Mutation screening of the *UBE3A* gene in Chinese Han population with autism

Xue Zhao
Shanghai Mental Health Center
Ran Zhang
Shanghai Mental Health Center
Shunying Yu (yushuny@yahoo.com)
Shanghai Mental Health Center

Research article

Keywords: ASD, 15q11-13, UBE3A, HRM, sequencing

Posted Date: July 27th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-40284/v1

Version of Record: A version of this preprint was published at BMC Psychiatry on December 11th, 2020. See the published version at https://doi.org/10.1186/s12888-020-03000-5.

Abstract

Background

15q11-13 region is one of the most complex chromosomal regions in the human genome. *UBE3A* is an important candidate gene of autism spectrum disorder (ASD), which located at the 15q11-13 region and encodes ubiquitin-protein ligase E3A. Previous studies about *UBE3A* gene and ASD have shown inconsistent results and few studies were performed in Chinese population. This study aimed to detect the genetic variation of *UBE3A* gene in Chinese Han population with ASD and analyze the genetic association between these variations and ASD.

Methods

The samples consisted of 192 patients with clinical diagnosis of autism according to the DSM-IV diagnostic criteria and 192 healthy controls. We searched for mutations at coding sequence (CDS) regions and their adjacent non-coding regions of *UBE3A* gene using the high resolution melting (HRM) and Sanger sequencing methods. We further increased sample size to validate the detected variants using HRM and analyzed the difference of allele and genotype frequencies between case and control groups.

Results

A known single nucleotide polymorphism (T > C, rs150331504) located at the CDS4 and a known 5 bp insertion/deletion variation (AACTC+/-, rs71127053) located at the intron region of the upstream 288 bp of the CDS2 of *UBE3A* gene were detected using Sanger sequencing method. The association analysis suggested that there were no significant difference about the allele and genotype frequencies of rs71127053 and rs150331504 between case and control groups after extending the sample size. Besides, rs150331504 is a synonymous mutation and we analyzed the minimum free energy (MFE) of mRNA coded by the different alleles of rs150331504. The result showed that MFE values of the allele T and allele C of rs150331504 were different and indicated that the variant might alter the mRNA secondary structure. We did not detect mutations in other coding regions of *UBE3A* gene.

Conclusions

These findings showed that *UBE3A* gene might not be a major disease gene in Chinese ASD cases.

Background

Autism spectrum disorder (ASD) is a set of childhood-onset neurodevelopmental disorders characterized by abnormal social interactions, impaired verbal and nonverbal communication, and the presence of restricted interests and repetitive behaviors with long-term persistence of core features and functional impairment and the onset of ASD is usually before 3 years old. ASD includes disorders that were previously diagnosed separately, such as autistic disorder, Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified in the fifth edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [1]. In the USA, the prevalence of ASD increased in the past decade according to the report of Centers for Disease Control and Prevention of USA [2]. The estimated prevalence of ASD in Chinese population ranged from 2.8 to 29.5 per 10,000 persons according to a review that summarized the findings in Chinese population from several areas [3]. Although epidemiological studies have found a number of risk factors for ASD, such as maternal pregnancy and pregnancy complications [4, 5], the cause of ASD is not clear. Multiple twin and family studies have confirmed that genetic factors play an important role in the development of ASD and the heritability is 90% [6]. Recent genetic studies have found that hundreds of genetic variants, including common and rare variants, contributing to the occurrence of ASD.

