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ORIGINAL RESEARCH

Multiple Recurrent Copy Number Variations (CNVs) in Chromosome 22 Including 22q11.2 Associated with Autism Spectrum Disorder

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Introduction: Autism spectrum disorder (ASD) is a developmental disorder that can cause substantial social, communication, and behavioral challenges. Genetic factors play a significant role in ASD, where the risk of ASD has been increased for unclear reasons. Twin studies have shown important evidence of both genetic and environmental contributions in ASD, where the level of contribution of these factors has not been proven yet. It has been suggested that copy number variation (CNV) duplication and the deletion of many genes in chromosome 22 (Ch22) may have a strong association with ASD. This study screened the CNVs in Ch22 in autistic Saudi children and assessed the candidate gene in the CNVs region of Ch22 that is most associated with ASD.

Methods: This study included 15 autistic Saudi children as well as 4 healthy children as controls; DNA was extracted from samples and analyzed using array comparative genomic hybridization (aCGH) and DNA sequencing.

Results: The aCGH detected (in only 6 autistic samples) deletion and duplication in many regions of Ch22, including some critical genes. Moreover, DNA sequencing determined a genetic mutation in the TBX1 gene sequence in autistic samples. This study, carried out using aCGH, found that six autistic patients had CNVs in Ch22, and DNA sequencing revealed mutations in the TBX1 gene in autistic samples but none in the control.

Conclusion: CNV deletion and the duplication of the TBX1 gene could be related to ASD; therefore, this gene needs more analysis in terms of expression levels.

Keywords: autism spectrum disorder, chromosome 22, copy number variations, Saudi autistic children, TBX1

Introduction

Autism spectrum disorder (ASD) refers to a heterogenous neurodevelopmental disorder that includes problems with social communication and behavior. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), the professional diagnosis of ASD is based on two areas—difficulties in social communication and restricted, repetitive and/or sensory behaviors.[1] Symptoms can vary depending on their various degrees of severity and the age of onset.[2] Furthermore, the symptoms of autism could arise early in childhood, with onset earlier than three years of age. However, the symptoms may not show clearly until school age or later, as not every child has the same symptoms or the same level of development.[3–6]

ASD has a complex etiology, and its mechanism remains largely unclear.[7] According to the Centers for Disease Control and Prevention (CDC) and the Autism and Developmental Disabilities Monitoring (ADDM) Network, the prevalence of ASD globally has increased. However, in the United States of America (USA), the prevalence of ASD was

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estimated to be 1 in 40 children in 2018,[8,9] while in the Kingdom of Saudi Arabia, the prevalence of ASD was estimated to be (2.51%, 1:40, 25 per 1000), with male-to-female ratio of 3:1.[10,11] Moreover, the prevalence of ASD is affected by gender, where the rate in males is four times greater than that in females.[12,13]

Several studies have indicated that genetic, epigenetic, and environmental factors are implicated in ASD.[14–20] Genetic factors, including mutations and various submicroscopic structural chromosome variations, such as CNVs, could increase the risk of ASD.[17] In addition, a previous study observed a consistently higher incidence of ASD in monozygotic twins than in dizygotic twins.[20,21]

CNVs are a type of structural variation that can affect chromosomal structure via duplications or deletions that alter DNA sequence.[22–27] CNVs are considered as a source of genetic variation that is important for genetic diversity in humans, gene evolution and phenotypic diversity.[4,22,25,27] CNVs can affect critical gene, which is susceptible to a specific disease and is associated with several neurodevelopmental disorders including ASD and other diseases,[28–30] for example microdeletion of CAPG reduced its expression level in ASD patients.[30] The effect of CNVs is based on their size and location, which are most strongly associated with neurodevelopmental disorders.[30,31] However, CNVs can be detected by various genome analysis platforms and cytogenetics techniques, such as fluorescent in situ hybridization (FISH), array comparative genomic hybridization (aCGH), SNP genotyping array, and next-generation sequencing.[2,32–34]

The acrocentric chromosome 22 (Ch 22) is the second smallest human autosomal chromosome and comprises 1.6–1.8% of the genomic DNA and carries about 50,818,468 bp. According to the European bioinformatics institute (EMBL-EBI), Ch 22 contains 495 protein-coding genes.[35–37] Previous studies have revealed that some genes on Ch 22, such as SHANK3, TOP3B, TANGO2, ADSL, and TBX1, are related to neurodevelopment disorders, including ASD.[4,38–42] Since 2006, CNVs have been recognized as important genetic factors in ASD.[43] In recent years, an increasing number of structural genomic variations including CNVs have arisen as significant risk factors for neurodevelopmental disorders and have been recognized as susceptibility loci for ASD.[13,23,44–46] Furthermore, many studies including copy number analysis have discovered many new variants, new transcripts and identified genetic risk loci.[4,47–51] CNVs in some chromosomal regions are common in ASD, including the 22q11.2 region.[17,25,50,51] DiGeorge syndrome (DS) is the most frequent chromosomal microdeletion syndrome, which is considered as the second most common chromosomal disorder after Down syndrome. Moreover, 22q11.2DS arises from an interstitial chromosomal microdeletion with a length of 1.5 to 3 Mb of DNA on 22q11.2 that may include 40 genes, which affect human health depending on size and gene containing.

Both deletions (22q11.2DS) and duplications (22q11.2DupS) are considered as CNVs that increase susceptibility to neurodevelopmental disorders, including ASD.[52] Although there are convincing data showing that CNVs in Ch 22 could be related to ASD, the detection and characterization of CNVs and mutated genes in Ch 22 still need more analysis.

A human study on 46 patients with nested deletions or duplications of 22q11.2 indicated 25 genes in the 22q11.2 region which are related to ASD. The list of genes included COMT, PRODH, and TBX1.[53] TBX1 is a protein coding gene, which is located in the long arm of Ch22. Furthermore, TBX1 plays an essential role in the regulation of developmental processes and the formation of tissues and organs during embryonic development, including heart and limb development.[54–56] In addition to neurodevelopmental disorders, TBX1 may be associated with other diseases, such as congenital heart defects and DiGeorge syndrome, in mouse models of 22q11 DS.[57–63] Mutant TBX1 sufficiently causes most of the physical features of 22q11.2 DS.[64] Previous studies have reported that TBX1 is strongly linked to behavioral disorders in mice and humans.[40,64]

Based on previous studies that revealed that the CNVs in Ch 22 could be more susceptible to autism, the detection and characterization of CNVs and the mutated genes in Ch 22 still need more analysis. The objectives of the present study are to screen the CNVs in Ch 22 in autistic Saudi children in addition to the candidate genes in the CNV regions that could be associated with ASD.

Materials and Methods

Participants

This study included 19 Saudi children as samples (male and female); 15 of the samples were from autistic children (12 male and 3 female), and four non-autistic children (3 male and 1 female) were the control group. The children were aged

between 3 and 12 years old. Peripheral blood samples were collected at an autism clinic in Jeddah, and the children's parents signed consent forms for the agreement of the participation of their children in this study. The children were diagnosed based on Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-5) and showed no symptoms of malnutrition, active infection, or known genetic disease (such as Down syndrome).[1] Anyone with ASD is further diagnosed with ASD levels 1, 2 or 3, depending on the severity of the disorder and how much support they need in their daily life, according to the (DSM-5). The levels range from least to most severe, where level 3 represents the most severe level of ASD symptoms, and level 1 represents the mild of the spectrum.

Array-Based Comparative Genomic Hybridization (aCGH)

aCGH was applied for 15 autistic and three neurotypical sibling samples according to the protocol of the manufacturer (Agilent Technologies, USA). DNA was extracted from 2 mL of blood from all samples (autistic and neurotypical children) using QIAamp DNA blood mini kit (QIAGEN, Germany) following manufacturer's instruction. Then, a SureTag DNA Labeling Kit was used for DNA fragmentation and DNA labeling. Then, restriction enzyme digestion was used to make appropriate DNA fragments suitable for hybridization and DNA labeled with Cyanine 3-dCTPs and Cyanine 5-dCTPs by random primer for test samples and reference samples, respectively. After that, a SureTag purification column kit was used, along with column purification with 30 KDa Amicon fillers to get rid of impurities (unlabeled sample). The hybridization was carried out on Oligo aCGH/ChIP-on-chip Hybridization Kit and washing was performed using Oligo aCGH/ChIP-on-chip Wash Buffer Kit. After that, feature extraction software was performed to scan images of the array and Cytogenomics software was carried out of aCGH data analysis. Aberration Detection Method 2 (ADM-2) algorithm with default settings was used for identification and detection of aberrant copy number segments in aCGH data that passed the Quality Control (QC) metrics. The p-values for each probe were calculated, providing additional objective statistical criteria to determine the deviation from zero (±0.25), while the most ideal and reliable mean log ratios for gain (duplication) and loss (deletion) were around +0.58 and −1, respectively. Furthermore, all the detected variants were filtered by overlapping with Agilent CNV reference and global healthy control participants (DGV database) and in Saudi control participants to avoid false discovery, and all CNVs except chromosome 22 CNV were excluded. The terms of amplification, gain, loss and deletion depend on the mean log ratio, where the normal range is between 0 to +0.25 and 0 to −0.25. However, the size of CNV amplification is greater than +0.60 while in gain case, the size of CNV ranged between +0.25 and +0.58. On the other hand, CNV deletion is greater in size than −1.0, while in loss case, CNV size ranged between −0.25 and −0.99.

