Diagnostic Yield of Molecular Karyotyping of Idiopathic Intellectual Disability Patients Ended with One Causative Anomaly; Duplication 9q34 Syndrome

İdiyopatik Entelektüel Yetersizliği olan Hastaların Moleküler Karyotiplemesinin Tanısal Kullanımı ile Saptanan bir Anomali Nedeni: 9q34 Duplikasyon Sendromu

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ABSTRACT

Objective: Clinical application of sequence comparative genomic hybridization has greatly contributed to the diagnosis of patients with multiple congenital anomalies, syndromic or non-syndromic intellectual disability. The idiopathic intellectual disability patients with normal karyotype and/or normal subtelomeric rearrangement analysis via Fluorescence in situ Hybridization (FISH), using genome-wide microarray platforms have detected chromosome abnormalities in up to 12% of cases. In this study, we aimed that evaluate the etiology of 9 patients with idiopathic intellectual disability and congenital malformations or dysmorphic features.

Methods: We performed genom wide SNP 2.7 array, in the evaluation of 9 patients with idiopathic intellectual disability and congenital malformations or dysmorphic features as well as normal karyotype and normal subtelomeric rearrangement analysis by the usage of FISH technique.

Results: As a causative anomaly, a 2.6 Mb microduplication on 9q34.2-q34.3 was observed only in one patient who has idiopathic mental retardation and multiple skeletal anomalies.

Conclusion: Microarray technology is a highly diagnostic method that is recommended for individuals with intellectual disability and multiple congenital anomalies. Microarray analysis revealed a causal anomaly in one of nine patients (11%) consistent with the literature.

Key Words: Intellectual disability, microarray analysis, chromosome 9q34 duplication, microduplication, FISH

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ÖZET

Amaç: Dizi karşılaştırmalı genomik hibridizasyonun klinik uygulaması, çoklu konjenital anomalileri, sendromik veya sendromik olmayan entellektüel yetersizliği olan hastaların teşhisine büyük ölçüde katkıda bulunmuştur. Tüm genom mikrodizi platformları kullanılarak; normal karyotipli ve/veya Floresans in situ Hibridizasyon (FISH) ile subtelomerik yeniden düzenlenmesi olmadığı saptanan, idiopatik entelektüel yetersizliği olan olguların yaklaşık %12'sine kromozomal anormallikler tespit edilmiştir. Bu çalışmada idiopatik entelektüel yetersizlik ve konjenital malformasyon ya da dismorfik özellikleri bulunan 9 hastanın etyolojisinin değerlendirilmesi amaçlanmıştır.

Yöntem: Idiopatik entelektüel yetersizliği olan ve konjenital malformasyonlar veya dismorfik özellikleri taşıyan; normal karyotipli, FISH tekniği ile subtelomerik yeniden düzenlenmesi olmadığı tespit edilen 9 hastanın değerlendirilmesinde tüm genom SNP array 2.7 tansal uygulaması yapılmıştır.

Bulgular: Nedensel bir anomali olarak, idiopatik entelektüel yetersizlik ve çok sayıda iskelet anomalisi olan bir hastada 9q34.2-q34.3 üzerinde 2.6 Mb’lik bir mikroduplikasyon gözlenmişdir.

Sonuç: Mikroarray teknolojisi, entelektüel yetersizlik ve birden fazla konjenital anomalisi bulunulun bireyler için önemli bir tespit edici aracıdır. Bu çalışmada literatürü uygulamalar olarak Mikroarray analizi ile dokuz hastanın birinde (%11) nedensel bir anomali ortaya çıkmıştır.