15q11-q13 is a very complex chromosomal region and one of the most likely to occur abnormal regions in the genome, which is associated with a variety of neuropsychiatric diseases such as mental retardation and obsessive compulsive disorder [7]. Duplications of the 15q11-q13 region were the most frequently reported chromosomal aberration in individuals with ASD and most of duplications in this interval were caused by supernumerary chromosomes formed by the inverted duplication of proximal 15q, known as isodicentric chromosome 15 [idic (15)] [8]. The 15q11-q13 region consists of a large proximal domain (~ 2 Mb) of paternally expressed genes, a smaller maternal expression domain (MED: ~500 kb), and a large distal region (~ 2 Mb) of apparently biallelic expression [9]. The MED contains two known imprinted, maternally expressed genes, *UBE3A* and *ATP10C*. *UBE3A* is the causative gene in Angelman syndrome (AS) and encodes the E6-AP ubiquitin-protein ligase. Previous studies about *UBE3A* gene and ASD have shown inconsistent results. Cook et al. [10] conducted multiallelic transmission disequilibrium test (MTDT) on multiple markers of *UBE3A* gene in autistic disorder families, no correlation between these markers and autism was found. Nurmi et al. [11] reported that *UBE3A* gene was associated with autism for the first time, the researchers detected a significant linkage disequilibrium between the D15S122 marker located in *UBE3A* gene and autism in 94 autistic families. Subsequently, Nurmi et al. [9] conducted linkage disequilibrium analysis on multiple SNPs and genetic markers of *UBE3*A and *ATP10C* genes in 100 families with autism, but the researchers failed to find evidence for D15S122 marker related to autism. In addition, although Kato et al. [12] did not find association evidence for single nucleotide polymorphism (SNP) of *UBE3A* gene and autism, there was association between *SNRPN* gene and autism, *SNRPN* gene regulated MED and further affected *UBE3A* gene. Glessner et al. [13] conducted genome-wide copy number variants (CNVs) screening in autism patients and healthy control, found that autism patients carried CNVs in multiple genes of ubiquitin system (*UBE3A*, *PARK2*, *RFWD2* and *FBXO40*). In terms of rare mutation screening, Veenstra-VanderWeele et al. [14] conducted mutation screening in the exons and promoters of *UBE3A* gene in 10 patients with autism, found no functional mutations that changed amino acid sequence. Schaaf et al. [15] screened mutations in 21 autism candidate genes including *UBE3A* gene in 339 high-functioning ASD, found non-synonymous mutations in *UBE3A* gene of ASD patients.

Given that there was few studies about *UBE3A* gene in Chinese Han people with ASD, we investigated mutation screening for coding region of *UBE3A* gene and explored the relationship between rare mutations of *UBE3A* gene and ASD in Chinese Han people.

Methods

Subjects

This case-control study was conducted on 192 children who were diagnosed as autism. The patients were fulfilling the criteria for the diagnosis of autism according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). The case group comprised 154 males and 38 females. They were recruited from the Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine. Their ages ranged from 2 to 18 years old (mean ± SD = 5.65 ± 3.04). Patients were excluded if they had known mental and physical illness or chromosomal abnormalities. The 192 healthy controls subjects were volunteers recruited by advertisement from Shanghai Mental Health Center and local community, who did not suffer from any severe physical diseases, as well as personal and family history of mental diseases according to the brief interviews by senior psychiatrists. The control group included 147 males and 45 females and their ages ranged from 2 to 65 years old (mean ± SD = 31.59 ± 19.01). All the subjects are Chinese Han descendant. Ethical approval was obtained from the ethics committees of Shanghai Mental Health Center and an informed written consent of participation in the study was signed by the parents or the legal guardians of the studied subjects.

Gene screening

Five millilitres of the whole blood was taken and ethylenediaminetetraacetic acid (EDTA) was used to be anticoagulant. DNA was isolated according to the established laboratory protocols.

Sequencing data were aligned to the annotated human genome sequence (hg19) using the BLAT tool of the UCSC Genome Browser (http://genome.ucsc.edu/). The *UBE3A* gene sequence was searched from the UCSC Genome Browser database, which showed that there were three spliceosomes (NM_130838, NM_000462 and NM_130839) of *UBE3A* gene. The sequence information of spliceosome 2 includes spliceosome 1 (NM_130838) and 3 (NM_130839). Therefore, the sequence information of spliceosome 2(NM_000462) was selected as the candidate sequence. *UBE3A* gene sequence has 14 exons, exon 1, 2, 3, initiation 36 bp of exon 4 and the terminal 1888 bp of exon 14 are non-coding regions (untranslated regions, UTR), the terminal 29 bp of the exon 4, exon 5, 6, 7, 8, 9, 10, 11, 12, 13 and initiation 121 bp of exon 14 are coding sequence (CDS) (Fig. 1). In this study, we performed mutation screening for all coding regions and their adjacent non-coding regions named by CDS1-CDS11.

The two methods high resolution melting (HRM) and Sanger sequencing were performed in our study. Finally, the HRM method was used to detect CDS 3, 8, 9, 10 and 11, and the remaining CDS were directly sequenced by Sanger method after a series of preliminary experiments.

