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Association of mtDNA Mutation with Autism in Iranian patients

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Abstract

Introduction:
The autism spectrum disorders (ASD) are amongst the most heritable complex disorders. Although there have been many efforts to locate the genes associated with ASD risk, many has been remained to be disclosed about the genetics of ASD. Scrutiny's have only disclosed a small number of de novo and inherited variants significantly associated with susceptibility to ASD. These only comprise a small number of total genetic risk factors. Some studies confirm the contribution of mitochondrial genome mutations to the pathophysiology of the autism, but some other studies rejected such a contribution. In the current study we tried to scrutinize the association between mitochondrial tRNA genes mutations and the risk of Autism.

Materials and Methods:
DNA was extracted from the blood of 24 patients with ASD and 40 age-matched healthy controls from Special Medical Center in Tehran. 22 tRNA genes of mitochondrial genome were PCR amplified using 12 primer pairs and sequenced. Sequencing results were searched for mutations using clustalW Progran and then the association of mutations with the autism risk was assessed by statistical analysis using SPSS version 15.

Results:
Many of the observed mutations were sporadic mutations without any significant relationship with the risk of autism, and the other mutations including those of high frequency showed no significant relationship with the risk of disease as well (P>0.05) except mutations 16126T>C (P=0.01), 14569G>A(P=0.02) and 1811A>G(P=0.04). These three mutations were in the noncoding regions of the mitochondrial genome near tRNA genes. The mutation 16126T>C was in the mtDNA control region.

Conclusion:
Our study showed a significant relationship between the point mutations 16126T>C, 14569G>A and 1811A>G of the mitochondrial genome and the risk of autism.

Keywords:
Autism, Mitochondrial genome, Mutation, TRNA.

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Introduction

The autism spectrum disorders (ASDs) contain a group of neuropsychiatric conditions that dramatically affects the language, social skills and mental flexibility of the patients. Affecting more than about 1 in 150 according to recent estimations, autism is now known to be more threatening than ever (1). The ASD is one of the most heritable complex disorders. Although there have been many efforts to locate the genes associated with ASD risk, many has been remained to be disclosed about the genetics of ASD. To date only a small number of de novo and inherited variants significantly associated with susceptibility to ASD have been disclosed. These only comprise a small number of total genetic risk factors. In some cases the acquisition of ASD has been attributed to single gene disorders like mutations in FMR1, TSC1, TSC2, MECP2, and PTEN (2-3). Chromosomal aberrations like maternal duplication in 15q11-q13 have also been suggested as a possible cause of autism acquisition (4). Mutations in synaptic genes are known to be in relation with ASD as well (5-6).

Although the direct pathophysiology of ASD has remained unclear, evidences are available about the contribution of the disturbance of mitochondrial metabolic pathways to the risk of ASD acquisition (7-12). Some studies confirm the contribution of mitochondrial genome mutations to the pathophysiology of the autism(8,11,13), but some other studies rejected such a contribution (14). Anyway, it has remained a matter of debate if mutations in mitochondrial genome play a role in the pathogenesis of ASD. One of the mutations in mitochondrial genome which is suggested to be involved in the increased risk of ASD is the mtDNA 8363 G>A transfer ribonucleic acid (tRNA) Lys mutation (13). Due to impairment in critical highly energy dependent neurodevelopment stages, these mutations can result in neurodevelopmental disorders like ASD. Although some studies suggest the association between this mutation and the risk for ASD, but a recent study in Europe reported no association(14). Therefore more studies in different populations are required to define potential relationship between mitochondrial genome mutations and ASD susceptibility. In the current study we examined the association of mutations in some mitochondrial genomic regions assumed to be in relationship with ASD in a group of Iranian population.

Materials and Methods

Patients and controls: Peripheral blood samples from 24 children diagnosed with Autism, who were outpatiented at the Special Medical Center in Tehran, were drawn. Autism was confirmed by psychiatric examination based on behavioral observation of the child and semi-structured interview with the parent, a score of ≥6 on the DSM IV diagnosis criteria for autism and clinical judgment. Healthy controls were those who are older than 11 years old and had not shown any symptoms of autism. All the parents of subjects signed the informed consent before the children enter the study.

