Copy number variations in autistic children

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Abstract. Autism spectrum disorder (ASD) manifests as a neurodevelopmental condition marked by challenges in social communication, interaction and the performing of repetitive behaviors. The prevalence of autism increases markedly on an annual basis; however, the etiology remains incompletely understood. Cytogenetically visible chromosomal abnormalities, including copy number variations (CNVs), have been shown to contribute to the pathogenesis of ASD. More than 1% of ASD conditions can be explained based on a known genetic locus, whereas CNVs account for 5-10% of cases. However, there are no studies on the Saudi Arabian population for the detection of CNVs linked to ASD, to the best of our knowledge. Therefore, the aim of the present study was to explore the prevalence of CNVs in autistic Saudi Arabian children. Genomic DNA was extracted from the peripheral blood of 14 autistic children along with four healthy control children and then array-based comparative genomic hybridization (aCGH) was used to detect CNVs. Bioinformatics analysis of the aCGH results showed the presence of recurrent and non-recurrent deletion/duplication CNVs in several regions of the genome of autistic children. The most frequent CNVs were 1q21.2, 3p26.3, 4q13.2, 6p25.3, 6q24.2, 7p21.1, 7q34, 7q11.1, 8p23.2, 13q32.3, 14q11.1-q11.2 and 15q11.1-q11.2. In the present study, CNVs in autistic Saudi Arabian children were identified to improve the understanding of the etiology of autism and facilitate its diagnosis. Additionally, the present study identified certain possible pathogenic genes in the CNV region associated with several developmental and neurogenetic diseases.

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Abbreviations: ASD, autism spectrum disorder; CNV, copy number variation; aCGH, array-comparative genomic hybridization; DSM-5, Diagnostic and Statistical Manual of Mental Disorders version 5; NBPF, Neuroblastoma breakpoint family; NBPF25P, NBPF member 25 pseudogenes; CHL1, cell adhesion molecule L1 like; UGT2B17, uridine diphosphoglucuronosyl transferase protein family; CSMD1, CUB and sushi multiple domains 1; ZIC2, Zic family member 2; OR11H12, olfactory receptor family 11 subfamily H member 12; OR4N3P, olfactory receptor family 4 subfamily N member 3 pseudogene

Key words: copy number variations, autism; array-comparative genomic hybridization, genetic variants, chromosomal abnormalities

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that manifests during early childhood usually in the first 3 years of life (1). ASD is characterized by difficulties in social communication and interactions and the performing of repetitive behaviors. For ~80 years researchers have sought to detect the causes and develop treatments for ASD (2). Genetics, environmental factors and the interactions between them are hypothesized to be involved in the incidence of autism. ASD varies in its effect and severity from individual to individual depending on symptoms, cognitive skills and adaptation. Therefore, the severity can be categorized into mild, moderate and severe (3). This is evidenced by the use of the term 'spectrum' which describes different symptoms and severity levels.

Researchers are interested in ASD due to its increasing prevalence; however, the etiology of autism remains incompletely understood. In the first epidemiological study, four cases of autism were detected for every 10,000 cases (4). Since then, estimates have increased considerably. In 2023, according to the World Health Organization, the prevalence of autism globally was 1 in 100 children (5). The statistics in Saudi Arabia conducted by the General Authority for Statistics in 2017, intended for scanning individuals with different disabilities

such as attention deficit hyperactivity disorder, ASD and visual disability in all regions of Saudi Arabia, reported the number of individuals who were diagnosed with autism was 53,282 (6). A study conducted in Riyadh, the capital of Saudi Arabia, estimated the prevalence of ASD among children between 2 and 4 years old was 25 cases per 1,000 children (7). In addition, a previous study reported that the prevalence of ASD in Arabian countries was higher compared with that of other developing countries (8). Several studies have reported that the incidence of ASD is increasing in the Middle East, with rates ranging from 1 in 300 to 1 in 150 children (9). A regional overview of autism in the Gulf countries reported that Saudi Arabia has a higher prevalence of ASD than most countries, with 59 cases per 10,000 children (10). However, the prevalence rate of ASD in Saudi Arabia has not yet been accurately determined due to the limited availability of research on this topic and the fact that several undiagnosed cases have not been identified yet due to a lower level of awareness among parents regarding ASD symptoms, thus reducing the opportunity to access appropriate care and a proper diagnosis.

ASD is typically diagnosed in the first 3 years of life, although certain symptoms may appear more prominently during school age (11). No efficient screening process or biomarker-based diagnostic examinations are available for the detection of autism. The current diagnostic approach for autism depends on cognitive, social and behavioral observations using the Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-5) (12). According to the DSM-5 criteria, two primary features are used to diagnose ASD. First, deficits in social communication and interaction and second, restricted, repetitive behavior (13).

