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Identification of genetic mutations associated with autism in *GABRB3* gene in Iraqi autistic patients

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Abstract

This study was aimed to detect and identify genetic mutations in γ-aminobutyric acid receptor β3 subunit encoding gene *(GABRB3)* and its association with autism spectrum disorders. Forty autistic patients and 25 non-autistic as control group (5 unaffected sibling and 20 unrelated) with age range from 3 – 10 years were included in this study. Chromosomal DNA was extracted from blood samples followed by polymerase chain reaction (PCR) amplification of two targeted regions which include: (exon2-intron2-exon3) region and (exon 6) region of *GABRB3* for subsequent DNA sequencing. Identical bands related to the targeted regions were present in all samples. A sample of PCR products of patients and controls were sequenced. Sequencing results revealed the presence of four different single nucleotide polymorphism (SNPs) in four autistic patients (one SNP in exon2, two SNPs in intron 1 and one SNP in the beginning of intron 3) while four different autistic samples and three from control group had no mutations along this region. Exon 6 showed 100 % sequence identity in all samples (seven autistic and three from control group). The translation of nucleotides sequences into amino acid sequence revealed that the SNP in exon 2 caused a change of amino acid in the polypeptide sequence by changing the codon (AGA) which codes for arginine to (AAA) which codes for lysine. The online software tool, RaptorX was used for the prediction of the three dimensional structure of the polypeptides in which exon 2 carrying one SNP (30 a.a.) showed that 5 **(16%)** positions predicted as disordered, while the structure prediction of polypeptide translated from exon 3 was not obtained due to its small length (22 a.a.). Structure prediction of polypeptide of exon 6 (46 a.a.) showed that **(0%)** positions predicted as disordered, considering its lack of mutations. These results suggest the association of the detected SNPs with autism, especially the SNP located in exon 2 considering that it changed the polypeptide three dimensional structure, thus it could possibly alter its function. All SNPs are reported for the first time in this study except the one in intron 3 (rs755863611) which was previously reported.

Keywords: autism, *GABRB3*, autism spectrum disorders, SNPs.

التحري عن الطفرات الوراثية ضمن الجين *GABRB3* و المرتبطة بالتوحد في مرضى توحد عراقيين

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الخلاصة

اجريت هذه الدراسة بهدف التحري عن الطفرات الوراثية في الجين *GABRB3* الذي يشفر للوحدة الثانوية بيتا 3 ضمن مستقبل الحامض كاما- امينوبيوتيريك في مرضى طيف التوحد. شملت الدراسة اربعين طفل مصاب بالتوحد و خمس و عشرون اخرين غير مصابين بالتوحد كمجموعة سيطرة (خمس منهم من اشقاء الاطفال المصابين بالتوحد و عشرون اخرون غرباء) بمعدل عمر يتراوح بين 3 – 10 سنوات. تم استخلاص الحامض النووي منقوص الاوكسجين DNA من عينات دم المشتركين بالدراسة ، و استعملت تقنية تفاعل البلمرة المتسلسل PCR لتضخيم منطقتين ضمن الجين و هي (exon2-intron2-exon3) و (exon 6) و حدد تسلسل القواعد النتروجينية لهذه المناطق لعدد من العينات و لوحظ ظهور اربع تباينات فردية متعددة الاشكال للنيوكليوتيدة (SNP) في اربع عينات لمرضى التوحد (الاول ضمن المنطقة المشفرة exon 2 و اثنان ضمن المنطقة الغير مشفرة intron 1 و الاخير في المنطقة الغير مشفرة intron 3) في حين لم يلاحظ وجود اي تباين من نوع SNP ضمن هذه المناطق في اربع عينات لمرضى التوحد و ثلاث عينات من مجموعة السيطرة. أما بالنسبة للمنطقة المشفرة exon 6 ، فان تتابع القواعد النتروجينية جاء مطابقا بنسبة 100 % مع قاعدة البيانات NCBI ضمن هذه المناطق و لجميع العينات التي تم تحديد التتابع لها (7 عينات مرضى توحد و 3 عينات من مجموعة السيطرة). كشفت عملية ترجمة تسلسل القواعد النتروجينية للاحماض الامينية المشفرة لها عن ان التباين ضمن منطقة exon 2 قد سبب حدوث تغيير في حامض اميني ضمن سلسلة الببتيد المتعدد حيث تغير الكودون (AGA) و الذي يشفر للحامض الاميني الأرجنين الى الكودون (AAA) و الذي يشفر للحامض الاميني اللايسين. استعمل برنامج RaptorX للتنبؤ بالتركيب الثلاثي الابعاد لسلاسل الببتيد المترجمة من كل من المناطق المشفرة و أظهرت نتائج التنبؤ وجود 5 مواقع (16%) غير منتظمة ضمن منطقة exon 2 والمكونة من 30 حامض اميني ، بينما لم تحتوي منطقة exon 6 و المكونة من 46 حامض اميني على اي مناطق غير منتظمة ضمن التركيب الذي تم التنبؤ به لكونها خالية من التباين او الطفرات، اما بالنسبة للمنطقة exon 3 فلكونها قصيرة السلسلة الببتيدية (22 حتامض اميني) فانه يصعب الحصول على تنبؤ لتركيبها الثلاثي الابعاد .من خلال هذه النتائج يمكن الإشارة الى وجود ارتباط بين التباينات و مرض التوحد و خصوصا الطفرة ضمن المنطقة المشفرة exon 2 بعد الاخذ بالاعتبار كونها سببت حدوث تغيير في تسلسل الببتيد المتعدد و بالتالي تغيير التركيب ثلاثي الابعاد للبروتين مما قد يؤثر على وظيفته. تم التوصل لهذه التباينات (SNPs) لاول مرة ضمن هذه الدراسة ما عدا التباين (rs755863611) ضمن منطقة intron 3 .

