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Prenatal and Postnatal SNP Microarray Analysis and Parental Follow-up of 16q24.1 Deletions/Duplications That Include the FOXF1 Gene

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I. Introduction and Results

Deletions of 16q24.1 including FOXF1 or upstream regulatory elements of FOXF1 (non coding RNA genes LIN01081 and LIN01082) and heterozygous point mutations of the FOXF1 gene have been reported in newborns with a lethal lung developmental disorder, alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV). Studies have shown the majority of reported deletions that include the FOXF1 gene or its upstream regulatory regions are de novo and arise on the maternal allele, suggesting that genomic imprinting may be involved in this disorder. A collection of 13 prenatal and 10 postnatal CNV that include the FOXF1 gene, with or without the upstream regulatory elements of FOXF1, were compared clinically. Follow-up testing and parent of origin testing was also assessed.

The prenatal deletions ranged in size from 353kb to 4.69 Mb, eight of which included the FOX gene cluster and upstream regulatory elements of FOXF1. Eight of the prenatal deletions had parental follow-ups that showed the abnormality to be *de novo* in origin. An in depth review of the series was made. Four of the deletions also had origin studies performed using SNP microarray analysis (**Table 1**). Case 3 was an amniotic fluid specimen referred for hypoplastic heart, thickened nuchal fold with septations and small omphalocele with a 3.37 Mb deletion of 16q24.1 that included FOX gene cluster and upstream regulatory elements of FOXF1. Case 7 was a CVS specimen referred for cystic hygroma, echogenic bowel, suspected cardiac defect, bladder obstruction, bilateral pyelectasis, hypoplastic left heart, and mitral valve stenosis and a 1.96 Mb deletion of 16q24.1 that included FOX gene cluster and upstream regulatory elements of FOXF1. The baby died shortly after birth and autopsy confirmed a diagnosis of ACD/MPV. Case 10 was an amniotic fluid specimen referred for advanced maternal age, increased nuchal translucency, positive serum screen for Down syndrome, and tetralogy of fallot. SNP microarray identified a 407 kb deletion of 16q24.1 that only contained the FOX gene cluster and no enhancer elements. The baby was born with a diagnosis of ACD/MPV. Case 11 was an amniotic fluid referred for coarctation of the aorta and bilateral moderate to severe pyelectasis, and an abnormal AFP with a 353 kb deletion of 16q24.1 and contained the FOX gene cluster and no enhancer elements. Origin studies using the SNP microarray determined that the deletions occurred in the maternal homologue in Case 3, 7 and 10. However, case 11 showed that the deletion occurred on the paternal homologue (see genotyping results on **Case 11**).

The postnatal duplications ranged from 431 kb to 5.76 mb, nine of which included FOX gene cluster and upstream regulatory elements of FOXF1. Follow-up was available on four of the cases, one case was inherited from a clinically normal mother and only included the FOX gene cluster, one was paternally inherited and included the FOX gene cluster and upstream enhancers, and two cases were *de novo* and included the FOX gene cluster and upstream enhancer elements.

Case 10 showing the genotyping calls for maternal (mat), Paternal (pat) and prenatal specimens. Regions highlighted in red with a ★ indicated maternal exclusion, indicating deletion occurred on maternal allele

	Chromosome	Position	Genotype Calls: Case 10 pat...	Genotype Calls: Case 10 prenatal...	Genotype Calls: Case 10 mat...
	16	86,552,713	AA	AA	AA
	16	86,568,824	AA	AA	AA
	16	86,542,845	BB	BB	AB
	16	86,546,887	BB	BB	AB
	16	86,558,013	BB	BB	AB
	16	86,537,032	BB	BB	BB
	16	86,569,399	BB	BB	BB
	16	86,585,905	BB	BB	BB
	16	86,604,364	BB	BB	BB
	16	86,608,786	BB	BB	BB
	16	86,623,781	BB	BB	BB
	16	86,629,376	BB	BB	BB
	16	86,631,479	BB	BB	BB
★	16	86,637,060	BB	BB	AA
★	16	86,637,489	BB	BB	AA
	16	86,570,907	AB	BB	AB
	16	86,576,331	AB	AA	AB
	16	86,576,473	AB	BB	AB
	16	86,581,398	AB	AA	AB
	16	86,584,005	AB	AA	AB
	16	86,590,080	AB	AA	AB
	16	86,590,430	AB	AB	AB
	16	86,609,056	AB	AA	AB
★	16	86,552,647	AB	AA	BB
	16	86,618,630	AB	BB	BB
	16	86,618,726	AB	BB	BB
	16	86,619,405	AB	BB	BB
	16	86,619,812	AB	BB	BB
	16	86,620,191	AB	BB	BB
	16	86,622,436	AB	BB	BB
	16	86,624,863	AB	BB	BB
	16	86,629,126	AB	BB	BB
	16	86,629,141	AB	BB	BB
	16	86,632,293	AB	BB	BB
★	16	86,555,873	AB	BB	AA
	16	86,608,609	AB	AA	AA

