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Case Report Open Access

Case Report: Interstitial Deletion 21q22.13-Q22.3 in a Male Patient with Developmental Delay, Holoprosencephaly, Dysmorphic Features, and Multiple Congenital Anomalies

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Abstract

We report on a new case with developmental delay, dysmorphic features, holoprosencephaly that showed deletion in long arm of chromosome 21 (21q22.13-q22.3). The patient is a male 3 months old presented with fronto-nasal dysplasia, scoliosis, abnormal ears, VSD, hypospadias, undescended testis. MRI has shown holoprosencephaly and agenesis of corpus callosum. Array-comparative genomic hybridization using the Agilent 2×400 oligoarray showed an interstitial deletion in chr21q22.13-q22.3, (start-end: 36,854,967-46,006,008 bp) deletion size is 9 Mb (9,151,042 bp) and includes 75 genes (Data base of genomic variants, hg18). The deletion was found to be maternal in origin. The findings from this report underscore the role of the genes at chromosome 21q22.13-q22.3 in brain development and indicate the usefulness of array-CGH in identification of the deletion size and detection of genes that could be correlated to the patient's phenotype.

Keywords: Holoprosencephaly; Agenesis of corpus callosum; Developmental Delay; Interstitial deletion 21q22.13-q22.3; Phenotype/Genotype correlation

Introduction

Partial monosomy in chromosome 21q is a rare genetic condition and about 50 cases are reported so far. In contrast to trisomy 21, the most common chromosome aneuploidy, complete monosomy of chromosome 21 is incompatible with life and only segmental monosomy has been reported. Previous reports indicated a wide range of phenotypes ranging from normal to severe phenotypes associated with different sizes of deletions in different positions on chromosome 21q22 [1,2]. The spectrum of phenotypes ranges from normal [2], mild [3] to severe phenotypes associated with multiple congenital malformations, dysmorphic features, seizures and delayed development [4]. The use of advanced techniques such as array comparative genomic hybridization (array-CGH) allowed for the accurate delineation of the position and size of deletions and identification of genes for phenotype/genotype correlation. We report on a case with de novo 9,151 Mbp deletion in chromosome 21q22.13-q22.3, presented with developmental delay, holoprosencephaly, agenesis of corpus callosum, dysmorphic features, scoliosis and seizures.

Clinical Report

Patient AE (BL-901-12) three months old Male with developmental delay and dysmorphic features, was referred from the Genetic Department, Children Hospital, al-Taif to the CEGMR for genetic diagnosis. He was the first child after a previous spontaneous abortion. The parents were non-consanguineous of Syrian origin. The mother was 19 years old and the father 30 years old at the birth of the child.

There was a positive family history of similar condition; a maternal uncle had three babies with same complaint who died at ages below 1 year. The mother and her 2 sisters have a fold of skin in the lower back in front of the coccyx similar to that found in the proband.

The pregnancy was uneventful and ended with a preterm spontaneous vaginal delivery (35 weeks gestation), he presented with tachypnea, weak cry, and was incubated for 2 days after birth. He could not breast feed because of difficulty in sucking milk, he had seizures twice daily, wheezing chest.

On examination at birth the weight was 1.980 g (<3rd centile), head circumference 32.5 cm, length: 49 cm (<3rd centile), A S 7/8. The patient has dysmorphic features, developmental delay, ventricular septal defect (VSD), inguinal hernia, seizures, and scoliosis. Dysmorphic features were described as thin lips, long philtrum, low set abnormal ears, scanty eyebrows, small eyes, depressed nasal root, prominent nose, retracted mandible, micrognathia (Figure 1a and b). Other malformations were observed: hypospadias, undescended testis, and a fold of skin in the lower back in front of the coccyx (Figure 1C).

ECHO: Ventricular septal defect. MRI: Non contrast MRI study of the brain with multiplanar multisequential images has shown a picture of holoprosencephaly with cystic dilatation of fused bodies and occipital horns of lateral ventricles, and absence of corpus callosum. The diagnosis of holoprosencephaly, agenesis of corpus callosum was proposed.