HRM analysis

The CDS 3, 8, 9, 10 and 11 were amplified using polymerase chain reaction (PCR) and the primers information summarized in Table 1. The primers were designed to generate amplicons of 200–400 bp. Then HRM were performed using LightCycler® 96 Instrument (Roche Diagnostics, Roche Instrument Center AG, Rotkreuz, Switzerland) in DNA samples from autism patients and healthy controls. The amplifications were performed in 10 μL volumes containing 10 ng of genomic DNA, 2 μmol/L primers, 2.5 mmol/L MgCl2 and 5 μL 2X LightCycler® 96 High Resolution Melting Master (Roche Diagnostics) buffer. PCR cycling included an initial preincubation at 95℃ for 10 min, followed by 45 cycles of 10 s at 95 ℃, from 65℃ to a “touchdown” at 55℃, and 30 s at 72℃. The melting program included three steps: denaturation at 95℃ for 10 s, renaturation at 65℃ for 1 min, and a subsequent melting cycle consists of a continuous fluorescent reading from 60℃ to 90℃ at a rate of 25 acquisitions per ℃. Mutations were confirmed with an independent PCR and bidirectional sequencing.

Table 1
The primer information for HRM.

CDS		primer sequence	amplicon length(bp)
3	Forward	TCCCACATGGTTTTCAGGCA	397
	Reverse	GAGAGCTGTACTAATCACTGTGC	
8	Forward	TTTTGCAGACACCTGCTTTCTTA	251
	Reverse	GCAGCCCAATAACTTGTGTTTTGT	
9	Forward	GTCTGAAGCAAAATCACACACCC	256
	Reverse	ATATGTGGAAGCCGGGTAAGAA	
10	Forward	ACGAGGAATGCAAGGTTTTCG	242
	Reverse	ATGAATGCCAAACTGAAACCAGTA	
11	Forward	GTACTGGGACACTATCACCACC	285
	Reverse	TTTCCCATGACTTACAGTTTTCCTG	

Sanger Sequencing

CDS 1, 2, 4, 5, 6 and 7 were directly sequenced using Sanger method, which could detect about 800 bp sequence length. The length of CDS4 was 1247 bp, which was sequenced forward and backward. The PCR primers information were listed in Table 2. Each PCR master mix included 2 μM of each primer, 1 μl mix dNTP, 1.5 μl MgCl2, 5 μl of 10 × PCR buffer, (5 U/μl) AmpliTaq Gold DNA polymerase, 3 μl of DNA sample, and double-distilled water to reach the total volume of 50 μl. PCR condition contained initial denaturation at 95 °C for 5 min, 30 cycles of 95 °C for 30 s and 62 °C-65 °C for 30 s and 72 °C for 45 s, then 72 °C for 5 min as final extension was performed. After purification of PCR products using TaKaRa® Shrimp Alkaline Phosphatase (SAP) and Exonucleasel (ExonI), sequencing was performed by BigDye Terminator Kit (Applied Biosystems, USA) according to the method by Sanger F, et al [16]. The sequence information were read using Applied Biosystems (ABI) 3130 Genetic Analyzer (Applied Biosystems, USA). Mutations were confirmed with an independent PCR and bidirectional sequencing.

Table 2
The primer information for Sanger Sequencing.

CDS		primer sequence	amplicon length(bp)
1	Forward	GGTCTTGATTTGAATCGCAGAAA	779
	Reverse	CATTGACACCTAATTTGAAGCTTTG	
2	Forward	ATTTGCTTCTGCATCTTTCACTCT	639
	Reverse	TGTTGTATGGCCACCTGATCT	
4	Forward	TCCATGTGTTCCTATGCTATATGGT	1466
	Reverse	TGAGCCTAGAATGTTTGGCTGT	
5	Forward	CAGTCATGATGTGTGATTCTGGGT	603
	Reverse	TTCCATGTCCTGTGTAGTCCAG	
6	Forward	AGGCACACTCGTTGTAACTACC	800
	Reverse	CCGATGCCACCAAATTACTTACT	
7	Forward	GGGCTTTAGTGCCCAACTGTG	555
	Reverse	GGGACATCACAGTGACTGACAAT	

Association Analysis

For each detected variant, we further expanded the sample size of case and control subjects and case-control genotyping studies and association analysis were performed. Each detected variant of expanded samples were genotyped using HRM method.