Introduction:

Autism or (Autism Spectrum Disorders (ASDs) is a complex neurodevelopmental disorders characterized by severely impaired communication and a limited range of interests and behavior [1]. It was first described by Kanner in 1943. A diagnosis of autism can typically be made by 3 years of age [2]. The prevalence of diagnosed ASDs was continuously increasing since it was first described so that by the year 2014, it was estimated to be ~1% with male to female ratio of approximately 4:1, suggesting a possible imprinting effect and involvement of the sex chromosomes [3,4]. The exact cause of autism is unknown but it is believed to be multifactorial with a strong genetic influence [5], so it is considered as a genetically influenced neurodevelopmental disorder, with abundant evidence pointing to dysfunction at the level of the synapse. There is extensive genetic heterogeneity, with perhaps hundreds of genetic variants involved [6]. Compelling evidence for a the genetic basis for autism has been provided by family studies, particularly twin studies, which demonstrate a significantly higher concordance rate for monozygous versus dizygous twins, with an overall heritability of 80%–90% [7]. Autism spectrum disorders (ASDs) are mostly synaptic disorders due to the fact that dendritic spines have been found to be reduced in neurons of autistic patients; moreover, nearly all the genes associated with ASDs are involved in the formation, regulation, or normal function of the synapse [8,9]. Many studies have centered on genetic changes in ASD patients based on single-nucleotide polymorphisms (SNPs) analysis since they are considered genetic markers in studies of gene association with complex diseases [10]. Genetic complexity of ASDs has been investigated using genetic approaches such as cytogenetic analysis, genome-wide linkage and

association scans, and candidate gene analysis [11]. *GABRB3*, mapped to chromosome 15q12, encodes the subunit β3 in the GABAA receptor (Gamma-aminobutyric acid receptor type A) where GABA (Gamma-aminobutyric acid) is the main inhibitory neurotransmitter in the brain [12]. Based on many family studies, chromosome 15q11-q13 which includes ASD-related genes, such as *GABRB3*, *GABRA5* has been considered to be an autism candidate region [13]. The human *GABRB3* gene has 10 exons and contains two promoter regions upstream of the alternative first exons, exon 1a and exon 1. Both alternative first exons are predicted to encode a signal peptide [14]. The aim of this study was to detect and identify genetic mutations association with autisim in specific regions of *GABRB3* by direct sequencing.

Materials and Method

A sample of forty patients included 33 male and 7 female with age range from 3-10 years and diagnosed with ASDs were taken from two local institutes specialized in caring of autistic children which are; Happy Family Specialist Center (Baghdad / Mansour), Rahman Specialist Centre (Baghdad/Yarmouk) and from Central Hospital of Pediatrics (Baghdad). All parents received a comprehensive description of the study, and gave written informed consent for their children's participation. Control group included twenty five non-autistic children, five unaffected relatives (siblings) of patients which were all females and twenty unrelated children (10 females and 10 males) selected from Central Hospital of Pediatrics (Baghdad) in which they were attending for regular medical care. It was ensured that they had no neurological or psychological abnormalities as well as contagious diseases.