Polymerase Chain Reaction (PCR)

To validate the aCGH results, PCR was applied to five samples, four samples from autistic children and one sample from control samples. Primer A (forward: 5'-AGGCACCTCAAGTAGTCAGA-3', reverse: 5′TGCAAACTGTGGA T GATCTAGG-3') and Primer B (forward: 5'-TGCCAAAGTGTCCATCCCAT-3', reverse: 5'- TTTGCCTTTT CCCAGACAGC-3') were designed for different locations on TBX1 to find out if there are CNVs < 1Kb or genetic mutations in these locations. PCR amplification products were obtained using a final volume of 25 µL by (GoTaq® Green Master Mix, 2X) and gel electrophoresis was used to show the PCR results.

DNA Sequencing

DNA sequencing was applied with forward primers A and B of TBX1 for 5 samples, four from autistic children and one control sample to detect mutations in the DNA sequence. After first purification of PCR products (ethanol precipitation), cycle sequencing was used with fluorescent dyes in the reactions followed by a second purification of cycle sequencing products (ethanol precipitation) and a denaturation step by Hi-Di formamide. The samples were loaded to 96-well PCR plates, then placed in a 3500 Genetic analyzer machine, and a 3500 Series Data Collection Software program was used to collect the data. Analysis of the sequencing data was applied using the Sequencing Analysis Software program. We used a DNA sequencing chromatogram to trace viewer using Finch TV software; then, DNA sequencing results were aligned to FASTA format using the BLAST sequence alignment tool.

Characteristics of ASD Patients

Each family that cooperated in sampling in the study has signed the consent agreement form and filled a questionnaire that indicates the participants' characteristics (Table 1).

Results

The Results of aCGH

Detection of Amplification

The results reported, according to GRCh37, that amplification in two autistic samples varied in size. However, one female sample (Autistic Sample-1) showed 171.922 Kb amplification, which starts from region 21,386,562 to 21,558,483 on 22q11.22, while the male sample (Autistic Sample-2) showed 1.544 Kb amplification, which starts from region 18,127,933 to 18,129,476.

Detection of CNV Gain

The results showed CNV gain in two male autistic samples (Autistic Sample-2), where one sample had a gain of 1744.668 Kb on the 22q13.31- q13.32 region, which starts from region 46,288,660 to 48,033,327, while another sample (Autistic Sample-3) has a gain of 248.671 Kb, which starts from region 19,584,758 to 19,833,428 and includes the TBX1 gene.

Detection of CNV Loss

The study detected a loss of CNVs in three autistic samples, varying in size. However, one male sample (Autistic Sample-4) showed a loss of 1.983Kb, which starts from region 19,747,494 to 19,749,476, including the TBX1 gene. Moreover, in one female sample (Autistic Sample-5), there was a loss of 4.131Kb starting from region 19,746,363 to 19,750,493 on 22q11.21, including the TBX1 gene. In addition, this sample has another significant loss of 6051.996Kb, which starts from region 27,189,774 to 33,241,769 on 22q12.1 - q12.3. Furthermore, in another male sample (Autistic Sample-6), there was loss in two regions; the first loss was 6.035 Kb on 22q11.21, including the TBX1 gene, which starts from region 19,748,684 to 19,754,718, and the second loss was 852.313 Kb on 22q13.33, including the SHANK3 gene. There was another loss from 50,307,561 to 51,159,873 including other genes. The results of aCGH (Table 2) and (Figure 1) revealed CNV duplication and deletion in many regions, including some genes located in Ch22.

Detection of Mutation in TBX1

DNA sequencing was applied with primers A and B of TBX1 for four autistic samples and one non-autistic control sample. DNA sequencing was performed on ASD to validate the result of the aCGH that there is a mutation on TBX1. The detection limit of sequencing allowed for detecting insertion, deletion and substitution mutations in DNA sequences of less than 1Kb in samples with autism.

Table 1 Demographic Characteristics of ASD Children

Patient Number	Gender	Age (Years)	Birth Type	Severity of ASD	Onset of Symptoms	Family History
Autistic Sample-1	Male	9	Natural	Mild/level 2	After 2 years	No
Autistic Sample-2	Male	8	Caesarean	Mild/level 2	After 2 years	No
Autistic Sample-3	Male	7	Natural	Mild/level 2	After first year	Yes
Autistic Sample-4	Male	8	Caesarean	Severe/level 3	After 2 years	Yes
Autistic Sample-5	Female	6	Caesarean	Mild/level 1	After 2 years	No
Autistic Sample-6	Female	10	Natural	Simple/level 1	After 2 years	No

Table 2 Results of aCGH

Samples ID	Gender	Autism Level	Region	Gene	Type of CNVs	Size of CNVs (kb)
Autistic Sample-1	Female	Level 1	22q11.22	MIR650 MIR5571	Amplification	171.922
Autistic Sample-2	Male	Level 2	22q11.21	TBX1	Amplification	1.544
			22q13.31-q13.32	LINC00898 LOC284930/MIR3201/FAM19A5/ LOC284933/MIR4535/LINC01310	Gain	1744.668
Autistic Sample-3	Male	Level 1	22q11.21	SEPT5/SEPT5-GP1BB/GP1BB/TBX1 /GNB1L	Gain	248.671
Autistic Sample-4	Male	Level 2	22q11.21	TBX1	Loss	1.983
Autistic Sample-5	Female	Level 2	22q11.21	TBX1	Loss	4.131
			22q12.1- q12.3	LOC110091768/LINC01422/LOC284898/ LOC105372977/LINC01638/LINC02554/ MN1/PITPNB/TTC28-AS1/ MIR3199-1 / MIR3199-2/TTC28 /MIR5739/CHEK2/ HSCB/CCDC117/XBP1/ ZNRF3/ZNRF3-AS1/ C22orf31/ KREMEN1/EMID1/RHBDD3/ EWSR1/ GAS2L1/RASL10A/ AP1B1/MIR3653/ SNORD125/RFPL1S/ RFPL1/NEFH/THOC5/ NIPSNAP1/NF2/ CABP7/ ZMAT5/ UQCR10/ ASCC2/MTMR3/MIR6818/HORMAD2-AS1/ HORMAD2/LIFAS1/ LIF/LOC91370/OSM/ CASTOR1/TBC1D10A/SF3A1/CCDC157/ KIAA1656/RNF215/SEC14L2/MTFP1/ LOC105372990/SEC14L3/SDC4P/SEC14L4/ SEC14L6/GAL3ST1/PES1/TCN2/SLC35E4/ DUSP18/ OSBP2/MIR3200/LOC107985544/MORC2-AS1/ MORC2/ TUG1/SMTN/SELENOM/ INPP5J/ PLA2G3/MIR3928/RNF185/LIMK2/ PIK3IP1/PATZ1/PIK3IP1-AS1/ LINC01521/ DRG1/EIF4ENIF1/ SFI1/PISD/MIR7109/ PRR14L/DEPDC5 /C22orf24/YWHAH/ LINC02558/ SLC5A1/AP1B1P1/C22orf42/RFPL2/SLC5A4-AS1/ SLC5A4/ RFPL3/RFPL3S/ LOC339666/ RTCB/BPIFC/FBXO7/ SYN3/ TIMP3	Loss	6051.996
Autistic Sample-6	Male	Level 2	22q11.21	TBX1	Loss	6.035
			22q13.3	ALG12/CRELD2/PIM3/MIR6821/IL17REL/ TTLL8/MLC1/MOV10L1/PANX2/TRABD/ SELENOO/TUBGCP6/HDAC10/MAPK12/ MAPK11/PLXNB2/DENND6B/PPP6R2/SBF1/ ADM2/MIOX/LMF2/NCAPH2/SCO2/TYMP/ ODF3B/KLHDC7B/SYCE3/CPT1B/CHKB-CPT1B/CHKB/CHKB-AS1/MAPK8IP2/ ARSA/ SHANK3	Loss	852.313