Laboratory findings had shown hypochromic microcytic anemia (Hb: 9.5, RBCs: 3.12, WBC: 188, Platelet: 362 × 10^6) Na: 125, glucose: 89, Creat: 0.2, K: 5.7, Ca: 9.1. Results of laboratory studies for the

mother for TORCH has shown positive result for a previous cytomegalovirus infection (IgG positive) and Rubella infection (IgG positive).

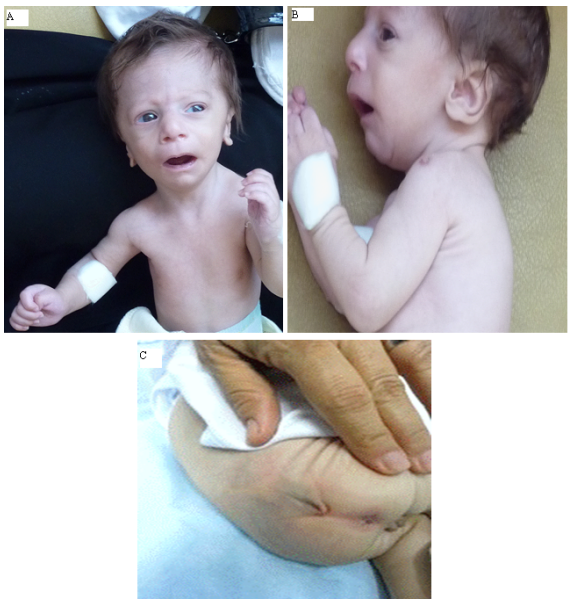

Figure 1: a) Patient with dysmorphic features, multiple congenital anomalies, b) Lateral view c) Lower back showing sacral dimple.

Blood samples were obtained from the patient and his parents after obtaining an informed consent. The work was a part of a project funded by the KACST (#09-BIO 695-03), King Abdulaziz University, and was approved by the Ethical Committee at the CEGMR (Center of Excellence in Genomic Medicine Research). Chromosomal analysis for the patient using conventional G-banding has shown apparently normal 46, XY karyotype, however, revision after obtaining array-CGH data had shown deleted chromosome 21q (Figure 2). Chromosomal analysis of the parents was normal. The patient died at 5 months old after surgery for correction of inguinal hernia.

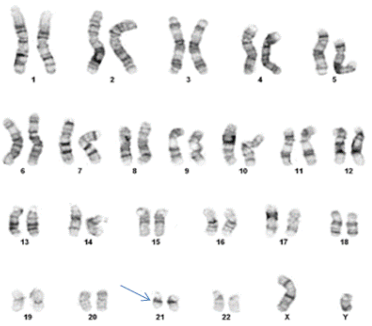

Figure 2: Karyotype of the patient showing del21q22.

Array-CGH

Array- comparative genomic hybridization (a-CGH) was done using the Agilent 2×400 oligoarray (Agilent Tecnologies, Paulo Alto, CA). DNA was extracted using blood mini kit (QIAGEN) following the manufacturer instructions. Experimental design for the array-CGH was done following the manufacturer guidelines with slight modifications. Analysis of the results of the array-CGH was done using the Cytogenomics Agilent software available free on line. The data base (http://www.genome.ucsc.edu) and (http:www.ncbi.nlm.nih.gov) were used for analysis of the data and for identification of genes.

PCR Analysis of STS Markers

To test for the origin of deletion, PCR amplification of the STS markers: D21S1921 (start: 35343951- end 35344296), D21S266 (start: 41606427- 41606641), D21S1890 (start: 43672584- 43672816), D21S1912 (start: 44402165- 44402509), D21S1903 (start: 44965473-44965750) was done for DNA samples from the proband and his parents followed by agarose gel electrophoresis.

