Diagnostic Value of Microarray Method in Autism Spectrum Disorder, Intellectual Disability, and Multiple Congenital Anomalies and Some Candidate Genes for Autism: Experience of Two Centers

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Abstract

Objective: This study aimed to demonstrate the diagnostic value of microarray testing in autism spectrum disorder, intellectual disability, and multiple congenital anomalies of unknown etiology, as well as to report some potential candidate genes for autism.

Methods: Microarray analysis records between January 2016 and December 2017 from two Genetic Diagnostic Centers in Turkey, Kanuni Sultan Suleyman and Adana Numune Training and Research Hospital, were compiled. Detected copy number variations (CNVs) were classified as benign, likely benign, variants of uncertain significance (VUS), likely pathogenic, and pathogenic according to American College of Medical Genetics and Genomics guidelines. The clinical findings of the some patients and the literature data were compared.

Results: In 109 (24.5%) of 445 patients, a total of 163 CNVs with reporting criterion feature were detected. Sixty-nine (42%) and 8 (5%) of these were evaluated as pathogenic and likely pathogenic, respectively. Fifteen (9%) CNVs were also evaluated as VUS. Pathogenic or likely pathogenic CNVs were detected in 61 (13.6%) of 445 patients.

Conclusions: We found that the probability of elucidating the etiology of microarray method in autism spectrum disorder, intellectual disability, and multiple congenital anomalies is 13.6% with a percentage similar to the literature. We suggest that the MYT1L, PXDN, TPO, and AUTS2 genes are all strong candidate genes for autism spectrum disorders. We detailed the clinical findings of the cases and reported that some CNV regions in the genome may be associated with autism.

Keywords: Microarray, autism spectrum disorders, autism genes and CNV regions.
INTRODUCTION

Rare diseases, which affect 6% to 8% of the European Union population and 10% of the US population, are a major health issue worldwide. Each country allocates a significant portion of its health expenditures to the diagnosis and treatment of rare diseases. For effective medical management and genetic counseling, definitive etiological diagnosis is crucial in rare diseases. It has been reported that approximately 80% of rare diseases have genetic variations in the etiology, and these diseases are often chronic and life-threatening. However, for this group, who were mostly evaluated by many health specialists, the diagnostic rates are not at the desired level. There are several different reasons for this situation. Some of these reasons include lack of information about the diseases, challenges in the availability of genetic tests, and high-priced tests. For two decades, through advances in genetic techniques, diagnostic rates in rare diseases have significantly increased. Microarray-based copy number variation (CNV) analysis and next-generation sequencing techniques, in particular, have provided significant contributions to diagnostic rates. Other factors that increase this rate include the application of genetic tests in laboratories of genetic diagnosis centers in many parts of the world, as well as the decrease in test prices.

Microarray is one of the important diagnostic tools that we frequently use in molecular genetics for intellectual disability (ID), developmental delay (DD), autism spectrum disorder (ASD), and multiple congenital anomalies (MCAs), which are common findings of rare diseases. The International Standards for Cytogenomic Arrays (ISCA) have recommended comparative genomic hybridization and single nucleotide polymorphism analysis as first-line diagnostic tests in patients with these conditions. The discovery of 10%-15% of diagnostic yield using microarray analysis in these populations has been supported in the literature. Many criteria are considered to classify CNVs. Many CNVs are classified as of uncertain clinical significance, despite the fact that many databases have been evaluated, such as DECIPHER, ISCA, ClinVar, Online Mendelian Inheritance in Man (OMIM), and Database of Genomic Variants. This problem can be overcome by bringing more microarray data into the literature and clinical databases, as well as by standardizing existing databases. As a result, it is possible to understand the real diagnosis rate of microarray testing in related diseases. For this purpose, we analyzed the microarray results and clinical findings of cases with mental retardation and MCAs, especially ASD, who applied to two different genetic diagnosis centers in Turkey.

MATERIALS and METHODS

Between January 2016 and December 2017, microarray analysis records from two genetic diagnosis centers in Turkey, Kanuni Sultan Suleyman and Adana Numune Training and Research Hospital, were compiled. The files of the patients who had any microarray analysis findings were examined. In addition, parental kinship and, if available, microarray results were compiled. This study was retrospectively designed, and all patient files between January 2016 and December 2017 were examined. Written informed consent for the study was obtained from all patients or their parents. The study was approved by University of Health Sciences Turkey, Kanuni Sultan Suleyman Training and Research Hospital Clinical Research Ethics Committee (decision no: 04, date: 28.09.2018). Every patient underwent a detailed evaluation by a medical geneticist, which included prenatal and birth history, pedigree, family history, and detailed clinical and dysmorphology examinations.

Statistical Analysis

According to the manufacturer’s instructions, genomic DNA was extracted from peripheral blood samples kept in EDTA tube. Affymetrix CytoScan Optima 315K arrays (Affymetrix, Santa Clara, CA, USA) were used in the microarray study of all patients. The data obtained were evaluated with the Chromosome Analysis Suite for the CHAS 3.1 program and reference GRCh 37/hg19. CNVs were classified as benign, likely benign, variants of uncertain significance (VUS), likely pathogenic, and pathogenic according to American College of Medical Genetics (ACMG) guidelines. In the study, descriptive biostatistical analysis was used.

RESULTS

CNV with reporting criteria was detected in 109 (24.5%) of 445 patients (229 men and 216 women). The mean age of the patients was 5.1 yr. Some patients had more than one CNV finding, a total of 163, of which 75 (46%) were gains, and 88 (54%) were losses. According to the ACMG criteria, 69 (42%) of them were considered pathogenic and 8 (5%) likely pathogenic, while 26 (16%) of them were benign, and 45 (27%) were considered likely benign. Fifteen (9%) CNVs were also classified as VUS (Figure 1). Pathogenic or likely pathogenic CNV was observed in 61 (13.6%) of 445 patients (Table 1).

