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Gebelik Kayıplarında 54 Genin Array CGH Methoduyla Yapılan Tanısal Sonuçları: İki Yıllık Retrospektif Çalışma

Diagnostic Outcomes For Genetic Testing Of 54 Genes in Pregnancy Loss Using Array CGH Method: A Two-Year Retrospective Study

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ÖZ

Amaç: Bu çalışma, 2016 ve 2017 yılları arasında tıbbi genetik kliniğine gelen tüm gebelik trimesterlerinde, fetal kayıp olgularına farklı bir tanısal yaklaşım olarak uygulanmış olan array-CGH genetik analizinin retrospektif olarak değerlendirmesini incelemeyi amaçlamıştır. 50 örnek üzerinde Kantitatif Floresan Polimeraz Zincir Reaksiyonu (QF-PCR) testi yapıldı ve test sonucunda 11 örnekte anöploidi saptandı ve QF-PCR normal olan 39 örneğe array-CGH analizi gerçekleştirildi. Bu amaçla, embriyonik dönemde hücre bölünmesi, doku farklılaşması aşamalarında etkili delesyon ve duplikasyonları, olası kopya sayısı varyasyonlarını (CNV) analiz etmeyi ve belirlemeyi amaçladık.

Gereç ve Yöntemler: Bu retrospektif çalışmada vakaların abortus ve fetal biopsi örneklerinden yapılan DNA izolasyonunda PureLink Genomik DNA izolasyon kiti kullanıldı. DNA numuneleri daha sonra oligonükleotid array-CGH yöntemi (aCGH, 60 K ISCA tasarımı, Agilent, Almanya) ile moleküler etiyolojik nedenler açısından incelendi. Olgu ve referans DNA'ların hibridize prob korelasyonları, intrauterin kayıplarla ilişkili 54 fonksiyonel gen CNV açısından genomik varyasyon analizinde kullanılan veri tabanları (Genomik Varyantlar Analizi Veritabanı) ile değerlendirildi.

Bulgular: Araştırma kapsamında analiz edilen 39 fetal örneğin 30'unda (% 77) CNV saptandı. CNV'lerin yüzde elli beşi duplikasyon (% 55) ve yüzde kırk beşi delesyon (% 45) şeklinde bulundu. Değerlendirme sonucunda 54 genin 19'unda (% 35) delesyon, 26'sında (% 48) duplikasyon, 3'ünde (% 6) hem delesyon hem de duplikasyon saptandı. Otozomal kromozomlarda CNV tespit edilmesine rağmen (kromozom 1, 2, 3, 4, 5, 7, 8, 10, 12, 13, 14, 15 ve 20), en sık CNV X kromozomunda saptandı. Çalışmamızda COX7B, ZIC1, MECP2, FMR1, HOXD 13, JAG 1, MSX 2, NEXN ve SIX 3 genleri ile ilişkili CNV 'le- rin fetal kayıp etiyolojisi açısından daha sık olduğu bulunmuştur.

Sonuç: Deneyimlerimize dayanarak, array-CGH yöntemi, fetal kayıp vakalarında QF-PCR ile normal sonuçlanmış vakaların etiyolojisini araştırmak için kullanılabilir. Array-CGH yöntemi uygulama kolaylığı ve elde edilen veriler nedeniyle giderek daha fazla tercih edilecektir. Literatüre baktığımızda, array-CGH yöntemi üzerinde fetal kayıplar hakkında yeterli araştırma olmadığı ve bu alandaki deneyimi arttırmak için daha fazla çalışmaya ihtiyaç olduğu görülmektedir.

Anahtar Kelimeler: fetal kayıp, array-CGH, DNA, QF-PCR, CNV

ABSTRACT

Objective: The current study aimed to retrospectively evaluate different diagnostic approaches for the array genetic analysis of the cases from all trimester fetal loss in the medical genetics clinic between 2016 and 2017. The Quantitative Fluorescent Polymerase Chain Reaction(QFPCR) test was performed on 50 samples, and aneuploidy was detected in 11 samples as a result of the test, and the array-CGH was performed when 39 QF-PCR resulted in normal test results. Under this purpose, we aimed to analyze and determine the possible copy number variation(CNV), gene deletions, and/or duplications involved in embryonic cell division, tissue differentiation, intended.

Materials and methods: DNA isolation from cases of this retrospective study was completed using the PureLink Genomic DNA isolation kit. DNA samples were then genoty - ped for molecular etiological reasons by oligonücleotid microarray -CGH method (aCGH , 60 K ISCA design , Agilent , Germany). Hybridized probe correlations of the case and reference DNAs were evaluated with databases (Database of Genomic Variants Analysis) used in genomic variation analysis in terms of 54 functional genes CNVs associated with intrauterine losses.