Anahtar Sözcükler: Entelektüel yetersizlik, mikroarray analizi, kromozom 9q34 duplikasyonu, mikroduplikasyon, FISH

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INTRODUCTION

The frequency of chromosome abnormalities and/or genomic rearrangements detected in patients with developmental delay/intellectual disability is quietly high, in the presence of additional malformations or dysmorphic features. Practice guidelines for the cytogentic evaluation of patients with developmental delay/intellectual disability recommend high-resolution chromosome banding, Fluorescence in situ hybridization (FISH) analysis consisting subtelomeric regions and specific amplification methods such as multiplex ligation-dependent probe amplification (MLPA) to search primarily for the imbalances of the subtelomeric regions, and also array comparative genomic hybridization (aCGH) analysis. There are advantages and disadvantages of the techniques compared to each other. While the rate of visible chromosome abnormalities in patients with intellectual disability is about three and half percent by the usage of conventional cytogenetic techniques, this ratio is come up to six percent (3–6%) by the usage of molecular techniques (1, 2). Array CGH has a 15–20% percent overall rate of detecting the genomic abnormalities that is mainly interstitial deletions and duplications, in patients with intellectual disability (3).

The detection ratio depends on the resolution of the techniques as conventional cytogenetic techniques is limited to approximately >5 Megabases (Mb) while array based methods can be useful to detect anomalies smaller than 3 Mb to kilobase levels.

Table 1. Clinical and laboratory findings of investigated patients.

| Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 | Case 8 | Case 9 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Age, sex | 2 years old, female | 7 years old, female | 11 years old, female | 1 years old, female | 15 years old, male | 7 years old, female | 13 years old, male | 22 years old, female |
| Weight | <3p | 10-25p | 25-50p | 25-50p | 10-25p | 25-50p | 3-10p | 50-75p |
| Length | <3p | <3p | <3p | <3p | 10-25p | 25-50p | 10-25p | 10-25p |
| Head circumference | - | 3rd degree | - | - | 3rd degree | - | 5th degree | ? |
| Intellectual disability (ID) | + | + | + | + | + | + | + | + |
| Other family members with ID | - | - | + | + | - | - | - | Unknown |
| Hypotonia | + | - | - | - | - | - | - | Unknown |
| Short stature | + | + | + | - | - | + | - | - |
| Microcephaly | + | + | + | - | - | + | - | - |
| Clinical Findings | Growth retardation, Brachycephaly, Downsplatal palpebral fissures, Dyplastic ears, Hydronephrosis | Febril convulsion history, Sleep disturbance, Narrow forehead, Short philtrum, Pointed teeth, Short neck, Clinodactyly | Attention deficit / hyperactivity disorder, Antverted nostrils, Long philtrum, Thin upper lip | Epilepsy, Short neck, Hemangiona on the back of the neck | Aggressive behaviors, Speech delay, Long face, Broad eyebrows, Synophrys, Anteverted nostrils, Mitral valve prolapse | Hearing loss, Speech delay, Epicanthus, Lacral duct obstruction, Bilateral short fifth fingers, Ingual hernia | Upsweep, Flat forehead, Curved and sparse eyebrows, Distinctive columnella, Long philtrum, Small mouth and ears, Thin upper lip | Epilepsy, Behavioral disturbance, Pain insensitivity, Narrow-long face, Starabismus, Prominent glabella, High nasal root and bridge, Short philtrum, Narrow and high palate, Crowded teeth, Retroglosection Joint contractures, Broad hallux, Aracnodactyly, Scoliosis and Kyphosis |
| Cranial MRI | Cerebral atrophy, Corpus callosum hypoplasia | Agenesis of corpus callosum | Normal | Not performed | Not performed | Agenesis of corpus callosum, in Pontine hypoplasia | Not performed | Normal |

In this study, nine idiopathic intellectual disability patients with normal karyotype and normal subtelomeric rearrangement analysis via Fluorescence in situ Hybridization (FISH), had been investigated for small deletions or duplications with “Genome Wide SNP 2.7 Array”. The analyses ended with a causative anomaly in one of the patient, namely 9q34.2-34.3 duplication anomaly, and in this report the discussion of the similar cases in the literature was held on.

MATERIAL and METHODS

Patients

Nine patients with idiopathic intellectual disability and congenital anomaly or dysmorphic features who were referred to Medical Genetics Department of Gazi University Faculty of Medicine between 2005 and 2009 were included in the study. All the patients with normal cytogenetic and subtelomeric FISH analysis, whose clinical findings were summarized on Table 1, underwent to whole-genome microarray analysis. There were satisfying birth and postnatal informations to evaluate their clinical condition except Patient 8 (Table 1). Regarding the history of orphanage living, there was no prenatal, natal and postnatal information related with this patient.