Three milliliters of blood were collected by vein puncture in EDTA anticoagulant tubes from all patients and control group then stirred gently for few seconds to avoid blood's clotting. Chromosomal DNA from blood samples were extracted using Wizard® Genomic DNA purification kit (Promega, USA) depending on the manufacturer's protocol. Agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA [15]. PCR reaction was used to amplify the two targeted regions: (exon2-intron2-exon3) region using the specific primers (Forward: 5' – AAGGCTTTTCGGCATCTTCT - 3' and Reverse: 3' – CACTGTGGACGCCTGTGAT - 5') and exon 6 using the specific primers (Forward: 5'- AAACATAGCAGAGCCGCAAC-3' and Reverse: 3'-AGAGAGGAGGGGTGTGTGTG-5') (Alpha DNA Company, Canada), these primers were designed by Chen *et al*., (2014) [11]. Components of PCR reaction and mixing amounts for both exon 1a promoter and exon 8 regions are shown in Table-1.

Table 1- Components of reaction for amplification of **(exon2-intron2-exon3)** region and **exon** 6 of *GABRB3*

Component	Concentration	Component of one reaction (µL)
Distilled water	---	12
Go Taq® green master mix	1 X	15
Forward primer	10 picomols/µL	0.5
Reverse primer	10 picomols/µL	0.5
DNA Sample	100 ng/µL	2
Total Volume	---	30

***DNA template of 2 µL for each sample**

Optimization of PCR reaction was achieved after several trials to reach for the optimum conditions. The final Programs used are shown in Tables-2 and 3.

Table 2- PCR program **(exon2-intron2-exon3)** region of *GABRB3*.

No. of steps		Steps	Temperature (C°)	Time (min.)	No. Of cycles
1		Initial denaturation	95	5	1
2	A	Denaturation	95	1	35
	B	Annealing	61	1	
	C	Extension	72	1	
3		Final extension	72	10	1

Table 3- PCR program for **exon 6** of *GABRB3.*

No. of steps		Steps	Temperature (C°)	Time (min.)	No. Of cycles
1		Initial denaturation	95	5	1
2	A	Denaturation	95	1	35
	B	Annealing	64	1	
	C	Extension	72	1	
3		Final extension	72	10	1

The PCR products were separated by 2 % agarose gel electrophoresis, stained with ethidium bromide and visualized by ultraviolet light (302 nm) using (Gel Documentation System).

Sequencing and sequence alignment

Sequencing of (exon2-intron2-exon3) region and exon 6 of *GABRB3* gene was performed by Macrogen company, South Korea for samples of patients with related autistic phenotypes, along with control samples. Eleven samples for exon2-intron2-exon3 region and ten samples for exon 6 were selected for DNA sequencing. Alignment was conducted using alignment tool, BLASTn for nucleotide sequence and BLASTp which are available online at the National Center Biotechnology Information (NCBI) at (http://www.ncbi.nlm.nih.gov). Translation of exons to polypeptide was achieved using EMBOSS Transeq tool which translates nucleic acid sequences to their corresponding peptide sequences available online at (http://www.ebi.ac.uk/Tools/st/emboss_transeq/) and the three dimensional structure was predicted using RaptorX tool which is a protein structure prediction server available online at (http://raptorx.uchicago.edu/).

Statistical analysis

All data was analyzed using Statistical Analysis System- SAS (2012). Chi-square was used to test the significant difference between patients and control groups.

Results and Discussion

Description of study sample

The results presented in this study were based on analyses of data from a total of 65 cases, 40 autistic children, 5 non-autistic (siblings) and 20 non-relatives healthy children. The results of this study pointed to the higher risk of autism in males 33 (82.5 %) than in females 7 (17.5%) with a ratio of 4:1 Table-4.

Table 4- Percentage of patients and control groups according to gender.