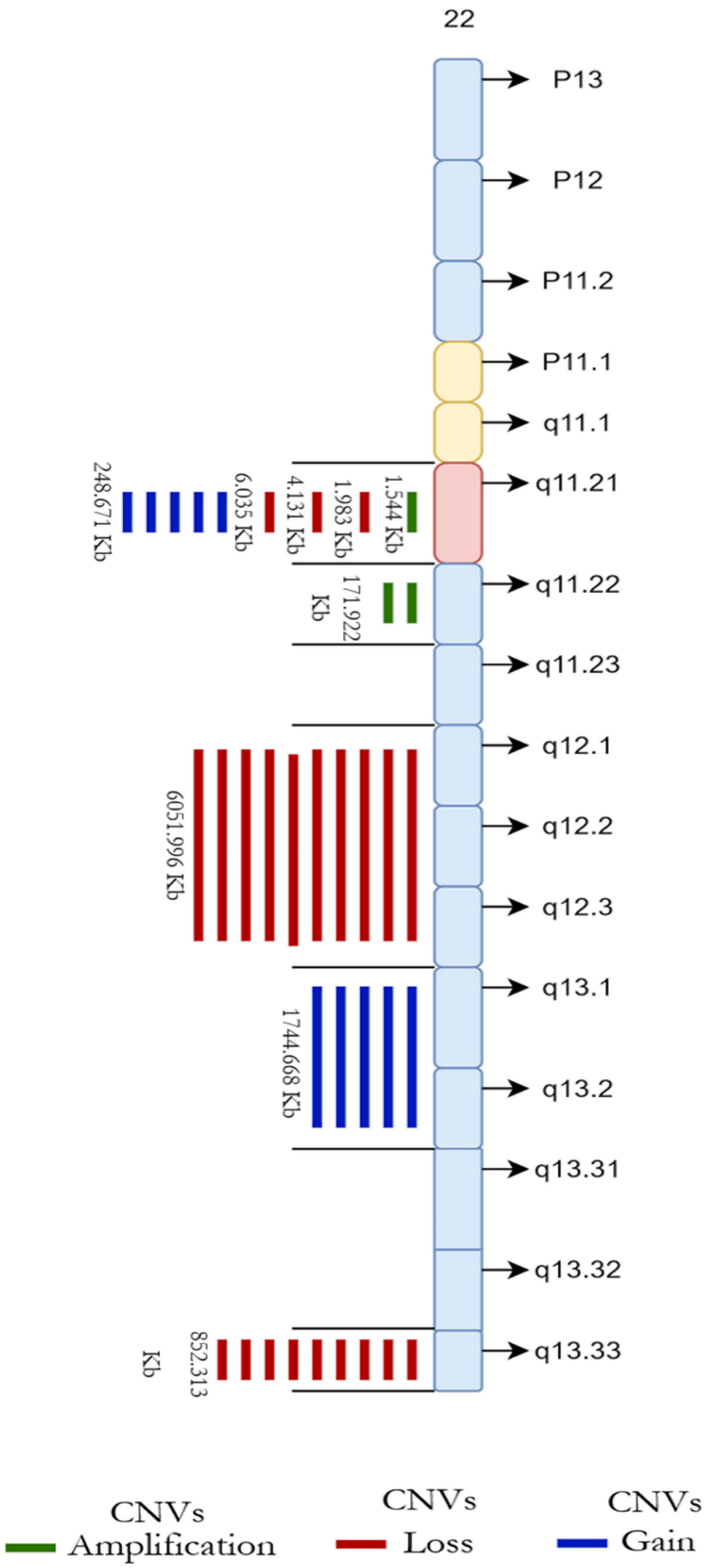

Figure 1 The number of predicted locations of CNV in Ch22 for 6 autistic samples. The majority of variants are found in 22q11.2.

The results for Primer A and B of TBX1 in control samples showed no mutations in the target sequences (Figure 2), while all autistic samples revealed mutations in many regions in the target sequences (Figure 3) (Figure S1,S2, and S3 in Supplementary Materials). As shown in Table 3 which revealed the type of mutations in each autistic sample and Table 2, mutation severity is related to the size of CNVs (Primer A and B sequences in Figure S4 in the supplementary).

A

Primer A

Score	Expect	Identities	Gaps	Strand
1206 bits(653)	0.0	653/653(100%)	0/653(0%)	Plus/Plus

```
Query  9    GGGGATTGTTAGGGACTGGGGGAGAGGAATGGGGAGTTGGCGTCTAGTGGGGACAGAGGT  68
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  96   GGGGATTGTTAGGGACTGGGGGAGAGGAATGGGGAGTTGGCGTCTAGTGGGGACAGAGGT  155

Query  69   TCCATTTTGCGTTCATCTGGAGGTGGACGCACAGCATCGGGAATACACAATGCCACTGAA  128
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  156  TCCATTTTGCGTTCATCTGGAGGTGGACGCACAGCATCGGGAATACACAATGCCACTGAA  215

Query  129  TTGTGTACTTCAACATGGTCACAATGGCACATTCTTTATTTTTTGTAGAGACAGGGTGTC  188
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  216  TTGTGTACTTCAACATGGTCACAATGGCACATTCTTTATTTTTTGTAGAGACAGGGTGTC  275

Query  189  ACTGTGTTGCCCAGGCTAGCCTCGAACTCGGGTTCAGGCTCTCCTTCCACCTCGGCCTCC  248
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  276  ACTGTGTTGCCCAGGCTAGCCTCGAACTCGGGTTCAGGCTCTCCTTCCACCTCGGCCTCC  335

Query  249  CAAAGTGCTGGGATTATAGGTGTGAGCCACCACACCTGGCCAAATTTGTGGGtttttggg  308
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  336  CAAAGTGCTGGGATTATAGGTGTGAGCCACCACACCTGGCCAAATTTGTGGGTTTTTTGG  395

Query  309  tttttatgttttGAAACAGAGTTTCTCCCTTGTCACCTAGGCTGGAGTACAGTGGCGCG  368
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  396  TTTTTATGTTTTTGAAACAGAGTTTCTCCCTTGTCACCTAGGCTGGAGTACAGTGGCGCG  455

Query  369  ATCTTGGCTCACTGCAACCTTTACCTCCTGGGTTCAAGCGATTCTCCTGCCTCAGCCTCC  428
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  456  ATCTTGGCTCACTGCAACCTTTACCTCCTGGGTTCAAGCGATTCTCCTGCCTCAGCCTCC  515

Query  429  CAAGTAGCTGGGATTACAGGCACCTGCCAACACACCCAGCTAATTTTTTCTATTTTTAGT  488
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  516  CAAGTAGCTGGGATTACAGGCACCTGCCAACACACCCAGCTAATTTTTTCTATTTTTAGT  575

Query  489  AGAGACGGGGTTTCACCATGTTGGCCAGGCTGGTCTCGAACTCCTGACCTCAGGTGATCC  548
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  576  AGAGACGGGGTTTCACCATGTTGGCCAGGCTGGTCTCGAACTCCTGACCTCAGGTGATCC  635

Query  549  GCCCACCTCAGCCTCCCAAAGTGCTGGGATTACAGGAGTGAGGCGCTGCGCCCAGCCAAA  608
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  636  GCCCACCTCAGCCTCCCAAAGTGCTGGGATTACAGGAGTGAGGCGCTGCGCCCAGCCAAA  695

Query  609  ATTTGTGTTTTTAAATGTATGTTTAACAATTTAAAAATCCTAGATCATCCACA  661
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  696  ATTTGTGTTTTTAAATGTATGTTTAACAATTTAAAAATCCTAGATCATCCACA  748
```

Figure 2 Continue.