This study was approved by the Gazi University Faculty of Medicine Ethics Committee in June 23, 2010 with the decision number 057.
DNA extraction and molecular analysis

Peripheral blood samples were collected from the patients. Genomic DNA was extracted from blood, using a salting-out isolation method according to standard protocols. Whole-genome microarray was performed using The Affymetrix® Cytogenetics Whole-Genome 2.7M Array (Affymetrix, Santa Clara, CA, USA) system. DNA digestion, labeling, and hybridization were performed following the manufacturer instructions.

Each array contains approximately 2,761,979 oligonucleotide markers, including 2,316,876 CNV markers, 400,103 SNP markers and 2,370,000 non-polymorphic markers. SNPs used in this study were obtained from The Single Nucleotide Polymorphism Database (dbSNP) and are able to detect loss of heterozygosity (LOH). Non-polymorphic markers contain RefSeq genes, cytogenetically critical points and frequent CNVs. In the study, a limit of 100 kb was determined for the losses and gains. CEL files obtained by scanning the arrays were analyzed using the Chromosome Analysis Suite (ChAS) v1.2.1 Software and Cytogenetics Array NetAffx Analysis fileset version NA32 (hg19) (Affymetrix). Variants found in the results were evaluated with online databases and libraries, including DECIPHERv4.1 (DatabasE of Chromosome Imbalance and Phenotype in Humans using Ensembel Resources), Ensemble (Genome Browser), DGV (Database of Genomic Variants) and ECARUCA (European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations) OMIM (Online Mendelian Inheritance in Man) and PubMed (the U.S. National Library of Medicine).

RESULTS

In all cases investigated, some changes were found clinically insignificant. But no literature-supported evidence was available at the clinical disclosure point except for the Patient 8. All the variants detected in the patients were analyzed with the algorithms specified in the literature (4). Some of these variants were classified as benign (polymorphism), and further studies have been planned for variants with uncertain significance. In patient 8, the detected variant was thought to be responsible of the phenotype (Figure 1, Table 2).

Table 2. Results of the microarray analyses of all investigated patients

| Patient | Chromosomal position | Variant type | Size(kb) | The genes in the context | Marker count | Result           |
|---------|----------------------|--------------|----------|--------------------------|--------------|-----------------|
| 1       | 1:3002202-3163805    | Gain         | 161,603  | PRDM16, MIR4251, GPR62, PCBP4, ABHD14B, LOC283914, LOC146481 | 42           | Polymorphism    |
| 2       | 16:34476235-34731571 | Gain         | 255,536  | HERC2P3, GOLGA6L6, GOLGARc, BCL8, POTE8, NF1P1, LOC646214, CXADR2P2, ORAM2, ORAM4, ORAM3P, RERE3G, GOLGA8DP, GOLGA6L1 | 66           | Polymorphism    |
| 3       | 15:20175623-22752987 | Loss         | 2,577.364 | HERC2P3, GOLGA6L6, GOLGARc, BCL8, POTE8, NF1P1, LOC646214, CXADR2P2, ORAM2, ORAM4, ORAM3P, RERE3G, GOLGA8DP, GOLGA6L1 | 53           | Parental testing was planned |
| 4       | 14:22408697-2296172  | Loss         | 553,015  | -                        | -            | Parental testing was planned |
| 5       | 15:20175623-22749949 | Gain         | 2,574,326 | HERC2P3, GOLGA6L6, GOLGARc, BCL8, POTE8, NF1P1, LOC646214, CXADR2P2, ORAM2, ORAM4, ORAM3P, RERE3G, GOLGA8DP, GOLGA6L1 | 220          | Polymorphism    |
| 6       | 12:129329020-130654618 | Loss        | 1,325,598 | GLT1D1, TMEM132D, LOC100190940, FLJ31485, FZD10 | 65           | Polymorphism    |
| 7       | 7:64621287-65090561  | Loss         | 469,274  | INTS4L1, ZNF92            | 133          | Polymorphism    |
| 8       | 9:135920384-138520804 | Gain         | 2,600.42 | GTF3C5S, CEL, CEP, RALGDS, GBGT1, OPBP2, ABO, SURF6, MED22, RPL7A, SNOARD2, SNOARD36B, SNOARD36A, SNOARD36C, SURF1, SURF2, SURF4, C9orf96, REXO4, ADAMTS13, C9orf7, SLC2A6, TMEM8C, ADAMTSL2, FAM1638, DBH, SARDH, VAV2, NCRNA00094, BDR3, WDR5, RNU6ATAC, RXRA, COLSA1, FCN2, FCN1, OFLMT1, KIAA0649, C9orf116, MRPS2F, LCN1, OPBP2A, PAEP, LOC100130954, GLT6D1, MIR3180-1, MIR3180-3, MIR3180-2, MIR3179-3, MIR3179-2, MIR3179-1, NOMO2, ABC6G1 | 61           | Likely Pathogenic |