22q11.21 region was the most common pathogenic CNV region in our report. 22q11 deletion and duplication were observed in four and two patients, respectively. In addition, deletion and duplication in this region were detected in a patient (P71) together. 15q11.2 region was
the second most common pathogenic CNV region. Three patients had a deletion of the region, whereas one patient had duplication. One of the other pathogenic variants was 8q24.3 duplication detected in three patients. Other frequent pathogenic variant regions observed in our two genetic diagnosis centers were 8p23, 16p13, 1p36, 1q21.1, 2p25.3, 9q34.3, 4p16, 6p25.3, 22q13.3, and 17q11.2.

CNVs in the Yq11.2 region were the most common benign variant and were observed in seven patients. CNVs in Xp22, Xq28, 10q11.22, and 15q11.2 regions were other common benign variants shown in this study (Table 2).

Fifteen VUS in 15 patients whose size ranged from 400 to 1,200 kb were detected (Table 3). Four of these patients’ pathogenic CNVs were also present.

**DISCUSSION**

In many rare disease groups, arrayCGH method has started to be used as a first-line diagnostic test. The arrayCGH test has a diagnostic value of 10%-15% in this population, and this rate in the literature was observed to be similar in our study (13.6%). The most significant issue in the arrayCGH results of these patient groups today is the difficulty in evaluating the data. Parental studies may contribute to the solution of this problem, but this brings the additional cost of using arrayCGH. There are many suggestions for evaluating arrayCGH data in these large patient groups more effectively and efficiently, including enriching the data content of relevant clinical databases and increasing healthy population studies. In this article, we aimed to share the arrayCGH results of patients who were referred to two genetic diagnosis centers in Turkey.