B

Primer B

Score	Expect	Identities	Gaps	Strand
918 bits(497)	0.0	497/497(100%)	0/497(0%)	Plus/Plus

```
Query  3    CTGCTCTAGAAGAGCTCATGTTCCAGCAGGGACAGCTAACACCCAGGGGCCTGCACGGGG  62
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  46   CTGCTCTAGAAGAGCTCATGTTCCAGCAGGGACAGCTAACACCCAGGGGCCTGCACGGGG  105

Query  63   TGGAGGGCTCTTCAGAGGCAGGATCACCTCGGAGATGGGTGCAGGGGACCTCGGGCCACC  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  106  TGGAGGGCTCTTCAGAGGCAGGATCACCTCGGAGATGGGTGCAGGGGACCTCGGGCCACC  165

Query  123  CCAGGGCCAGGCCTGCTTGCCCTGCTTAGGAGCTGGGTCTCCCACTCAGGGAGGGAGGGG  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  166  CCAGGGCCAGGCCTGCTTGCCCTGCTTAGGAGCTGGGTCTCCCACTCAGGGAGGGAGGGG  225

Query  183  TCATGTCTCAAGGGCAGCCACTGGGCCTGAAAAGCAGAACCGCATGTGATCAGTCTGGGT  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226  TCATGTCTCAAGGGCAGCCACTGGGCCTGAAAAGCAGAACCGCATGTGATCAGTCTGGGT  285

Query  243  GGGCAAGACTTCAAGAGAGCACGCCTACCCTCAAGGGATGGAAGCAGAGGCTGGTAGCCG  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  286  GGGCAAGACTTCAAGAGAGCACGCCTACCCTCAAGGGATGGAAGCAGAGGCTGGTAGCCG  345

Query  303  ACGTCCATGCCAGCCACCAACTCAGAGCCAGCACACTACCCGCATGTGCCTCTGCCAATG  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  346  ACGTCCATGCCAGCCACCAACTCAGAGCCAGCACACTACCCGCATGTGCCTCTGCCAATG  405

Query  363  AGTGGATGAAAAGCAGCGACCTACAGATGCAGGCAACCTGGATGAGTCTTGACTTTATCT  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406  AGTGGATGAAAAGCAGCGACCTACAGATGCAGGCAACCTGGATGAGTCTTGACTTTATCT  465

Query  423  CACGGAGGGAAGGAAGCAGGCTCGGGAGGTGCCGTGCCGCGTGGCTCTGTTTATAAGCTG  482
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466  CACGGAGGGAAGGAAGCAGGCTCGGGAGGTGCCGTGCCGCGTGGCTCTGTTTATAAGCTG  525

Query  483  TCTGGGAAAAGGCAAAA  499
            |||||||||||||||||
Sbjct  526  TCTGGGAAAAGGCAAAA  542
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Figure 2 DNA sequencing for Primer A and B of TBX1 in healthy control showed no mutation in sequence thus the identity is 100%.

Discussion

Children with ASD suffer from problems with social communication and behavior, which can range from minor difficulty to a disability that needs full-time care. The prevalence of ASD has increased worldwide over time, and the etiology of ASD is complex and still under research. In recent years, many studies have highlighted that increasing CNV deletion or duplication could be a risk factor for psychiatric and neurodevelopmental disorders.[65–68]

Our findings revealed that six of the autistic samples have multiple CNVs in Ch22, which included significant genes (TBX1, LIF, SEPT5, GNB1L, YWHAH, SHANK3, PPARA, SYN3, TUG1, MLC1, PANX2, and HDAC10). The result of this study revealed that the 22q11.2 region contains CNVs that include genes that are essential for brain and cognitive

A

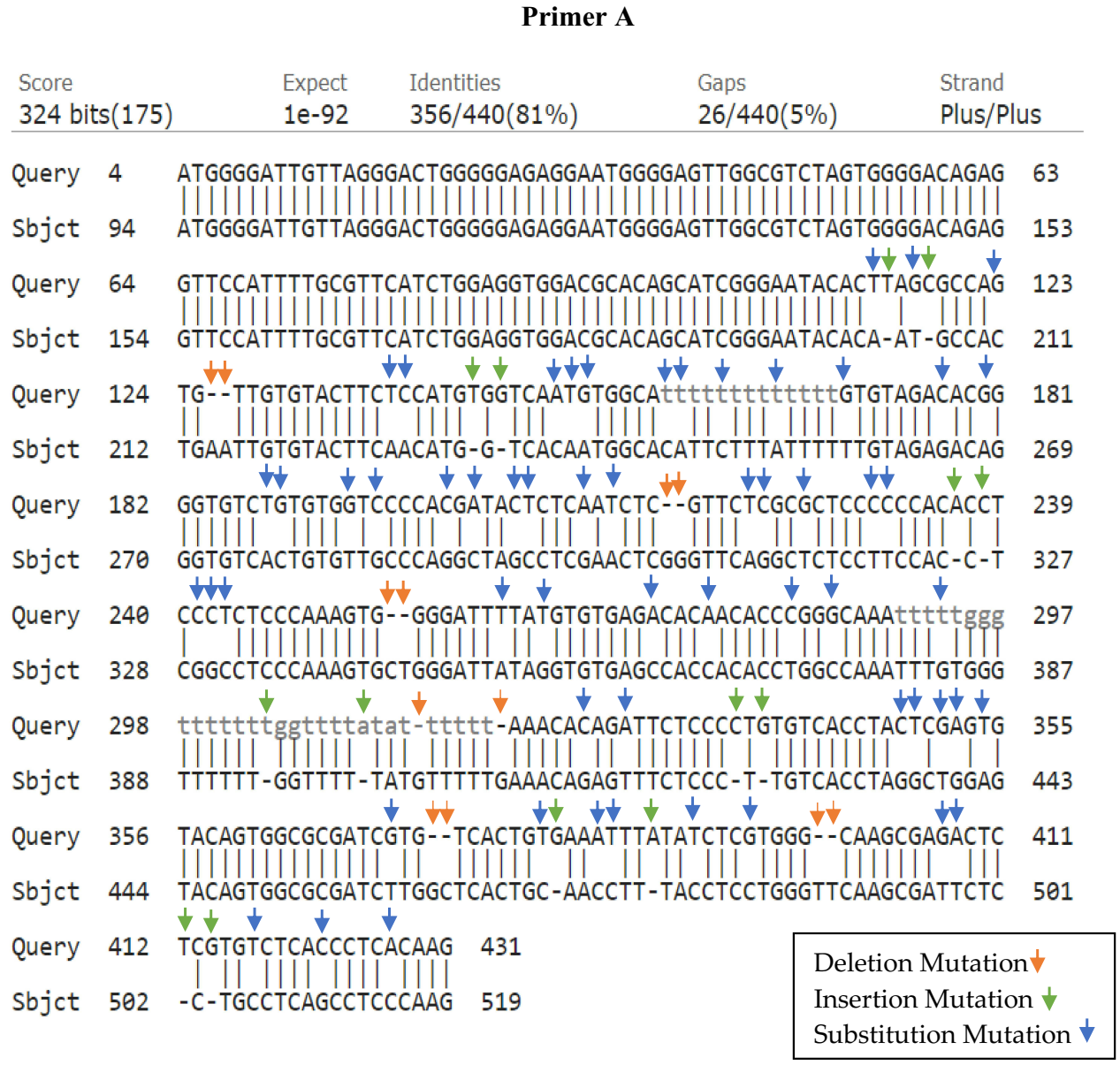

Figure 3 Continue.

development and may be related to ASD, such as TBX1 that could play a significant role in neurodevelopmental disorders.[17,21,39,50–52] Our study found CNV loss in the TBX1 gene in three samples, while two samples showed amplification. This finding is consistent with previous studies, which reported that several cases of CNVs in TBX1 are associated with ASD.[40,64] The results also showed CNV loss in LIF gene, which is related to neurodevelopmental disorders where the protein encoded by this gene is involved in induction of neuronal cell differentiation. A previous study has indicated that the LIF gene impairs brain development in mice related to the mature and immature nervous system.[69] Butler and Moudi's studies showed that genetic variations in the LIF gene may be related to neurodevelopmental disorders and related to increased susceptibility to schizophrenia and the degeneration of working memory function.[69,70] Thus, we suggest that the TBX1 and LIF genes may be candidates for ASD and deserve more focus and analysis.

B

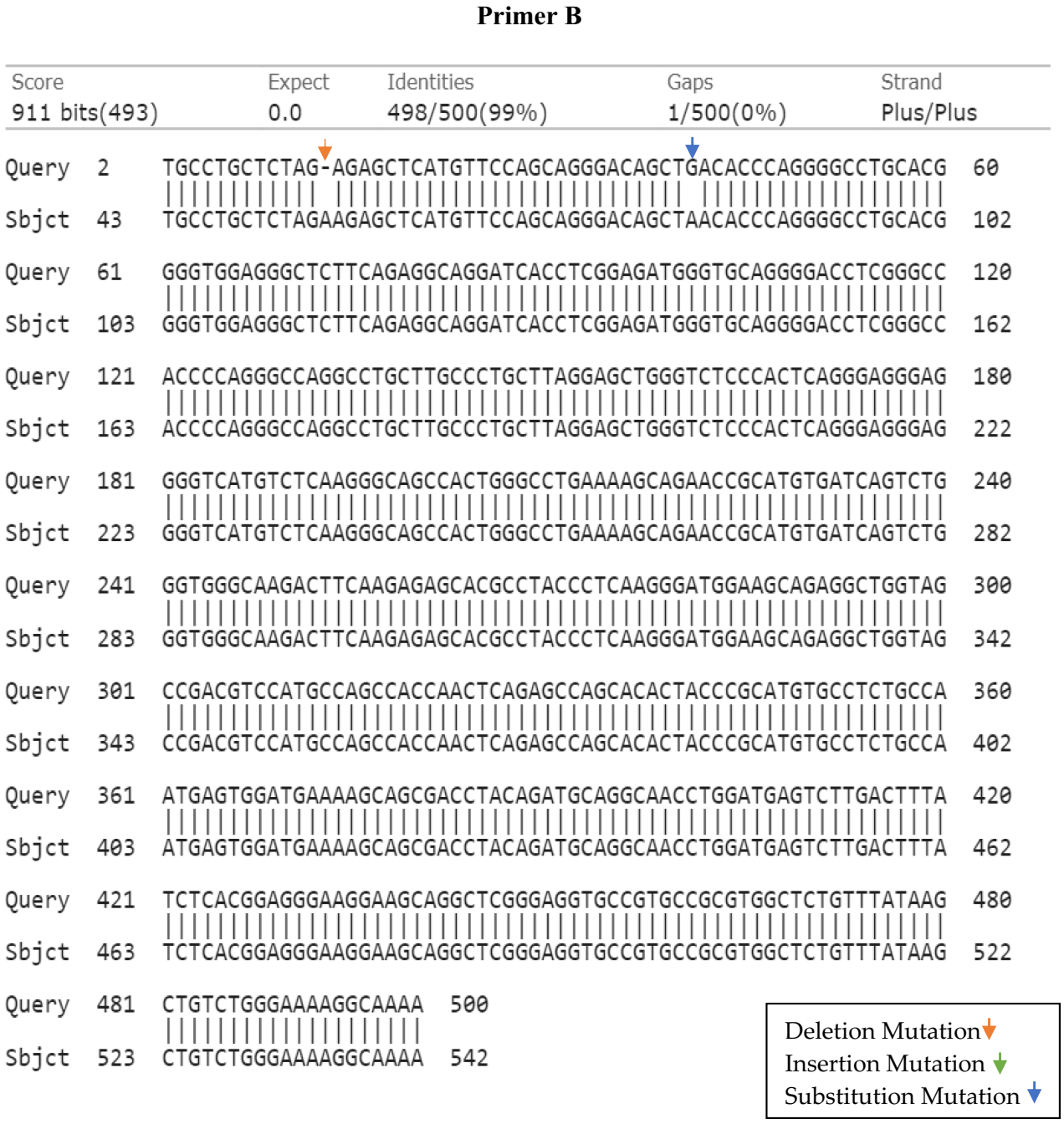

Figure 3 DNA sequencing for autistic sample 5 (Primer A and B of TBX1) revealed mutations in many regions at target sequences. (**A**) For primer A, there are 12 bases deletion,14 bases insertion, and 58 substitution mutations. (**B**) For primer B, there is one base deletion and one substitution mutation.

In addition, previous studies reported overexpressing of SEPT5 in ASD and suggested that SEPT5 is one of the candidate drivers of 22q11.2 synaptic pathology.[30] Moreover, Chen et al reported the involvement of GNB1L in ASDs.[41] Furthermore, many studies have revealed the possible implications of YWHAH in psychiatric disorders including ASD, schizophrenia, and bipolar disorder.[71–73] The result of the identification of CNV duplication in region 22q11.2 is consistent with those of Woodward et al who also found that nested 22q11.2 duplications between LCR22B and LCR22D could play a significant role in neurodevelopmental phenotypes that are associated with autism, such as

Table 3 Type of Mutations in Autistic Samples

Samples ID	Type of Mutations	
	Primer A	Primer B
Autistic Sample-2	Frameshift (two bases insertions) and three substitution mutations.	Frameshift (two bases deletions and one base insertion) and one substitution mutations.
Autistic Sample-4	Frameshift (five bases insertions) and two substitution mutations.	Frameshift (one base deletion) and two substitution mutations.
Autistic Sample-5	Frameshift (12 bases deletions and 14 bases insertions) and 58 substitution mutations	Frameshift (one base deletion) and one substitution mutations.
Autistic Sample-6	Frameshift (20 bases deletions and 17 bases insertions) and 62 substitution mutations.	Frameshift (one base deletion and two bases insertions) and two substitution mutations.

language delay and abnormal behaviors.[74] However, it is not yet known which gene in the 22q11.2 region is responsible for the observed autistic behavioral phenotypes.