Results: CNV was detected in 30 (77%) of 39 fetal samples analyzed within the scope of the research. Fifty-five percent of CNVs were found to be duplication (55%) and forty-five percent were deletions (45%). As a result of the evaluation, deletion was detected in 19 (35%) of 54 genes, duplication was detected in 26 (48%), while in 3 (6%) both deletion and duplication were detected. Although CNV detected in autosomal chromosomes (chromosome 1, 2, 3, 4, 5, 7, 8, 10, 12, 13, 14, 15 and 20), CNV was established the most common in X chromosome. In our study, CNVs associated with COX7B, ZIC1, MECP2, FMR1, HOXD13, JAG1, MSX2, NEXN, and SIX3 genes were found to be more frequent in terms of fetal loss etiology.

Conclusions: Based on our experience, the array-CGH method can be used to investigate the etiology of the normal results of QF-PCR in cases of fetal loss. The array CGH method will be preferred more and more due to the ease of application and the data obtained. When we look at the literature, it is seen that there is not enough research on array CGH about fetal loss and more studies are needed to increase the experience in this field.

Key words :fetal loss, array CGH, DNA, QF-PCR, CNV

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INTRODUCTION

It is estimated that approximately 15-20% of recognized pregnancies result in abortion and 3,5 per 1000 in stillbirth. There are numerous causes of spontaneous abortion and fetal death, including genetic conditions, placental abnormalities, infections and feto-maternal hemorrhage. Valuable diagnostic procedures used to evaluate the cause of pregnancy loss including fetal autopsy, placental examination, genetic analysis, and detection test of fetomaternal hemorrhage(1,2).

Recently, the etiology of fetal loss has not been clarified due to inaccurate birth records and incomplete post-mortem evaluation. Therefore, 60% of pregnancy losses are classified as unknown(3). However, identifying the cause of pregnancy losses is important for families to plan their future pregnancy and recurrence risk prediction, reduction of parental anxiety, diminish antenatal tests cost (4). The American Society of Reproductive Medicine, the College of Obstetricians and Gynecologists and the Royal College of Obstetricians and Gynecologists all offers chromosomal analysis of fetal tissue(2). Traditional studies to identify genetic etiology in pregnancy loss have used G-banded chromosome analysis, fluorescence in situ hybridization(FISH), Multiplex ligation-dependent probe amplification (MLPA) and Quantitative Fluorescence - Polymerase Chain Reac - tion (QF -PCR)(5). Chromosome analysis was used to investigate pregnancy losses and involved the culture of fetal tissue. However, to perform karyotyping, active, dividing live cells require for culturing and it results in 35-55% culture failure due to the possibility of non-li- ving cells in fetal loss samples. Karyotyping in pregnancy losses is limited due to only informing about numerical and major structural chromosome abnormalities. The reason for the low success of kar- yotyping in pregnancy losses depends on the requires for live cells for culture, low culture success rate, overgrowth of maternal cells, microbial contamination, and poor chromosome resolotion(6–8). MLPA and QF-PCR methods that do not require culturing are useful in the diagnosis of common aneuploidies. The QF-PCR test has been used for prenatal rapid aneuploidy detection for the last 25 years. In contrast to karyotyping, QF-PCR can be done in a small amount and in a short time(9). Deletions and duplications in the chromosome segments are defined as Copy Number Variation(CNV)(10). The array CGH can be examined directly from the fetal tissue and does not require a living cell. Moreover, the array CGH can detect submicroscopic changes that cannot be detected by karyot - yping, even when poor fetal chromosomal morphology(7).

MATERIALS AND METHODS

In our study, fetal deaths samples included pregnancy losses after the tenth week of pregnancy collected between 2016 and 2017 in the genetic diagnostic center. The approval for this study was obtained from the ethics committee of Canakkale Onsekiz Mart University Faculty of Medicine with the decision numbered 2017-E.66893. All patients gave informed consent for genetic analysis and examinations on the miscarriage and fetal tissue. Samples are collected from miscarriage by curettage and from fetal death after birth then analyses were performed on fetal tissue skin. All samples were macroscopically examined, removing blood and decidua.

DNA was isolated from 200 µl uncultured fetal tissue samples which were incubated overnight by proteinase K digestion, followed by isopropyl alcohol exctraction using pure link genomic DNA isolation kit(Qiagen DNA mini kit, lot: 157037013, Cat no: 51304, Germany) according to the manufacturer's instructions. DNA quality and quantity were determined at the 230, 260 and 280 nm absorbance measurements by the nanodrop spectrophotometer (IMPLEN, P 330 Nanophotometer, Germany). Samples with degraded DNA were investigated by QF-PCR or if necessary tested by array CGH.

QF-PCR analysis was performed on all samples to the detection of common aneuploidies involving chromosomes 13, 18, 21, X and Y using a set of STR markers by Aneufast QF-PCR kit according to the manufacturer's instructions (Genomed AG, Switzerland). After QF-PCR analysis, only samples with normal results continued to genome-wide clinical array CGH testing on DNA from intra uterine fetal loss. Thirty-nine samples were tested using the oligonucleotide array-based CGH(SurePrint G3 Human CGH ISCA v2 Kit 8×60K) according to the manufacturer's instructions(Agilent Technologies, Santa Clara, CA, USA). This is a commercially available, 8x60 K oligonucleotide array that contains approximately sixty-mer probes with a 60 kb average probe area, within region the International Standard Cytogenomic Array(ISCA) Consortium. Scanned microarray images and translated into logratios is the process named feature extraction using the Agilent Feature Extraction software. Quality control reports were reviewed after the extraction process. In Feature Extraction quality control report; Schematic image showing the distribution of spots on the array glass, the spatial distribution of probes, positive and negative LogRatios spatial distribution, metrics evaluation values, the histogram of red and green spots. Obtained raw data were analyzed using the Agilent Cyto Genomic 3.0.2.11

software program.

