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A CGH array study in nonsyndromic (primary) autism patients: deletions on 16p13.11, 16p11.2, 1q21.1, 2q21.1q21.2, and 8p23.1

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Background/aim: To detect specific molecular changes of DNA level in primary autism patients by using whole genome CGH array technology.

Materials and methods: A cohort of 35 primary autism patients received clinical genetic testing by using an oligonucleotide-based CGH array platform to test for submicroscopic genomic deletions and duplications. Fluorescent in situ hybridization was performed in seven patients for confirmation of the results.

Results: We found 16p13.11 deletion in thirteen patients, 16p11.2 deletion in twelve patients, 1q21.1 deletion in ten patients, 2q21.1q21.2 deletion in eight patients, and 8p23.1 deletion in seven patients.

Conclusion: Our study indicates that genes in 16p13.11, 16p11.2, 1q21.1, 2q21.1q21.2, and 8p23.1 loci are potential predisposition and new suspicious regions for primary autism. Deletions in these regions should be investigated in further studies to understand pathogenesis of primary autism.

Key words: Autism spectrum disorders, primary autism, CGH-array, deletion

1. Introduction

Autism spectrum disorders (ASDs) are known as a group of neurodevelopmental disorders with a strong genetic etiology. Cytogenetic abnormalities have been detected in 5%–10% of autism patients. Chromosomal abnormalities, by disrupting a gene or providing a permissive genetic environment for mutations elsewhere in the genome, may be the cause of autism. However, all chromosomes have defined suspected regions for autism development, and chromosomal regions 2q, 7q, and 15q are observed more than others (1).

CGH array (aCGH) technology is a method that analyzes DNA copy number variations that cause genetic deformities in humans. Ability to compare different genomes, no need for metaphase plaque, analysis made with low amounts of DNA, and analysis of small changes in DNA are the main advantages of this method (2–4).

Twin (5–7) and family studies and reports of chromosomal aberrations in ASD individuals have suggested a role for genes in ASD development. While a number of ASD susceptibility-associated genes have been observed in different studies, variants in a single gene cannot explain more than a small percentage of cases. Indeed, recent estimates suggest that there may be around 400 chromosomal regions involved in ASD predisposition.

In our study, we performed a whole-genome aCGH study in primary autism patients. The study was performed using DNA material isolated from peripheral blood. It was important to understand specific molecular changes at DNA level with this study. By using these new diagnostic tools, new treatment approaches can be defined in primary autism patients.

2. Materials and methods

We evaluated 35 primary autism patients (both sexes, aged between 0 and 18 years). Eight of these patients (22.9%) had minor dysmorphic features with/without additional neurodevelopmental abnormalities like mental retardation or epilepsy, while one of the patients (2.9%) had only epilepsy additional to the autistic features. We tried to create a patient population in which environmental factors were as homogeneously distributed as possible due to the multifactorial or polygenic character of the disease. The study thus included only the children of families

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living in Kocaeli, Turkey. Again, for the same reason, primary autism patients with no major syndromes were chosen. All patients were diagnosed in our Department of Child Psychiatry according to DSM-IV criteria (8) and ethical permission was granted by the local authority (11/23/2009).

The CytoSure microarray (Oxford Gene Technology, Oxford, UK) platform was used for aCGH analysis. Genomic DNA was extracted from the blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's protocol. Commercially bought DNA (Promega Corporation, Madison, WI, USA) was used as the reference/control. DNA was digested with *Rsa*I and *Alu*I restriction enzymes, labeled by incorporation of Cy-dUTP using exo-Klenow polymerase (Agilent Technologies, Palo Alto, CA, USA), and purified by columns (Kreatech Diagnostics, Amsterdam, the Netherlands). Fluor-labeled DNA samples were hybridized to microarray slides using 2X aCGH hybridization buffer (Agilent Technologies, Santa Clara, CA, USA). Following 42 h of hybridization, the arrays were washed. The arrays were scanned on an Agilent G2505B scanner at 100% PMT. The scanned arrays were quantified using Agilent's Feature Extraction software. Data were then normalized and analyzed using CytoSure software, which uses a standard CBS method (9).

Fluorescent in situ hybridization (FISH) was performed using BlueFish tile BAC probes RP11-100A24 and RP11-158L15 from the deleted region according to the manufacturer's instructions (BlueGnome, Cambridge, UK).