Quantitative Real Time PCR (qPCR)

The findings were confirmed by real-time quantitative PCR (qPCR) using STS markers located on the long arm of chromosome 21q: D21S1921, D21S1903, D21S1912, D21S1890, and D21S266. The PCR was carried out using an ABI StepOne Plus (Applied Biosystems) in a 96-well optical plate with a final reaction volume of 10 µl. The reaction was done using KAPA SYBR® FAST qPCR Kit (KAPA Biosystems, USA), comprising of (5 µl) of 2X SYBR-Green PCR Master mix, 0.2 µl High ROX (50 X), 10 pmol forward and reverse primers for each STS marker and endogenous gene B2-microglobulin (B2M) as reference gene, 10 ng genomic DNA (1 µl) and RNase-grade water up to 10 µl, samples were run in triplicates. The PCR conditions were initial denaturation step of 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Analysis of the results was done by using StepOne Software V2.3 (Applied Biosystem). A higher or lower starting copy-number of input DNA as a sign for a deletion or a duplication will result in an earlier or later increase of fluorescence. ΔCt represent the mean Ct value of each sample, the ΔΔCt ratio was calculated for each DNA sample using Livak [5] method as follow:

$$\Delta\Delta Ct=[\Delta Ct(\text{target gene})-\Delta Ct(\text{Reference gene})]^{\text{Unknown sample}}-[\Delta Ct(\text{target gene})-\Delta Ct(\text{Reference gene})]^{\text{Control sample}}$$

$$\text{Normalized copy number of STS marker} = 2^{-\Delta\Delta Ct}$$

Results

High resolution analysis using the Agilent 2×400 whole human genome CGH microarray revealed an interstitial deletion size of 9,151 Mb spanning from position 36,854967 to -46,006008 bp encompassing cyto- band: q22.13 - q22.3, however, the deleted segment was shown as complex regions of deletions and duplications (Figure 3). Analysis of the arrays was done using the Cytogenomics software available on line. The deleted region contains ~75 genes (Data base of genomic variants, hg18). Three regions were showing amplification: Cytoband q22.11 (start-end: 33,316,640-34,203,048), Amp cytoband: q22.12 (Start-end: 35,126,979-35,396,718), and Amp cytoband: q22.2 - q22.3 (start-end: 41,367,753-41,838,468). Four regions were showing deletion: cytoband 21q22.13 (36,860,867-37,937,316), cytoband 21q22.2 (start-end: 39,569,960-41,357,412), cytoband: 21 q22.3 (start- end:

41,843,363-44,602,935), and cytoband 21q22.3 (45143028-45993425) (Figure 3).