**Cases Diagnosed with ASD**

In five patients with ASDs, pathogenic or likely pathogenic CNVs located on 2p25.3, 7q11.22, 15q11.2q13.1, and 17p11.2 were observed. In addition, 1p36.32, 8q24.3, and 9q34.2 duplications were seen together in P4. Deletions involving chromosome band 2p25.3 are associated with a nonspecific clinical phenotype that includes ID, obesity, and various dysmorphic features. Deletion of the 2p25.3 region, including the disease-causing MYT1L, PXDN, and TPO genes, was observed in P76. Our patient had ASDs, ID, and epileptic seizures. When we evaluated the patient at the age of 6, she did not have obesity, but we have no information about her current condition. Major clinical findings as 7q11.22 deletion, mental motor retardation (MMR), ASD, and atypical facial appearance were detected in P109. One of the important genes in this region is AUTS2, which has been reported to be linked to autism, ID, and juvenile myoclonic epilepsy.
| Patient no | Gender | Age  | Consanguinity | Major findings                                                                 | Chromosomal location | Deletion/duplication | Size (Kb) | Evaluation of pathogenicity | Inheritance |
|-----------|--------|------|---------------|-------------------------------------------------------------------------------|----------------------|----------------------|-----------|-----------------------------|-------------|
| P4        | M      | 7 yr | NA            | Autism spectrum disorders, delayed speech development                          | 1p36.32              | Gain                 | 798       | LP                          | De novo     |
|           |        |      |               |                                                                                | 8q24.3               | Gain                 | 1.297     | LP                          | De novo     |
|           |        |      |               |                                                                                | 9q34.3               | Gain                 | 1.145     | LP                          | De novo     |
| P5        | M      | 3 yr | NA            | MCA, epilepsy, atypical facial appearance, growth retardation, intellectual disability, atrial septal defect, hypospadias | 2q35q37.3           | Gain                 | 24.480    | P                           | Unknown     |
|           |        |      |               |                                                                                | 9p24.3p24.1         | Loss                 | 4.850     | P                           | Unknown     |
| P6        | M      | 2 yr | NA            | Congenital cardiac anomalies                                                   | 22q11.21            | Loss                 | 2.843     | P                           | Unknown     |
| P7        | M      | 1 yr | NA            | Microcephaly, cerebellum hypoplasia, hypotonia, growth retardation, intellectual disability | 2q36.3q37.3         | Gain                 | 15.162    | P                           | De novo     |
|           |        |      |               |                                                                                | 10q26.2q26.3        | Loss                 | 7.518     | P                           | De novo     |
| P9        | F      | 9 yr | NA            | Corpus callosum agenesis, mental retardation, atypical facial appearance, iris abnormality | 18q21.31q21.32      | Loss                 | 1.755     | LP                          | De novo     |
| P10       | F      | 10 yr| NA            | MMR, epilepsy                                                                   | 22q13.31q13.33      | Loss                 | 4.964     | P                           | De novo     |
| P12       | M      | 5 yr | NA            | MCA/MR, microcephaly, cardiomyopathy, IUGR                                     | 9q21.11q22.32       | Gain                 | 27.304    | P                           | De novo     |
| P16       | F      | 2 yr | NA            | Autism Spectrum Disorders, MMR, atypical facial appearance                      | 17p11.2             | Gain                 | 3.511     | P                           | De novo     |
| P18       | M      | 2 yr | No            | Growth retardation (prenatal onset), microcephaly, hypotonia, corpus callosum hypoplasia | 15q11.2q12         | Loss                 | 3.924     | P                           | Unknown     |
| P20       | F      | 3 yr | NA            | MCA, Growth retardation                                                         | 4p16.3p16.1         | Loss                 | 6.799     | P                           | De novo     |
|           |        |      |               |                                                                                | 4p16.1p15.1         | Gain                 | 22.306    | P                           | De novo     |
| P23       | M      | 2 yr | No            | MMR, atypical facial appearance, microcephaly, myoclonic seizure, pes valgus, flexion contractures | 1p36.33p36.31       | Loss                 | 5.207     | P                           | De novo     |
|           |        |      |               |                                                                                | 4p16.3              | Gain                 | 3.800     | P                           | De novo     |
| P24       | M      | 2 yr | NA            | IUGR, atypical facial appearance, hypotonia, cataract, cryptorchidism, pes equinovarus | 4p16.3p15.1        | Loss                 | 31.803    | P                           | De novo     |
| P29       | F      | 3 yr | NA            | Intellectual disability, growth retardation, microcephaly, severe short stature | 19p13.3             | Gain                 | 3.153     | P                           | Unknown     |
| P30       | M      | 1 yr | NA            | Epilepsy, microcephaly, developmental delay                                     | 1p34.2              | Loss                 | 3.033     | P                           | Unknown     |
| P31       | F      | 8 yr | Yes           | MMR, atypical facial appearance, ASD, cleft palate, cataract                   | 6q25.1q27           | Loss                 | 15.072    | P                           | De novo     |
| P33       | F      | 9 yr | NA            | MMR                                                                          | 16p13.3p13.2        | Gain                 | 9.084     | P                           | Unknown     |
| P38       | M      | 17 yr| NA            | West syndrome, MR                                                             | 5q14.3              | Loss                 | 4.588     | P                           | De novo     |
| P39       | F      | 2 yr | NA            | MMR, premature                                                                 | 2q37.1q37.3         | Loss                 | 7.703     | P                           | De novo     |
| P40       | M      | 2 yr | NA            | Atypical facial appearance, coarse face, frequent upper respiratory infections, truncus arteriosus | 22q11.21           | Loss                 | 1.800     | P                           | De novo     |
| P41       | M      | 2 yr | Yes           | Autism spectrum disorders (early onset), developmental delay                  | 15q11.2q13.1        | Gain                 | 6.058     | P                           | De novo     |
| P43       | M      | 1 yr | NA            | MCA/MR                                                                       | 9q34.3              | Loss                 | 3.015     | P                           | Unknown     |
|           |        |      |               |                                                                                | 22q13.31q13.33      | Gain                 | 3.859     | P                           | Unknown     |
| Patient no | Gender | Age     | Consanguinity | Major findings                                                                 | Chromosomal location | Deletion/duplication | Size (Kb) | Evaluation of pathogenicity | Inheritance |
|------------|--------|---------|---------------|---------------------------------------------------------------------------------|----------------------|----------------------|-----------|-----------------------------|-------------|
| P48        | M      | 5 yr    | NA            | Congenital cardiac anomaly, intellectual disability                             | 22q11.21             | Loss                 | 2.549     | P                           | De novo     |
| P50        | F      | 1 yr    | No            | Mental retardation, unilateral renal agenesis, ASD, periferic pulmonary stenosis, blue sclerae, atypical facial appearance, overlapping fingers | 15q24.1q24.2         | Loss                 | 2.920     | P                           | De novo     |
| P51        | M      | 8 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 16p11.2              | Loss                 | 659       | P                           | Unknown     |
| P53        | M      | 3 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 17p13.3p13.2         | Loss                 | 1.590     | P                           | De novo     |
| P55        | F      | 12 yr   | NA            | MR, obesity, hyperthyroidism                                                    | 22q11.21             | Gain                 | 2.888     | P                           | Paternal    |
| P56        | M      | 9 months| NA            | Congenital cardiac anomaly, intellectual disability                             | 5p15.33              | Loss                 | 1.557     | P                           | Paternal    |
| P57        | M      | 5 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 17p13.3q11.22        | Loss                 | 4.034     | P                           | De novo     |
| P58        | M      | 4 yr    | No            | MR, aortic coartation, PDA, VSD, left ventricular hypertrophy                   | 1q33.3q34            | Loss                 | 4.824     | P                           | Unknown     |
| P59        | M      | 3 yr    | Yes           | Congenital cardiac anomaly, intellectual disability                             | 17q11.2              | Loss                 | 1.364     | P                           | De novo     |
| P60        | F      | 2 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 1q33.3q13.43         | Gain                 | 9.515     | P                           | De novo     |
| P61        | F      | 3 yr    | Yes           | Congenital cardiac anomaly, intellectual disability                             | 19q13.25             | Loss                 | 916       | P                           | De novo     |
| P62        | M      | 1 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 19q13.3q13.43        | Gain                 | 9.515     | P                           | De novo     |
| P65        | M      | 11 yr   | No            | Congenital cardiac anomaly, intellectual disability                             | 4q28.3q31.21         | Loss                 | 4.898     | P                           | De novo     |
| P66        | M      | 5 yr    | Yes           | Congenital cardiac anomaly, intellectual disability                             | 5q11.23              | Loss                 | 1.609     | P                           | De novo     |
| P69        | F      | 1 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 15q11.2q13.3         | Loss                 | 10.144    | P                           | De novo     |
| P71        | M      | 1 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 22q11.21             | Loss                 | 2.888     | P                           | Unknown     |
| P74        | M      | 4 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 17q11.2              | Loss                 | 1.285     | P                           | De novo     |
| P76        | F      | 6 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 2p25.3               | Loss                 | 3.327     | P                           | Unknown     |
| P77        | M      | 16 yr   | Yes           | Congenital cardiac anomaly, intellectual disability                             | Yp11.2               | Gain                 | 945       | LP                          | Unknown     |
| P78        | M      | 3 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 6p25.3               | Loss                 | 1.137     | P                           | Paternal    |
| P79        | M      | 3 yr    | No            | Congenital cardiac anomaly, intellectual disability                             | 1q21.1               | Gain                 | 2.100     | P                           | Unknown     |