Epidemiologic investigations demonstrate that parental age, preterm birth, prenatal exposure to air pollution and genetic abnormalities are factors that may play a role in ASD (14-16).

Copy number variations (CNVs) are a significant genetic structural variation; they are chromosomal rearrangements that are present as duplications, deletions, inversions, or translocations and they may contribute to the instability of the genome (17). CNVs have been demonstrated to play a significant role in the pathogenesis of several diseases and disorders such as ASD through the alterations of gene expression levels (16). One of the most challenging issues in genomics is the method of detecting disease-related genes. CNVs markedly exacerbate this issue and have received significant interest as a type of genetic variation participating in genomic diversity, while also being involved in complex diseases and drug responses (17). CNVs have shown to be useful in the molecular diagnosis of several diseases and disorders including several types of cancer, neuropsychiatric disorders and diabetes (18). Most of these genetic disorders are the result of a defect linked to variations in multiple genes rather than a defect in a single gene. Initially, 22q11.2 deletions were linked to the risk of schizophrenia (19) and, since then, studies have focused on the contribution of CNVs to neuropsychiatric disorders such as ASD. Several studies noted the role of CNVs in the etiology of autism (14,16,18,20-23). Theoretically, CNVs are more reliable than gene expression profiles for the diagnosis of a disease as these CNVs serve as more robust biomarkers than gene expression signatures, the latter of which are more readily disturbed. CNVs are estimated to cover 12-15% of the genome and comprise at least 1,000 genes (24). Therefore, CNVs markedly contribute to the genotypic and phenotypic variability in a population. CNVs have been demonstrated to be common susceptibility factors, with value for the detection of several diseases including autoimmune diseases, HIV infection and asthma (25-27). A review of the impact of CNVs on ASD identified 1,632 protein-coding genes and long non-coding RNAs within candidate CNVs that contribute to ASD, where 552 of the included genes were markedly expressed in the brain (28). Indeed, the identified CNVs may serve as valuable resources for personalized medicine and research on the genetic diversity of humans, as well as for diagnostic purposes. Consequently, the results presented in the present study may facilitate improvement in detecting genetic biomarkers for the diagnosis of ASD and predicting the response to therapy that could be used for the treatment of autism.

Materials and methods

Participants. In the present study, 14 Saudi Arabian children (12 males and two females), aged between 3 and 12 years old, who were diagnosed as autistic children by a physician at Pediatric Clinics at King Abdulaziz University Hospital in Jeddah, Saudi Arabia, based on the DSM-5 diagnostic criteria (29) were recruited over a period of the first six months (from January to June) of 2022 and all exhibited marked delayed speech development, poor eye contact and limited verbal communication and skills. In addition, four healthy children (three males and one female) aged between 3-12 years were included. A total of 2 ml of blood was collected in the autistic clinic in Jeddah. Blood samples were stored in EDTA tubes (Lavender-top tubes) to prevent clotting. Children with other genetic diseases or infections were excluded from the present study. The present study was approved by the Ethics Committee of Biomedical Research-Centre of Excellence in Genomic Medicine Research at King Abdulaziz University (approval no. 02-CEGMR-Bioeth-2018). It was performed in accordance with the guidelines in King Fahd Center for Medical Research (Jeddah, Saudi Arabia) and in accordance with Declaration of Helsinki. The children's parents signed consent forms for the participation of their children in the present study.

Array Comparative Genomic Hybridization (aCGH). aCGH was performed on 14 autistic and four healthy sibling samples according to the manufacturer's protocol (Agilent Technologies, Inc.). Using a QIAamp DNA blood mini kit (Qiagen GmbH). DNA was extracted from the 2 ml blood samples according to the manufacturer's protocol. Next, DNA was fragmented using restriction digestion and the appropriate fragments were prepared for hybridization with probes after which DNA labeling was used to distinguish between the test and the reference samples. Random primers and two types of dyes were used, red cyanine 5-dUTP (Cy5) for autistic (test) samples and green cyanine 3-dUTP (Cy3) for the reference samples, all samples were incubated at 37˚C for two hours, followed by 10 min at 65˚C. The previous two steps were performed using the SureTag DNA labeling kit (Agilent Technologies, Inc.) according to the manufacturer's protocol. Using 30 KDa Amicon filters (Agilent Technologies, Inc.), unlabeled samples

Table I. Demographic characteristics of ASD and healthy children.