Furthermore, 22q11.2DS affects brain development in different ways, one of them being brain dysfunction that may lead to ASD in cooperation with genetic, epigenetic, or environmental factors.[45] Moreover, individuals with 22q11.2DS present behavioral features associated with ASD.[51] In addition, 22q11.2DS has an effect on intellectual abilities and social cognition.[52] Furthermore, 22q11.2DS poses a disproportionate risk for the development of schizophrenia, congenital cardiac defects, congenital malformations, palatal abnormalities, immune deficiency, characteristic facial features, learning difficulties, and neurodevelopmental disorders including ASD.[45,52,65,75–78]

In the 22q13.3 region, this study found CNV deletion in SHANK3. This gene is a member of the Shank gene family that plays a role in synapse formation and dendritic spine maturation, and plays a role in normal neurodevelopment, where many studies have revealed that this gene is associated with neurodevelopment disorders including ASD.[79–84] However, SHANK3 is strongly related to ASD, and many studies have shown a relation between CNV deletion in SHANK3 and the ASD phenotype in autistic children.[80–83] CNV duplication in SHANK3 can lead to various neurodevelopment symptoms.[82] SHANK3 mutations are significantly associated with ASD; they are found in approximately 2% of ASD cases and can cause autism-like behavioral changes.[84] Moreover, previous studies have found large deletions in 22q13.3, which contains the autism gene SHANK3 and causes Phelan–McDermid syndrome.[85] For instance, PPARA on 22q13.3 is involved in regulating cellular energy metabolism, as well as playing a role in neuroprotection and synaptic plasticity.[64,86,87] Moreover, mutation and gene dysregulation were reported for SYN3 and TUG1 on 22q12.1 and q12.3, which strongly suggest their involvement in autism.[88,89] The SYN3 implicated in synaptogenesis and the modulation of neurotransmitter release, suggesting a potential role in several neuropsychiatric diseases.[88] While TUG1 is one of the long non-coding RNAs (lncRNAs) currently known as essential regulators that have been implicated in ASD, and a previous study revealed that some lncRNAs show altered expression levels in autistic brains.[89] Moreover, sample-6 showed CNVs loss on Ch22q13.3 which including many genes such as MLC1, PANX2, and HDAC10 were also found to be involved in ASD through various mechanisms. MLC1 mutation has been linked to brain disorders, such as epilepsies and autism.[90] PANX2 and HDAC10 were both found to be differentially expressed in the brains or blood of individuals with autism compared to controls in humans and rodents.[91,92]

Furthermore, the findings of the DNA sequencing of TBX1, which revealed different mutations in the region out of those in detected CNVs by aCGH, suggested that CNVs may cause/or consequence of genome instability leading to imbalances affect at other regions of the gene; thus, it may affect the gene expression. In addition, there is no relation between the severity of mutation and the size of CNVs in this study. These findings are in line with those of previous studies, which suggested that CNVs act as contributors to chromosome instability and that the relative contribution of CNV size to the mutation rate may vary across the genome.[23,93–95] However, contrary to previous study which demonstrated that CNV size has a significant effect on the severity of the phenotype across the spectrum of

neurodevelopmental diseases including ASD,[96] there is no relation between the severity of autism and the CNVs size as well as mutations type in our study.

Conclusions

Recently, the most significant topic studied in genetics is ASD because of the increasing prevalence of ASD worldwide. ASD is associated with many factors, such as genetic, epigenetic, and environmental effects. Therefore, the purpose of this study was to screen CNVs in Ch22 in autistic children and identify significant mutated genes in those regions. The findings of this study identified many CNVs in Ch22 in autistic patients. Our findings supported our hypothesis that there is a correlation between CNVs in Ch22 and ASD, and the study identified some mutated genes in those regions, such as TBX1, which could play a significant role in neurodevelopmental disorders including ASD. However, TBX1 is one of the candidate genes in Ch22 that needs more analysis, and it has an impact on social interaction and behavioral phenotypes that are related to ASD.

Finally, for future research in this field, we recommend using a large sample size of the Saudi population and conducting more genetic studies through the use of more advanced technical tools and analysis. In addition, we recommend applying gene expression analysis and studying protein levels and the function of TBX1 in autistic children.

Abbreviations

ASD, Autism Spectrum Disorder; Ch22, Chromosome 22; ADHD, attention deficit hyperactivity disorder; CDC, Centers for Disease Control and Prevention; ADDM, Autism and Developmental Disabilities Monitoring Network; CNVs, Copy number variations; (FISH), Fluorescent in situ hybridization; ADM-2, Aberration Detection Method 2.

Ethics Statement

This study was designed in correspondence to the codes of the guidelines for the Ethics Committee of Biomedical Research-Centre of Excellence in Genomic Medicine Research at King Abdul Aziz University, ethical approval number (02-CEGMR-Bioeth-2018). The study was executed in consensus with the guidelines followed in King Fahd Center for Medical Research, KAU, Jeddah, Saudi Arabia, which were in accordance with the Declaration of Helsinki.

Consent Statement

Informed consent forms were signed by the parents of the participants.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in relation to this work.

References

1. American Psychiatric Association. *DSM-5 Task Force. Diagnostic and Statistical Manual of Mental Disorders: DSM-5™*. 5th ed. American Psychiatric Publishing, Inc; 2013. doi:10.1176/appi.books.9780890425596
2. Persico AM, Napolioni V. Autism genetics. *Behav Brain Res*. 2013;251:95–112. doi:10.1016/j.bbr.2013.06.012
3. Almandil NB, Alkuroud DN, AbdulAzeez S, AlSulaiman A, Elaissari A, Borgio JF. Environmental and genetic factors in autism spectrum disorders: special emphasis on data from Arabian studies. *Int J Environ Res Public Health*. 2019;16(4):658. doi:10.3390/ijerph16040658