Table 1. continued
| Patient no | Gender | Age | Consanguinity | Major findings | Chromosomal location | Deletion/duplication | Size (Kb) | Evaluation of pathogenicity | Inheritance |
|------------|--------|-----|--------------|----------------|----------------------|---------------------|----------|-----------------------------|-------------|
| P81        | F      | 7 yr | No           | Developmental delay, short stature, corpus callosum hypoplasia | 13q21.33q31.1 | Loss                | 11.842   | P                           | Unknown     |
| P82        | M      | 2 yr | No           | Intellectual disability, nystagmus, megacisterna magna, VSD | 16p13.12p12.3 | Loss                | 2.139    | P                           | De novo     |
| P84        | M      | 8 yr | Yes          | Deafness, atypical facial appearance, cryptorchidism | 6q14.3 | Gain                | 2.307    | LP                          | Unknown     |
| P86        | F      | 2 yr | No           | Intellectual disability, developmental delay, corpus callosum agenesis, hydrocephaly | 8p23.3p23.1 | Loss                | 6.812    | P                           | De novo     |
| P87        | F      | 12 yr | No        | Epilepsy, MMR, microgliosis, asymmetric hippocampus, and frontal lobes | 13q14.1q21.2 | Loss                | 18.822   | P                           | De novo     |
| P88        | F      | 2 yr | No           | Atypical facial appearance, hydrocephaly, corpus callosum agenesis, colpocephaly | 17q21.31 | Loss                | 590      | P                           | De novo     |
| P89        | F      | 5 yr | No           | MMR, atypical facial appearance, delayed myelination white matter | 18p11.2p11.21 | Gain               | 15.034   | P                           | De novo     |
| P91        | F      | 3 yr | No           | IUGR, MMR, microcephaly | 7q36.1q36.3 | Loss                | 9.553    | P                           | De novo     |
| P92        | F      | 4 yr | Yes          | Motor retardation, overgrowth, difficulty swallowing foods | Xp22.2p11.3 | Loss                | 28.140   | P                           | De novo     |
| P93        | F      | 3 yr | No           | Atypical facial appearance, MMR, deafness, microcephaly, delayed myelination white matter | 18q22.3q23 | Loss                | 8.252    | P                           | De novo     |
| P94        | M      | 5 yr | No           | Atypical facial appearance, VSD, PDA, splenomegaly, vesicoureteral reflux, hallux valgus, renal ectopia | 18q11.1q12.3 | Gain               | 19.882   | P                           | De novo     |
| P96        | M      | 2 yr | No           | MMR, ASD, atypical facial appearance, cryptorchidism | 15q11.2q13.1 | Loss                | 6.265    | P                           | De novo     |
| P98        | M      | 4 yr | Yes          | Intellectual disability, microcephaly | 7p11.2q11.21 | Loss                | 4.547    | LP                          | De novo     |
| P103       | M      | 7 yr | No           | MR (mildly), atypical facial appearance, cryptorchidism, prematurity, pectus excavatum, preauricular pit | 22q11.1q11.21 | Gain               | 4.577    | P                           | Unknown     |
| P104       | F      | 8 yr | No           | MR (mildly), short stature | 8p23.3p23.2 | Loss                | 5.149    | P                           | De novo     |
| P107       | F      | 7 yr | No           | MMR, atypical facial appearance | 8p23.3 | Loss                | 5.149    | P                           | De novo     |
| P108       | M      | 2 yr | No           | MMR, autism spectrum disorder, atypical facial appearance | 7q11.22 | Loss                | 495      | P                           | Unknown     |
| P109       | F      | 12 yr | No          | Short stature, delayed puberty, cleft palate, bifid uvula, pectus excavatum (mildly) | 1q21.1q12.1 | Loss                | 2.016    | P                           | Unknown     |
| P110       | F      | 8 yr | No           | Intellectual disability, severe short stature, atypical facial appearance | 1q44 | Loss                | 2.952    | LP                          | De novo     |