Sample	Sex	Age (Years)	Severity of ASD	Onset of symptoms
1	Male	12	Simple/level1	After 2 years
2	Female	9	Mild/level2	After 2 years
3	Male	10	Simple/level1	After 2 years
4	Male	8	Mild/level2	After 2 years
5	Male	8	Mild/level2	After 6 months
6	Male	6	Mild/level2	After 2 years
7	Male	7	Simple/level1	After 2 years
8	Male	12	Mild/level2	After 2 years
9	Male	8	Sever/level3	After 2 years
10	Male	7	Mild/level2	After 1 year
11	Male	10	Mild/level2	After 2 years
12	Female	10	Simple/level1	After 2 years
13	Male	9	Mild/level2	After 2 years
14	Male	8	Mild/level2	After 2 years
Cont. 1	Male	10	-	-
Cont. 2	Male	9	-	-
Cont. 3	Male	8	-	-
Cont. 4	Female	6	-	-

were purified. An Oligo aCGH/ChIP-on-chip Hybridization kit (Agilent Technologies. Inc.) was used for hybridization and washing was performed using the Oligo aCGH/ChIP-on-chip Wash Buffer kit (Agilent Technologies. Inc.). Microarray slides were scanned using the Feature Extraction software (v8.1) (Agilent Technologies. Inc.) after inserting the slides into a scanner connected to a computer. From the scanned microarray image, a quality control report and feature extraction file were extracted and translated into log ratios using the Agilent CytoGenomics software (Agilent Technologies, Inc.).

Results

Clinicopathological characteristics of the children with ASD. The characteristics of the children with ASD children are presented in Table I. The autistic patients consisted of 14 children including 12 males and two females.

aCGH analysis. aCGH was used to detect CNVs in the extracted DNA from the patients (22) along with four healthy children. The results of aCGH showed the type and size of common CNVs in the different genomic regions of the autistic and healthy samples (Table II). In addition, certain possible pathogenic genes in the CNV regions were identified in at least two autistic cases that were associated with several developmental and neurogenetic diseases (Table III). The remainder of the results on CNVs in each sample are presented in Table SI.

Analysis of the most frequent CNVs

Chromosome 1. The results showed a CNV loss of 234.112 Kb starting from 149,041,013-149,275,124 on cytoband 1q21.2 in a male autistic patient (sample 6) and in sample 14 in the same 1q21.2 region, there was a CNV gain of 169.303 Kb starting from 149,063,179-149,232,481; both CNVs containing the neuroblastoma breakpoint family member 25 pseudogene (NBPF25P) gene.

Chromosome 3. There was a CNV loss on cytoband 3p26.3 in two male samples that varied in size. One sample (sample 6) had a CNV loss of 244.267 Kb which started from ~62,199-306,465 and the other sample (sample 7) had a CNV loss of 155.533 Kb, which started from ~127,520-283,052 and contained LINC01986, cell adhesion molecule L1 like (CHL1)-AS2 and CHL1 genes.

Chromosome 4. aCGH revealed CNVs in cytoband 4q13.2 in five autistic patients and two healthy patients, which differed in size and mutation type. In four male autistic samples (samples 1, 4, 7 and 8) and one healthy patient (Cont. 4), there was a CNV deletion or loss of 90.702 Kb which started from 69,075,171-69,165,872 that contained the UGT2B17 gene. There was a CNV amplification in the same cytoband with a size of 90.702 Kb in a female autistic sample (sample 2) as well. In addition, a healthy patient (Cont. 2) had a CNV loss at the same cytoband although it differed in size (216.167 Kb).

Chromosome 6. There was a CNV loss on cytoband 6p25.3 in two male patients varying in size. One sample (sample 4) had a CNV of 130.165 Kb, which started from ~204,318-334,482 and contained the DUSP22 gene and another sample (sample 8) had a CNV loss of 619.488 Kb, which started from ~221,768-841,255 and contained DUSP22, IRF4, EXOC2 and HUS1B. In addition, CNV loss/or deletion on cytoband 6q24.2 in three male samples varied in size. One sample (sample 7) had a CNV loss of 13.413 Kb, which started from ~144,315,564-144,328,976 where this site contained two genes: PLAGL1 and HYMA and another sample (sample 14) had a CNV loss of 1.434 Kb which started from ~144,328,437-144,329,870. In addition, there was a CNV deletion on the same cytoband 6q24.2 in one male sample (sample 9) of 0.235Kb which started from ~144,328,742-144,328,976.

Table II. Type and size of common CNVs in the samples.