DNA extraction and PCR: DNA extracted from the peripheral blood mononuclear cells by Gene JET™ Genomic DNA Purification Kit (Ferment as, Lithuania) according to the manufacturer's guide. 12 pairs of specific primer pairs (15). Table 1 were used to amplify mitochondrial tRNA genes containing regions. We used 2X PCR Master Mix (Ferment as, Lithuania) for PCR. 25µl of PCR reaction mixtures, containing 0.3µM of both forward and reverse primers and 100 pg of template DNA, were thermal-cycled using the following thermal profile for the exons ONP 82.164, ONP 84.65, ONP 25.185, ONP 93.70, ONP 71.46 and ONP 21.90: initial denaturation 95˚C for 5 minutes, denaturation 95˚C for 30s, annealing 55˚C for 30s, extension 60s at 72˚C, and final extension 8 minutes at 72˚C. The conditions

were the same for other exons except that T_a was 64˚C for ONP 78.79, 48˚C for ONP 60.136 and 58˚C for ONP 97.208 and ONP 98.99.

Sequencing and sequence analysis: Sequencing was done on 3100 ABI Machine (Kowsar Tech Exploration Company). Sequencing results were aligned with reference sequence to determine if any mutations are present. Multiple sequence alignment by MEGA5 software using ClustalW algorithm were used to determine the rate of mutations in patients and healthy controls for each genomic region.

Statistical analysis: frequency of each mutation in both of patients and healthy controls were determined. SPSS software version 15 was used for statistical analysis of the results. Chi-square test performed for evaluating the significance of the association between the disease and the genetic variations. P-value was determined for each mutation**.** Relative risk of acquiring the disease was assessed for each mutation by odds ratio (OR) analysis.

Table 1: Primer pairs used for amplification of mtDNA and their target sequences on mtDNA

Target Sequence	Sequence of forward primer (name and sequence of the primers)	Sequence of reverse primer (name and sequence of the primers)
511- 780	ONP78 5’-CAG CAC ACA CAC ACC GCT GC-3’	ONP79 5’-GAG ACT GCA GTG CTG CGT GCT-3’
141- 1980	ONP60 5’-AGT AGA GTG CTT AGT TGA GC-3’	ONP136 5’-TAT AAA TCT TCC CAC TAT TT-3’
3187- 3550	ONP82 5’-CTC AAC TTA GTA TTA TAC CC-3’	ONP164 5’-GAT GGT GAG AGC TAA GGT CG-3’
4211- 4650	ONP84 5’-TAT GAT ATG TCT CCA TAC CC-3’	ONP65 5’-GGA AAT ACT TGA TGG CAG CT-3’
5461- 6020	ONP86 5’-CCC TTA CCA CGC TAC TCC TA-3’	ONP67 5’-GGC TCG AAT AAG GAG GCT TA-3’
7377- 7650	ONP21 5’-CTG GAG TGA CTA TAT GGA TG-3’	ONP90 5’-GTG ATA AGC TCT TCT ATG AT-3’
8161- 9239	ONP25 5’-CTACGGTCA ATG CTC TGA AA -3’	ONP185 5’-TAC TAT ATG ATA GGC ATG TGA-3’
9851- 10150	ONP91 5’-CAC TAT CTG CCT CAT CCG CC-3’	ONP92 5’-ATG TAG CCG TTG AGT TGT GG-3’
10361- 10582	ONP93 5’-TCT GGC CTA TGA GTG ACT AC-3’	ONP70 5’-AGT ATT ATT CCT CTC AGG CA-3’
11901- 12420	ONP71 5’-TGC TAG TAA CCA CGT TCT CC-3’	ONP46 5’-TTT GTT AGG GTT AAC GAG GG-3’
14184- 14840	ONP208 5’-CACCAACAAACAATGGTCAA-3’	ONP97 5’-TTCATCATGCGGAGAATGTTG-3’
15791- 16150	ONP98 5’-ATCATTGGACAAGTAGCATC-3’	ONP99 5’-GTG GTC AAG TAT TTA TGG TA-3’

Table 2: Point mutations found in different parts of mtDNA in 24 autistic patients and controls.