The current array platform did not have Loss of Heterozygous(-LOH) evaluation feature. Therefore, data on clinically relevant LOH changes could not be produced. When selecting cases for the study, those with chromosomal abnormalities such as aneuploidy and polyploidy in QF-PCR analysis over 10 weeks were not included. 11 of the 50 cases were excluded from the current study due to QF-PCR analysis results were aneuploidy. For the genetic analysis approach, in the first approach, all CNVs with mean Log-Ratio value below -0.5 for deletions and above +0.5 for duplications were included in the study. Next, CNVs were not included which without currently chosen genes or were defined as benign variation in the Database of Genomic Variants(DGV) or our in- house array databases containing more frequently in the population.

In this study, we investigated genes named BMP7, CHRNA1, CHRND, CHRNG, COL1A, COX7B, FAM20C, FLNA, FLNB, FGFR1-23, FGF 8, FOXD 3, FOXG 1, FMR 1, EPHB 4, GPC 3, HAND 1, HAND2, HCCS, HOXA, HOXB, HOXC, HOXA2, HOXB3, HOXA 13, HOXD 3, HOXD 13, IKBKG , LBR , MECP 2, MITF , MSX 2, MYOD, OTX2, PAX1, PAX2, PAX3, PDX1, PHEX, PORCN, PRPS 1, RET, SHH, SIX3, SOX2, SOX9, TAF1, TBX4, TBX5, TWIST1, WNT 3A, WNT 4, WNT 6, WNT 9B which have effects in the embryological period, in terms of fetal loss etiology.

RESULTS

Between 2016 and 2017, fifty fetal loss samples were received for genetic analysis. Of these, 11 samples were excluded from array CGH analysis when QF- PCR results were positive. Array CGH analysis was successfully performed to 39 fetal loss samples. All samples were obtained from frozen tissue consisting of feal solid tissue.

All the 39 cases were included in the array CGH evaluation because the DNA quality and analysis criteria were appropriate. In 30 cases abnormal array results found. CNV was detected in 30 (77%) of 39 fetal samples analyzed within the scope of the research. Fifty-five percent of CNVs were found to be duplication (55%) and forty-five percent were deletions (45%). As a result of the evaluation, the deletion was detected in 19 (35%) of 54 genes, duplication was detected in 26 (48%), while in 3 (6%) both deletion and duplication were detected. Although CNV detected in autosomal chromosomes (chr1, 2, 3, 4, 5, 7, 8, 10, 12, 13, 14, 15 and 20), CNV was established the most common in X chromosome. In our study, CNVs associated with COX7B, ZIC1 , MECP2, FMR1, HOXD13, JAG1, MSX2, NEXN, and SIX3 genes were found to be more frequent in terms of fetal loss etiology(Figure 1). CNVs without ge- nes in 1, 2, 4, 6, 8, 9, 14, 15 , 21 and X chromosomes were found in 12 cases. Array CGH results revealed that Chromosome 3 duplications(3q24) and Chromosome X deletions(Xq21 and Xq24) were more frequent in this study (Table 1).

Table 1. The distribution of the detected CNVs in our study

Chromosome	Locus	CNV type	Number of cases(n)	İncidence (%)
1	p13	Del	2	5
	p22	Del	1	3
	p31	Del	3	8
	p32	Del	1	3
	q32	Del	1	3
2	q31	Del	1	3
	p21	Dup	3	8
	q31	Dup	3	8
3	p14	Del	1	3
	q24	Dup	7	**18***
	q28	Dup	1	3
4	q21	Dup	1	3
5	q31	Del	1	3
	q35	Dup	3	8
7	p21	Dup	2	5
8	q24	Dup	1	3
10	q26	Dup	1	3
12	q13	Del	1	3
	q24	Dup	2	5
13	q12	Dup	1	3
14	q23	Del	1	3
	q12	Dup	2	5
	q22	Dup	1	3
15	q26	Del	2	5
	q26	Dup	2	5
20	p12	Dup	3	8
X	q13	Del	1	3
	q21	Del	11	**28***
	q24	Del	6	**15***
	p11	Del	1	3
	q22	Del	1	3
	p11	Dup	2	5
	q22	Dup	1	3
	p22	Dup	2	5
	q27	Dup	4	10
	q28	Dup	1	3

CNVs detected in cases above -0.5 / + 0.5 MeanLog Ratio are listed in Table 2. This table summarizes the mutation type, chromosomal localization, size, start and end codons, and gene-clinic relationships detected in fetal materials with chromosomal Array-CGH analysis.