M: Male, F: Female, P: Pathogenic, LP: Likely pathogenic, MR: Mental retardation, MMR: Mental motor retardation, MCA: Multiple congenital anomaly, IUGR: Intrauterin growth retardation, ASD: Atrial septal defect, VSD: Ventricular septal defect, PDA: Patent ductus arteriosus, EMC: Electromyography.
| Patient no | Gender | Age   | Consanguinity | Major findings                                           | Chromosomal location | Deletion/duplication | Size Kb | Evaluation of pathogenicity | Inheritance |
|------------|--------|-------|---------------|---------------------------------------------------------|----------------------|----------------------|---------|-----------------------------|-------------|
| P1         | F      | 18 yr | NA            | Delayed pubertal development, ovarian agenesis, small uterus, obesity | Xq28                 | Loss                 | 286     | LB                          | Unknown     |
| P2         | M      | 3 yr  | NA            | MCA/MR, contracture, hypotonia                           | 3p26.1               | Loss                 | 961     | LB                          | Unknown     |
| P3         | F      | 1 yr  | Yes           | Pulmonary atresia/hypoplasia, ventricular septal defect  | 4q34.1               | Gain                 | 1,045   | B                           | Unknown     |
| P5         | M      | 3 yr  | NA            | MCA, epilepsy, atypical facial appearance, growth retardation, intellectual disability, atrial septal defect, hypoplasia | 9q32                 | Gain                 | 280     | B                           | Unknown     |
| P6         | M      | 2 yr  | NA            | Congenital cardiac anomalies                             | Yq11.223             | Loss                 | 133     | B                           | Unknown     |
| P8         | M      | 14 yr | Yes           | Autism spectrum disorders                                 | 3p25.2               | Loss                 | 243     | B                           | Paternal    |
| P10        | F      | 10 yr | NA            | MMR, epilepsy                                            | 18p11.31             | Gain                 | 1,200   | LB                          | Paternal    |
| P11        | M      | 2 yr  | No            | Developmental anomaly, MCA (mildly), obesity             | 7q35                 | Loss                 | 534     | B                           | Maternal    |
| P13        | M      | 1 yr  | No            | IVF pregnancy, MCA, hydronephrosis                       | 7q35                 | Loss                 | 124     | B                           | De novo     |
| P14        | M      | 4 yr  | NA            | Growth retardation (prenatal onset), microcephaly, congenital cardiac anomalies, poor eyesight | 8p24.3               | Gain                 | 241     | LB                          | De novo     |
| P15        | M      | 2 yr  | Yes           | MMR, atypical facial appearance, subaortic ventricular defect, prematurity, spasticity | 9q32                 | Gain                 | 280     | B                           | Unknown     |
| P17        | F      | 5 yr  | NA            | Psychomotor retardation, epilepsy                        | 15q26.3              | Loss                 | 161     | B                           | De novo     |
| P18        | M      | 2 yr  | No            | Growth retardation (prenatal onset), microcephaly, hypotonia, corpus callosum hypoplasia | Yq11.223             | Loss                 | 133     | B                           | Unknown     |
| P19        | M      | 3 yr  | No            | Microcephaly, iris coloboma, cataract, strabismus        | 2q21.1               | Loss                 | 215     | B                           | De novo     |
| P20        | F      | 3 yr  | NA            | MCA, growth retardation                                  | 7q36.3               | Gain                 | 529     | LB                          | De novo     |
| P21        | M      | 5 yr  | Yes           | Atypical facial appearance, pes equinovarus, VSD         | 8p23.3               | Gain                 | 288     | B                           | De novo     |
| P22        | M      | 2 yr  | NA            | Autism spectrum disorders                                 | Yq12                 | Loss                 | 150     | B                           | Paternal    |
| Patient no | Gender | Age | Consanguinity | Major findings                                                                 | Chromosomal location | Deletion/duplication | Size Kb | Evaluation of pathogenicity | Inheritance |
|------------|--------|-----|---------------|-------------------------------------------------------------------------------|----------------------|----------------------|--------|-----------------------------|-------------|
| P25        | M      | 4 yr| Yes           | Autism spectrum disorders, choanal atresia, cleft lip and palate, delayed speech development | 16p13.3              | Loss                 | 518    | LB                          | Unknown     |
| P27        | M      | 6 yr| Yes           | Epilepsy, delayed speech development, atypical facial appearance, abnormal EEG | 17q21.31             | Loss                 | 372    | LB                          | De novo     |
|            |        |     |               |                                                                                | Xp22.31              | Gain                 | 1.600  | LB                          | Maternal    |
| P28        | M      | 2 yr| Yes           | MCA/MR, hypotonia, microcephaly                                               | 7q21.12q21.13        | Gain                 | 1.765  | LB                          | Paternal    |
| P29        | F      | 3 yr| NA            | Growth retardation, microcephaly                                               | 17q25.3              | Loss                 | 1.099  | LB                          | Unknown     |
| P32        | M      | 14 yr| NA            | Autism spectrum disorders, MMR, epilepsy                                       | 10q23.31             | Gain                 | 522    | LB                          | Unknown     |
| P35        | M      | 6 months| NA | IUGR (prenatal onset), short extremities, polyhydramnios, duodenal atresia, ASD (secundum) | 19q13.42             | Loss                 | 314    | B                           | De novo     |
| P37        | M      | 17 yr| NA            | Atypical facial appearance, learning disability, scoliosis, hydrachyposias    | 1q31.3               | Loss                 | 345    | B                           | De novo     |
| P45        | M      | 1 yr| No            | Atypical facial appearance, cutis laxa, low birth weight, cryptorchidism, deafness, cleft palate, multiple dislocation, congenital hip dislocation, aortic root dilatation | 3p26.3               | Gain                 | 716    | LB                          | De novo     |
| P46        | M      | 3 yr| NA            | Autism spectrum disorders                                                     | Yq11.23              | Loss                 | 1.320  | LB                          | Unknown     |
| P47        | M      | 5 yr| NA            | Autism spectrum disorders                                                     | 3q21.3               | Loss                 | 908    | LB                          | Unknown     |
| P48        | M      | 5 yr| NA            | Congenital cardiac anomaly, intellectual disability                          | Yq11.23              | Loss                 | 1.831  | LB                          | De novo     |
| P49        | M      | 5 yr| Yes           | Atypical facial appearance, learning disability, short stature, short neck, delayed speech development | 15q11.2              | Loss                 | 506    | LB                          | Paternal    |
| P52        | F      | 1 yr| NA            | MCA/MR                                                                       | 17q11.2              | Loss                 | 832    | LB                          | Unknown     |
| P54        | F      | 10 yr| No            | Micrognathia, ASD, pulmoner atresia, tricuspid valve hypoplasia              | 17p13.3              | Loss                 | 212    | LB                          | Paternal    |
| P58        | M      | 4 yr| No            | MR, aortic coarctation, PDA, VSD, left ventricular hypertrophy               | 3q23                 | Gain                 | 486    | LB                          | Unknown     |
| P59        | F      | 17 yr| NA            | MR, scoliosis                                                                | 4q21.21q21.22        | Loss                 | 691    | LB                          | Unknown     |
|            |        |     |               |                                                                                | 10q11.22             | Gain                 | 2.048  | LB                          | Unknown     |
| P64        | F      | 18 yr| No            | MR, seizures, hypothyroidism                                                 | 10q11.22             | Gain                 | 1.959  | B                           | Paternal    |
| Patient no | Gender | Age | Consanguinity | Major findings                                      | Chromosomal location | Deletion/duplication | Size (Kb) | Evaluation of pathogenicity | Inheritance |
|-----------|--------|-----|---------------|-----------------------------------------------------|----------------------|----------------------|----------|----------------------------|-------------|
| P68       | M      | 6 yr | No            | Autism spectrum disorders, IVF pregnancy, delayed speech development | Yq11.223q11.23      | Gain                 | 4.042    | LB                         | Paternal    |
| P70       | M      | 6 months | No     | Unilateral cleft palate, high nasal bridge, skin tag on ear lobe | 8q23.3               | Gain                 | 861      | LB                         | De novo     |
| P72       | M      | 6 yr | NA            | Common developmental disorder | 16p13.3              | Loss                 | 518      | LB                         | Paternal    |
| P75       | F      | 9 yr | Yes           | Facial asymmetry, hemivertebrae, cafe-au lait, low back hairline | 1p32.2p32.1          | Loss                 | 1.122    | LB                         | Paternal    |
| P77       | M      | 16 yr | Yes          | Epilepsy, short stature, MR (mildly) | Yq11.23              | Loss                 | 525      | LB                         | Unknown     |
| P81       | F      | 7 yr | No            | Developmental delay, short stature, corpus callosum hypoplasia | 1q21.3               | Loss                 | 200      | LB                         | Unknown     |
| P83       | F      | 2 yr | Yes           | Developmental delay, motor retardation, cerebellar atrophy, irregular teeth | 4q28.3              | Loss                 | 226      | B                          | Paternal    |
| P87       | F      | 12 yr | No            | Epilepsy, MMR, microgliosis, asymmetric hippocampus, and frontal lobes | 15q11.2              | Gain                 | 518      | LB                         | De novo     |
| P90       | M      | 2 yr | No            | MMR, congenital cataract | Xp22.2               | Gain                 | 262      | LB                         | De novo     |
| P91       | F      | 3 yr | No            | IUGR, MMR, microcephaly | 1q21.2               | Loss                 | 522      | B                          | De novo     |
| P95       | M      | 10 yr | No            | Seizures, MR (mildly) | 14q21.2              | Gain                 | 423      | B                          | De novo     |
| P97       | M      | 14 yr | No            | Epilepsy, MR | Xq21.31            | Gain                 | 485      | LB                         | De novo     |
| P98       | M      | 4 yr | Yes           | Intellectual disability, microcephaly | 6q12                 | Loss                 | 332      | B                          | Paternal    |
| P99       | M      | 2 yr | No            | Atypical facial appearance, MMR, inguinal hernia, | 4q13.2               | Loss                 | 184      | B                          | Paternal    |
| P100      | F      | 19 yr | Yes           | MMR, epilepsy, self-destructive behavior | 22q11.22             | Gain                 | 433      | LB                         | Unknown     |
| P101      | F      | 8 yr | Yes           | MMR, epilepsy | 2q13               | Loss                 | 266      | LB                         | Paternal    |
| P102      | M      | 12 yr | Yes           | MMR | Yq11.223q11.23    | Gain                 | 3.841    | LB                         | Unknown     |
| P105      | M      | 4 yr | No            | Delayed speech development, aortic coarctation, VSD, ASD, neuroblastoma, Meckel diverticulum | 16q23.3              | Gain                 | 415      | B                          | Paternal    |
| P106      | M      | 4 yr | Yes           | Perimembranous VSD, motor retardation, facial asymmetry, skin tag | Xq22.2              | Gain                 | 190      | LB                         | Unknown     |