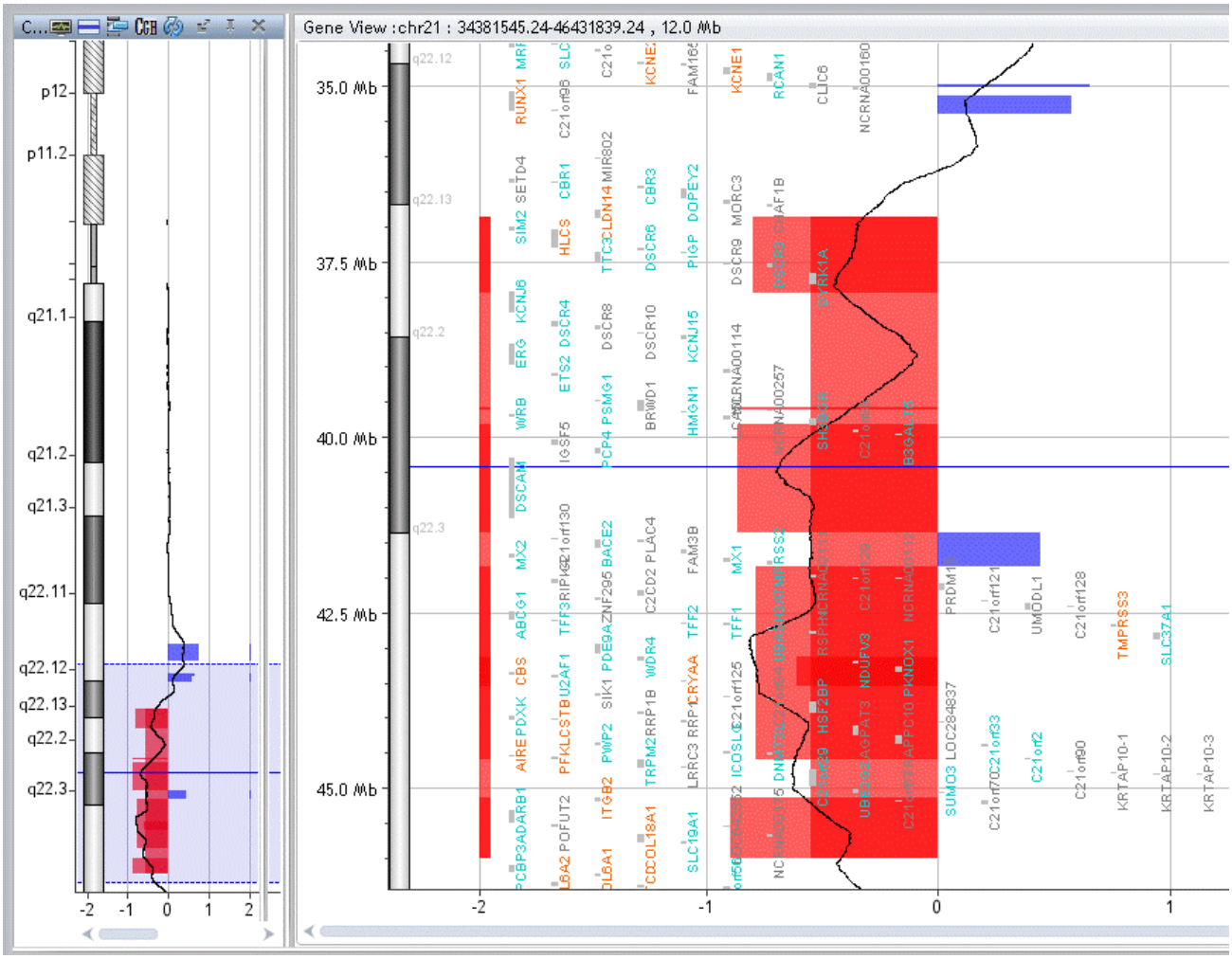

Figure 3: Array-CGH showing regions of deletions/duplications and the annotated genes at chromosome 21q22.11- q22.3.

Del/ Dup	Start- End (Mb)	Annotation
Duplication 21q22.11	33316640-34203048	OLIG2, OLIG1, C21orf54, IFNAR2, IL10RB, IFNAR1, IFNGR2, TMEM50B, DNAJC28, GART, SON, DONSON, CRYZL1, ITSN1, ATP5O
Duplication 21q22.12	34965869-35396718	CLIC6, RUNX1, C21orf96
Deletion 21q22.13	36860867-37937316	CLDN14, SIM2, HLCS, DSCR6, PIGP, TTC3, DSCR9, DSCR3, DYRK1A, KCNJ6
Deletion 21q22.2	39569960-41357412	BRWD1, SH3BGR, C21orf88, B3GALT5, IGSF5, PCP4, DSCAM
Duplication	41367753-41838468	C21orf130, BACE2, PLAC4, FAM3B, MX2, MX1, TMPRSS2
Deletion 21q22.3	41843363-44,602,935	NCRNA00111, C21orf129, NCRNA00112, RIPK4, PRDM15, C2CD2, ZNF295, C21orf121, UMODL1, C21orf128,ABCG1,TFF3,TFF2,TFF1, TMPRSS3,UBASH3A, RSPH1, SLC37A1, PDE9A, WDR4, NDUFV3, PKNOX1, CBS, U2AF1, CRYAA, SIK1, C21orf125, C21orf84, HSF2BP,RRP1B,PDXK,CSTB,RRP1, LOC284837, AGPAT3, TRAPPC10, PWP2, C21orf33, ICOSLG, DNMT3L, AIRE, PFKL, C21orf2, TRPM2

Deletion 21q22.3	45143028-45993425	ITGB2, C21orf67, C21orf70, NCRNA00163, NCRNA00162, C21orf122, ADARB1, POFUT2, LOC642852, COL18A1, NCRNA00175, SLC19A1

Table 1: Results of array-CGH showing regions of deletions/duplications and the annotated genes at chromosome 21q22.11- q22.3.

Table 1 summarizes the results of array-CGH showing the regions of deletion/duplication in chromosome 21 and the annotated genes. Array-CGH analysis for both parents has shown no deletion in chromosome 21q, however, copy number variants were observed in other chromosomes.