4. Hayretdag C, Algedik P, Ekmekci CG, et al. Determination of genetic changes in etiology of autism spectrum disorder in twins by whole-exome sequencing. *Gene Reports*. 2020;19:100618. doi:10.1016/j.genrep.2020.100618
5. Ergaz Z, Weinstein-Fudim L, Ornoy A. Genetic and non-genetic animal models for autism spectrum disorders (ASD). *Reprod Toxicol*. 2016;64:116–140. doi:10.1016/j.reprotox.2016.04.024
6. Ecker C, Bookheimer SY, Murphy DG. Neuroimaging in autism spectrum disorder: brain structure and function across the lifespan. *Lancet Neurol*. 2015;14(11):1121–1134. doi:10.1016/S1474-4422(15)00050-2
7. Li X, Zou H, Brown WT. Genes associated with autism spectrum disorder. *Brain Res Bull*. 2012;88(6):543–552. doi:10.1016/j.brainresbull.2012.05.017
8. Kogan MD, Vladutiu CJ, Schieve LA, et al. The prevalence of parent-reported autism spectrum disorder among US children. *Pediatrics*. 2018;142(6):e20174161. doi:10.1542/peds.2017-4161
9. CDC. Centers for Disease Control and prevention (CDC) and Autism and Developmental Disabilities Monitoring (ADDM) network. Available from: https://www.cdc.gov/ncbddd/autism/addm.html. Accessed July 15, 2022.
10. AlBatti T, Alsaghan L, Alsharif M. Prevalence of autism spectrum disorder among Saudi children between 2 and 4 years old in Riyadh. *Asian J Psychiatr*. 2022;71:103054. doi:10.1016/j.ajp.2022.103054
11. General Authority for Statistics; Kingdom of Saudi Arabia. 2017 نتائج مسح ذوي الإعاقة لعام [Disability Survey 2017]; 2017. Available from: https://www.stats.gov.sa/ar/904. Accessed July 15, 2022. Arabic.
12. Baio J, Wiggins L, Christensen DL, et al. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2014. *MMWR Surveill Summ*. 2018;67(6):1. doi:10.15585/mmwr.ss6706a1
13. Oikonomakis V, Kosma K, Mitrakos A, et al. Recurrent copy number variations as risk factors for autism spectrum disorders: analysis of the clinical implications. *Clin Genet*. 2016;89(6):708–718. doi:10.1111/cge.12740
14. Fine SE, Weissman A, Gerdes M, et al. Autism spectrum disorders and symptoms in children with molecularly confirmed 22q11. 2 deletion syndrome. *J Autism Dev Disord*. 2005;35(4):461–470. doi:10.1007/s10803-005-5036-9
15. Sealey L, Hughes B, Sriskanda A, et al. Environmental factors in the development of autism spectrum disorders. *Environ Int*. 2016;88:288–298. doi:10.1016/j.envint.2015.12.021
16. Geschwind DH. Genetics of autism spectrum disorders. *Trends Cogn Sci*. 2011;15(9):409–416. doi:10.1016/j.tics.2011.07.003
17. Cheroni C, Caporale N, Testa G. Autism spectrum disorder at the crossroad between genes and environment: contributions, convergences, and interactions in ASD developmental pathophysiology. *Mol Autism*. 2020;11:69. doi:10.1186/s13229-020-00370-1
18. Stefano V, Viviana C, Deny M, et al. Copy number variants in autism spectrum disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;92:421–427. doi:10.1016/j.pnpbp.2019.02.012
19. Sahin M, Sur M. Genes, circuits, and precision therapies for autism and related neurodevelopmental disorders. *Science*. 2015;350(6263):aab3897. doi:10.1126/science.aab3897
20. Masini E, Loi E, Vega-Benedetti AF, et al. An overview of the main genetic, epigenetic and environmental factors involved in autism spectrum disorder focusing on synaptic activity. *Int J Mol Sci*. 2020;21(21):8290. PMID: 33167418; PMCID: PMC7663950. doi:10.3390/ijms21218290
21. Takumi T, Tamada K, Hatanaka F, Nakai N, Bolton PF. Behavioral neuroscience of autism. *Neurosci Biobehav Rev*. 2020;110:60–76. doi:10.1016/j.neubiorev.2019.04.012
22. Yu P, Wang C, Xu Q, et al. Detection of copy number variations in rice using array-based comparative genomic hybridization. *BMC Genomics*. 2011;12(1):372. doi:10.1186/1471-2164-12-372
23. Zhang F, Gu W, Hurles ME, Lupski JR. Copy number variation in human health, disease, and evolution. *Annu Rev Genomics Hum Genet*. 2009;10(1):451–481. doi:10.1146/annurev.genom.9.081307.164217
24. Henrichsen CN, Chaignat E, Reymond A. Copy number variants, diseases and gene expression. *Hum Mol Genet*. 2009;18(R1):R1–R8. doi:10.1093/hmg/ddp011
25. Nakai N, Otsuka S, Myung J, Takumi T. Autism spectrum disorder model mice: focus on copy number variation and epigenetics. *Sci China Life Sci*. 2015;58(10):976–984. doi:10.1007/s11427-015-4891-7
26. Grayton HM, Fernandes C, Rujescu D, Collier DA. Copy number variations in neurodevelopmental disorders. *Prog Neurobiol*. 2012;99(1):81–91. doi:10.1016/j.pneurobio.2012.07.005
27. Scherer SW, Lee C, Birney E, et al. Challenges and standards in integrating surveys of structural variation. *Nat Genet*. 2007;39(7s):S7–S15. doi:10.1038/ng2093
28. Magini P, Scarano E, Donati I, et al. Challenges in the clinical interpretation of small de novo copy number variants in neurodevelopmental disorders. *Gene*. 2019;706:162–171. doi:10.1016/j.gene.2019.05.007
29. Bacchelli E, Loi E, Cameli C, et al. Analysis of a Sardinian multiplex family with autism spectrum disorder points to post-synaptic density gene variants and identifies CAPG as a functionally relevant candidate gene. *J Clin Med*. 2019;8(2):212. PMID: 30736458; PMCID: PMC6406497. doi:10.3390/jcm8020212
30. Forsyth JK, Nachun D, Gandal MJ, et al. Synaptic and gene regulatory mechanisms in schizophrenia, autism, and 22q11. 2 copy number variant–mediated risk for neuropsychiatric disorders. *Biol Psychiatry*. 2020;87(2):150–163. doi:10.1016/j.biopsych.2019.06.029
31. Mehta D, Iwamoto K, Ueda J, et al. Comprehensive survey of CNVs influencing gene expression in the human brain and its implications for pathophysiology. *Neurosci Res*. 2014;79:22–33. doi:10.1016/j.neures.2013.10.009
32. Korbel JO, Urban AE, Affourtit JP, et al. Paired-end mapping reveals extensive structural variation in the human genome. *Science*. 2007;318(5849):420–426. doi:10.1126/science.1149504

33. Mills RE, Walter K, Stewart C, et al. Mapping copy number variation by population-scale genome sequencing. *Nature*. 2011;470(7332):59–65. doi:10.1038/nature09708
34. Sudmant PH, Kitzman JO, Antonacci F, et al. Diversity of human copy number variation and multicopy genes. *Science*. 2010;330(6004):641–646. doi:10.1126/science.1197005
35. Dunham I, Hunt A, Collins J, et al. The DNA sequence of human chromosome 22. *Nature*. 1999;402(6761):489–495. doi:10.1038/990031