Sample	Sex	Chromosome band	CNVs type	Size (kb)
AUT1	Male	4q13.2	Deletion	90.702
		7p21.1	Gain	707.325
		14q11.1-q11.2	Loss	1044.756
AUT2	Female	4q13.2	Amplification	90.702
		14q11.1-q11.2	Amplification	1044.756
AUT3	Male	7q34	Loss	154.48
		Xp22.33	Gain	26.169
		Yp11.32	Gain	26.169
AUT4	Male	4q13.2	Loss	90.702
		6p25.3	Loss	130.165
		14q11.1-q11.2	Loss	571.927
AUT5	Male	-	-	-
AUT6	Male	1q21.2	Loss	234.112
		3p26.3	Loss	244.267
		7q34	Loss	0.795
		8p23.2	Gain	828.449
		15q11.1-q11.2	Loss	2142.513
		Xp22.33	Gain	224.216
		Yp11.32	Gain	224.216
		Yp11.2	Loss	215.607
AUT7	Male	3p26.3	Loss	155.533
		4q13.2	Loss	90.702
		6q24.2	Loss	13.413
		8p23.2	Gain	655.942
		15q11.1-q11.2	Loss	2142.513
		Yq11.21	Gain	586.292
AUT8	Male	4q13.2	Deletion	90.702
		6p25.3	Loss	619.488
		15q11.1-q11.2	Loss	2142.513
AUT9	Male	6q24.2	Deletion	0.235
		13q32.3	Loss	731.71
		Yp11.2	Gain	1399.514
AUT10	Male	13q32.3	Loss	731.71
		Xp22.33	Amplification	37.061
		Yp11.32	Amplification	37.061
		Yp11.2	Deletion	210.584
AUT11	Male	-	-	-
AUT12	Female	7q11.1	Loss	345.203
		15q11.1-q11.2	Loss	2142.513
		Yq11.21	Gain	318.887
AUT13	Male	7q11.1	Gain	345.203
		15q11.1-q11.2	Loss	2142.513
AUT14	Male	1q21.2	Gain	169.303
		6q24.2	Loss	1.434
		7p21.1	Loss	1.909
		13q32.3	Loss	3.94
		Yp11.2	Gain	1269.05
Cont. 1	Male	-	-	-
Cont. 2	Male	4q13.2	Loss	216.167
Cont. 3	Male	-	-	-
Cont. 4	Female	4q13.2	Deletion	90.702

CNVs, copy number variations; kb, kilobases; AUT, Autism; Cont., control.

Table III. Most frequent CNVs and genes in cases of autism.

Chromosome	Cytoband	Number of affected samples	Genes	Developmental and neurogenetic diseases that are related to the pathogenic genes
1	1q21.1	2	NBPF25P	Microcephaly, autism, schizophrenia, cognitive disability (32,33)
3	3p26.3	2	LINC01986, CHL1-AS2, CHL1	Learning and language difficulties (37-40)
4	4q13.2	5	UGT2B17	Autism (42-55)
6	6p25.3	2	DUSP22, IRF4, EXOC2, HUS1B	-
	6q24.2	3	PLAGL1, HYMAI	Prenatal growth defects (58-60)
7	7p21.1	2	AHR, KCCAT333, LOC101927630, SNX13, TWIST	-
	7q34	2	MTRNR2L6, PRSS1	-
8	8p23.2	2	CSMD1	Speech delay, autism, epilepsy and learning difficulties (71-73). ASD (74)
13	13q32.3	3	ZIC2, LINC00554, LOC105370333, PCCA, PCCA-AS1, GGACT, TMTC4, NALCN-AS1	ASD (78-80)
14	14q11.1-q11.2	3	OR11H12, LINC02297, POTEG, LOC101929572, POTEH-AS1, DUXAP10, LINC01296, BMS1P18 BMS1P17, BMS1P22, POTEM, LOC100508046 OR11H2, OR4Q3, OR4M1, OR4N2, OR4K3, OR4K2, OR4K5, OR4K1	Seizures, motor and cognitive delays, speech impairment and autistic traits (83,84)
15	15q11.1-q11.2	5	CHEK2P2, HERC2P3, GOLGA6L6, GOLGA8CP, NBEAP1, MIR3118-2, MIR3118-3, MIR3118-4, POTEB, POTEB2, POTEB3, NF1P2, MIR5701-1, MIR5701-2, MIR5701-3, LINC01193, LINC02203, FAM30C, LOC646214, CXADRP2, LOC101927079, OR4M2, OR4N4, OR4N3P, IGHV1OR15-1, LOC102724760, IGHV1OR15-3, LOC642131, MIR1268A, REREP3	ASD (86), neurological diseases and cognition defects (88)
X	Xp22.33	4	AKAP17A, ASMT, XG, GYG2, PPP2R3B	-
Y	Yp11.32	4	AKAP17A, ASMT, PPP2R3B	-
	Yp11.2	4	TTTY16, TTTY12, LINC00279, TTTY18, TTTY19, TTTY11, TGIF2LY	-
	Yq11.21	2	TTTY15, USP9Y	-

CNVs, copy number variations; ASD, autism spectrum disorder.