Table 2. Details of CNV of fetal death studied.

Case no	Mutation	Allele	Chromosome	Locus	Size (Kb)	Position	Genes	Clinical relevance
1	Del	--	--	--	--	--	--	--
	Dup	--	--	--	--	--	--	--
2	Del	--	--	--	--	--	--	--
	Dup	het	15	q26.2	11.8	96,869,390-96,881,219	**NR2F2**	**NR2F2:** Congenital heart defects, multiple types
3	Del	het	X	q21.1	502.2	76,776,651-77,368,137	**ATRX**, MAGT 1, **COX7B**, **ATP7A,** PGAM4, PGK1	**COX7B:** Linear skin defect- multiple congenital anomaly 2 **ATRX:** alpha thalassemia myelodysplastic syndrome, somatic Mental retardation - hypotonic facial syndrome, X-linked **ATP7A:** Spinal muscular atrophy, Occipital horn syndrome, Menkes disease
	Dup	--	--	--	--	--	--	--
4	Del	--	--	--	--	--	--	--
	Dup	het	2	q31.1	1.5	176,958,034-176,959,625	**HOXD13**	**HOXD13**: Brachydactyly-syndactyly syndrome
	Dup	het	3	q24	404	146,729,902-147,133,996	ZIC4, **ZIC1**	**ZIC1:** Craniosynostosis 6
	Dup	het	7	p21.1	1.1	19,154,855-19,157,193	**TWIST1**	**TWIST1**: Craniosynostosis syndrome
5	Del	het	X	q21.1	1,6	77,157,760-77,159,406	**COX7B**	**COX7B:** Linear skin defect- multiple congenital anomaly 2
	Dup	het	14	2	9.8	54,417,617-54,427,486	**BMP4**	**BMP4:**Microophthalmic syndromic, Orofacial kleft 11
	Dup	het	2	p21	3.5	45,168,836-45,172,394	**SIX3**	**SIX3**: Holoprosencephaly 2, schizencephaly
	Dup	het	14	q22.2	10	57,267,408-57,276,927	**OTX2**	**OTX2**: Mikroftalmi sendromik 5, hipofiz hormon eksikliği, kombine tip 6
	Dup	het	X	q28	13	153,770,700-153,783,639	**IKBKG**	**IKBKG**: Incontinentia pigment
	Dup	het	X	p11.23	17	48,361,164-48,379,190	**PORCN**	**PORCN**: Focal dermal hypoplasia
6	Del	--	--	--	--	--	--	--
	Dup	het	2	P21	2.8	45,169,517-45,172,394	**SIX3**	**SIX3**: Holoprosencephaly 2, schizencephaly
	Dup	het	2	Q35.2	6	174,151,663-174,157,924	**MSX2**	**MSX2:** Craniosynostosis, Parietal foramina cleidocranial dysplasia
	Dup	het	7	p21.1	2.3	19,154,855-19,157,193	**TWIST1**	**TWIST1**: Craniosynostosis 1, Robinow- Sorauf syndrome Saethre-Chotzen syndrome with eyelid anomaly Sweeney-Cox syndrome
	Dup	het	14	14q12	1041	28,304,052-29,345,932	**FOXG1**	**FOXG1:** Rett syndrome, congenital variant
	Dup	het	12	12q24.21	329	114,791,887-115,121,468	**TBX5, TBX3**	**TBX5:** Holt-Oram syndrome **TBX3:** Ulnar-mammari syndrome

7	Del	het	X	q24	81	153,287,517-153,359,700	MECP2	**MECP2:** Rett syndrome, Encephalopathy, severe in newborn
	Dup	het	4	q21.3-q22.1	1098	87,869,469-88,968084	AFF1, KLHL8, HSD17B13, HSD17B11, **DSPP**, **DMP1**, IBSP, MEPE, SPP1, **PKD2**	**DMP1:** Hypophosphotemic rickets, AR **PKD2:** Polycystic kidney disease 2 **DSPP:** deafness, autosomal dominant 39, dentinogenesis
8	Del	--	--	--	--	--	--	--
	Dup	--	--	--	--	--	--	--
9	Del	--	--	--	--	--	--	--
	Dup	het	X	q27.3	36.2	146,994,804-147,032,524	**FMR1**	**FMR1**: Fragile X tremor / ataxia syndrome
	Dup	het	5	q35.2	4.1	174,153,810-174,157,924	**MSX2**	**MSX2:** Craniosynostosis, Parietal foramina cleidocranial dysplasia
	Dup	het	X	q22.3	13.4	106,877,900-106,891,393	**PRPS1**	**PRPS1:** Arts syndrome, Charcot-Marie-Tooth disease, X-linked recessive, 5
10	Del	het	X	q24	81	153,287,517-153,359,700	**MECP2**	**MECP2:** Rett syndrome, Encephalopathy,
	Dup	--	--	--	--	--	--	--
11	Del	het	1	P31 p22 p13	387 1723 526	78,107,120-78,494,303 92,333,192-94,056,303 109,465,048-109,991,413	USP33, FAM73A, **NEXN**, FUBP1------TGFBR3, BRDT, RPAP2, GFI1, EVI5, **RPL5**, FAM69A, MTF2, DR1, FNBP1L, BCAR3 ---- **GPSM2**, WDR47, KIAA1324, SARS, CELSR2, PSRC1, SORT1, PSMA5	**NEXN:** Cardiomyopathy **RPL5:** Diamond-Blackfan anemia 6 **GPSM2:** Chudley-McCullough syndrome
	Del	het	X	q24	81	153,287,517-153,359,700	**MECP2**	**MECP2:** Rett syndrome, Encephalopathy, severe in newborn
	Del	het	X	q21.1	1169	76,124,793-77,294,633	FGF16, **ATRX**, MAGT1, **COX7B, ATP7A**, PGAM4	**COX7B:** Linear skin defect- multiple congenital anomaly 2 **ATRX:** Alpha thalassemia myelodysplastic syndrome, somaticMental retardasyon- hipotonik yüz sendromu, X-linked **ATP7A:** Menkes disease, occipital horn syndrome, Spinal muscular atrophy, distal X-linked 3
	Del	het	3	p14.3	1863	56,598,066-58,461,365	FAM208A, ARHGEF3, SPATA12, IL17RD, **HESX1**, APPL1, DNAH12, PDE12, ARF4, **FLNB**, DNASE1L3, RPP14, PXK, PDHB,	**HESX1:** Growth hormone deficiency - with pituitary anomaly **FLNB:** Athelosteogenesis, type I Larsen's syndrome, Boomerang dysplasia
	Del	het	X	q22.3	20	106,874,245-106,894,224	**PRPS1**	**PRPS1:** Arts syndrome