3. Results

Our observations revealed 16p13.11 deletion in thirteen patients, 16p11.2 deletion in twelve patients, 1q21.1 deletion in ten patients, 2q21.1q21.2 deletion in eight patients, and 8p23.1 deletion in seven patients. Deletion of the 16p13.11 region in one of our patients is shown in the Figure. This region contains a candidate region for autistic development. In addition, we found 2p21 deletion in six

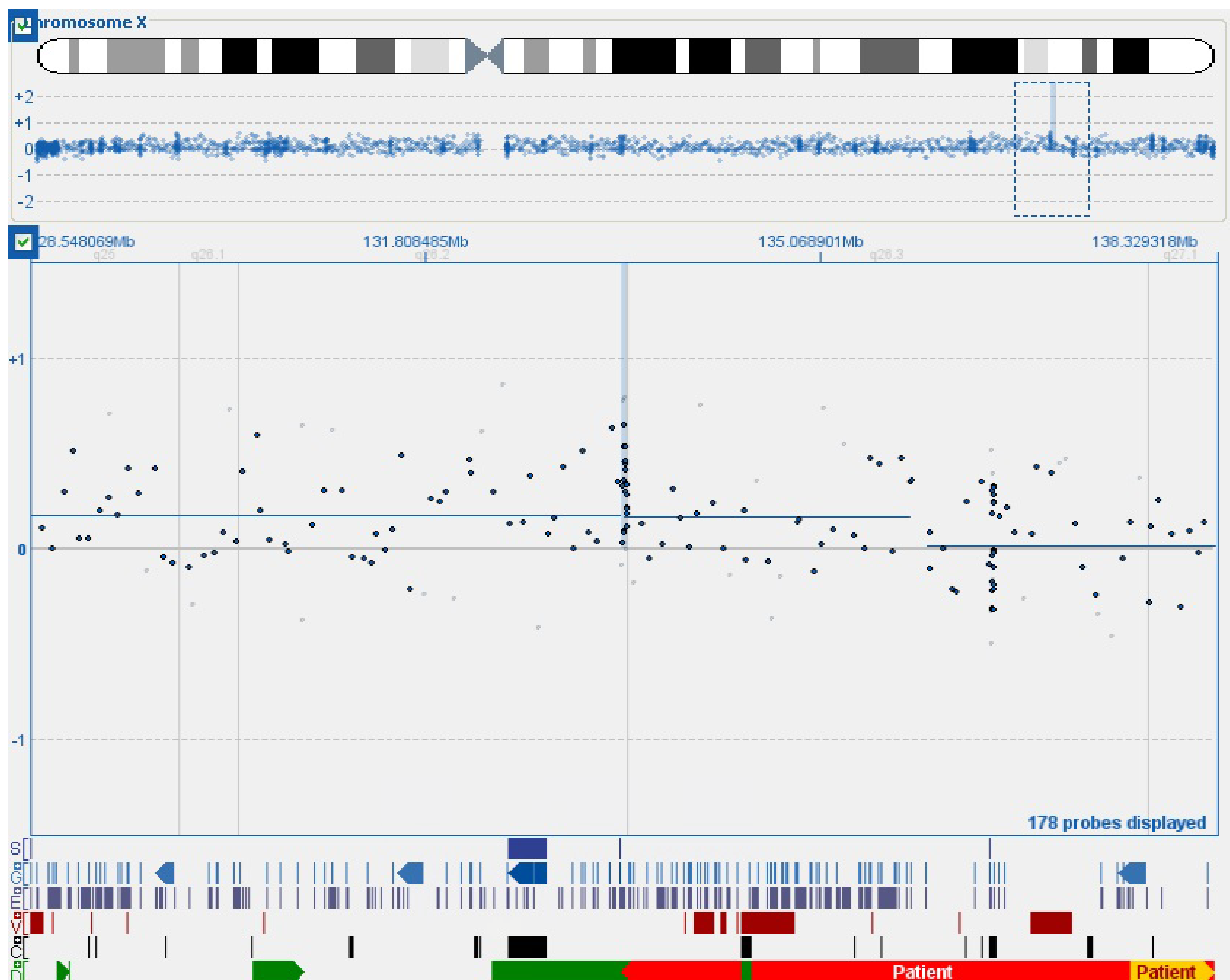

Figure. Deletion of 16p13.11 region in one of our patients.

patients, 7q36.3 deletion in three patients, and 16p13.3 deletion in two patients. A full list of deleted regions in our 35 patients is shown in Table 1. Seven selected patients were studied also by BlueGnome BlueFish probes. We used the 16p13.11 (RP11-100A24) probe in four patients with 16p13.11 deletion and the 8p23.1 (RP11-158L15) probe in three patients with 8p23.1 deletion. All FISH results of the seven patients were normal (Table 2). Array CGH aberrations are shown individually in Table 3 with descriptions of clinical findings.