PCR amplification of the STS markers on chromosome 21 from position 21q22.12 to 21q22.3 has shown that the deleted region was of maternal origin (Figure 4). Results of Real Time PCR qPCR have confirmed the position of deletion and deletion size on the chromosome (data not shown). A comparison of the size and position of deletions on chromosome 21q and the associated phenotypes in our patient and other reported cases is presented in (Table 2). Comparison of the phenotype in our patient with other cases sharing deletion in the segment spanning from the region 37.55 to 46,006 shows that they all suffer from developmental delay, microcephaly, epilepsy, dysmorphic features and large low set ears.

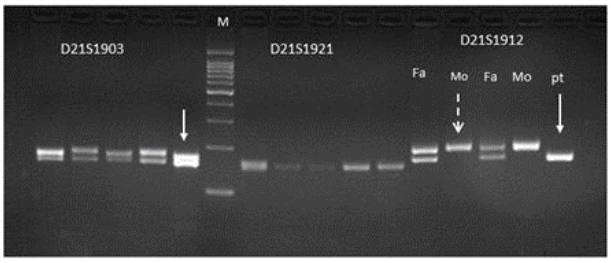

Figure 4: Gel electrophoresis of STS/PCR amplified products showing absence of maternal alleles in the patient. Fa-father, Mo-mother, pt-patient, M-marker 100bp plus ladder (QIAGEN)

	Present case	**Braddock Case (1)**	**Byrd [28]**	**Katzaki Case (1)**	**Guion-Almeida [8]**	**Yamamoto [14] case (2)**	**Valetto et al., [4] (1)**	**Izumi et al.,**	**Oemega, [7]**	**Roberson[1] case (1)**
Chromosomal deletion	36,854967 46,006008	30.02-37.55 Mb	30.69-37.00 Mb	33.45-36.36 Mb	46.625-46.884	36,156,827 46,944,323	37,713441-42,665162	32,273, -34,.168,	37053-41102	42.047-46.909
Size of the deletion	9.151 Mb	7.5 Mb	6.3 Mb	2.29 Mb	219 Kb	9,897 Mb	4.95 Mb	1.9 Mb	4.049 Mb	4.86 Mb
Age/sex	2 mn/M					16 yr/ F	11 yr/M	2 mn/ F	M	3.8 yr/ M
Curly or/ sparse hair	-/ +	+ /+			-/ +	---		+/-		
High forehead	+	+				+		-		-
Downslanted palpebral fissure		+	+	+		hypertelorism	Long palpeb. fissure	+		Epicanthic fold
Posteriorly rotated (dysplastic) ears	+	+	+			+	Large ears	-	Low set dysplastic	Simple ears
Broad nasal root	+	+		+	+			+	Deep nasal bridge	
Prominent nose	+					+	+			
Cleft lip/palate/ Thin upp lip	-/Thin lips	+	+	-	+	+	Thin upp. lip	+		Large mouth
Microcephaly	+				+	+	+	+		dolicocephaly
Micrognathia/ retrognathia	+	+	+	-		+/ +	+/ +	+	+/ +	---
Cong. Heart disease	VSD	VSD	ASD	-		PDA	no	ASD		no
Hematology thrombocytopenia	anemia	+	+	+			normal	-		

CNS: holoprosencephaly	+	-	-		encephalocele	colpocephaly	No holoprose ID severe	-	ID	--
Agenesis/ hypoplasia of corpus callosum	+	+	+	+	+		hypoplastic corpus callosum	-	Abn. Rt. Frontal cortex Polymicrogiri	--
Developmental delay/delay speech	+	+	+	+	-	+/severe delayed speech	+/absent speech	+	+	Speech delay
Epilepsy	+	-				+	+	-	+	--
Inguinal hernia	+	-						-		+
Undesended testis hypospadias	+	-						-		--
Scoliosis/sacral dimple	+	-				scoliosis		-		Sacral dimple

Table 2: Comparison of the phenotypes and deletion size in chromosome 21q among different reported cases.

Discussion

We report a new case with deletion of chr21q22.13-q22.3 presented with developmental delay (DD), holoprosencephaly and multiple congenital malformations. Other authors reported infants with multiple abnormalities and a deletion in the long arm of chromosome 21 from band 21q22.1–qter [6]. They suggested that the critical region of deletion lies within 21q22.1-q22.2. More sever phenotypes associated with 21q22 deletion were reported such as intrauterine growth retardation, microcephaly, seizures, corpus callosum abnormalities, eye anomalies, micrognathia, abnormal/dysplastic ears, intellectual disability [1,7,8].