For the association analysis, the online software SHEsis [17] (http://analysis2.bio-x.cn/myAnalysis.php) was used to compare the allelic and genotypic frequencies between the case and control groups. Another online software SNPStats [18] (http://bioinfo.iconcologia.net/snpstats/start.htm) was used to calculate the association between the detected variants and the risk of autism under 5 inheritance models, including codominant, dominant, recessive, overdominant and log-additive models. All of the statistical tests were two-sided, and $p < 0.05$ was defined as statistically significant.

Results

Results of mutation of screening

HRM method was used to detect mutations of CDS 3, 8, 9, 10, 11 and the results indicated that there were not mutations in CDS 3, 8, 9, 10 and 11 in our tested samples. The known insertion deletions (AACTC+/-, rs71127053) in the upstream 288 bp of CDS 2 and the synonymous mutation located on CDS4, rs150331504(T□C)were found in the both case and control groups using Sanger sequencing method. The frequencies of rs71127053 in the case and control groups were both 16/192 (0.083), 4/192 (0.02) in the case group and 1/192 (0.005) in the control group carried the rs150331504 mutation in our screening samples.

Results of association analysis

The case and control subject numbers were respectively 391 and 384 for rs71127053, were both 384 for rs150331504 after expanding the sample size in the association analysis. The association analysis for rs71127053 and rs150331504 revealed there was no significant differences in the distribution of allele (rs71127053: $p = 0.697$, OR = 1.096, 95%CI = 0.691–1.739; rs150331504: $p = 0.194$, OR = 2.013, 95%CI = 0.68–5.918) and genotype (rs71127053: $p = 0.134$;

rs150331504: p = 0.192) frequencies between the case and control groups (Table 3). The genotypes frequencies of rs71127053 showed no significant association with autism under 5 inheritance models (p > 0.05) (Table 4).

Table 3
The allele and genotype distributions of rs71127053 and rs150331504 in ASD patients and healthy controls.

	N	Allele N(%)		OR	95%CI	χ2	P	Genotype N(%)			χ2	P	P of HWE
rs71127053		AACTC-	AACTC+					11	12	22			
case	391	40(0.051)	742(0.949)	1.096	0.691–1.739	0.152	0.697	355(0.908)	32(0.082)	4(0.010)	4.014	0.134	0.858
control	384	36(0.047)	732(0.953)					349(0.909)	34(0.089)	1(0.003)			
rs150331504		T	C					TT	CT	CC			
case	384	758(0.987)	10(0.013)	2.013	0.68–5.918	1.683	0.194	374(0.974)	10(0.026)	0	1.699	0.192	0.898
control	384	763(0.993)	5(0.007)					379(0.987)	5(0.013)	0			
11 represents the wild-type homozygote, 12 represents the mutant heterozygote, and 22 represents the mutant homozygote.													

Table 4
Genotype comparison of rs71127053 under different inheritance models.

Model	genotype	OR(95%CI)	P-value	AIC
Codominant	1 1 vs 1 2	0.92(0.54–1.58)	0.31	1001.6
	1 1 vs 2 2	4.83(0.50-47.07)		
Dominant	1 1 vs 1 2–2 2	1.02(0.61–1.72)	0.94	1002
Recessive	1 1–1 2 vs2 2	4.87(0.50-47.39)	0.13	999.7
Overdominant	1 1–2 2 vs1 2	0.91(0.53–1.56)	0.74	1001.9
Log-additive	—	1.11(0.69–1.78)	0.66	1001.8
11 represents the wild-type homozygote, 12 represents the mutant heterozygote, and 22 represents the mutant homozygote				

Bioinformatics Analyses

The rs150331504 is a synonymous mutation, which has been shown to be functional according to alter the mRNA secondary structure[19]. Although association analysis results showed that there were no significant differences about the allele and genotype distributions of rs150331504 between the case and control groups, the case group carried the mutation frequency had obvious increasing trend. Therefore, the secondary structure and minimum free energy (MFE) of mRNA encoded by different alleles were predicted using the on-line analysis software RNAfold (http://rna.tbi.univie.ac.at//cgi-bin/RNAfold.cgi). The prediction results of MFE were shown in Table 5 and the results showed that the MFE of allele T and C of rs150331504 coding mRNA were different, indicated that the secondary structure of mRNA might be changed.

