Published in final edited form as:

Am J Med Genet A. 2012 January ; 0(1): 103–108. doi:10.1002/ajmg.a.34375.

Duplication of the *ZIC2* gene is not associated with holoprosencephaly

Vaidehi Jobanputra¹, Alanna Burke², Anyane-Yeboah Kwame³, Anita Shanmugham², Maryam Shirazi¹, Stephen Brown⁴, Peter E. Warburton⁵, Brynn Levy¹, and Dorothy Warburton⁶

¹Department of Pathology, Columbia University Medical Center, New York, NY

²Division of Clinical Genetics, Children's Hospital of New York-Presbyterian, New York, NY

³Department of Pediatrics, Columbia University Medical Center, New York, NY

⁴Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT

⁵Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY

⁶Department of Genetics and Development, Columbia University Medical Center, New York, NY

Abstract

Cytogenetic testing using genomic microarrays presents a clinical challenge when data regarding the phenotypic consequences of the genomic alteration are not available. We describe a chromosome 13q32.3 duplication discovered by microarray testing in a fetus with a prenatally detected apparently balanced *de novo* translocation 46,XY,t(2;13)(q37;q32). Microarray analysis on the fetal DNA showed duplications of 384 kb and 564 kb at the breakpoint regions on chromosomes 2q37.3 and 13q32.3, respectively. There were no disease-associated genes in the duplicated region on chromosome 2q37. The duplicated region on chromosome 13q contains the *ZIC2* gene. Haploinsufficiency of *ZIC2* is known to cause holoprosencephaly and other brain malformations. Studies in the mouse models have suggested that over expression of *ZIC2* may also lead to brain malformations. Fetal MRI of the brain was normal and the family elected to continue the pregnancy. An apparently normal baby was born at term. At 3 months of age a physical exam showed no abnormalities and no developmental delay. This report shows that duplication of *ZIC2* is not necessarily associated with brain malformations. We also describe the phenotype from four additional patients with duplications of the region of chromosome 13 containing *ZIC2* and three previously described patients with supernumerary marker chromosomes derived from distal chromosome 13. None of the eight patients had holoprosencephaly or brain malformations, indicating that duplication of *ZIC2* is not associated with brain anomalies. This information will be useful for counseling in other occurrences of this duplication identified by microarray.

Keywords

Holoprosencephaly; *ZIC2*; chromosome 13q32; duplication; microarray; CNV; prenatal diagnosis

INTRODUCTION

Microarray analysis allows for genome-wide screening at a resolution not achievable by conventional cytogenetic techniques. The finding of a copy number variant (CNV) by microarray testing often creates a counseling dilemma because of lack of information about the clinical consequences of the genomic alteration. The identification of CNVs in genomic regions associated with recurrent microdeletion/duplications syndromes such as 7q11.2 and 22q11.2 and in the previously well defined chromosomally abnormal regions are interpreted as pathogenic. However many CNVs containing genes are particularly challenging to interpret because accurate clinical information concerning the consequences of dosage changes is lacking. These include rare CNVs that (i) have been described in very few affected individuals, (ii) are inherited from a parent who is not affected or with unknown phenotype or (iii) contain dosage sensitive gene(s) where disease association is known for the deletion (haploinsufficiency) but the consequences of duplication have not been described. Finding of these rare CNVs is more exigent in a prenatal setting because of the inability to predict phenotype and time pressure for the parents to make a decision whether to terminate or continue the pregnancy.

Here we report on a challenging situation of interpretation of CNVs identified by prenatal microarray testing in a fetus with an apparently balanced, *de novo* translocation 46,XY,t(2;13)(q37;q32) that includes duplication of the *ZIC2* gene. Loss of function of this dosage sensitive gene is associated with holoprosencephaly type 5 (HPE5, OMIM: 609637) and possibly other brain malformations seen in distal 13q deletion patients [Brown et al., 2001; Brown et al., 1998]. Because the phenotype associated with this gene duplication is unknown, we present genotype-phenotype data from this prenatal patient and additional postnatal patients with duplication of chromosome 13q32.2 region characterized with microarray analyses. None of the patients had holoprosencephaly or other brain malformations described in patients with complete trisomy of chromosome 13.