Case-control	Gender				Total	Chi-square (χ^2)	P-value
	Male	%	Female	%			
Autism	33	82.5	7	17.5	40 (61.54%)	12.69 **	0.0076
Non autistic (siblings)	0	0	5	100	5 (7.69%)	4.92 *	0.0393
Control (non-relatives)	10	50	10	50	20 (30.77%)	0.00 NS	1.00
Total	43	66.15	22	33.85	65	10.52 **	0.0047
Chi-square (χ^2)	13.693 **		9.448 **		12.07 **		
P-value	0.00217		0.0129		0.0084		

*** (P<0.05), ** (P<0.01), NS: Non-significant.**

The ASDs prevalence being four times higher in males (which have one copy of X chromosome XY) than in females (which have two copies of X chromosome XX) is thought to be due to the X-linked genes involved in brain development and cognition which are higher in proportion when compared with autosomal genes [16]. Werling and Geschwind observed that there is sex differences in autism spectrum disorders [4].

Polymerase chain Reaction (PCR) Results

Successful PCR amplification reaction for the targeted regions was confirmed by (2%) agarose gel electrophoresis when DNA bands of a molecular size related to the targeted regions were detected. Molecular sizes for amplified (exon2-intron2-exon3) region and exon 6 were 613 bp and 390 bp respectively as shown in Figure-1 and 2.

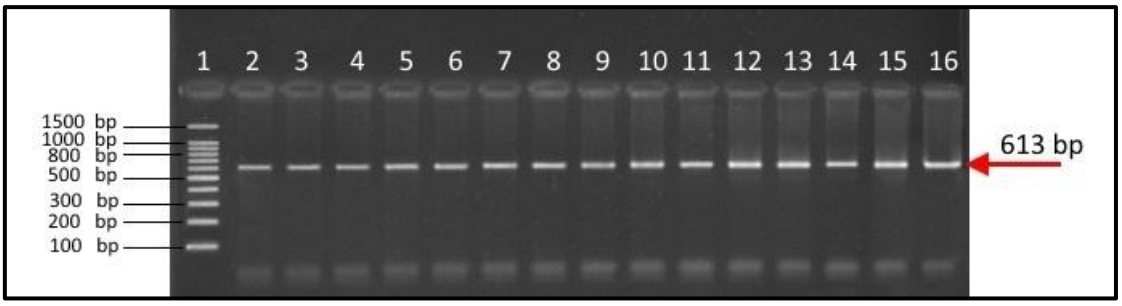

Figure 1- Agarose gel electrophoresis of PCR products of ***GABRB3* gene –** (exon2-intron2-exon3) region with a molecular size of 613 bp indicated by red arrow. Bands were separated by electrophoresis on 2 % agarose gel at 90 voltages for one hour and visualized by gel documentation system after 30 minutes of staining with EtBr. Lane 1: DNA ladder (100-1500 bp). Lane (2-10): PCR products of autistic children. Lane (11-13): PCR products of relatives. Lane (14-16): PCR products of healthy children.

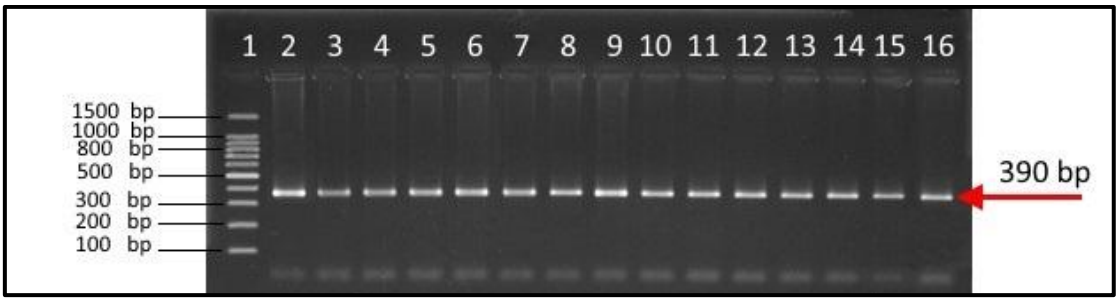

Figure 2- Agarose gel electrophoresis of PCR products of ***GABRB3* gene – exon 6** with a molecular size of 390 bp indicated by red arrow. Bands were separated by electrophoresis on 2 % agarose gel at 90 voltages for one hour and visualized by gel documentation system after 30 minutes of staining with EtBr. Lane 1: DNA ladder (100-1500 bp). Lane (2-10): PCR products of autistic children. Lane (11-13): PCR products of relatives. Lane (14-16): PCR products of healthy children.