Chromosome 7. The results of aCGH revealed CNVs in chromosome 7 on various cytobands and of different sizes and mutation types. There was a gain of 707.325 Kb that started from 17,192,079-17,899,403 on cytoband 7p21.1 containing the genes AHR, KCCAT333, LOC101927630 and SNX13 in 1 male autistic sample (sample 1) while in sample 14, the same region 7p21.1 had a CNV loss of 1,909 Kb starting from 19,155,285-19,157,193, which contained the TWIST1 gene.

Additionally, CNV losses on cytoband 7q34 in two male samples that varied in size were identified. One sample (sample 3) had a CNV of 154.48 Kb which started from ~141,985,598-142,140,077 and contained the genes MTRNR2L6 and PRSS1, while the other sample (sample 6) had a CNV loss of 0.795 Kb, which started from ~142,459,709-142,460,503 and contained the PRSS1gene.

Furthermore, there were CNVs in cytoband 7q11.1 in two autistic patients that were the same size; 345.203 Kb from 61,274,531-61,619,733 but of different mutation types. A CNV loss occurred in a female sample (sample 12) and a CNV gain was observed in the male sample (sample 13), but no affected gene was identified in this CNV.

Chromosome 8. There was a CNV gain in cytoband 8p23.2 in two male autistic patients varying in size. One sample (sample 6) had a gain of 828.449 Kb which started from ~337,9054-4,207,502 and another sample (sample 8) had a CNV

gain of 655.942 Kb which started from ~3,413,488-4,069,429 and contained the CSMD1 gene.

Chromosome 13. There was a CNV loss on cytoband 13q32.3 in three male samples that varied in size; two samples (samples 9 and 10) had the same CNV loss of 731.71 Kb which started from ~100,638,020-101,369,729 and included the Zic family member 2 (ZIC2), LINC00554, LOC105370333, PCCA, PCCA-AS1, GGACT, TMTC4 and NALCN-AS1 genes and another sample (sample 14) had a CNV loss of 3.94 Kb which started from ~100,634,636-100,638,575 and contained the ZIC2 gene.

Chromosome 14. There were CNVs detected in cytoband 14q11.1-q11.2 in three autistic patients of different sizes and mutation types. In two male autistic patients, there was a CNV loss of 1,044.756 Kb which started from 18,446,762-19,491,517 in sample 1 and a loss of 571.927 Kb which started from 18,919,591-19,491,517 in sample 4; these two CNVs contained the LINC01296, DUXA P10, BMS 1P22, BMS1P18, BMS1P17, POTEM, LOC100508046, olfactory receptor family 11 subfamily H member 12 (OR11H2), OR4Q3, OR4M1, OR4N2, OR4K3, OR4K2, OR4K5 and OR4K1 genes. In addition to these genes, the CNV in sample 1 also included the OR11H12, LINC02297, POTEG, LOC101929572 and POTEH-AS1 genes. There was a CNV amplification in the same cytoband with a size of 1,044.756 Kb which started from ~18,446,762-19,491,517 in a female autistic patient (sample 2).

Chromosome 15. There was a CNV loss in four male autistic patients (samples 6, 7, 8 and 13) and in one female autistic patient (sample 12) with the same CNV size of 2,142.513 Kb starting from 20,416,244-22,558,756 on cytoband 15q11.1-q11.2 that contained several genes including CHEK2P2, HERC2P3, GOLGA6L6, GOLGA8CP, NBEAP1, MIR3118-2, MIR3118-3, MIR3118-4, POTEB, POTEB2, POTEB3, NF1P2, MIR5701-1, MIR5701-2, MIR5701-3, LINC01193, LINC02203, FAM30C, LOC646214, CXADRP2, LOC101927079, OR4M2, OR4N4, OR4N3P, IGHV1OR15-1, LOC102724760, IGHV1OR15-3, LOC642131, MIR1268A and REREP3.