In our case we noticed the presence of three deletions and three duplications, two duplications located upstream of the deleted region, one corresponding to cytoband q22.11 and the other at cytoband q22.12, and the third was observed inside the deleted region at cytoband 21q22.2. Complexity of the rearrangements was reported by Lindstrand et al. [9] in patients with DD and congenital malformations, one of them has four deletions and four duplicated segments with 16 breakpoints. Another patient had deletion in two different positions (21q21.1-21q22.11) and (21q11.2-21q21.3), these regions are upstream (more proximal) from the regions in our patient. Similar observation of concomitant deletion and duplication in chr21q was reported by Qi et al. [10] in a fetus with Down syndrome [10]. Array-CGH technique is expected to detect more cases with complex rearrangements. It was observed that proximal deletion of 21q is associated with mild or no cognitive impairment and problems with balance [9]. Deletion sizes of 4.86 Mb, 5.68 Mb and 16.4 Mb in three different patients were reported by Roberson et al. [1] and 4.9 Mb in another patient by Valetto et al. [4] compared to deletion size 9,151 Mb in our patient.

The duplicated segment from 33,316,640 to 35,396,718 included the ITSN1and RUNX1 genes. The Intersectin 1(ITSN1) gene was reported to be expressed in the brain and could be involved in synaptic vesicle recycling, overexpression of the ITSN1 gene results in sex dependent decrease in locomotor activity and was implicated in the neurodegenerative disorders such as Down syndrome [11]. Runt-related transcription factor 1(RUNX1) gene is thought to be involved in the development of normal hematopoiesis, our patient had anemia but there was no thrombocytopenia which was reported by others [12]. The deleted segment included 75 genes, some of these are: the Holoprosencephaly-1 (HPE1) gene located at 41400001-46944323, (OMIM: 236100), DYRKIA, BRWD1, DSCAM (Down syndrome cell adhesion molecule), ADARB1 (adenosine deaminase, RNA-specific, B1), SIM2, HMGN1, RIPK4, PRDM15 among others.

Mapping the phenotypes to regions of deletions is useful to identify genes associated with specific phenotype and to provide better understanding of the pathogenesis. Three broad regions are implicated to the phenotype of monosomy 21. The first from the centromere to 31.2 Mb contains 50 genes and produces severe phenotype [13]. The second from 31.2 to 36 Mb contain 80 genes but deletion is rare in this region indicating intolerance in monosomic state [13]. The third from 36- 37.5 Mb to the telomere contain 130 genes and result into a milder phenotype [3,13]. The deletion in our patient spans from 36,854 to 46,006 Mb, downstream from the intolerant deletion region, but have more sever phenotype than that reported by Lyle et al. Similar deleted segment, albeit smaller, was reported by Valetto et al. [4] from position (37,713,441) (q22.13) to position 42,665,162 (q22.3) in a patient with dysmorphic features, intellectual disability and epilepsy [4]. The patient had microcephaly, sparse hair, thin upper lip, long philtrum, retrognathia and epilepsy similar to that observed in our patient.

Comparison of the region of deletion in our patient with those reported by others (Table 2) has shown an overlapping region of deletion that spans from position 37,047 to 41,102 Kb. The annotated genes are CLDN14, SIM2, DYRK1A, KCNJ6, BRWD1, and DSCAM. All patients show microcephaly, epilepsy, brain atrophy and retro-micrognathia [4,7,14] which emphasize the role of these genes in brain development. Holoprosencephaly (HPE1) (MIM 236100) gene was mapped to chromosome 21q22.3.

Estabrooks [15] described an infant with HPE and normal facies and minute deletion in chromosome 21q22.3. Muenke et al., [16] reported 3 cases with HPE and defined the HPE minimal critical region in chromosome 21q22.3. However, he ruled out SIM2 as a candidate gene for HPE1. HPE was previously reported in cases with ring chromosome 21 [17,18]. However, Chen et al. [19] reported a patient with microcephaly, borderline ventriculomegaly and cerebellar

hypoplasia, micrognathia, and ventricular septal defect associated with duplication of 12q24.32-->qter and a deletion of 21q22.2-->qter. Genetic marker analysis of their patient showed a deletion at 21q22.2, the deleted segment was measured about 4.5 Mb encompassing the HPE critical region. They suggested that haplo insufficiency of the HPE critical region at 21q22.3 may not cause an HPE phenotype [19].