M: Male, F: Female, B: Benign, LB: Likely benign, MR: Mental retardation, MMR: Mental motor retardation, MCA: Multiple congenital anomaly, IUGR: Intrauterin growth retardation, ASD: Atrial septal defect, VSD: Ventricular septal defect, PDA: Patent ductus arterious, EEG: Electroencephalography
The clinical findings of our patient with a 495 kbp deletion region in AUTS2 are compatible with the literature. The region we detected as duplication in another patient (P41) referred to our department due to autism, and DD was 15q11.2q13.1. The association between approximately 6 Mb duplication of this region and autism is well known, and this phenotype is registered at OMIM with #608636. The other region, also known as
Potocki-Lupski syndrome (PTLS), which is well known to be associated with autism and identified in OMIM with the #610883 phenotype, is the 17p11.2 duplication region. In a 2-yr-old female patient referred to us due to early-onset autism, MMR and atypical facial appearance and duplication of 3.511 kbp in the 17p11.2 region was shown. Her clinical findings were consistent with PTLS. A 7-yr-old male patient (P4) referred for ASD and delayed speech development had three possible pathogenic CNV sites, 1p36.32, 8q24.3, and 9q34.3 duplications. To our knowledge, duplication of regions 1p36.32 and 9q34.2 has been reported in two individuals with autism at 292900 and 256523, respectively, in DECIPHER, although there is no proven association between duplication of these regions and autism.