12	Del	het	1	q32.1	425	203,625,291-204,050,423	ATP2B4, ZC3H11A, ZBED6, **SNRPE**, SOX13	**SNRPE:** Hypotrichosis 11
	Del	het	X	q24	81	153,287,517-153,359,700	**MECP2**	**MECP2:** Rett syndrome, Encephalopathy, severe in newborn
	Del	het	X	q21.1	1,6	77,157,760-77,159,406	**COX7B**	**COX7B:** Linear skin defect- multiple congenital anomaly 2
	Del	het	X	q22.3	20	106,874,245-106,894,224	**PRPS1**	**PRPS1:** Arts syndrome
	Dup	--	--	--	--	--	--	--
13	Del	het	14	q23.2-q23.3	837	64,054,041-64,891,348	SGPP1, **SYNE2**, ESR2	**SYNE2**: Emery-Dreifuss muscular dystrophy 5, autosomal dominant
	Del	het	X	q21.1	1,6	77,157,760-77,159,406	**COX7B**	**COX7B:** Linear skin defect- multiple congenital anomaly 2
	Dup	het	12	q24.22	33	114,791,887-114,824,183	**TBX5**	**TBX5**: Holt-Oram syndrome
	Dup	het	X	p22.2	289	11,056,336-11,345,765	**HCCS**, ARHGA P6, AMELX	**HCCS**: Linear skin defect- multiple congenital anomaly 1
	Dup	het	X	q27.3	36.2	146,994,804-147,032,524	**FMR1**	**FMR1**: Fragile X tremor / ataxia syndrome
	Dup	het	8	q24.22	195	133,879,951-134,075,815	TG, SLA,	**SLA**: Pontocerebellar hypoplasia type 2D
14	Del	het	X	p11.22	0.1	53,460,311-53,460,442	**HSD17B10**	**HSD17B10 HSD10:** Mitochondrial disease. Mental retardation. X linked syndromic
	Del	het	X	q24	81	153,287,517-153,359,700	**MECP2**	**MECP2:** Rett syndrome, Encephalopathy, severe in newborn
	Dup	het	X	p22.2	511	10,834,439-11,345,765	**MID1**, **HCCS**, ARHGAP6, AMELX	**MID1**: Opitz GBBB syndrome, type I **HCCS**: Linear skin defect- multiple congenital anomaly 1
15	Del	het	X	q24	81	153,287,517-153,359,700	**MECP2**	**MECP2:** Rett syndrome, Encephalopathy, severe in newborn
	Dup	--	--	--	--	--	--	--
16	Del	--	--	--	--	--	--	--
	Dup	--	--	--	--	--	--	--
17	Del	--	--	--	--	--	--	--
	Dup	het	3	q28	1058	188,389,234-189,447,715	LPP, MIR28, **TP63**	**TP63** Ectrodactyly, ectodermal dysplasia and cleft lip / palate syndrome 3 Hay-Wells syndrome ADULT syndrome
	Dup	het	20	p12.2	131	10,534,477-10,665,574	SLX4IP, **JAG1**,	**JAG1** Alagille syndrome Fallot tetralogy Congenital heart defect and posterior embryotoxone
18	Del	het	X	q21.1	605	76,762,631-77,367,966	ATRX, MAGT1, **COX7B**, ATP7A, PGAM4, PGK1	**COX7B:** Linear skin defect- multiple congenital anomaly 2
	Dup	--	--	--	--	--	--	--