Chromosome X. The results revealed CNVs in cytoband Xp22.33 in four male autistic patients of different sizes and mutation types. There was a CNV gain in three samples: Sample 3 with a size of 26.169 Kb which started from 232,942-259,110 and contained the Protein phosphatase 2 regulatory subunit B'β (PPP2R3B) gene; sample 6 with a size of 224.216 Kb which started from 1,711,433-1,935,648 and contained the AKAP17A and ASMT genes; and sample 13 with a size of 99.105 Kb starting from 2,700,316-2,799,420 and contained the XG and GYG2 genes. Additionally, in the same sample, there was a CNV loss with a size of 7.315 Kb starting from 284,015-291,329. There was a CNV amplification in sample 10 with a size of 37.061 Kb starting from ~302,004-339,064 that contained the PPP2R3B gene.

Chromosome Y. Different sizes and mutation types of CNVs were detected on cytoband Yp11.32 in four autistic patients. There was a CNV gain in two samples: Sample 3 with a size of 26.169 Kb starting from ~232,942-259,110 and containing the PPP2R3B gene and sample 6 with a size of 224.216 Kb starting from 1,661,433-1,885,648 that contained the AKAP17A and ASMT genes. In addition, there was a CNV amplification in sample 10 with a size of 37.061 Kb starting from 252,004-289,064 that contained the PPP2R3B gene. In addition, there was a CNV loss in sample 13 with a size of 7.315 Kb which started from 234,015-241,329.

Moreover, different sizes and mutation types of CNVs were detected in four autistic patients on cytoband Yp11.2. There was a gain in two samples: Sample 9 with a size of 1,399.514 Kb which started from 7,259,988-8,659,501 and contained the genes TTTY16, TTTY12, LINC00279, TTTY18, TTTY19 and TTTY11 and in sample 14 with a size of 1,269.05 Kb which started from 3,102,385-4,371,434 and contained the TGIF2LY gene. In addition, there was CNV loss in sample 6 with a size of 215.607 Kb which started from ~3,065,579-3,281,185. There was a CNV deletion in sample 10 with a size of 210.584 Kb which started from 4,142,943-4,353,526.

Finally, there was a CNV gain in cytoband Yq11.21 in two autistic patients varying in size. One sample (sample 7) had a gain of 586.292 Kb which started from ~13,675,923-14,262,214 and another sample (sample 12) had a CNV gain of 318.887 Kb which started from ~14,692,143-15,011,029 and contained the genes TTTY15 and USP9Y.

Discussion

In the present study, the results identified CNVs in Saudi Arabian children with autism to improve the understanding of the etiology of autism, as this may facilitate its diagnosis. The results of the present study illustrated the importance of aCGH in autism diagnosis since it could be used to characterize a patient sample in terms of potential cytogenetic aberrations through the detection of CNVs.

Bioinformatics analysis reported the most frequent CNVs in several regions of the genome of autistic children by aCGH. The most frequent CNVs were 1q21.2, 3p26.3, 4q13.2, 6p25.3, 6q24.2, 7p21.1, 7q34, 7q11.1, 8p23.2, 13q32.3, 14q11.1-q11.2 and 15q11.1-q11.2.

Regarding chromosome 1, the CNV loss of 1q21.2, which was identified in the present study in two samples, is considered a rare recurrent CNV that has been revealed as being a significant risk factor for ASD (30). This region includes the NBPF25P gene (31). Several developmental and neurogenetic diseases, including microcephaly, autism, schizophrenia, cognitive disability, congenital heart disease, neuroblastoma and congenital kidney and urinary tract anomalies have been linked to CNVs in 1q21.2, where the majority of the DUF1220 domain resides (32,33). According to the characteristics of patients with ASD, the two samples in the present study showed a severity of ASD of mild/level 2, which is consistent with the findings of the previous studies that link CNVs in 1q21.2 with autism severity (30,31).

Regarding the CNV loss on cytoband 3p26.3, evidence in recent years has accumulated that the p-arm of chromosome 3 is a risk locus for ASD (34). Similarly, studies have verified the existence of an overlap between neurological and psychological symptoms and loss or gain on 3p26.3. The 3p26.3 region contains the LINC01986, CHL1-AS2 and CHL1 genes. The CHL1 gene is highly expressed in the central and peripheral nervous systems and encodes a protein that belongs to the L1 gene family of neural cell adhesion molecules, which contributes to the development of the nervous system and synaptic plasticity (35-36). Learning and language difficulties

have been associated with heterozygous loss of the CHL1 gene in humans (37-40).