Sequencing results of exon2-intron2-exon3 and exon 6 of *GABRB3*

For exon2-intron2-exon3 region of *GABRB3*, BLASTn local and global alignment with the reference gene (GenBank: NG_012836.1) for all 11 (8 autistic and 3 control samples) of DNA sequence showed that only four autistic samples appeared to have SNPs in this region while the remaining autistic and control group showed 100% identity. Four different SNPs were identified in four autistic male samples, one of them is located in exon 2 and another two are located in the end of intron 1, while the fourth SNP is located in the beginning of intron 3 as shown in Table-5.

Table 5- SNP analysis for Exon2-intron2-exon3 region

no.	Sample type	SNP	BP Seq*	Region	RefSNP**	Sequence 5'→ 3'	Allelic origin
1	Autism	G>A	6086	Exon2	-	TAA[A/G]ACC	Heterozygote
		C>G	6402	Intron3	rs755863611	GGG[G/C]GGT	Heterozygote
2	Autism	C>T	5961	Intron1	-	CGG[T/C]TGA	Heterozygote
		G>C	5965	Intron1	-	TGA[C/G]CCG	Heterozygote
		G>A	6086	Exon2	-	TAA[A/G]ACC	Heterozygote
3	Autism	C>T	5961	Intron1	-	CGG[T/C]TGA	Heterozygote
		G>A	6086	Exon2	-	TAA[A/G]ACC	Heterozygote
4	Autism	C>T	5961	Intron1	-	CGG[T/C]TGA	Heterozygote
		G>A	6086	Exon2	-	TAA[A/G]ACC	Heterozygote

*** Sequence of SNP in reference gene in NCBI.**
**** SNP name in SNP database in NCBI.**
(-) SNP reported for the first time in this study (novel mutations).

All SNPs are found to be of heterozygote allelic origin. Each SNP and its alleles is demonstrated in Figure-3 in which the peaks obtained from the sequencing results show the type of alleles in each sample as mentioned in the previous table.

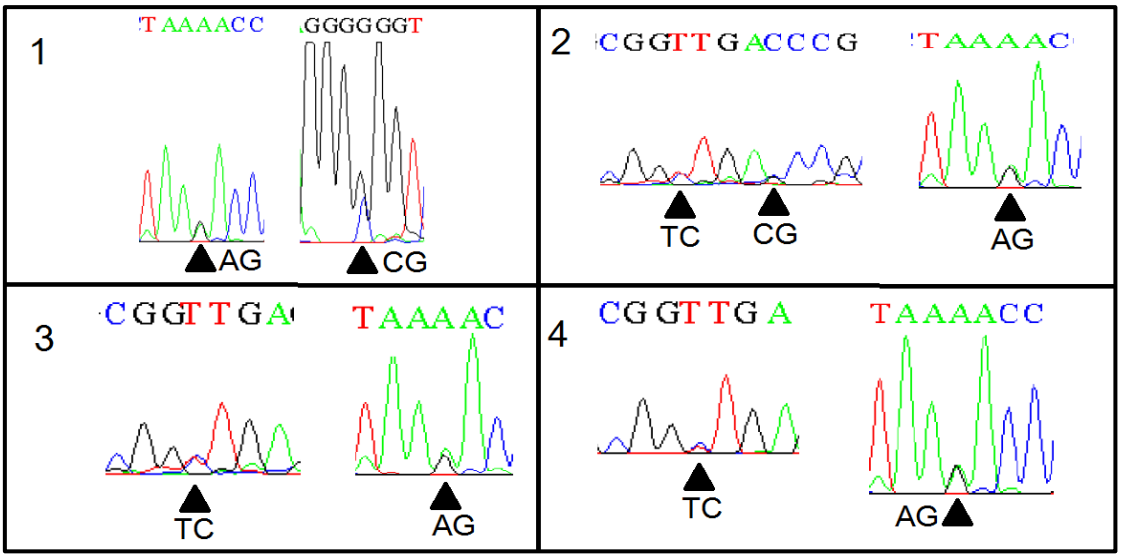

Figure 3- Allelic origin for SNPs in exon2-intron2-exon3 region in four samples with the black triangle indicating the location of each SNP as well as the alleles.