19	Del	het	1	31.1	429	78,107,120-78,536,731	USP33, FAM73A, **NEXN**, FUBP1	**NEXN:** cardiomyopathy
	Del	het	X	q21.1	1,6	77,157,760-77,159,406	**COX7B**	**COX7B:** Linear skin defect- multiple congenital anomaly 2
	Dup	het	X	q27.3	36.2	146,994,804-147,032,524	**FMR1**	**FMR1**: Fragile X tremor / ataxia syndrome
20	Del	het	2	q31.1	81	176,944,823-177,025,840	EVX2, **HOXD1 3**, HOXD12, H OXD11, HOXD 10, HOXD9, HOX D8, MIR10B, HOXD4,	**HOXD13**: Brachydactyly-syndactyly syndrome
	Dup	het	3	q24	404	146,729,902-147,133,996	ZIC4, **ZIC1**	**ZIC1:** Craniosynostosis 6
21	Del	het	X	q21.1	1,6	77,157,760-77,159,406	**COX7B**	**COX7B:** Linear skin defect- multiple congenital anomaly 2
	Dup	het	X	q27.3	36.2	146,994,804-147,032,524	**FMR1**	**FMR1**: Fragile X tremor / ataxia syndrome
22	Del	het	X	q21.1	1,6	77,157,760-77,159,406	**COX7B**	**COX7B:** Linear skin defect- multiple congenital anomaly 2
	Del	het	12	q13.2-q13.3	804	56,159,652-56,974,447	SARNP, ORMDL2, MMP19, DGKA, CDK2, RAB5B, SUOX, IKZF4, RPS26, **ERBB3**, PA2G4, RPL41, ESYT1, MYL6 B, MYL6, SMARCC2, SL C39A5, CS, PA N2, IL23A, STAT2, APOF, TIMELESS, MI P, GLS2,	**ERBB3:** ölümcül konjenital konotrunkal sendrom 2
	Dup	het	2	q31.1	305	176,719,953-177,025,840	EVX2, **HOXD1 3**, HOXD12, H OXD11, **HOX D10**, HOXD9, HOX D8, MIR10B, HOXD4	**HOXD13**: Brachydactyly-syndactyly syndrome **HOXD10:** Charcot-Marie-Tooth disease, foot deformity-v-rical talus, congenital
	Dup	het	10	q26.11	36	119,302,225-119,339,004	EMX2OS, **EMX2**	**EMX2:** schizencephaly
	Dup	het	3	q24	404	146,729,902-147,133,996	ZIC4, **ZIC1**	**ZIC1:** Craniosynostosis 6
	Dup	het	14	q12	2.3	29,236,277-29,238,620	**FOXG1**	**FOXG1:** Rett syndrome, congenital variant
23	Del	--	--	--	--	--	--	--
	Dup	--	--	--	--	--	--	--

24	Del	het	5	Q31.2	1803	137,221,748-139,025,407	**MYOT**, PKD2L 2, WNT8A, NME5, KIF20A, CDC23, GFRA3, CDC25C, KDM3B, **REEP2**, EGR1, ETF1, HSPA9, CTNNA 1, LRRTM2, **SIL1**, MATR3, PAIP2, SLC23A1, MZB1, ECSCR, TMEM 173, UBE2D2,	**MYOT:** Muscular dystrophy **REEP2:** Spastic paraplegia **SIL1:** Marinesco-Sjogren syndrome
	Dup	--	--	--	--	--	--	--
25	Del	--	--	--	--	--	--	--
	Dup	--	--	--	--	--	--	--
26	Del	het	X	Xq21.1	1,6	77,157,760-77,159,406	**COX7B**	**COX7B:** Linear skin defect- multiple congenital anomaly 2
	Dup	hom	13	Q12.13	238	26,207,304-26445,861	**ATP8A2**	**ATP8A2**: cerebellar ataxia, mental retardation, and disecu-librum syndrome 4
27	Del	het	1	P13.3	879	109,512,869-110,392,345	WDR47, KIAA1324, SARS, CELSR2, PSRC1, SORT1, PSMA5, AMIGO1, GPR61, **GNAI3**, MIR197, GNAT2, **AMPD2**, GSTM4, GSTM2, GSTM1, GSTM5, GSTM3, EPS8L3,	**GNAI3:** Auriculocondiler syndrome 1 **AMPD2:** Pontocerebellar hypoplasia
	Dup	--	--	--	--	--	--	--
28	Del	--	--	--	--	--	--	--
	Dup	het	3	q24	404	146,729,902-147,133,996	ZIC4, **ZIC1**	**ZIC1:** Craniosynostosis 6
29	Del	--	--	--	--	--	--	--
	Dup	--	--	--	--	--	--	--
30	Del	het	15	q26.2	1.7	96,873,441-96,875,178	**NR2F2**	**NR2F2:** Congenital heart defects, multiple types, 4
	Dup	--	--	--	--	--	--	--
31	Del	--	--	--	--	--	--	--
	Dup	--	--	--	--	--	--	--
32	Del	het	1	p32.3	1003	52,474,062-53,477,097	TXNDC12, ZFYVE9, **ORC1**, ZCCHC11, GPX 7, COA7, **SCP2**	**ORC1:** Meier-Gorlin syndrome 1 **SCP2:** Leukoencephalopathy-dystonia and motor neuro-pathy
	Del	het	1	P31.1	470	78,107,120-78,577,692	USP33, FAM73A, **NEXN**, FUBP1	**NEXN:** cardiomyopathy
	Del	het	X	q13.1	251	70,543,060-70,794,385	**TAF1**, INGX, OGT	**TAF1:** Mental retardation, X-linked, syndromic 33
	Dup	--	--	--	--	--	--	--