MATERIALS AND METHODS

Subjects

Prenatal and postnatal patients reported in this manuscript were recruited in our research study, approved by the Institutional Review Board of Columbia University Medical Center. Cases were recruited for the study when they had a cytogenetically visible chromosome abnormality. Cases 2, 3, 4, and 7 in Table I were recruited through the Chromosome Deletion Network (<http://www.chromodisorder.org>). Written informed consent was obtained from the patients and/or parents to participate in the study. DNA and chromosome preparations were obtained from peripheral blood from postnatal patients or residual cultured cells after prenatal diagnosis. Clinical information from enrolled study patients was reviewed by authors (AB and KY). DNA was prepared from lymphoblastoid or fibroblast cell lines from three previously published patients [Warburton et al., 2000] with a neocentromeric supernumerary marker containing a duplication of *ZIC2* (patients 5, 6, and 8 in Table I).

Microarray Analyses

SNP oligonucleotide microarray analysis was performed using Affymetrix GeneChip Human Mapping 6.0 SNP array. Sample preparation, hybridization, and scanning were performed according to manufacturer's specifications (Affymetrix, Santa Clara, CA). Analyses were performed using the Affymetrix Chromosome Analysis Suite 1.0 (ChAS) and BioDiscovery's Nexus Copy Number (5.1) software.

FISH confirmation

Chromosome preparations were made using standard methods. To confirm the duplication on chromosome 13 we selected one BAC (633J24) containing the *ZIC2* gene and to confirm the duplication on 2q37 we selected two BACs within the proximal (RP11-155J6 on 2q37.2q37.3; from base position 236879817 to 237043120) and distal regions (RP11-368H15 on 2q37.3; from base position 237121764 to 237291631) of the duplication. Probes were labeled and FISH was performed as described [Jobanputra et al., 2005].

RESULTS

Prenatal Case

The family was referred for counseling in our Genetics Clinic because an amniocentesis showed an apparently balanced, *de novo* translocation 46,XY,t(2;13)(q37;q32) in their fetus. (Fig 1). The woman, a 38-year-old G4P1, had an amniocentesis because of her age, as well as the finding of a two-vessel umbilical cord on ultrasound. Karyotype analysis from the amniotic fluid showed an apparently balanced translocation between chromosomes 2q and 13q. Parental chromosome studies were normal. The family was quoted a risk of approximately 6% [Warburton, 1991] for an abnormal outcome associated with a *de novo*, balanced translocation. The family elected to enroll in our research study, and, as a part of the study, microarray was performed on the fetal DNA and peripheral blood DNA from the parents. Prior to testing, the couple was counseled that microarray testing may not help to resolve questions about the translocation chromosome. A SNP-microarray analysis showed duplications of 384 kb and 564 kb at the breakpoint regions on chromosomes 2q37.3 and 13q32.3, respectively. The fetal karyotype was thus 46,XY,t(2;13)(q37;q32).arr2q37.2q37.3(236,910,048-237,294,055)x3,13q32.2q32.2(99,309,736-99,873,865)x3. Microarray analyses of the parents were interpreted as normal with no clinically significant CNVs. A map of the duplication interval on chromosomes 2 and 13 is shown in Figure 2. Table II lists the genes in the two duplication regions, with what is known of their function.

The FISH analysis confirmed the presence of duplicated material from both chromosomes 2 and 13. However, the location of the probes was unexpected and showed that the apparently balanced translocation was complex. At both breakpoints a copy of the duplicated segment was found on both derivative chromosomes, and the position of the probe suggested an inversion had occurred. It appeared that a duplication occurred first on both chromosomes, followed by an inversion that separated the two copies. A subsequent translocation moved part (chromosome 2) or all (chromosome 13) of the duplicated region to the other chromosome. This complex rearrangement is shown diagrammatically and by FISH in Figures 3 and 4.

There are no published human phenotype data on duplications of the *ZIC2* gene. The microarray results were conveyed to the patient, with an explanation of the fact that the pregnancy outcome could not be predicted. A fetal cranial MRI was normal and normal fetal anatomy was reported by sonogram. The family elected to continue the pregnancy and the baby was born at term by cesarean, birth weight 6 lbs 9oz, length-18.5 inches, APGAR scores of 9 and 9 at one and five minutes, respectively. At 3 months of age, the baby slept well, grabbed objects, and rolled over front to back. Head circumference was 40.5 cm (10th – 25th centile), length was 61.5 cm (~50th centile), and weight was 6.35 kg (~50th centile). There was mild micrognathia, but no overt dysmorphism. The baby was interactive, smiled and laughed, and was overall felt to be developing normally, although formal developmental testing was not performed.