Table 5
The prediction results of MFE of allele T and C of rs150331504.

rs150331504	MFE of the optimal secondary structure	MFE of the centroid secondary structure
Allele T	-74.00 kcal/mol	-47.00 kcal/mol
Allele C	-73.30 kcal/mol	-52.80 kcal/mol

Discussion

UBE3A gene is a maternal imprinted gene located in 15q11-13, which encoded ubiquitin-protein ligase E3A that played an important role in the ubiquitin pathway, and it is also an important candidate gene of autism. Based on the genetic model of common disease rare variant (CDRV), our study investigated the role of *UBE3A* gene variation in the pathogenesis of autism. In our study, 192 patients meeting the diagnostic criteria of DSM-IV about autism and 192 healthy controls were included, *UBE3*A gene was selected as the candidate gene to conduct mutation screening on its coding region and adjacent non-coding regions.

We found a known variant rs71127053 (AACTC+/-) on the upstream 288 bp of CDS2. The frequencies of rs71127053 were both 0.083 (16/192) in the case and the control groups in our screening samples. The 1000 Genomic Project database showed that the Minor Allele Frequency (MAF) of rs71127053 was 0.0412 in the Chinese Han population, the NCBI database displayed the Global Minor Allele Frequency (GMAF) of rs71127053 was 0.1276 worldwide, rs71127053 was a common mutation. Subsequently, we expanded the sample size and conducted an association analysis. The results revealed that there

was no significant difference on the distribution frequencies of allele and genotype of rs71127053 between the case and control groups, rs71127053 did not increase the risk of autism under different inheritance models.

Another known variant rs150331504 (T > C) was detected in the CDS4 region. The 1000 genome project database showed that the MAF of rs150331504 in the Chinese Han population was 0.0103, and the GMAF of rs150331504 in the NCBI database was 0.0056 worldwide, rs150331504 was a rare variation (MAF < 5%) and synonymous mutation. Similarly, we expanded the sample size and subsequent association analysis showed no significant difference in allele and genotype distribution frequencies of rs150331504 between the case and control groups. It was generally believed that the non-synonymous mutations can change the amino acid coding sequence, which were related to disease. However, previous study found that non-synonymous SNPs and synonymous SNPs had the same effect on diseases incidence [20]. Nackley et al. [19] found that synonymous mutations would lead to mRNA structure change, and the most stable structure was associated with the lowest protein level and reduced enzyme activity. In our study, the frequency of rs150331504 was significantly increased in the case group. Considering the role of synonymous mutations in transcription and translation, we compared the MFE and secondary structure of mRNA encoded by different alleles of rs150331504.The results showed that there was a certain different MFE between the allele T and C of rs150331504, suggesting that this variation might change the secondary structure of mRNA.

We did not find any variation other than the two known variants, which might be due to the following reasons. First of all, we detected mutations for *UBE3A* gene in 192 samples of autism and 192 healthy controls in this study, although we expanded the sample size, which was undoubtedly very small for the association study. Secondly, we searched the results of genome-wide association analysis (GWAS) for autism in the Psychiatric Genomics Consortium database, no positive sites for *UBE3A* gene were found to be associated with autism at the genome-wide level. These results might suggest that common variations in the *UBE3A* gene were not associated with autism. In addition, we used DnaSP 5.0 software to calculate the nucleotide diversity of the 2628 bp coding region of *UBE3A* gene, and the π value was only 3.8×10^{-4}, indicating that the mutation probability of *UBE3A* gene was very low. At last, previous studies have confirmed that CNVs in region 15q11-13 was related to the risk of autism, indicating that compared with point mutations, structural variations might more easily change the gene function and lead to the occurrence of diseases. Though the results were negative, this study would extend our understanding about the role of *UBE3A* gene in ASD in different populations.