36. NCBI. Homo sapiens chromosome 22. Available from: https://www.ncbi.nlm.nih.gov/nucleotide/CM000684.2. Accessed July 15, 2022.
37. (EMBL-EBI) TEBI. Chromosome 22: 1-50,818,468; 2022. Available from: http://asia.ensembl.org/Homo_sapiens/Location/Chromosome?chr=22;r=22:1-50818468. Accessed July 15, 2022.
38. Riley JD, Delahunty C, Alsadah A, Mazzola S, Astbury C. Further evidence of GABRA4 and TOP3B as autism susceptibility genes. *Eur J Med Genet*. 2020;63(5):103876. doi:10.1016/j.ejmg.2020.103876
39. Betancur C. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. *Brain Res*. 2011;1380:42–77. doi:10.1016/j.brainres.2010.11.078
40. Hiramoto T, Kang G, Suzuki G, et al. Tbx1: identification of a 22q11. 2 gene as a risk factor for autism spectrum disorder in a mouse model. *Hum Mol Genet*. 2011;20(24):4775–4785. doi:10.1093/hmg/ddr404
41. Chen YZ, Matsushita M, Girirajan S, et al. Evidence for involvement of GNB1L in autism. *Am J Med Genet Part B Neuropsychiatr Gene*. 2012;159(1):61–71. doi:10.1002/ajmg.b.32002
42. Jonas RK, Montojo CA, Bearden CE. The 22q11. 2 deletion syndrome as a window into complex neuropsychiatric disorders over the lifespan. *Biol Psychiatry*. 2014;75(5):351–360. doi:10.1016/j.biopsych.2013.07.019
43. Jacquemont M-L, Sanlaville D, Redon R, et al. Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. *J Med Genet*. 2006;43(11):843–849. doi:10.1136/jmg.2006.043166
44. O'donovan MC, Kirov G, Owen MJ. Phenotypic variations on the theme of CNVs. *Nat Genet*. 2008;40(12):1392–1393. doi:10.1038/ng1208-1392
45. Vorstman JA, Breetvelt EJ, Thode KI, Chow EW, Bassett AS. Expression of autism spectrum and schizophrenia in patients with a 22q11. 2 deletion. *Schizophr Res*. 2013;143(1):55–59. doi:10.1016/j.schres.2012.10.010
46. Gamsiz ED, Sciarra LN, Maguire AM, Pescosolido MF, van Dyck LI, Morrow EM. Discovery of rare mutations in autism: elucidating neurodevelopmental mechanisms. *Neurotherapeutics*. 2015;12(3):553–571. doi:10.1007/s13311-015-0363-9
47. Safari MR, Ghafouri-Fard S, Noroozi R, et al. FOXP3 gene variations and susceptibility to autism: a case–control study. *Gene*. 2017;596:119–122. doi:10.1016/j.gene.2016.10.019
48. Noroozi R, Taheri M, Movafagh A, et al. Glutamate receptor, metabotropic 7 (GRM7) gene variations and susceptibility to autism: a case–control study. *Autism Res*. 2016;9(11):1161–1168. doi:10.1002/aur.1640
49. Taraska JW, Zagotta WN. Fluorescence applications in molecular neurobiology. *Neuron*. 2010;66(2):170–189. doi:10.1016/j.neuron.2010.02.002
50. Wenger TL, Miller JS, DePolo LM, et al. 22q11. 2 duplication syndrome: elevated rate of autism spectrum disorder and need for medical screening. *Mol Autism*. 2016;7(1):27. doi:10.1186/s13229-016-0090-z
51. Ousley O, Evans A, Fernandez-Carriba S, et al. Examining the overlap between autism spectrum disorder and 22q11. 2 deletion syndrome. *Int J Mol Sci*. 2017;18(5):1071. doi:10.3390/ijms18051071
52. Lin A, Vajdi A, Kushan-Wells L, et al. Reciprocal copy number variations at 22q11. 2 produce distinct and convergent neurobehavioral impairments relevant for Schizophrenia and Autism Spectrum Disorder. *Biol Psychiatry*. 2020;88(3):260–272. doi:10.1016/j.biopsych.2019.12.028
53. Clements CC, Wenger TL, Zoltowski AR, et al. Critical region within 22q11.2 linked to higher rate of autism spectrum disorder. *Mol Autism*. 2017;8(1):58. doi:10.1186/s13229-017-0171-7
54. Paylor R, Lindsay E. Mouse models of 22q11 deletion syndrome. *Biol Psychiatry*. 2006;59(12):1172–1179. doi:10.1016/j.biopsych.2006.01.018
55. Yagi H, Furutani Y, Hamada H, et al. Role of TBX1 in human del22q11. 2 syndrome. *Lancet*. 2003;362(9393):1366–1373. doi:10.1016/S0140-6736(03)14632-6
56. El Omari K, De Mesmaeker J, Karia D, Ginn H, Bhattacharya S, Mancini EJ. Structure of the DNA-bound T-box domain of human TBX1, a transcription factor associated with the DiGeorge syndrome. *Proteins Struct Funct Genet*. 2012;80(2):655–660. doi:10.1002/prot.23208
57. Wang H, Chen D, Ma L, et al. Genetic analysis of the TBX1 gene promoter in ventricular septal defects. *Mol Cell Biochem*. 2012;370(1):53–58. doi:10.1007/s11010-012-1397-5
58. Xu Y-J, Chen S, Zhang J, et al. Novel TBX1 loss-of-function mutation causes isolated conotruncal heart defects in Chinese patients without 22q11. 2 deletion. *BMC Med Genet*. 2014;15(1):1–9. doi:10.1186/1471-2350-15-78
59. Jaouadi A, Tabebi M, Abdelhedi F, et al. A novel TBX1 missense mutation in patients with syndromic congenital heart defects. *Biochem Biophys Res Commun*. 2018;499(3):563–569. doi:10.1016/j.bbrc.2018.03.190
60. Li D, Gordon CT, Oufadem M, et al. Heterozygous mutations in TBX1 as a cause of isolated hypoparathyroidism. *J Clin Endocrinol Metab*. 2018;103(11):4023–4032. doi:10.1210/jc.2018-01260
61. Jerome LA, Papaioannou VE. DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. *Nat Genet*. 2001;27(3):286–291. doi:10.1038/85845
62. Lindsay EA, Vitelli F, Su H, et al. Tbx1 haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature*. 2001;410(6824):97–101. doi:10.1038/35065105
63. Merscher S, Funke B, Epstein JA, et al. TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell*. 2001;104(4):619–629. doi:10.1016/S0092-8674(01)00247-1
64. Paylor R, Glaser B, Mupo A, et al. Tbx1 haploinsufficiency is linked to behavioral disorders in mice and humans: implications for 22q11 deletion syndrome. *Proc Natl Acad Sci*. 2006;103(20):7729–7734. doi:10.1073/pnas.0600206103
65. Schneider M, Debbané M, Bassett AS, et al. Psychiatric disorders from childhood to adulthood in 22q11. 2 deletion syndrome: results from the International Consortium on Brain and Behavior in 22q11. 2 Deletion Syndrome. *Am J Psychiatry*. 2014;171(6):627–639. doi:10.1176/appi.ajp.2013.13070864
66. Monks S, Niarchou M, Davies AR, et al. Further evidence for high rates of schizophrenia in 22q11. 2 deletion syndrome. *Schizophr Res*. 2014;153(1–3):231–236. doi:10.1016/j.schres.2014.01.020

67. Girirajan S, Eichler EE. Phenotypic variability and genetic susceptibility to genomic disorders. *Hum Mol Genet*. 2010;19(R2):R176–R87. doi:10.1093/hmg/ddq366
68. Crespi BJ, Crofts HJ. Association testing of copy number variants in schizophrenia and autism spectrum disorders. *J Neurodev Disord*. 2012;4 (1):15. doi:10.1186/1866-1955-4-15