Several studies on chromosome 4 reveal the existence of a relationship between 4q chromosomal aberrations that vary in type, size and breakpoints and encompassed a variety of chromosome bands and genes, with neurodevelopmental phenotypes (41-44). Notably, the aCGH results detected a deletion/or loss in 4q13.2 in four male autistic samples and amplification in one female autistic sample, including the UGT2B17 gene, which encodes a member of the uridine diphosphoglucuronosyl transferase protein family. The encoded enzyme is involved in the metabolism of steroids by catalyzing glucuronidation. Autism has been associated with higher androgen (testosterone or dihydrotestosterone) levels in several studies (42-54). Testosterone/epitestosterone ratios in autistic children may thus be used as biomarkers to diagnose autism (55).

Regarding chromosome 6, CNVs in the short arm are rare and are associated with developmental delay, hypotonia, brain malformations, congenital anomalies, dysmorphic characteristics, language impairment and delayed motor development (56,57). The effect of CNVs in chromosome 6 has been verified by the South Carolina Autism Project in participants suffering from ASDs (57). The cytobands in chromosome 6 that may be associated with autism contain several genes that have been identified as strong candidates for autism or autistic features due to their role in brain function or development, or their involvement in neurological disorders (58). PLAGL1 like zinc finger 1 gene encodes the C2H2-type zinc finger protein (C2H2-ZNF) that functions as a transcription factor or cofactor for several regulator proteins (59). Abnormal expression of PLAGL1 is involved in prenatal growth defects (60). Evidence has increasingly demonstrated that C2H2-ZNFs are important targets for neurodevelopmental disorders and pathogenic processes (61). Moreover, C2H2-ZNFs are implicated in the pathogenesis and pathophysiology of ASDs (62-65).

Chromosome 7q rearrangement was identified in a patient with mental retardation, anxiety disorder and autistic characteristics (66). A previous study provided evidence that the 7q11.23 duplication may play a role in a wide range of clinical phenotypes, from developmental delay to mental retardation and autism (67). In addition, these findings demonstrate that there may be one or multiple genes on 7q11.23 involved in language and social interactions that are sensitive to gene dosage (67). CNV gains at 7q11.23 have been demonstrated as rare CNVs that are implicated in ASD pathogenesis (68). Short arm duplication of chromosome 7 is extremely rare and the duplication's phenotype spectrum varies depending on the area duplicated. A previous study revealed the existence of a *de novo* duplication of the 7p21.1p22.2 chromosomal region in a 3-year-old autistic male child (69). A study by Schuch *et al* (70) reported that the 7p21 cytoband was implicated as a pathogenic CNV linked with ASD.

According to the results of the present study, a CNV gain on cytoband 8q23.2 was reported and varied in size between two autistic samples. Similarly, several studies showed that CNVs of 8p23.1 and 8p23.2 were linked with speech delay, autism, epilepsy and learning difficulties (71,72). This region contains CUB and sushi multiple domains 1 (CSMD1) gene. A previous study revealed the role of CSMD1 in the activation and inflammation of the central nervous system (73). Another study performed genome-wide association analysis using ASD families and healthy control samples obtained from East Asian populations to investigate ASD candidate genes and found that the CSMD1 gene was markedly associated with ASD (74). A study consisting of 144 Brazilian individuals with ASD found a total of 39 rare CNVs in 39 patients where CSMD1 was classified pathogenetically with another 14 genes (FAT1, CAMK4, BIRC6, DPP6, CTNNA3, CDH8/CDH11, CDH13, OR1C1, CNTN6, CNTNAP4, FGF2 and PTPRN2) (75).

Chromosome 13 abnormalities in autism cases have been reported in previous studies (76,77) as well as in the Collaborative Linkage Study of Autism (78). In the search for gene loci associated with autism, chromosome 13 is an interesting candidate (79). In one case study of an 18-year-old male with autistic disorder, an interstitial deletion of chromosome 13 was observed (80). The results of the present study identified CNV loss on cytoband 13q32.3 in three autistic samples, which affected the ZIC2 gene located on 13q32.3.

Chromosome 14 alterations are frequently associated with neurodevelopmental impairments (81). Anomalies on chromosome 14 (both single-gene disorders and submicroscopic changes, such as deletions or duplications) are markedly associated with ASD (82). Rare disorders associated with interstitial deletions of the long arm of chromosome 14 are characterized by facial dysmorphism, neurological disorders such as seizures, motor and cognitive delays, speech impairment and autistic traits (83,84). A case study of an 11-year-old girl with autism identified a *de novo* deletion in the 14q11.2 region using aCGH (85). OR11H12 triggers neuronal activity by interacting with an odorant molecule in the nose. Genes associated with olfactory receptors in cytoband 14q11.2 are hypothesized to be the most affected by CNVs (79). Research has revealed that autistic patients react differently to human odors than their typical peers, suggesting that their inability to sense odor may explain their impairment in social interaction (80).