Partial trisomy 13 patients ascertained postnatally

We performed microarray analyses in three previously described cell lines with distal chromosome 13-derived marker chromosomes [Warburton et al., 2000] to accurately characterize the chromosome abnormality at a higher resolution. Along with these previously described patients with a 13q supernumerary marker, we describe the phenotype from four additional postnatal patients with duplication of chromosome 13q characterized using microarray analyses (Table I and Fig 5). None of these patients were known to have gross brain malformations.

DISCUSSION

The duplicated region of chromosome 13 in the prenatal patient reported here is 384 kb and contains the *ZIC2* gene. Haploinsufficiency of this gene is associated with HPE and brain malformations. Rosenfeld et al. [2010] reviewed the phenotype associated with deletions of various HPE-related genes. The HPE phenotype was also described in patients with full trisomy 13, reported in 17–39% of patients [Lehman et al., 1995; Lin et al., 2007; Papp et al., 2006]. Using an *in vitro* assay, Brown et al. [2005] showed that the majority of HPE-associated *ZIC2* mutations resulted in loss of transcriptional activity; however, one mutation resulted in increased activity. This result suggested that HPE could potentially result from an increase in *ZIC2* activity.

Based on findings of this *in vitro* study and the characteristics of patients with full trisomy 13, there was a concern for the present prenatal patient that the fetus could have a brain malformation. We were unable to provide any definitive answer to the family about the possibility of brain anomalies in the fetus because of lack of published human phenotype data for the *ZIC2* gene duplication. Reassured by the finding of normal anatomy by sonography as well as MRI, the family elected to continue the pregnancy; a normal baby was born at term. There was no evidence of a brain anomaly at three months of age.

Partial duplications of the chromosome 13 are rare. A review of the phenotypes in eight patients with supernumerary inversion duplications of partial chromosome 13 [Warburton et al., 2000] and an additional three patients with neocentromere-containing inverted duplications of chromosome 13 [Li et al., 2002] found that none of the patients with the distal chromosome 13 duplications had holoprosencephaly or mid-brain anomalies. The supernumerary markers in these patients were characterized using G-banding and FISH and showed several specific clinical features seen in patients with full trisomy 13 (vascular malformations, seizures, coloboma, and microphthalmia) but no HPE.

The clinical descriptions provided by Warburton et al. [2000] and the present prenatal patient support the idea that holoprosencephaly and the other midbrain anomalies that are hallmarks of full trisomy 13 are not associated with the duplication of the distal chromosome 13. Genotype-phenotype data from the patients evaluated here provide strong evidence that simple duplication of *ZIC2* (and *ZIC5*) does not commonly cause brain malformation. *ZIC2* is dosage sensitive when haploinsufficient, but over-dosage is less detrimental. The present prenatal patient suggests that an extra copy of *ZIC2* may be benign. This information should be helpful for counseling for other families in which this duplication occurs.

Acknowledgments

We thank the families and members of the Chromosome Disorders Outreach (CDO) for participation in this study. VJ received support from a grant (KL2 RR024157) from the National Center for Research Resources. We also thank Ailene Muhlhauser and Chih-yu Yu for helping with DNA preparation and FISH.