69. Moudi M, Sargazi S, Heidarinia M, et al. Polymorphism in the 3′-UTR of LIF but not in the ATF6B gene associates with schizophrenia susceptibility: a case-control study and in silico analyses. *J Mol Neurosci*. 2020;70(12):2093–2101. doi:10.1007/s12031-020-01616-6
70. Butler MG, Mcguire AB, Masoud H, Manzardo AM. Currently recognized genes for schizophrenia: high-resolution chromosome ideogram representation. *Am J Med Genet Part B Neuropsychiatr Gene*. 2016;171:181–202. doi:10.1002/ajmg.b.32391
71. Grover D, Verma R, Goes FS, et al. Family-based association of YWHAH in psychotic bipolar disorder. *Am J Med Genet Part B Neuropsychiatr Genet*. 2009;150:977–983. doi:10.1002/ajmg.b.30927
72. Torrico B, Antón-Galindo E, Fernàndez-Castillo N, et al. Involvement of the 14-3-3 gene family in autism spectrum disorder and schizophrenia: genetics, transcriptomics and functional analyses. *J Clin Med*. 2020;9:1851. doi:10.3390/jcm9061851
73. Cormand B, Torrico B, Ghorbani S, et al. M13 - contribution of The 14-3-3 gene family to autism spectrum disorder. *Eur Neuropsychopharmacol*. 2017;27:S374–S375. doi:10.1016/j.euroneuro.2016.09.404
74. Woodward KJ, Stampalia J, Vanyai H, et al. Atypical nested 22q11.2 duplications between LCR22B and LCR22D are associated with neurodevelopmental phenotypes including autism spectrum disorder with incomplete penetrance. *Mol Genet Genomic Med*. 2019;7(2):e00507. doi:10.1002/mgg3.507
75. McDonald-McGinn DM, Sullivan KE, Marino B, et al. 22q11. 2 deletion syndrome. *Nat Rev Dis Primers*. 2015;1(1):1–19. doi:10.1038/nrdp.2015.71
76. Hoeffding LK, Trabjerg BB, Olsen L, et al. Risk of psychiatric disorders among individuals with the 22q11. 2 deletion or duplication: a Danish Nationwide, register-based study. *JAMA Psychiatry*. 2017;74(3):282–290. doi:10.1001/jamapsychiatry.2016.3939
77. Olsen L, Sparsø T, Weinsheimer SM, et al. Prevalence of rearrangements in the 22q11. 2 region and population-based risk of neuropsychiatric and developmental disorders in a Danish population: a case-cohort study. *Lancet Psychiatry*. 2018;5(7):573–580. doi:10.1016/S2215-0366(18)30168-8
78. McDonald-McGinn DM, Hain HS, Emanuel BS, et al. 22q11.2 deletion syn-drome. In: Adam MP, Ardinger HH, Pagon RA, editors. *GeneReviews®*. Seattle: University of Washington, Seattle; 1999.
79. Moreira ES, Silva IM, Lourenco N, et al. Detection of small copy number variations (CNVs) in autism spectrum disorder (ASD) by custom array comparative genomic hybridization (aCGH). *Res Autism Spectr Disord*. 2016;23:145–151. doi:10.1016/j.rasd.2015.12.012
80. Yi F, Danko T, Botelho SC, et al. Autism-associated SHANK3 haploinsufficiency causes Ih channelopathy in human neurons. *Science*. 2016;352: aaf2669.
81. Moreira DP, Griesi-Oliveira K, Bossolani-Maetins AL, et al. Investigation of 15q11-q13, 16p11. 2 and 22q13 CNVs in autism spectrum disorder Brazilian individuals with and without epilepsy. *PLoS One*. 2014;9:e107705. doi:10.1371/journal.pone.0107705
82. Meguid NA, Eid OM, Reda M, Elalfy DY, Hussein F. Copy number variations of SHANK3 and related sensory profiles in Egyptian children with autism spectrum disorder. *Res Autism Spectr Disord*. 2020;75:101558. doi:10.1016/j.rasd.2020.101558
83. Durand CM, Betancur C, Boeckers TM, et al. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet*. 2007;39:25–27. doi:10.1038/ng1933
84. Chen J, Yu S, Fu Y, Li X. Synaptic proteins and receptors defects in autism spectrum disorders. *Front Cell Neurosci*. 2014;8:276. doi:10.3389/fncel.2014.00276
85. Griswold AJ, Ma D, HN C, et al. Evaluation of copy number variations reveals novel candidate genes in autism spectrum disorder-associated pathways. *Hum Mol Genet*. 2012;21(15):3513–3523. doi:10.1093/hmg/dds164
86. Barone R, Rizzo R, Tabbì G, Malaguarnera M, Frye RE, Bastin J. Nuclear Peroxisome Proliferator-Activated Receptors (PPARs) as therapeutic targets of resveratrol for autism spectrum disorder. *Int J Mol Sci*. 2019;20(8):1878. PMID: 30995737; PMCID: PMC6515064. doi:10.3390/ijms20081878
87. Pierrot N, Ris L, Stancu IC, et al. Sex-regulated gene dosage effect of PPARα on synaptic plasticity. *Life Sci Alliance*. 2019;2(2):e201800262. doi:10.26508/lsa.201800262
88. Giovedí S, Corradi A, Fassio A, Benfenati F. Involvement of synaptic genes in the pathogenesis of autism spectrum disorders: the case of synapsins. *Front Pediatr*. 2014;2:94. PMID: 25237665; PMCID: PMC4154395. doi:10.3389/fped.2014.00094
89. Ghafouri-Fard S, Noroozi R, Brand S, et al. Emerging role of non-coding RNAs in autism spectrum disorder. *J Mol Neurosci*. 2022;72(2):201–216. doi:10.1007/s12031-021-01934-3
90. Brignone Maria S, Angela L, Serena C, et al. MLC1 protein: a likely link between leukodystrophies and brain channelopathies. *Front Cell Neurosci*. 2015;9. doi:10.3389/fncel.2015.00106
91. Davis LK, Gamazon ER, Kistner-Griffin E, et al. Loci nominally associated with autism from genome-wide analysis show enrichment of brain expression quantitative trait loci but not lymphoblastoid cell line expression quantitative trait loci. *Mol Autism*. 2012;3(1):3. PMID: 22591576; PMCID: PMC3484025. doi:10.1186/2040-2392-3-3
92. Qin L, Ma K, Wang ZJ, et al. Social deficits in Shank3-deficient mouse models of autism are rescued by histone deacetylase (HDAC) inhibition. *Nat Neurosci*. 2018;21(4):564–575. doi:10.1038/s41593-018-0110-8
93. Yuan B, Wang L, Liu P, et al. CNVs cause autosomal recessive genetic diseases with or without involvement of SNV/indels. *Genet Med*. 2020;22 (10):1633–1634. doi:10.1038/s41436-020-0864-8
94. Iourov IY, Vorsanova SG, Kurinnaia OS, et al. Causes and consequences of genome instability in psychiatric and neurodegenerative diseases. *Mol Biol*. 2021;55(1):37–46. doi:10.1134/S0026893321010155
95. Andriani GA, Vijg J, Montagna C. Mechanisms and consequences of aneuploidy and chromosome instability in the aging brain. *Mech Ageing Dev*. 2017;161:19–36. doi:10.1016/j.mad.2016.03.007
96. Chung B, Tao V, Tso W. Copy number variation and autism: new insights and clinical implications. *J Formos Med Assoc*. 2014;113(7):400–408. doi:10.1016/j.jfma.2013.01.005

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