The three SNPs (G>A, C>T and G>C) are reported for the first time in this study and they are located in the end of intron 1 and in exon 2 while the one within intron 3 is previously reported in NCBI (rs755863611). Exon 2, comprising 90 nucleotides (30 codons) that encode 30 amino acids, starts from base 6009 till base 6099 in the reference gene (NG_012836.1), while amino acid sequence within the polypeptide starts from the residue 29 to 58 (NCBI). A SNP in this exon was found to cause a change in amino acid sequence when translated to a polypeptide because of the changing of the codon (AGA) which codes for arginine to (AAA) which codes for lysine. Translation of nucleotide sequence for exon 2 was achieved by EMBOSS Transeq tool available online at the European Molecular Biology Open Software Suite which allows translation of nucleotide sequence to their corresponding peptide sequence by giving the single letter symbol of amino acids. This tool belongs to the European Bioinformatics Institute which is part of the European Molecular Biology Laboratory (EMBL-EBI).

Following translation, the peptide sequence was aligned with the protein alignment tool, BLASTp which revealed the effect of SNP on sequence of amino acid in the polypeptide as arginine (R), the 53rd amino acid, was changed to lysine (K). SNPs in coding regions (cDNA SNPs or cSNPs) and regulatory regions are most likely to affect gene function [17]. The three dimensional structure was predicted using RaptorX [18-20] tool available online and there was 5(16%) positions predicted as disordered with a slight modification in the folding of the α-helix as well as a slightly different coiling of the β-sheet as shown in Figure-4. Nonsynonymous SNPs (nsSNPs), which lead to substitution of amino acids, are likely to affect the function of the peptide, and may contribute to disease risk. Many nsSNPs have been found to be associated with altered disease risk and variable treatment response [21].

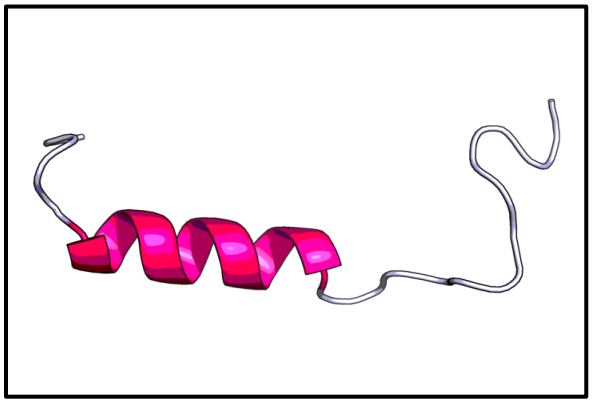

Figure 4- Three dimensional structure prediction of the 30 amino acid polypeptide sequence obtained from exon 2 showing the alpha helix in white color and the beta sheet in pink color.

As for exon 6, BLASTn results for all ten samples of DNA sequence for this region appeared to have 100% identity, meaning that the sequence of DNA in these samples was completely matching the original reference sequence. There were no deletions or insertions in all samples. Exon 6 contains 138 nucleotides (46 codons) that encode 46 amino acids. It starts from base 198327 till base 198464 in the reference gene (NG_012836.1), while amino acid sequence within the polypeptide starts from residue 124 to 169. Translation was also achieved using Transeq tool available online at the European Molecular Biology Open Software Suite (EMBOSS) and the translated peptide was aligned using protein alignment tool, BLASTp as shown in Figure-5. All samples showed 100 % identity with the original peptide reference sequence meaning there is no change in amino acid sequence.