Nucleotide position	Locus	Amino Acid change	Frequency in patients	Frequency in controls	Report	Reported in mtDNA base substitution disease	Reference
A750G	MT-RNR1	noncod	23/24	33/40	No	-	-
A1811G	MT-RNR2	noncod	2/24	10/40	Yes	Oral cancer	(Basak et al. 2003)
G1888A	MT-RNR2	noncod	5/24	1/40	Yes	-	(Voets et al. 2010)
A8860G	MT-ATP6	T-A	21/24	36/40	Yes	Abdominal aortic aneurysm	(Tilson et al. 2008)
A12308G	MT-TL2	noncod	6/24	13/40	Yes	-	(Voets et al. 2010)
G14569A	MT-ND6	Syn	1/24	11/40	Yes	-	(Voets et al. 2010)
C14766T	MT-CYB	T-I	17/24	32/40	Yes	-	(Voets et al. 2010)
T16126C	D-Loop	noncod	9/24	0/40	Yes	Oral cancer	(Saranathet al. 2003)

Results

Several point mutations were identified in the regions harboring tRNA genes on the mtDNA in our study (Tables 3, 4 and 5). Many of them were sporadic point mutations with low frequency and it was impossible to determine if their association with the ASD is statistically significant. Mutations 4596G>A, 5790C>A, 12330A >G, and 14395T>C were identified for first time in the current study and they have not been reported to the mitochondrial databases yet. Among those of high significance, were mutations 16126T>C (P=0.001) and 14569G>A (P=0.02). Mutations 750A>G, 1811A>G, 1888G>A, 8860A>G, 12308A>G, 14569G>A, 14766C>T and 16126T>C were found in high frequencies in both patients and healthy controls and did not shown to be statistically significant associated with the disease.

Table 3: Variations which found only in controls.

3480	15933	4320	12372	7380	10371	14791	8251
8256	15954	1459	644	7598	754	15884	8996
10551	10398	14199	10572	15936	1463	8702	8615
10420	5814	1463	9058	8271	10420	9056	5792

Table 4: Variations which found only in Patients.

14476	14395	16126	16038	5656	4353	12330	1703
14798	15924	15928	16086	5790	4454	12297	8472
14687	15929	19954	10463	5495	4561	1811	8865
14364	15973	16129	10550	4596	3480	1598	7581

Table 5: Variations which found in both controls and patients.

709	1719	1888	8697	9055	12372	14783	16069
750	1721	3480	8701	10463	14766	14793	
1700	1811	8684	8860	12308	14569	15930	

Discussion

ASD includes a series of developmental disorders ranging from impairments in language skills to variable degrees of mental retardation as well as unusually repetitive and abnormal patterns of behavior (16). So far, many explorations have been done to uncover the mitochondrial problems relationship with the Autism. Plethora of evidences supports the claim that autism is associated with and has relation to mitochondrial disorders (10-11). In fact it is well established that many of the people with neurological disorders show pathological signs of mitochondrial disorders and vice versa (7,16-17). Also pathogenicity of some of the mutations in mitochondrial DNA, including tRNA genes mutations, is known at the time (13,17). Although many have tried to uncover the relationship between mtDNA mutations and the ASD, it has been a matter of debate whether mtDNA tRNA genes mutations are involved in the pathogenesis of the autism (7-8,10,12,14). However, the available data are contradictory. A few of recent studies suggested that there is no relationship between the disease and mutations in mitochondrial genome, while others denied these results (14). Our study suggests that there is a significant relationship between mutations 16126T>C and 14569G>A in the mitocho- ndrial genome with the susceptibility to the autism, although more studies should be

conducted to consolidate the results. It is evident from previous studies that ASD is associated with altered mitochondrial function and cellular energetics. As reviewed by Luigi Palmieri, et al. (16) the etiology of ASD and its associated clinical, biochemical, or neuropathological outcomes may lie in the mitochondrial genome and be the results of rare mutations in mtDNA or mutations or rearrangements of the genomic DNA affecting loci involved in the mitochondrial function. In fact many of the biochemical alterations and aberrations observed in mitochondrial disease, has been shown to alter to great extent in the same manner in the ASD (16).