Chen et al., [20] reported a malformed infant with ring chromosome 21 but not showing any central nervous system abnormality. However, the presence of holoprosencephaly in our case confirms the critical region for HPE in 21q22.3.

Agenesis of corpus callosum in our patient was associated with HPE, similarly other reports considered agenesis of corpus callosum as part of the HPE spectrum. A critical region for agenesis of the corpus callosum has been suggested on chromosome 21q22.2-q22.3 [21] and was previously reported to be associated with satellite chromosome 21q [22]. Thin corpus callosum and mild brain atrophy was reported by Valetto et al. [4] in the same deleted segment. The association between the 21q22.3 region and the spectrum of agenesis of corpus callosum/ holoprosencephaly can be confirmed by our finding and the previous observations of thin corpus callosum in patients with deletion in the overlapping region spanning from (37,713 to 39,585) reported by Oemega [7], Valetto [4] and callosal agenesis described by Guion-Almeida et al. [8] with partial 21q22.3 deletion.

The deleted region (36,860,867-37,937,316) (21q22) included the DYRKIA and KCNJ6 genes among others. Previous reports indicated that 21q22 micro deletions including only DYRK1A (dual-specificity tyrosine- (Y)-phosphorylation-regulated kinase 1A) gene was found in patients presented with microcephaly, growth retardation, epilepsy, delayed speech and similar features such as low set ears, long philtrum, micrognathia [4,14,23]. Deletion including the 5` region of the DYRK1A gene was observed in another patient with microcephaly, seizures and dysmorphic features [24]. It was suggested that the DYRK1A gene might be the causative gene responsible for most of the clinical features observed in interstitial deletion of chromosome 21. The DYRK1A gene is highly conserved in mammals and was implicated in brain development and causative gene for microcephaly in humans [4]. Mouse models heterozygote for the mutation Dyrk1a +/-showed developmental delay, hypo activity and reduced size of the brain [25] which confirm the role of this gene in brain development in humans.

The other gene KCNJ6 (potassium inwardly-rectifying channel, subfamily J, member 6) is one of the ion channel genes, its role in causing seizure phenotype was suggested by the haploinsufficiency in KCNJ6 gene in the patients with epilepsy reported by Yamamoto et al. [14] and Valetto et al. [4]. The role of this gene in epilepsy needs larger study on selected samples.

Another gene located in this site, the CLDN14 (Claudin 14) gene is a protein coding gene, a member of the claudin family; it is an integral membrane protein and is a component of tight junction strands. Defects in this gene can cause autosomal recessive form of nonsyndromic sensorineural deafness [26].

The DSCAM (Down syndrome cell adhesion molecule) is a protein-coding gene. DSCAM is located within the deleted segment (chr21:40,306,213-41,140,909), it is a member of the immunoglobulin superfamily of cell adhesion molecules, and is involved in human central and peripheral nervous system development. This gene encodes a cell adhesion molecule and is expressed in the heart during cardiac development and was proposed as a candidate gene for Down syndrome and congenital heart disease (DS-CHD) [27]. Congenital heart defect in the form of (VSD) was observed in our patient, and in other reported cases, however they reported deleted segment in more proximal region between (30,02-37,55Mb) [12,28] and (34,796 and 35,363) [9] including genes CLIC6 and RUNX1. Lindstrand [9] suggested that a critical region of 0.5 Mb between 34,796 and 35,363 including CLIC6 and RUNX1 and another 2 genes is associated with congenital heart disease, however, this region has shown duplication in our patient which might indicate different mechanism in causing heart defect.

Conclusion

The findings of this report underscore the role of the genes at chromosome 21q22.13-q22.3 (36,860,867-45993425) in causing developmental delay, holoprosencephaly, agenesis of corpus callosum, and epilepsy. Whole genome array- CGH analyses help in determining the microdeletion size and the annotated genes that could be related to the phenotype.

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