4. Discussion

Among our findings, the diffuse variant genotypic distribution initially stands out. Variations of 16p13.11, 16p11.2 , 1q21.1, 2q21.1q21.2, 8p23.1, 2p21, 7q36.3, and 16p13.3 loci were defined in 27 cases that were scanned. These variations could not be related to any known diseases in our patient group. The high number of copy number variations is thought to be due to diversity in ethnic backgrounds of the patient group.

A 1.5-Mb duplication was detected in the 16p13.11 region in 4 autistic patients using high-resolution CGH array in previous studies (10,11). The same duplication was found in many relatives, as well. These duplications and deletions in the 1.5-Mb interval could not be shown in over 600 additional patients not defined in the Genomic Variant Database analyzed using high-resolution CGH array. Therefore, these deviations were considered to show a recurrent genomic imbalance causing autism and/or mental retardation predisposition (11). Hannes et al. (12) suggested that the recurrent duplication at 16p13.11 may be a benign variant. However, other reports associated this CNV region with a variety of neuropsychiatric and neurobehavioral disorders, including autism, schizophrenia, intellectual disabilities, cognitive impairment, attention deficit-hyperactivity disorder, and epilepsy, as well as congenital heart defects, skeletal manifestations, and thoracic aortic aneurysms and dissections (13). We suggest that this duplication may contribute to the autistic features seen in our 13 patients.

Table 1. List of the deleted regions in our 35 patients.

Region of deletion	Number of patients	Percentage
16p13.11	13	36.1%
16p11.2	12	33.3%
1q21.1	10	27.7%
2q21.1q21.2	8	22.2%
8p23.1	7	19.4%
2p21	6	16.6%
7q36.3	3	8.3%
16p13.3	2	5.5%

Table 2. FISH results of the selected seven patients.

Patient	FISH probe	Result
aCGH-429	16p13.11 (RP11-100A24)	Nuc ish(16p13.11×2) Negative
aCGH-439	16p13.11 (RP11-100A24)	Nuc ish(16p13.11x×2) Negative
aCGH-474	16p13.11 (RP11-100A24)	Nuc ish(16p13.11×2) Negative
aCGH-449	16p13.11 (RP11-100A24)	Nuc ish(8p23.1×2) Negative
aCGH-448	8p23.1 (RP11-158L15)	Nuc ish(8p23.1×2) Negative
aCGH-468	8p23.1 (RP11-158L15)	Nuc ish(8p23.1×2) Negative
aCGH-447	8p23.1 (RP11-158L15)	Nuc ish(8p23.1×2) Negative

Table 3. Array CGH results and clinical findings of 35 primary autism patients.