Case 11 showing the genotyping calls for maternal (mat), Paternal (pat) and prenatal specimens. Regions highlighted in red with a ★ indicated paternal exclusion, indicating deletion occurred on paternal allele

	Chromosome	Position	Genotype Calls: Case 11 pat...	Genotype Calls: Case 11 prenatal...	Genotype Calls: Case 11 mat...
★	16	86,521,317	AA	BB	AB
★	16	86,521,590	BB	AA	AA
	16	86,523,727	AA	AA	AA
★	16	86,524,083	AA	BB	AB
	16	86,524,207	AA	AA	AA
	16	86,524,303	AA	AA	AA
	16	86,537,032	BB	BB	BB
	16	86,537,060	AA	AA	AA
	16	86,537,489	AA	AA	AA
	16	86,537,913	AB	BB	BB
	16	86,542,845	AB	BB	BB
	16	86,546,887	BB	BB	BB
	16	86,549,984	AB	BB	AB
	16	86,552,647	AB	BB	BB
★	16	86,552,713	AA	BB	AB
	16	86,555,873	AB	AA	AA
	16	86,558,013	BB	BB	BB
	16	86,558,152	AA	AA	AA
	16	86,568,824	AB	AA	AA
	16	86,569,399	AB	NoCall	BB
	16	86,570,907	BB	AB	AA
★	16	86,576,331	AA	BB	BB
★	16	86,576,473	BB	AA	AA
★	16	86,581,398	AA	BB	BB
	16	86,584,005	BB	BB	BB
	16	86,585,905	AB	BB	BB
★	16	86,590,080	AA	BB	BB
	16	86,590,430	AB	AA	AA
	16	86,604,364	BB	BB	BB
	16	86,608,609	AB	AA	AA