Conclusion

In conclusion our study supports the idea that mtDNA mutations are associated with increased risk of acquiring ASD and these mutations may actually play a role in the pathophysiology of ASD.

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References

1. Cave SF. The history of vaccinations in the light of the autism epidemic. Alternative therapies in health and medicine2008;14(6):54-7.
2. Bayou N, M'Rad R, Ahlem B, Bechir Helayem M, Chaabouni H. Autism: an overview of genetic aetiology. Tunis Med 2008;86(6):573-8.
3. Muhle R, Trentacoste SV, Rapin I. The genetics of autism. Pediatrics 2004;113(5):e472-86.
4. Dykens EM, Lee E, Roof E. Prader-Willi syndrome and autism spectrum disorders: an evolving story. Journal of neurodevelopmental disorders 2011;3(3):225-37.
5. Auerbach BD, Osterweil EK, Bear MF. Mutations causing syndromic autism define an axis of synaptic pathophysiology. Nature 2011;480(7375):63-8.
6. Fassio A, Patry L, Congia S, Onofri F, Piton A, Gauthier J, et al. SYN1 loss-of-function mutations in autism and partial epilepsy cause impaired synaptic function. Human molecular genetics 2011;20(12):2297-307.
7. Correia C, Coutinho AM, Diogo L, Grazina M, Marques C, Miguel T, et al. Brief report: High frequency of biochemical markers for mitochondrial dysfunction in autism: no association with the mitochondrial aspartate/ glutamate carrier SLC25A12 gene. J Autism Dev Disord 2006;36(8):1137-40.
8. Kent L, Gallagher L, Elliott HR, Mowbray C, Chinnery PF. An investigation of mitochondrial haplogroups in autism. Am J Med Genet B Neuropsychiatr Genet 2008;147B(6):987-9.
9. Pons R, Andreu AL, Checcarelli N, Vila MR, Engelstad K, Sue CM, et al. Mitochondrial DNA abnormalities and autistic spectrum disorders. J Pediatr 2004;144(1):81-5.
10. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Molecular psychiatry 2011.
11. Weissman JR, Kelley RI, Bauman ML, Cohen BH, Murray KF, Mitchell RL, et al. Mitochondrial disease in autism spectrum disorder patients: a cohort analysis. PLoS One 2008;3(11):e3815.
12. Zhang B, Angelidou A, Alysandratos KD, Vasiadi M, Francis K, Asadi S, et al. Mitochondrial DNA and anti-mitochondrial antibodies in serum of autistic children. J Neuroinflammation 2010;7:80.
13. Virgilio R, Ronchi D, Bordoni A, Fassone E, Bonato S, Donadoni C, et al. Mitochondrial DNA G8363A mutation in the tRNA Lys gene: clinical, biochemical and pathological study. Journal of the neurological sciences 2009;281(1-2):85-92.
14. Alvarez-Iglesias V, Mosquera-Miguel A, Cusco I, Carracedo A, Perez-Jurado LA, Salas A. Reassessing the role of mitochondrial DNA mutations in autism spectrum disorder. BMC Med Genet 2011;12:50.
15. Houshmand M, Larsson NG, Holme E, Oldfors A, Tulinius MH, Andersen O. Automatic sequencing of mitochondrial tRNA genes in patients with mitochondrial encephalomyopathy. Biochimica et biophysica acta 1994;1226(1):49-55.
16. Palmieri L, Persico AM. Mitochondrial dysfunction in autism spectrum disorders: cause or effect? Biochimica et biophysica acta 2010;1797(6-7):1130-7.
17. DiFabio R, Santorelli FM, Nola G, Cricchi F, Masi R, Ingrosso A, et al. Clinical and audiological follow up of a family with the 8363G>A mutation in the mitochondrial DNA. Neuromuscul Disord 2009;19(4):291-6.