No.	Age	Sex	Clinical manifestation	Array aberration	Start	Stop	Aberration size
aCGH-423	8	F	Autism	Del(1)(q21.1)	145846731	146812360	0.96 Mb
				Del(2)(q21.1q21.2)	131853615	132750662	0.897 Mb
aCGH-424	6	M	Autism	Del(16)(p11.2)	31855086	33868735	2.01 Mb
aCGH-427	9	F	Autism	No aberration	-	-	-
aCGH-430	9	F	Autism	No aberration	-	-	-
aCGH-433	14	M	Autism	Del(1)(q21.1)	145846731	146812360	0.96 Mb
				Del(2)(q21.1q21.2)	131891907	133037601	1.14 Mb
				Del(16)(p13.11)	14985781	15273532	0.28 Mb
				Del(16)(p11.2)	31974463	34054687	2.08 Mb
aCGH-439	13	M	Autism	Del(1)(q21.1)	145846731	146812360	0.96 Mb
				Del(2)(q21.1q21.2)	131794750	133037601	1.24 Mb
				Del(16)(p13.11)	14985781	15273532	0.28 Mb
				Del(16)(p11.2)	33414608	34054687	0.64 Mb
aCGH-441	14	M	Autism	Del(8)(p23.1)	6905456	7727586	0.822 Mb
				Del(16)(p11.2)	32191055	33769613	1.578 Mb
aCGH-444	14	M	Autism	Del(16)(p13.11)	14985781	15399114	0.41 Mb
aCGH-445	7	F	Autism	Del(8)(p23.1)	6905456	7707493	0.8 Mb
				Del(16)(p13.11)	14985781	15273532	0.28 Mb
				Del(16)(p11.2)	33414608	33868735	0.45 Mb
aCGH-447	2	F	Autism	Del(1)(q21.1)	145846731	146812360	0.96 Mb
				Del(2)(p21)	45022785	45025898	3.113 kb
				Del(7)(q36.3)	155288370	155295993	7.623 kb
				Del(8)(p23.1)	6928936	7727586	0.79 Mb
				Del(16)(p13.11)	14985781	15399114	0.41 Mb
aCGH-448	6	M	Autism	Del(2)(p21)	45001201	45025898	0.02 Mb
				Del(8)(p23.1)	6928936	7727586	0.79 Mb
				Del(16)(p13.3)	757452	1211797	0.48 Mb
				Del(16)(p13.11)	14956161	15399114	0.44 Mb
aCGH-449	5	M	Autism	Del(1)(q21.1)	145846731	146812360	0.96 Mb
				Del(2)(p21)	45022785	45025898	3.113 kb
				Del(7)(q36.3)	155288370	155295993	7.623 kb
				Del(16)(p13.11)	14985781	15399114	0.41 Mb
aCGH-459	9	F	Autism	Del(16)(p11.2)	31855086	33868735	2.01 Mb
aCGH-468	14	M	Autism	Del(2)(p21)	45023113	45025818	2.705 kb
				Del(8)(p23.1)	6928936	7727586	0.79 Mb

Table 3. (Continued).

No.	Age	Sex	Clinical manifestation	Array aberration	Start	Stop	Aberration size
aCGH-474	14	M	Autism	Del(16)(p13.11)	15087448	15273532	0.18 Mb
aCGH-477	7	M	Autism	Del(2)(p21)	44349018	45025818	0.67 Mb
				Del(16)(p13.11)	14956161	15538550	0.58 Mb
aCGH-487	7	M	Autism	Del(1)(q21.1)	145846731	146812360	0.96 Mb
				Del(2)(p21)	45023113	45025818	2.705 kb
aCGH-499	3	M	Autism	No aberration	-	-	-
aCGH-431	5	M	Autism	No aberration	-	-	-
aCGH-443	7	M	Autism	No aberration	-	-	-
aCGH-151	4	M	Autism	No aberration	-	-	-
10S 9	6	M	Autism	Del(2)(q21.1q21.2)	131891907	132753284	0.86 Mb
				Del(16)(p13.11)	14985781	15273532	0.28 Mb
aCGH-426	8	M	Autism	Del(2)(q21.1q21.2)	131891907	132753284	0.86 Mb
aCGH-425	7	M	Autism, dysmorphic features (microprognathia , short-thick fingers, prominent ears, depressed nasal bridge, long philtrum, down-slanting palpebral fissures)	Del(2)(q21.1q21.2)	131891907	132858722	0.96 Mb
				Del(16)(p11.2)	35254058	34350878	1.79 Mb
aCGH-429	11	M	Autism, dysmorphic features (hyperextensibility, hypertelorism, short philtrum, almond shape eyes, small-low set ears, unilateral inguinal hernia)	Del(8)(p23.1)	6928936	7727586	0.79 Mb
				Del(16)(p13.11)	14985781	15273532	0.28 Mb
				Del(16)(p11.2)	31974463	33868735	1.89 Mb
aCGH-436	6	M	Autism, mild MR, hyperextensibility	Del(16)(p13.11)	14985781	15273532	0.28 Mb
				Del(16)(p11.2)	31974463	33868735	1.89 Mb
aCGH-446	6	M	Autism, epilepsy, dysmorphic features (down-slanting palpebral fissures, prominent forehead, short-thick fingers, hypertelorism, thick lower lip)	Del(1)(q21.1)	146603223	147348365	0.74 Mb
				Del(16)(p11.2)	31855086	34054687	2.19 Mb
aCGH-458	13	M	Autism, severe mental retardation, dysmorphic features (prominent ears, hypertelorism, midfacial hypoplasia, down-slanting palpebral fissures, retrognathia, unilateral inguinal hernia)	Del(2)(p21)	45024516	45025818	1302 kb
aCGH-464	9	M	Autism, severe mental retardation, dysmorphic features (prognathia, flat forehead, long palpebral fissures, long philtrum, dysplastic ears, bilateral epicanthus)	Del(8)(p23.1)	6928936	7727586	0.79 Mb
				Del(16)(p13.3)	386286	1361169	0.97 Mb
aCGH-472	7	M	Autism, dysmorphic features (narrow forehead, retromicrognathia, dysplastic ears, short-thick fingers, hyperextensibility)	Del(1)(q21.1)	147311057	147349306	0.03 Mb
				Del(7)(q36.3)	155288370	155295993	7.623 kb
aCGH-20	11	M	Autism, epilepsy	No aberration	-	-	-
aCGH-567	8	M	Autism, mental retardation, dysmorphic features (tubular nose, hypotelorism)	Del(1)(q21.1)	145872615	147308632	1.43 Mb
				Del(2)(q21.1q21.2)	132023708	132942237	0.91 Mb
				Del(16)(p13.11)	14831450	16582412	1.75 Mb
aCGH-609	6	M	Autism	Del(1)(q21.1)	145846731	146812360	0.96 Mb
				Del(2)(q21.1q21.2)	131853615	132750662	0.897 Mb
				Del(16)(p11.2)	31855086	33868735	2.01 Mb
aCGH-6010	11	M	Autism	No aberration	-	-	-
aCGH-6011	8	M	Autism	Del(16)(p11.2)	31855086	33868735	2.01 Mb