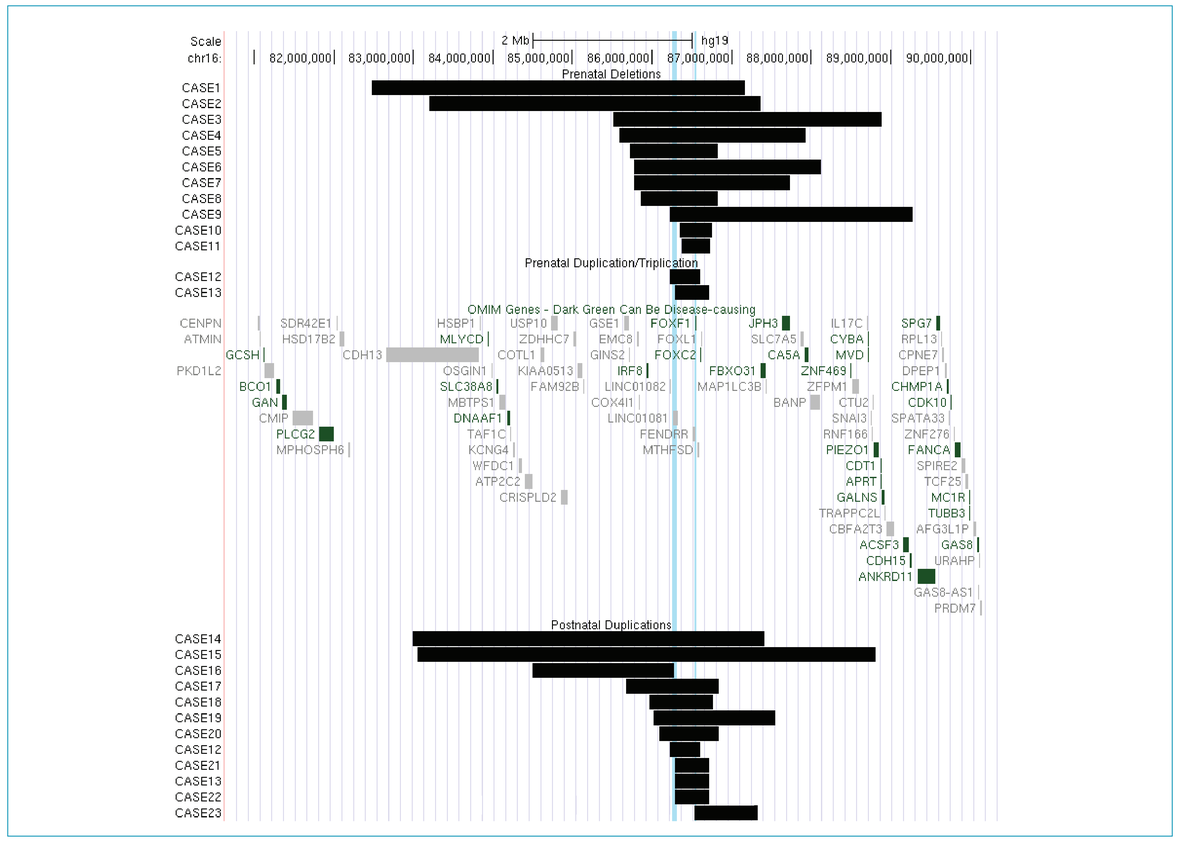

Table 1. Prenatal cases with interstitial 16q deletions involving the FOXF1 region

PRENATAL	GESTATION (WKS)	SPECIMEN	CNV	SIZE	BAND	START [hg19]	END [hg19]	INHERITANCE	ORIGIN	CLINICAL	FOX gene cluster +/ -	ENHANCERS LINC01082, LINC01081 +/ -
CASE 1	16	AMNIOTIC FLUID	DELETION	4.69 Mb	16q23.3q24.2	82,478,592	87,166,206	__	__	ASD, single umbilical artery, increased NT	+	+
CASE 2	20.4	AMNIOTIC FLUID	DELETION	4.16 Mb	16q23.3q24.2	83,196,442	87,359,648	DE NOVO	__	cystic hygroma, abnormal kidney	+	+
CASE 3	21.4	AMNIOTIC FLUID	DELETION	3.37 Mb	16q24.1q24.3	85,516,276	88,889,441	Maternal normal/father not tested	MATERNAL HOMOLOG	hypoplastic heart, thickened NT with septations, small omphalocele	+	+
CASE 4	22.0	AMNIOTIC FLUID	DELETION	2.33 Mb	16q24.1q24.2	85,594,403	87,932,471	DE NOVO	__	endocardial cushion defect, hydronephrosis, atriventricular canal defect, elevated AFP	+	+
CASE 5	19	AMNIOTIC FLUID	DELETION	1.1 Mb	16q24.1	85,728,812	86,831,579	__	__	Ascites, 2 vessel cord, LOH fourth degree consanguinity	+	+
CASE 6	N/A	CYSTIC HYGROMA FLUID	DELETION	2.35 Mb	16q24.1q24.2	85,776,097	88,123,598	__	__	cystic hygroma	+	+
CASE 7	N/A	CVS	DELETION	1.96 Mb	16q24.1q24.2	85,776,097	87,736,650	DE NOVO	MATERNAL HOMOLOG	cystic hygroma, echogenic bowel, suspected cardiac defect, bladder obstruction, bilateral pylectasis, hypoplastic left heart, mitral valve stenosis, passed away shortly after birth	+	+
CASE 8	21.2	POC	DELETION	978 Kb	16q24.1	85,853,985	86,831,579	DE NOVO	__	bilateral pyelectsis, partial fetal bladder obstruction, 19wks anhydramnios, bilateral moderate pyelectasis, fetal bladder obstruction, Autopsy atrial septal defect, pulmonary hypoplasia and misalignment of pulmonary veins consistent with ACD/MPV	+	+
CASE 9	21.6	AMNIOTIC FLUID	DELETION	3.1 Mb	16q24.1q24.3	86,230,158	89,273,883	DE NOVO	—	Micrognathia, complete AVSD, posterior urethral valves	+	partially includes LINC01081
CASE 10	16.7	AMNIOTIC FLUID	DELETION	407 Kb	16q24.1	86,353,606	86,756,619	DE NOVO	MATERNAL HOMOLOG	AMA, tetrology of fallot, abnormal maternal serum screen for Down syndrome, AFP within expected limits, increased NT, bilateral pyelectasis, echogenic intracardiac focus, 2 vessel cord, cystic hygroma	+	—
CASE 11	N/A	AMNIOTIC FLUID	DELETION	353 Kb	16q24.1	86,373,329	86,725,901	DE NOVO	PATERNAL HOMOLOG	severe coarctation of the aorta, moderate bilateral pyelectasis, elevated AFP, born with ACD/MPV	+	—
CASE 12	17.6	AMNIOTIC FLUID	DUPLICATION	379 Kb	16q24.1	86,229,000	86,608,077	DE NOVO	—	AMA, choroid plexus cyst, EIF, renal anomaly, single umbilical artery	+	includes LINC01081 and partially include LINC01082
CASE 13	21.3	AMNIOTIC FLUID	TRIPLICATION	423 Kb	16q24.1	86,287,540	86,710,169	MATERNAL	__	hydrops, fetal demise	+	LINC01081 and partially include LINC01082