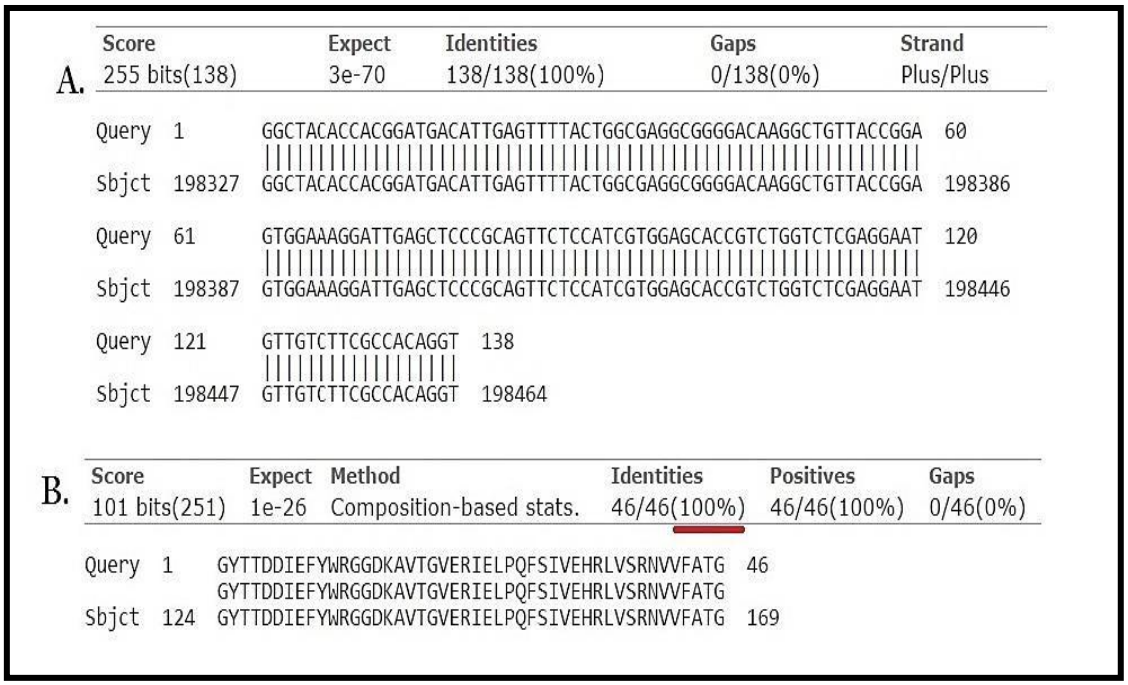

Figure 5- Alignment of exon 6 and its polypeptide product, A. BLASTn results for exon 6 showing 100% identity. B. BLASTp results showing 100% identity with no change in amino acids.

The three dimensional structure was also predicted for this region using RaptorX as shown in Figure-6 which revealed that **0** (**0%**) positions predicted as disordered.

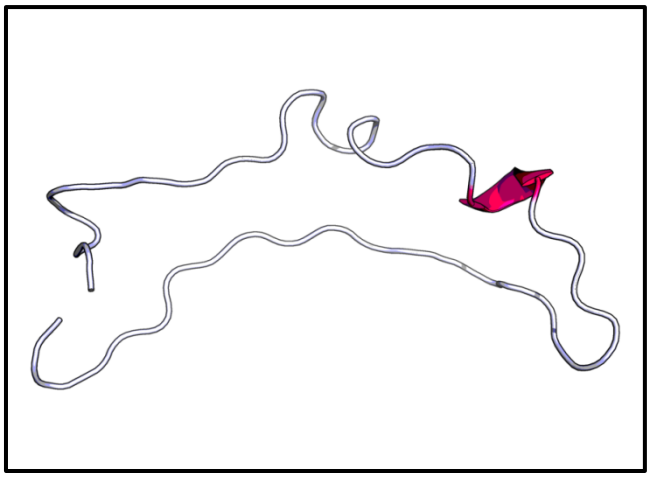

Figure 6-Three dimensional structure prediction of the 46 amino acid polypeptide sequence obtained from exon 6 showing the alpha helix in white color and the beta sheet in pink color.

The current study revealed that the SNP detected in the coding region exon 2 is found in four autistic patients, while it was not found in control group. This leads to the suggestion of association of this detected SNP with autism, considering that it changed the polypeptide three-dimensional structure, thus it could possibly affect on its function. As for intronic SNPs, it is possible to suggest

that they are not associated with autism since they are located far from the splicing site (intron-exon junction), while exon 6 had no mutations detected meaning it is not a candidate region for autism susceptibility.