Array analysis of the Patient 8 revealed a 2,6 Mb microduplication on the distal part of long arm of chromosome 9 and molecular karyotype of the patient was “arr[GRCh38]9p34.2q34.3 (135920384-138520804)x3” (Figure 2). This interval includes 2,730 probes and there are 45 OMIM genes located on this duplicated site which was detected with 61 markers (Table 2).
DISCUSSION

Conventional cytogenetic analysis allows for detection of numerical and structural chromosomal abnormalities present in the entire genome, but has a limited resolution of 5-10 megabases. Thus, submicroscopic aberrations cannot be detected, and interpretation of the test results remains subjective. FISH and MLPA can detect specific cytogenetic aberrations with a higher sensitivity than conventional cytogenetic techniques; but, they cannot cover entire regions of chromosomes (5). Array CGH has many advantages over conventional cytogenetic techniques in that it can provide rapid genome-wide assessments at a high resolution (≤1 Mb). It can also detect single-copy gains and losses across whole chromosomes, but balanced translocations cannot be detected. Nowadays, high-resolution array comparative genomic hybridization (array CGH) is a powerful and efficient method for diagnosis and research of intellectual disability because of it can be explain the etiology of intellectual disabilities in a rate of 15-20% (3). It has been suggested as a standard practice for children with diagnoses including unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASDs), and dysmorphic features (6-8).

In this study, nine cases were studied with molecular karyotyping method (2.7 SNP Array) and only in one case (Patient 8) a duplication of about 2.6 Mb was detected, which could be figured out the clinical findings, located on the long arm of chromosome 9 (chr: 135920384-138520804) (Table 1, Figure 1 and 2).

Duplication of 9q34 is usually based on unbalanced meiotic segregation of the gametes of balanced translocation career healthy parents.

First association between clinical features and isolated duplication of 9q34 on seven patients in a translocation career family was described by Allerdice et al. and it was called as “Duplication 9q34 Syndrome” (9). In the literature, there are a few patients with de novo and/or whole 9q duplication of the chromosome 9. Clinical features of 9q34 duplication syndrome vary depending on the size of the duplicated region but common characteristic clinical features are consist of intellectual disability, motor retardation, long narrow asymmetric face, short palpebral fissures, high nasal root, dysplastic ears, tooth abnormalities and skeletal findings including scoliosis, kyphosis, arachnodactyly, camptodactyly, and joint contractures. Previous studies have suggested that a large number of functional genes are located on the 9q34 region causing multi systemic clinical findings, but that there is not yet a definitive candidate gene to explore the abnormalities presented in this syndrome (10, 11).