NT-nuchal translucency; SUA- single umbilical artery; ASD-atrial septal defect; EIF-echogenic intracardiac focus; LOH-loss of heterozygosity; ACD/MPV-alveolar capillary dysplasia with misalignment of pulmonary veins

Table 2. Postnatal cases with interstitial 16q duplications involving the FOX cluster region

POSTNATAL	AGE (years)	CNV	SIZE	BAND	START [hg19]	END [hg19]	INHERITANCE	CLINICAL	ADDITIONAL ALTERATIONS	FOX gene cluster +/ -	ENHANCERS LINC01082, LINC01081 +/ -
CASE 14	3.37	DUPLICATION	4.41 Mb	16q23.3q24.2	82,997,025	87,408,717	__	failure to thrive, dysmorphic features, short stature, <3rd percentile for Head circumference, weight, height	__	+	+
CASE 15	0.31 (115 days)	DUPLICATION	5.76 Mb	16q24.1q24.3	83,052,231	88,815,024	__	N/A	__	+	+
CASE 16	11.99	DUPLICATION	1.78 Mb	16q24.1	84,494,522	86,273,615	__	generalized muscle weakness behavior problems	—	–	Enhance region only LINC01082 and partially include LINC01081
CASE 17	0.04 (16 days)	DUPLICATION	1.16 Mb	16q24.1	85,679,855	86,844,223	DE NOVO	hypospadias, extra nipple, hypothyroidism, cardiac defect, polydactyly, elevated TSH	—	+	+
CASE 18	2.55	DUPLICATION	794 kb	16q24.1	85,965,801	86,759,662	PATERNAL	Oligohydramnios in utero, intrauterine growth delay, short stature	—	+	+
CASE 19	0.01 (6 days)	DUPLICATION	1.5 Mb	16q24.1q24.2	86,020,114	87,551,687	DE NOVO	lack of coordination, other anomalies of the larynx, fetal and neonatal jaundice, stridor, esophageal reflux	arr[hg19] 2q23.3(151,892,909-152,387,511)x3 pat	+	+
CASE 20	6.94	DUPLICATION	756 kb	16q24.1	86,088,402	86,844,223	—	N/A	arr[hg19] 16p11.2(29,597,822-30,177,240)x3	+	+
CASE 21	23.02	DUPLICATION	438 kb	16q24.1	86,281,536	86,719,305	MATERNAL	Pervasive Developmental Disorder, hypotonia, Obsessive compulsive disorder/anxiety, mom is apparently normal	arr[hg19] 16q23.3(82,215,286-82,727,174)x3 mat	+	partially include LINC01081 only
CASE 22	7.62	DUPLICATION	431 kb	16q24.1	86,287,540	86,718,579	__	Complex syndactyly of the left hand, normal intelligence	arr[hg19] 16q23.3(82,215,286-82,731,711)x3	+	partially include LINC01081 only
CASE 23	1.64	DUPLICATION	797 kb	16q24.1q24.2	86,536,683	87,333,583	__	delayed milestones, Bell's palsy	__	+	—

II. Summary and Conclusions

- Our series of cases highlight that FOXF1 deletions/FOX cluster region deletions are more often identified prenatally and are associated with more significant malformations than duplications in which the majority have been ascertained postnatally.
- Prenatal microdeletions with parental follow-up were all found to be *de novo*. Whereas, the prenatal duplication and triplication were found to be inherited and *de novo*.
- Origin studies show the prenatal microdeletions occur on both the paternal and maternal homolog suggesting that imprinting may not be the underlying mechanism of disease. However, the data does suggest that deletions do have a maternal preponderance.
- The prenatal microdeletions of the FOX cluster with or without the presence of the enhancer elements appear to result in a similar phenotype. Furthermore, the parent of origin does not seem to result in different phenotypes .
- Prenatal microdeletions involving the 16q24.1 including the FOX cluster appear to have a phenotype that includes cystic hygroma, heart defect, and pyelectasis, which is similar to previously reported cases.
- Postnatal duplications were found to be both inherited and *de novo*. In case 21, inheritance was from a clinically normal mother suggesting that the duplications of this region may have no clinical significance or variable expression.
- Postnatal duplications of 16q24.1 appear to be variable in size and with no trend in phenotype or age. However, the clinical data available was limited and further studies need to be done.

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