References

1. Kanner, L. **1943.** Autistic Disturbances of Affective Contact. *Nervous Child*., 2, pp:217-250.
2. Limpraert, P. **2008**. Genetics of autism. *Siriraj Medical Journal*, 60(5), pp: 305-309.
3. Ronemus, M., Iossifov, I., Levy, D., and Wigler, M. **2014**. The role of de novo mutations in the genetics of autism spectrum disorders. *Nature Reviews Genetics*, 15(2), pp: 133-141.
4. Werling, D. M., and Geschwind, D. H. **2013**. Sex differences in autism spectrum disorders. *Current Opinion In Neurology*, 26(2), p:146.
5. Omar, S. K. **2012** Study of Prevasive developmental disorders among students in Mousil. *Ann. Coll. Med. Mosul* 2012, 38 (2), pp: 52-61.
6. Carter, M. T., & Scherer, S. W. **2013**. Autism spectrum disorder in the genetics clinic: a review. *Clinical genetics*, 83(5), pp:399-407.
7. Folstein, S.E. and Rosen-Sheidley, B. **2001**. Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat. Rev. Genet.* 2(12), pp:943-955.
8. Zoghbi, H.Y. **2003**. Postnatal neurodevelopmental disorders: meeting at the synapse. *Science.* 302(5646), pp:826-830.
9. Garber, K. **2007**. Neuroscience. Autism's cause may reside in abnormalities at the synapse. *Science.* 317(5835), pp:190-191.
10. Jiao, Y., Chen, R., Ke, X., Cheng, L., Chu, K., Lu, Z. and Herskovits, E. H., **2012**. Single nucleotide polymorphisms predict symptom severity of autism spectrum disorder. *Journal of autism and developmental disorders*, 42(6), pp:971-983.
11. Chen, C.H., Huang, C.C., Cheng, M.C., Chiu, Y.N., Tsai, W.C., Wu, Y.Y., Liu, S.K. and Gau, S.S.F. **2014**. Genetic analysis of GABRB3 as a candidate gene of autism spectrum disorders. *Molecular autism*, 5(1), p:1.
12. Sutcliffe, J. S., Nurmi, E. L., Lombroso, P. J. **2003**. Genetics of childhood disorders: XLVII. Autism, part 6: duplication and inherited susceptibility of chromosome 15q11-q13 genes in autism. *J Am Acad Child Adolesc Psychiatry*, 42, pp:253–256.
13. Kim, H.G., Kishikawa, S., Higgins, A.W., Seong, I.S., Donovan, D.J., Shen, Y., Lally, E., Weiss, L.A., Najm, J., Kutsche, K. and Descartes, M. **2008**. Disruption of neurexin 1 associated with autism spectrum disorder. *The American Journal of Human Genetics*, 82(1), pp:199-207.
14. Urak, L., Feucht, M., Fathi, N., Hornik, K., Fuchs, K. **2006**. A *GABRB3* promoter haplotype associated with childhood absence epilepsy impairs transcriptional activity. *Hum Mol Genet*, 15, pp:2533–2541.
15. Sambrook, J., Fritsch, E.F. and Maniatis, T. **1989**. *Molecular cloning: A laboratory manual.* Second Edition, Cold spring harbor laboratory press, New York.
16. Piton, A.**,** Gauthier, J., Hamdan, F.F., Lafreniere, R.G., Yang, Y., Henrion, E., Laurent, S., Noreau, A., Thibodeau, P., Karemera, L., Spiegelman, D., Kuku, F., Duguay, J., Destroismaisons, L., Jolivet, P., Côté, M., Lachapelle, K., Diallo, O., Raymond, A., Marineau, C., Champagne, N., Xiong, L., Gaspar, C., Rivière, J.B., Tarabeux, J., Cossette, P., Krebs, M.O., Rapoport, J.L., Addington, A., Delisi, L.E., Mottron, L., Joober, R., Fombonne, E., Drapeau, P. and Rouleau, G.A. **2011**. Systematic resequencing of X-chromosome synaptic genes in autism spectrum disorder and schizophrenia. *Mol. Psychiatry*., 16(8), pp:867-880.
17. Wang, Z. and Moult, J **2001**. SNPs, Protein Structure, and Disease. *Human Mutation*,17, p:263270.
18. Källberg, M., Wang, H., Wang, S., Peng, J., Wang, Z., Lu, H. and Xu, J. **2012**. Template-based protein structure modeling using the RaptorX web server. *Nature Protocols*, 7, pp:1511-1522.
19. Ma, J., Wang, S., Zhao, F. and Xu, J. **2013**. Protein threading using context-specific alignment potential. *Bioinformatics*, 29(13), pp: i257-i265.
20. Peng, J and Xu, J. **2011**. A multiple-template approach to protein threading. *Proteins*, 79(6), pp:1930-1939.
21. Jarjanazi, H., Savas, S., Pabalan, N., Dennis, J. W., and Ozcelik, H. **2008.** Biological implications of SNPs in signal peptide domains of human proteins.*Proteins: Structure, Function, and Bioinformatics*, 70(2), pp:394-403.