In 15 patients, 15 CNV regions that we interpreted as VUS were shown, and three of them had ASD, whether isolated or not. Each of these three patients had duplication of the following regions: 16q23.1, 4p16.1, and 1p36.33p36.32. Duplication of 16q23.1, known as the fragile region, is not associated with autism in the light of current knowledge. In a patient with mental retardation and midface hypoplasia, duplication of 16q22.1→q23.1 has been reported via karyotype15. However, although no array was used to detail this region, the duplication we detected as de novo in P22, who applied to our department with isolated ASD, covers the area with a high probability. 4p16.1 was the second VUS region we showed as duplication in patients with autism. Although the association between 4p16.1 duplication and some psychiatric conditions, such as schizophrenia, is known, there is no correlation between duplication of this region and autism. It is reported that DRD5, which encodes the D5 subtype dopamine receptor found in the duplication region of our patient, may be among the genes that have an important role in the etiopathogenesis of schizophrenia16,17. However, our 5-yr-old girl patient had no psychiatric disease excluding autism. The third VUS we found as duplication in patient with ASD, motor retardation, and atypical facial appearance was 1p36.33p36.32. In the light of current knowledge, there is no known relationship between duplication of this region and autism in the literature, DECIPHER, and ClinVar.

Other Special Cases

ArrayCGH is a significant diagnostic method for mental disability of unknown etiology, as well as ASDs of unknown etiology. The majority of patients with likely pathogenic or pathogenic CNV had an ID, and the deletion duplication sizes in the regions, excluding six CNV, were larger than 1 Mb. A 24 Mb duplication of 2q35 and a 4.8 Mb deletion of 9p24.3p24.1 were detected in P5, who was referred to our department due to ID, MCAs, and atypical facial appearance and epilepsy findings. Congenital heart defects, dysmorphic facial features, hypotonia, feeding difficulties, microcephaly, and psychomotor development were observed in a patient with trisomy 2q31.2-37.3 and monosomy 9p24.3 reported by Colangelo et al.18 (2018). When both cases are compared, it is seen that the findings other than epilepsy are common.

We identified 15 Mb duplication of 2q36.3q37.3 and 7.5 Mb deletion of 10q26.2q26.3, which were inherited from his father with a balanced translocation between 10q and 2q, in P7, 1-yr-old male, who had severe hypotonia, microcephaly, and cerebellum hypoplasia. Common findings of this contiguous gene deletion syndrome are short stature, microcephaly, and mental retardation, which are also present in our case19. Duplication of 2q36.3q37.3 was identified together with 10p15.3 deletion in a case with an ID or DD by Lee et al.20.

P10, a 10-yr-old female patient with MMR and epilepsy, has a 5 Mb deletion of 22q13.31q13.33 region. Deletion of this chromosomal location was known as Phelan-McDermid syndrome (PHMDS), which is caused by SHANK3 mutation. Although clinical findings of our patient, such as MMR and seizures, are also present in cases with PHMDS syndrome, the region we detected does not include this gene. Recently, cases without SHANK3 deletion but clinically compatible with PHMDS have been reported21.

P12 with MCAs, mental retardation, microcephaly, intrauterine growth retardation (IUGR), and cardiomyopathy had 9q21.11q22.32 duplication about 27 Mb in size. Microcephaly, mental retardation, MCAs, and facial dysmorphic findings were previously reported in duplication 9q22,23. As far as we know, cardiomyopathy was not reported in cases with duplication of this region before.

In P18, a 2-yr-old male patient who is referred to our department due to microcephaly, prenatal onset growth retardation, hypotonia, and corpus callosum hypoplasia, approximately 4 Mb of deletion was seen in the region of 15q11.2q12, which is also a critical region for Prader-Willi syndrome and Angelman syndrome (AS). We think that his clinical findings are more compatible with AS because of the presence of microcephaly and corpus callosum hypoplasia and the absence of obesity. In addition, he had hypopigmentation of skin and hair, brachycephaly and hyperreflexia seen mostly in those cases with AS.
Loss of 4p16.3p16.1, which is the critical region for Wolf-Hirschhorn syndrome, one of the well-known contiguous gene deletion syndrome, and gain of 4p16.1p15.1 were observed in P20. A 3-yr-old female patient had MMR, ventricular septal defect, low birth weight, and atypical facial appearance (prominent glabella, high forehead, strabismus, and beaked nose). About half of the deletions are isolated, while the other half may be accompanied by trisomy of a different region as a result of balanced translocation. We could not find the chromosome results of our patient’s parents, but one of the parents seems likely to have a translocation or inversion due to the presence of 4p16.1p15.1 gain. We found 4p16.3 duplication and 1p36.33p36.31 deletion in P23 who had MMR, atypical facial appearance, microcephaly, myoclonic seizure, pes valgus, and flexion contractures about 3.8 Mb and 5.2 Mb, respectively. Among the most common terminal deletions, monosomy 1p36 is characterized by MCA, mental retardation, typical craniofacial features, and hypotonia. His craniofacial findings, such as brachycephaly, straight eyebrows, thickened helices, and deep-set eyes were compatible with chromosome 1p36 deletion syndrome. To date, no other case has been found in these two chromosomal anomalies are detected together in the literature. In another patient with a large 4p16.3 deletion of approximately 32 Mb, also identified via cytogenetic before, IUGR, atypical facial appearance, hypotonia, cataract, cryptorchidism, and pes equinovarus were present (P24).

Duplication of 19p13.3 is one of the rarely reported microduplication syndromes, characterized by ID, dysmorphic facial appearance, and IUGR. We found about 3.2 Mb duplication in the region of 19p13.3 in P29 who had ID, growth retardation, microcephaly, and severe short stature. Orellana et al. reported three affected siblings with IUGR, global growth retardation, and dysmorphic features. The duplication of 4.95 Mb in the 19p13.3 region reported in this family overlaps with the second half of our duplication region. These approximately 1.5 Mb regions include AP3DI, AMH, LMNB2, TLE6, and GNA11 as disease-causing gene. Based on clinical findings, growth retardation, microcephaly, and ID were present both our case and aforementioned three cases.