Moreover, we found the 2p21 deletion containing the *SIX3* gene in 6 patients in our study and it is defined as holoprosencephaly region 2 by the analysis software. Similarly, we found the 7q36.3 deletion containing the *SHH* gene in our 3 patients and it is again defined as holoprosencephaly region 3 by the analysis software. The data should be analyzed with a method similar to exome sequencing to understand whether these deletions point to actual data or are because of a probe synthesis error during technological production.

The 16p13.3 region that we found in two patients is defined as a novel recurrent/overlapping CNV related with ASDs (14).

It is interesting to note that some de novo or inherited CNVs associated with ASD, which occur in the same locus among unrelated individuals, have so far resisted identification of specific ASD genes. One of the most frequent of these involves the 16p11.2 region that we saw in 12 (33.3%) of our patients. Although many of the duplications or deletions observed in ASD children occur as de novo variants, duplications, for example on chromosome 16p11.2, often are inherited from an asymptomatic parent. Moreover, both deletions and duplications encompassing a portion of the 16p11.2 region have been associated with ASD and 16p11.2 gains have been associated with attention deficit-hyperactivity disorder and schizophrenia. These findings indicate that the same genomic region can be involved in multiple developmental conditions.

We found 2q21.1q21.2 deletion in eight patients (22.2%). The G protein-coupled receptor 39 (*GPR39*) gene in this region is expressed in the body, especially in the central nervous system. Zinc is known as the natural ligand of this receptor. Zinc activates different signaling pathways via *GPR39*, mainly the phospho-CREB/CRE pathway. Phospho-CREB is involved in the physiological and pharmacological regulation of the expression of brain-derived neurotrophic factor (BDNF), which is involved in the process of neuron survival and the regulation of neuronal plasticity (15). Deletion of the *GPR39* gene may lower the expression of BDNF and decreased expressions of BDNF were detected in autism patients in previous studies (16).

Deletion in the 1q21.1 region was found in ten patients (27.7%) in the present study. Specific deletion at 1q21.1 was found in association with schizophrenia, mental retardation, autism, and attention deficit-hyperactivity disorder before (17).

8p23.1 was deleted in seven patients (19.4%) according to our results. 8p23.1 copy number loss was related to ASD in a previous study (18).

According to our study, all FISH results of the seven patients were negative. These findings show us the importance of the CGH array in clinical diagnosis in terms of sensitivity. This sensitive technology will give us opportunities to detect new breakpoints about autism pathogenesis.

In light of all these data, we can conclude that the 16p13.11 region and its associated genes represent a predisposition to autism. Our study also indicates genes of the 16p11.2, 1q21.1, 2q21.1q21.2, and 8p23.1 regions that we would like to spotlight in primary autism.

Our study is a whole-genome aCGH analysis and most of our findings are previously reported deletions in patients with primary autism. Deletions in these regions should be investigated in further studies to understand the pathogenesis of primary autism

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