A variety of stereotyped deletions and duplications in chromosome 15 on the long arm contribute to neurodevelopmental disorders and ASD (86). Deletions and duplications on 15q11-q13 are commonly identified as recurrent rearrangements that are consistently linked to autism (87). Deletion on 15q11.2 is one of the most commonly identified CNVs recurrently associated with autism (88). Olfactory receptors, such as olfactory receptor family 4 (OR4) subfamily N member 4, OR4 subfamily M member 2 and OR4 N member 3 pseudogene, which are located on the 15q11.1-q11.2 cytoband, play a major role in the most relevant biological processes and functional attributes (89). Previous research has associated olfactory receptors with neurological diseases and cognition defects (90).

Regarding the sex chromosomes, the PPP2R3B gene, which is located on chromosomes Xp22.33 and Yp11.32, is one of the four major Ser/Thr phosphatases implicated in the negative control of cell growth and division and opposes the action of kinases and phosphorylases involved in signal transduction. Several cellular functions, including signal transduction vesicle trafficking, membrane receptor localization and activity and determination of membrane identity, are

regulated by phosphoinositides, which are integral components of lipid membranes (91). These functions require finely balanced activity of specific phosphoinositide kinases and phosphatases that are dependent on dynamic and highly regulated phosphoinositide metabolism (92). However, emerging evidence suggests that certain forms of autism are also affected by phosphoinositide kinases and phosphatases, highlighting opportunities for disease-specific therapies (93). There are certain possibilities and challenges arising from these findings that may warrant further study of phosphoinositide kinase, as using specific drugs is of benefit for the treatment of ASDs caused by phosphoinositide metabolism defects. Genetic and functional studies show that these enzymes are often dysregulated in ASD (93-96). Understanding the dysregulation of phosphoinositides may offer opportunities to manage ASD in an improved way (96).

In general, the discovery of CNVs in ASD can have a significant effect on, and implications for, a patient depending on the various factors such as the specific CNVs involved, individual characteristics of the patient and available interventions. There are some effects and implications for identifying CNVs associated with ASD such as contributing to improving diagnosis and understanding the underlying genetic factors contributing to the disorder which can lead to more personalized treatment approaches tailored to the individual's genetic profile and potentially improving outcomes. In addition, knowledge of CNVs associated with ASD can inform decisions regarding family planning and facilitate genetic counseling for families for an improved understand the genetic basis of ASD and make informed decisions regarding future pregnancies. Moreover, certain CNVs may be associated with specific clinical features or outcomes in patients with ASD therefore understanding these associations can provide prognostic information that may guide treatment planning and interventions to address the needs of patients in an improved way.

CNVs play a major role in neurological disorders as well as in the genetic etiology of ASDs. The prevalence of autism increases in different regions of the Kingdom of Saudi Arabia. The purpose of the present study was to determine the CNVs in Saudi Arabian children with autism to increase our understanding of the etiology of autism, which may facilitate its diagnosis. In the present study, certain CNVs/genes associated with autism in Saudi children were investigated. Based on the findings, more awareness of genetic variations in ASD is required and may provide a deeper understanding of the vital genomic regions that contribute to the development of ASD. Indeed, improving the diagnosis of ASD requires a more comprehensive description of each patient and identification of possible pathogenic genome variants and genes in the CNV region. Additional studies are required with a larger cohort of ASD patients to identify the CNVs/genes that contribute to autism and may thus serve as biomarkers. There is still much to learn regarding the pathogenetic mechanisms by which a genetic adjustment results in ASD. Understanding these mechanisms may provide important insights into the prevention and/or treatment of ASD.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

SA and MH conceived and designed the study. SA, MH, MZ and AE collected samples and patient information. SA, MZ, AE, AA and MH analyzed the data. MH, AR, AE, HA and MZ performed the experiments. AC, AA and AE provided the resources. MH, MZ and SA wrote the manuscript. SA, MH, MZ, AA, FB and AM reviewed and edited the manuscript. SA and MH confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Biomedical Research-Centre of Excellence in Genomic Medicine Research at King Abdulaziz University (approval no. 02-CEGMR-Bioeth-2018). It was performed in accordance with the guidelines in King Fahd Center for Medical Research (Jeddah, Saudi Arabia) and in accordance with Declaration of Helsinki. The children's parents signed consent forms for the participation of their children in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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