analyses, except one, preserved statistically significant association between polymorphism in rs2292813 and autism spectrum disorder. **b** "One-study removed" procedure applied to sensitivity analysis of family-based design studies of rs229813 has demonstrated that all the sensitivity analyses preserved the significance of the association. **c** "One-study removed" sensitivity analysis of rs2056202 demonstrated that six out of 11 preserved the significant association between polymorphism in rs2056202 and autism spectrum disorder. **d** "One-study removed" procedure applied to sensitivity analysis of family-based design studies of rs2056202 showed that two out of the six sensitivity analyses have replicated the significant association

NAAG levels in individuals with ASD [16–18, 60, 61]. It is assumed that abnormality in AGC1 activation may result in abnormal aspartate/glutamate exchange rate, which induces abnormal respiratory chain activity and enhances oxidative stress, which eventually results in neuronal neurofilamentous accumulations and myelination deficits (reviewed in [12, 33]).

Although the general meta-analysis and sensitivity analysis focusing only on family-based design studies demonstrated significant association between polymorphism in SLC25A12 and ASD, sensitivity analysis focusing only on case-control design studies did not show any significant association. This may have been accounted for by the fact that family-based design studies compare individuals with ASD to their relatives (mainly their parents), who may share common genetic characteristics. In contrast, in case-control design studies, it is required to conduct population stratification analysis to ensure that cases and controls share common genetic characteristics other than the SNPs of interest [62]. Not all case-control design studies included in the current meta-analysis have implemented population stratification analysis. Thus, in comparison to case-control design studies, it might be possible that family-based design studies had more statistical power to detect the potential effect of SLC25A12.

Our results should be considered in the light of some limitations. First, although I^2 scores of the two general meta-analyses (the one on rs2292813 and the other on rs2056202, including all available studies) were small, the meta-analyses might have been hampered by considerable inherent between-study heterogeneity, since they integrated studies with two different study designs, namely family-based design and case-control design. Second, in addition to differences in design, included studies were also heterogeneous in terms of type of tissue collecting. Particularly, one study with case-control design by Lepagnol-Bestel et al. collected brain tissue, rather than blood or buccal swabs, to investigate variants in SNPs [44]. In addition, from the forest plot, this study may be considered as an outlier. However, it is worth noting that in the "one-study removed" sensitivity analysis that excluded the study by Lepagnol-Bestel et al. [44], the association between ASD and polymorphisms of rs2292813 (OR=1.186, 95 % CI 1.051–1.337, P=0.006, Fig. 3a) or rs2056202 (OR=1.199, 95 % CI 1.035–1.389, P=0.016, Fig. 3b) remained significant. Furthermore, we note that our approach of pooling studies based on different tissues is consistent with a recent meta-analysis of SNPs of oxytocin receptor in ASD [9]. Third, with regard to one study by Blasi et al. [25], we used datasets of both family-based design and case-control design. However, as shown in one-study removed sensitivity analysis, it should be emphasized that both the sensitivity analysis discarding family-based design dataset from Blasi et al. [25] and the sensitivity analysis excluding case-control design dataset from the study preserved the statistical conclusion that polymorphism in rs2292813 (OR=1.184, 95 % CI 1.028–1.363, P=0.019 and OR=1.209, 95 % CI 1.070–1.366, P=0.002, respectively, Fig. 3a) and rs2056202 (OR=1.219, 95 % CI 1.024–1.452, P=0.026 and OR=1.235, 95 % CI 1.061–

1.438, P=0.007, Fig. 3b) in SLC25A12 is associated with ASD. Fourth, although we have demonstrated statistically robust association between variants in SNPs in SLC25A12 and ASD, it was not possible to conduct sensitivity analysis based on ethnicity due to insufficient information from the retained studies.

Conclusion

The present meta-analysis suggests that polymorphism in SLC25A12 deserves further attention as possible mechanism involved in the etiopathophysiology of ASD.

Author contributions YA conceived the study design. YA and SC have independently screened and extracted the data. YA and SC wrote the paper.

Conflict of Interest Dr. Samuele Cortese has received royalties from Aargon Healthcare Italy.

Dr. Yuta Aoki declares no conflict of interest.

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