Comparison of the clinical findings of our case who has characteristic duplication 9q34 syndrome and other cases with 9q34 duplication diagnosed by microarray method which has overlapping duplication region are summarized in Table 3. As seen in the Figure 3 and Table 3 while clinical findings in the presented case and other cases in the literature with 9q34 duplication are quite similar, the duplicated regions do not overlap completely (11-13). Developmental delay and intellectual disability were found in four cases, but phenotypic features and skeletal findings were not found in all of them (Table 3). The case which is described by Mizuno et al. has the most different phenotypic findings from the other three reported ones, possibly because of the Japanese lineage (Table 3) (11).
Although the case reported by Gijbers et al. was clinically similar to other cases, mentioned patient had a heavier phenotype (severe mental retardation) and this can be attributed to possible sub-regional gene duplications that are triplicated within the dupplex region at 9q34 (13). In the literature most phenotypically similar case to our patient, which is not represented on the Table 3 due to having an extra chromosomal deletion. This patient reported by Young et al. who had 13.79 Mb duplication (126.42–140.20 Mb) at 9q33.3-q34.3 and a 155 kb deletion at 12p13.33 that contains only IQSEC3 gene. Features of monosomy chromosome 12p13.33 reported in the literature include developmental delay, protruding tongue, strabismus, slightly unusual facies, slight micrognathia, and speech delay (14). The neurological findings of the case described by Young et al. may have been caused by both genomic alterations. However, dysmorphic facial findings and skeletal system anomalies of their and our patients may thought to be due to 9q34 duplication. As seen at Figure 3 in a total of four cases including our case, there are a total 8 common duplicated genes including OLFM1, PPP1R26, C9ORF116, MRPS2, LCN1, PAEP and GLT6D1 genes in the common duplicated region. OLFM1 gene (MIM *605366) was found to encode a deduced 135-amino acid protein, which the authors termed AMY that may have an essential role in nerve tissue (15). Overexpression of PPP1R26 gene (Protein Phosphatase 1, Regulatory Subunit 26; MIM * 614056) has been shown to be associated with proliferation and tumor formation (16). Other common genes are not associated with any phenotypes and their functions are as follows: C9ORF116 gene (MIM * 614502) as a target of P53; MRPS2 gene (MIM * 613971) as a protein component of mitochondrial ribosomes that are encoded by the nuclear genome; LCN1 gene (MIM * 151675) as having a role in the non-immunologic defense; OBP2A gene (MIM * 164320) as odorant-binding protein which is found in nasal epithelium; PAEP gene (MIM * 173310) as inducer for angiogenesis; GLT6D2 gene (MIM * 613699) as a susceptibility locus for periodontitis.

There are 45 genes within the 2.6 Mb region in 9q34.2-q34.3 which is duplicated in our case; the functions of the 38 of them were determined and seven of these were associated with a phenotype/disease. These genes and their associated phenotypes are; ADAMTS13 with Thrombotic Thrombocytopenic Purpura (OMIM#274150), ADAMTS12 with Geleophysic Dysplasia 1 (OMIM#21050), CEL with MODY8 (Maturity-Onset Diabetes of the Young, Type 8, with Exocrine Dysfunction, OMIM#609812), COLS2A1 with Ehler Danlos Syndrome Type 1 and 2 (OMIM#130000-130010), Dbh with Congenital Beta Hydroxylase Deficiency (OMIM#223360), SARDAH with Sarcosinemia (OMIM#268900) and SURF1 with Leigh Syndrome (OMIM#256000). Although the deletion and mutation of these genes are known to cause related diseases, there is no information in case of duplication occurred. The clinical findings of the Patient number 8, do not compatible with the reported diseases.

Table 3. Comparison of the clinical features of other cases whose duplicated region of chromosome 9q coincide with the present case.

| Our patient (Patient 8) | Shalinder et al. | Mizuno et al. | Gijbers et al. |
|------------------------|-----------------|--------------|---------------|
| Size of 9q duplication | 2.6 Mb          | 2.3 Mb       | 8.5 Mb        |
| Start and endpoints    | 9:135920384-1385208041 | 9:137,864,059-140,171,337 | 9:131.7-140.2 |
| Neurodevelopmental findings |                        |              |               |
| Developmental delay    | +                | +            | +             |
| Hypotonia              | ?                | +            | +             |
| Intellectual disability| +                | +            | +             |
| Dysmorphic features    |                 |              |               |
| Long face              | +                | +            | -             |
| Facial asymmetry       | +                | +            | +             |
| Beaked nose            | +                | -            | -             |
| High nasal root        | -                | +            | +             |
| High palate            | +                | +            | -             |
| Retrogynatia/micrognatia| +              | +            | +             |
| Skeletla anomalies     |                 |              |               |
| Aracnodactyly/camptodactyly | +           | -            | +             |
| Scoliosis              | +                | +            | -             |
| Long hallux            | +                | +            | +             |

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