HRM and Sanger sequencing were used in our study simultaneously. Currently, HRM has been widely used in SNP typing, mutation screening and epigenetic studies, which is convenient, fast and much cheaper than sequencing technology. Tindall et al. [21] reported that sequences carrying one or more heterozygotes could be effectively distinguished from wild-type by HRM. HRM is widely used in the research of various diseases, especially tumor diseases. In the study of ASD, Kovac et al. [22] used this method to study the three genes SOD1, SOD2 and SOD3 and analyzed the relationship between these genes and ASD successfully. However, HRM also has some limitations. For example, the length of amplicons cannot be too long. Different from sequencing methods, the results of HRM experiment is the melting curve, which needs to be analyzed by software and draw a conclusion, so there would be some deviation in the interpretation of the result. Sequencing technology is the "gold standard" for mutation screening research at present. With the continuous emergence of second-generation sequencing and third-generation sequencing, it has been more and more favored by researchers. If the two methods were combined in a study, the total cost will be lower.

Conclusions

In this study, we investigated the role of *UBE3A* gene in ASD by comprehensive screening for coding sequence regions and their adjacent non-coding regions of *UBE3A* gen for mutations. There was no association between the two detected known variation rs150331504 and rs71127053 in *UBE3A* gene and ASD. These findings showed that *UBE3A* gene may not play an important role in our ASD cohort.

Abbreviations

ASD: autism spectrum disorder; CDS:coding sequence; HRM:high resolution melting; MFE:minimum free energy; DSM-5:the fifth edition of Diagnostic and Statistical Manual of Mental Disorders; [idic (15)]:isodicentric chromosome 15; MED:maternal expression domain; AS:Angelman syndrome; MTDT:multiallelic transmission disequilibrium test; SNP:single nucleotide polymorphism; CNVs:copy number variants; DSM-IV:the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders; EDTA:ethylenediaminetetraacetic acid; UTR:untranslated regions; PCR:polymerase chain reaction; CDRV:common disease rare variant; MAF:Minor Allele Frequency; GMAF Global Minor Allele Frequency; GWAS:genome-wide association analysis

Declarations

Funding

The research was supported by National Program on Key Basic Research Project (973 Program) (Grant No. 2012CB517900).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest. All the authors certify that the submission is an original work and it is not under review at any other journal.

Authors' contributions

ZX were responsible for writing and revising the article, as well as the experiment design and statistical methods. ZR were responsible for the guidance on experiment design. YS were responsible for the participant enrolment and the management and quality control of the research process. All authors gave their scientific contribution and have approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the ethics committees of Shanghai Mental Health Center and all participants and the parents or the legal guardians have written informed consent.

Consent for publication

Not applicable.

Acknowledgements

Not applicable.

Author details

[1]Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, 600 Wanping Nan Road, Shanghai 200030, China.