33	Del	--	--	--	--	--	--	--
	Dup	het	20	p12.2	24.1	10,629,661-10,653,828	**JAG1,**	**JAG1:** Alagille syndrome, Fallot tetralogy, Congenital heart defect and posterior embryotoxone
	Dup	het	3	q24	1109	146,362,487-147,472,385	ZIC4, **ZIC1**	**ZIC1:** Craniosynostosis 6
34	Del	het	X	Xq21.1	0.7	77,157,760-77,159,406	**COX7B**	**COX7B:** Linear skin defect- multiple congenital anomaly 2
	Del	hom	15	q26.2	1.7	96,873,441-96,875,178	**NR2F2**	**NR2F2:** Congenital heart defects, multiple types, 4
	Dup	hom	2	q31.1	81	176,958,852-176,959,346	**HOXD13**	**HOXD13**: Brachydactyly-syndactyly syndrome
35	Del	--	--	--	--	--	--	--
	Dup	het	15	q26.2	10.1	96,869,902-96,880,063	**NR2F2**	**NR2F2:** Congenital heart defects, multiple types, 4
	Dup	het	20	p12.2	577	10,620,343-11,197,525	**JAG1**	**JAG1:** Alagille syndrome, Fallot tetralogy, Congenital heart defect and posterior embryotoxone
	Dup	het	3	q24	771	146,362,487-147,134,223	ZIC4, **ZIC1**	**ZIC1:** Craniosynostosis 6
36	Del	--	--	--	--	--	--	--
	Dup	het	X	p11.4	4.5	39,950,434-39,954,957	**BCOR**	**BCOR:** Microophthalmos, syndromic 2
37	Del	--	--	--	--	--	--	--
	Dup	--	--	--	--	--	--	--
38	Del	--	--	--	--	--	--	--
	Dup	het	2	p21	3.5	45,168,836-45,172,394	**SIX3**	**SIX3:** Holoprosencephaly2
	Dup	het	3	q24	124	147,008,963-147,133,632	ZIC4, **ZIC1**	**ZIC1:** Craniosynostosis 6
	Dup	hom	5	q35.2	50.4	174,151,663-174,202,136	**MSX2**	**MSX2:** Craniosynostosis 2
	Dup	het	X	q27.1	200.8	139,384,622-139,585,454	**SOX3**	**SOX3:** Panhipopituarism, X-Linked, Mental retardation, X-linked, with isolated growth hormone deficiency
39	Del	--	--	--	--	--	--	--
	Dup	--	--	--	--	--	--	--

DİSCUSSİON

In the study of genetic factors in etiology, parental and fetal chromosome analysis, QF-PCR, FISH, MLPA, DNA sequence analysis for gene diseases, thrombophilia are performed, and studies in the literature reveal that genetic etiology is still not fully elucidated today. For this reason, new diagnostic technologies are needed to determine genetic etiology. As a result of the increase in the rate of genetic diagnosis, treatment and preimplantation at the gene level will have a chance to benefit from genetic diagnosis(11,12).

In terms of the resolution spectrum, genome sequence analysis and array CGH are the most soluble genetic methods. There are rules and algorithms to consider when applying the array-CGH test. As a result of the application, CNVs are detected at a certain location of chromosomes. The CNVs obtained as a result of the array CNV detection algorithm must be further analyzed and verified by other molecular techniques(13). In our study, array CGH data of 39 fetuses were analyzed retrospectively to investigate the etiology of fetal loss. The fetuses included in our study, all cases are included in the unexplained fetal loss group except for genetic factors and the mean gestational week is the 25th gestational week. In our study, when 54 genes that are effective in the development and growth stages of embryogenesis are examined, it is seen that there are non-mortal conditions in terms of Protein / Disease / Syndrome. Although the clinical conditions associated with these genes are related to the postnatal period and are compatible with life, we can explain the answer to the question of how these genes are involved in the etiology of fetal loss.

Studies investigating the etiology of fetal loss by aCGH method in the literature in terms of CNV changes are summarized together

with their features in Table 3. In these studies; It is seen that the frequency of CNV is at most 22%. The frequency of CNV in our study is 77%, and we think that difference is due to the analysis method. In our study, besides the CNVs obtained from the analysis program, the fact that data was created as a result of analyzing the genes that had roles in embryological periods caused more data output and thus a difference.