One-year-old male patient (P30) with epilepsy, microcephaly, and DD had a 3.0 Mb deletion in the 1p34.2 encompassing SLC2A1 gene. Glucose transporter type 1 deficiency syndrome, caused by mutations in the SLC2A1 gene, is a neurometabolic disorder typically characterized by acquired microcephaly, progressive encephalopathy, and drug-resistant epilepsy. Sequence analysis detects 81% of the SLC2A1 mutations, while gene-targeted deletion/duplication analysis detects 14%. In this article, SLC2A1 deletion was demonstrated by the arrayCGH method in a patient with P30.

Common findings observed in 15q24 microdeletion cases are global growth retardation, short stature, skeletal abnormalities, and joint laxity. We detected a 2.9 Mb deletion of the 15q24 region involving SIN3A, responsible for Witteveen-Kolk syndrome, in P50. She had motor retardation, unilateral renal agenesis, ASD, peripheric pulmonary stenosis, blue sclerae, atypical facial appearance, and overlapping fingers when she was 1 yr old. Atypical facial findings, such as microretrognathia and high arched palate, and digital abnormalities identified in Witteveen-Kolk syndrome were present in our case. Although peripheric pulmonary stenosis was reported before in 15q24 microdeletion syndrome, renal agenesis and atrial septal defect were not reported before.

Miller-Dieker lissencephaly syndrome, which is caused by the deletion of the lissencephaly 1 gene and tyrosine 3-monoxygenase/tryptophan 5 monoxygenase activation protein (YWHAE), is a severe neurological disease characterized by typical facial features, ID, and epileptic seizures. Positive findings of patient P53, 3-yr-old male, were MMR, epilepsy, microcephaly, dolichocephaly, strabismus, atypical facial appearance, hypermetropia, and lissencephaly. Approximately 1.6 Mb deletion of 17p13.3p13.2 region we showed in the aforementioned patient includes LIS, one of the crucial genes for cerebral development, not YWHAE. A de novo 17p13.3 deletion is observed in approximately 80% of affected individuals. The remaining 20% are inherited from a parent with balanced chromosomal rearrangement. Chromosomal analysis of his parent were normal.

Neurofibromatosis type 1 (NF1) associated with monoallelic NF1 gene mutations is characterized by multiple cafe-au lait spots, freckling in the axillary and inguinal, multiple cutaneous neurofibromas, and lisch nodules in the iris. In P61 referred to our department due to NF1 prediagnosis, atypical facial appearance, ID, cafe-au lait, feeding difficulties, hypothyroidism, and eosinophilia were present. After his NF1 sequence analysis had been detected as normal, arrayCGH was planned and 1.4 Mb deletion of 17q11.2 region involving NF1 gene was found. While no obvious intellectual development problem is observed in most of the affected cases, learning difficulties or behavioral problems is observed in some of the cases. Even if NF1 sequencing is normal, the possibility of deletion should not be ignored if the patient’s findings are compatible with NF1. In addition, cases with NF1 gene deletion are more risky in terms of...
Monoallelic NAA15 (N-alpha-acetyltransferase 15) gene mutations have been reported with the phenotype “intellectual developmental disorder, autosomal dominant 50, with behavioral abnormalities” in the OMIM database. This phenotype is characterized by varying degrees of ID, ASD, and global DD. Craniofacial dysmorphism, congenital heart anomalies, and epileptic seizures are other findings observed. We showed 4.9 Mb deletion of 4q28.3, where the NAA15 was localized, in P65 who had a learning disability, unilateral deafness, overcrowded teeth, cachetic, and atypical facial appearance. PCDH18 (protocadherin 18), the other possible candidate gene is located in the deletion region for ID, is expressed in the brain, heart, kidney, lung, and trachea. The association of other genes in the deletion region with deafness and dental malocclusion has not been previously reported to date.

We detected 15 VUS regions in 15 patients, but four of these (P48, P58, P87, and P89) also had pathogenic variants. In addition, VUS in six patients (P22, P26, P36, P42, P48, and P87) was detected de novo. While 911 kb VUS on 2q13 inherited from her father was showed in one patient (P89), no parental study was performed in eight patients. As a negative aspect of our study, we can show that parental array studies are insufficient, especially in this group. In these disease groups, clinical exome analysis, whole exome analysis, or whole genome analysis can be recommended for cases that cannot be diagnosed by microarray analysis.

CONCLUSIONS

With this paper, we aim to bring the array of results of two genetic diagnosis centers serving in Turkey to literature together with the genotype-phenotype relationship. We mentioned the importance of the array technique in the etiology of unknown autism, ID, and MCAs once again. In the evaluation of the pathogenesis of array results, it is important to obtain more data into the literature, especially together with parental results. In addition, functional studies, protein-protein interaction studies and more literature data are needed to elucidate candidate genes.

Ethics

Ethics Committee Approval: The study was approved by University of Health Sciences Turkey, Kanuni Sultan Suleyman Training and Research Hospital Clinical Research Ethics Committee (decision no: 04, date: 28.09.2018).

Informed Consent: Written informed consent for the study was obtained from all patients or their parents.

Peer-review: Externally and internally peer-reviewed.

Author Contributions

Surgical and Medical Practices: A.A., A.G., E.Y.G., O.O., S.Y., Concept: A.A., E.Y.G., S.Y., Design: A.A., S.Y., Data Collection and/or Processing: A.A., A.G., E.Y.G., O.O., S.Y., Analysis and/or Interpretation: A.A., E.Y.G., O.O., S.Y., Literature Search: A.A., A.G., A.H.K., Z.D., Writing: A.A., E.Y.G., A.H.K., Z.D., S.Y.

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