References

1. Doernberg E, Hollander E. Neurodevelopmental Disorders (ASD and ADHD): DSM-5, ICD-10, and ICD-11. CNS Spectr. 2016;21(4):295–9.
2. Baio JWL, Christensen DL, Maenner MJ, Daniels J, Warren Z, Kurzius-Spencer M, Zahorodny W, Robinson Rosenberg C, White T, Durkin MS, Imm P, Nikolaou L, Yeargin-Allsopp M, Lee LC, Harrington R, Lopez M, Fitzgerald RT, Hewitt A, Pettygrove S, Constantino JN, Vehorn A, Shenouda J, Hall-Lande J, Van Naarden Braun K, Dowling NF. <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):1–23.
3. Feng L, Li C, Chiu H, Lee TS, Spencer MD, Wong JC. Autism spectrum disorder in Chinese populations: a brief review. Asia Pac Psychiatry. 2013;5(2):54–60.
4. Brown AS, Sourander A, Hinkka-Yli-Salomaki S, McKeague IW, Sundvall J, Surcel HM. Elevated maternal C-reactive protein and autism in a national birth cohort. Mol Psychiatry. 2014;19(2):259–64.
5. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. Br J Psychiatry. 2009;195(1):7–14.
6. Ratajczak HV. Theoretical aspects of autism: causes–a review. J Immunotoxicol. 2011;8(1):68–79.
7. Delorme R, Moreno-De-Luca D, Gennetier A, Maier W, Chaste P, Mossner R, Grabe HJ, Ruhrmann S, Falkai P, Mouren MC, et al. Search for copy number variants in chromosomes 15q11-q13 and 22q11.2 in obsessive compulsive disorder. BMC Med Genet. 2010;11:100.
8. Depienne C, Moreno-De-Luca D, Heron D, Bouteiller D, Gennetier A, Delorme R, Chaste P, Siffroi JP, Chantot-Bastaraud S, Benyahia B, et al. Screening for genomic rearrangements and methylation abnormalities of the 15q11-q13 region in autism spectrum disorders. Biol Psychiatry. 2009;66(4):349–59.
9. Nurmi EL, Amin T, Olson LM, Jacobs MM, McCauley JL, Lam AY, Organ EL, Folstein SE, Haines JL, Sutcliffe JS. Dense linkage disequilibrium mapping in the 15q11-q13 maternal expression domain yields evidence for association in autism. Mol Psychiatry. 2003;8(6):624–34, 570.
10. Cook EH Jr, Cox CR, Lord NJ, Gonen C, Guter D, Lincoln SJ, Nix A, Haas K, Leventhal R, Courchesne BL. E.: Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. Am J Hum Genet. 1998;62(5):1077–83.
11. Nurmi EL, Bradford Y, Chen Y, Hall J, Arnone B, Gardiner MB, Hutcheson HB, Gilbert JR, Pericak-Vance MA, Copeland-Yates SA, et al. Linkage disequilibrium at the Angelman syndrome gene UBE3A in autism families. Genomics. 2001;77(1–2):105–13.
12. Kato C, Tochigi M, Ohashi J, Koishi S, Kawakubo Y, Yamamoto K, Matsumoto H, Hashimoto O, Kim SY, Watanabe K, et al. Association study of the 15q11-q13 maternal expression domain in Japanese autistic patients. Am J Med Genet B Neuropsychiatr Genet. 2008;147B(7):1008–12.
13. Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, Zhang H, Estes A, Brune CW, Bradfield JP, et al. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature. 2009;459(7246):569–73.
14. Veenstra-VanderWeele J, Gonen D, Leventhal BL, Cook EH Jr. Mutation screening of the UBE3A/E6-AP gene in autistic disorder. Mol Psychiatry. 1999;4(1):64–7.
15. Schaaf CP, Sabo A, Sakai Y, Crosby J, Muzny D, Hawes A, Lewis L, Akbar H, Varghese R, Boerwinkle E, et al. Oligogenic heterozygosity in individuals with high-functioning autism spectrum disorders. Hum Mol Genet. 2011;20(17):3366–75.
16. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A. 1977;74(12):5463–7.
17. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res. 2005;15(2):97–8.
18. Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006;22(15):1928–9.
19. Nackley AG, Shabalina SA, Tchivileva IE, Satterfield K, Korchynskyi O, Makarov SS, Maixner W, Diatchenko L. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. Science. 2006;314(5807):1930–3.
20. Sauna ZE, Kimchi-Sarfaty C. Understanding the contribution of synonymous mutations to human disease. Nat Rev Genet. 2011;12(10):683–91.

21. Tindall EA, Petersen DC, Woodbridge P, Schipany K, Hayes VM. Assessing high-resolution melt curve analysis for accurate detection of gene variants in complex DNA fragments. Hum Mutat. 2009;30(6):876–83.
22. Kovac J, Macedoni Luksic M, Trebusak Podkrajsek K, Klancar G, Battelino T. Rare single nucleotide polymorphisms in the regulatory regions of the superoxide dismutase genes in autism spectrum disorder. Autism Res. 2014;7(1):138–44.

Figures

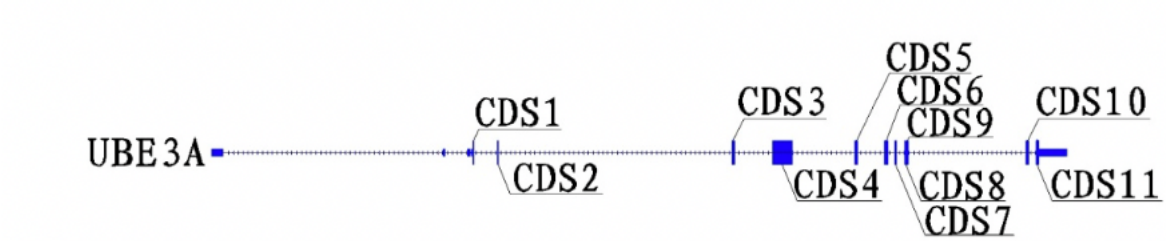

Figure 1

The illustration of coding regions of UBE3A gene.