Table 3 : Studies investigating the etiology of fetal loss by aCGH method in the literature

Reference	Cases studied	Number of mutations	Detected CNV rate	CNV type and rate of CNVs	Gene/Locus	Chromosome with the highest CNV detection
K. Wou and at all.	1071	330	36 (10.9%)	Del 20 (%6.1) Dup 16 (%4,8)	unspecified	unspecified
Björn and at all.	100	28	4 (%4)	Del (%3) Dup (%1)	dup(13)(q32.1qter) del(20)(pterp12.1), male del(7)(q36qter), female del(X)(q28qter), female	7,20,X
Rosenfeld and at all.	515	48	21 (%4)	Del 17 (%3.3) Dup 4 (%0.7)	NRXN1 gene deletion in 1 case	unspecified
Harris and at all.	54	12	12 (%22)	Del 8 (%15) Dup4 (%7)	ANKRD19,ASPN,AUH, BICD2, C9orf44, C9orf89, CENPP, DIRAS2, ECM2, FGD3, IARS, IPPK, NFIL3, NINJ1, NOL8, OGN, OMD, ROR2, SNORA84, SPT-LC1, SUSD3, SYK, WNK2, ZNF484, 2R1, OR51F2, OR51S1, OR51T1, CLLU1 OS, CLLU1	9
Reddy and at all.	532	37	37 (%7)	Del 9 (%1,6) Dup28 (%5,4)	unspecified	CNV was most commonly detected on the 19. Chr. 8q24-SLA gene dup, Xp22-HCCS ve MID1 gene del

Del: deletion, Dup: Duplication, Chr: Chromosome n: Number of cases

While there were more deletion variants in the literature, it was observed that duplication variants were higher in our study. The chromosome distribution of CNVs varies in the literature. In our study, CNV is more in the X chromosome, but more studies are needed in this regard. With the introduction of comparative genome hybridization in the early 1990s and new technological developments in CGH, it is understood that literature studies have been carried out with CGH in many areas of medicine but there are few articles on fetal loss.

In our study, CNVs were detected equally in all trimesters and are different from the literature in this respect. The fact that the number of cases in our study was 39, the distribution of the cases was not distributed equally to the trimesters and the lack of CNV in 9 cases may have caused a difference in the distribution of CNVs according to trimester. To create a clearer result, the total number of cases and cases should be distributed to trimesters homogeneously. Björn et al; In his study of 100 cases including spontaneous abortion and fetal losses; 72% of the cases were found to be normal in terms of genetic abnormalities and it was reported that only 3 cases had CNV which is (q36qter), del (X) (q28qter), del (20) (pterp12.1), dup (13) (q32.1qter) (14). Rosenfeld et al; 515 cases of fetal loss were analyzed and deletions were detected in 17 cases and duplication in 4 cases, and a total of 21 CNV (4%) cases were reported(7). In this study, non-CNV genetic abnormalities were 48 cases (9%), and in our study, the rate of CNV was higher than this study. In the Rosenfeld et al. study, only statistical data was presented, and chromosome locus with CNV was not presented as information. In an unexplained fetal loss study involving 54 fetuses over 22 weeks of age, Harris et al. detected 24 new CNVs, and further analysis of these CNVs revealed that 18 (75%) were placental tissue(3). When fetal CNVs were analyzed; ANKRD19, ASPN, AUH, BICD2, C9orf44, C9orf89, CENPP, DIRAS2, ECM2, FGD3, IARS, IPPK, NFIL3, NINJ1, NOL8, OGN, OMD, ROR2, SORA, SORA, SORA SYK, WNK2, ZNF484(completely deleted), C9orf129(partially deleted) on the chromosome 9 and

OR52R1, OR51F2, OR51S1, OR51T1(completely deleted) on the chromosome 11, 9th chromosome PAPP-A gene and CLLU1 OS, CLLU1 genes on the chromosome 12 detected pathogenically CNV of these genes which were not observed in our study. In a study analyzed by Reddy et al., aCGH was analyzed with 532 fetuses and revealed that aCGH detected 41.9% more CNV than karyotyping. CNV was reported in 7% of the cases. Although these CNVs are not called genes, chromosome loci information is available(15). When evaluated in terms of loci, SLA gene duplication in 8q24 locus and HCCS gene deletion in Xp22 locus in case 13 and HCCS and MID1 gene deletions in case 14 is compatible with the current study.

In the current study, genes detected as 8% and above were selected as candidates for the etiology of fetal loss due to the low number of cases. As the rate of detection of genes in our study decreases, the possibility of false positivity increases with the reflection of CNVs seen in one or two cases. For this research to have a stronger result, it is recommended to increase the number of cases to be studied and to discuss with the data in the current literature.

In conclusion; array CGH analysis has become more preferable in laboratories because it can detect smaller chromosomal abnormalities in detecting cytogenetic abnormalities. Because the array CGH method does not require culture, less labor, increasing more information, data is obtained more automatically. The current study demonstrates the benefits of fetal loss analysis by oligonucleotide array in terms of genes affecting the embryonic period. With more studies on this subject at the gene level, the rate of unexplained causes in fetal loss etiology will decrease.

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