References

- Brown L, Paraso M, Arkell R, Brown S. In vitro analysis of partial loss-of-function ZIC2 mutations in holoprosencephaly: alanine tract expansion modulates DNA binding and transactivation. *Hum Mol Genet.* 2005; 14:411–420. [PubMed: 15590697]
- Brown LY, Odent S, David V, Blayau M, Dubourg C, Apacik C, Delgado MA, Hall BD, Reynolds JF, Sommer A, Wieczorek D, Brown SA, Muenke M. Holoprosencephaly due to mutations in ZIC2: alanine tract expansion mutations may be caused by parental somatic recombination. *Hum Mol Genet.* 2001; 10:791–796. [PubMed: 11285244]
- Brown SA, Warburton D, Brown LY, Yu CY, Roeder ER, Stengel-Rutkowski S, Hennekam RC, Muenke M. Holoprosencephaly due to mutations in ZIC2, a homologue of Drosophila odd-paired. *Nat Genet.* 1998; 20:180–183. [PubMed: 9771712]
- Jobanputra V, Sebat J, Troge J, Chung W, Anyane-Yeboa K, Wigler M, Warburton D. Application of ROMA (representational oligonucleotide microarray analysis) to patients with cytogenetic rearrangements. *Genet Med.* 2005; 7:111–118. [PubMed: 15714078]
- Lehman CD, Nyberg DA, Winter TC 3rd, Kapur RP, Resta RG, Luthy DA. Trisomy 13 syndrome: prenatal US findings in a review of 33 cases. *Radiology.* 1995; 194:217–222. [PubMed: 7997556]
- Li S, Malafiej P, Levy B, Mahmood R, Field M, Hughes T, Lockhart LH, Wu Z, Huang M, Hirschhorn K, Velagaleti GV, Daniel A, Warburton PE. Chromosome 13q neocentromeres: molecular cytogenetic characterization of three additional cases and clinical spectrum. *Am J Med Genet.* 2002; 110:258–267. [PubMed: 12116235]
- Lin HY, Lin SP, Chen YJ, Hsu CH, Kao HA, Chen MR, Hung HY, Ho CS, Chang JH, Huang FY, Tsai TC, Lin DS, Chan WT. Clinical characteristics and survival of trisomy 13 in a medical center in Taiwan, 1985–2004. *Pediatr Int.* 2007; 49:380–386. [PubMed: 17532840]
- Papp C, Beke A, Ban Z, Szigeti Z, Toth-Pal E, Papp Z. Prenatal diagnosis of trisomy 13: analysis of 28 cases. *J Ultrasound Med.* 2006; 25:429–435. [PubMed: 16567430]
- Rosenfeld JA, Blake BC, Martin DM, Aylsworth AS, Bejjani BA, Torchia BS, Shaffer LG. Clinical characterization of individuals with deletions of genes in holoprosencephaly pathways by aCGH refines the phenotypic spectrum of HPE. *Hum Genet.* 2010; 127:421–440. [PubMed: 20066439]
- Warburton D. De novo balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: clinical significance and distribution of breakpoints. *Am J Hum Genet.* 1991; 49:995–1013. [PubMed: 1928105]
- Warburton PE, Dolled M, Mahmood R, Alonso A, Li S, Naritomi K, Tohma T, Nagai T, Hasegawa T, Ohashi H, Govaerts LC, Eussen BH, Van Hemel JO, Lozzio C, Schwartz S, Dowhanick-Morrisette JJ, Spinner NB, Rivera H, Crolla JA, Yu C, Warburton D. Molecular cytogenetic analysis of eight inversion duplications of human chromosome 13q that each contain a neocentromere. *Am J Hum Genet.* 2000; 66:1794–1806. [PubMed: 10777715]

Figure 1.
Partial fetal karyotype showing t(2;13)(q37;q32).

Figure 2.

UCSC Genome browser screen shot showing the duplications at the breakpoint regions on chromosome 2 and 13. (a): Chromosome 2: 384 Kb region, base positions 236,910,048 – 237,294,055. (b): Chromosome 13: 564 Kb region, base positions 99,309,736–99,873,865.

Figure 3.

Tandem duplication of a 564 kb segment containing *ZIC2* in 13q32, followed by inversion, which separated the duplicated segments and moved *ZIC2* distally on 13q. As seen by FISH with BAC 633J24 (red), translocation of one copy to 2q left one copy distally on the der(13) and one copy on distal 2q.

Figure 4.

Tandem duplication of a 384 kb segment containing a proximal BAC RP11-155J6 (red) and a more distal BAC RP11-368H15 (green) in 2q27, followed by inversion which separated the copies and inverted the red-green sequence. Translocation moved the distal region containing two copies of BAC RP11-155J6 and one copy of BAC RP11-368H15 to distal 13q. One copy of BAC RP11-368H15 remains at 2q27, which is just proximal to the translocation breakpoint.

Figure 5. Duplications of chromosome 13. Each black line represents chromosome 13 for a patient. The numbers correspond to the patient number in Table I. Duplicated regions are shown in blue and the deleted region in red. Patient 3 had a non-continuous duplication.

Table 1

Genomic microarray analysis in Duplication 13 patients

Patient No.	Patient Recruitment	Chromosome Abnormality	Phenotype	CNS findings	Chromosome Bands	Copy No. Change
1	CUMC	46,XY,t(2;13)(q37;q32) <i>de novo</i>	Prenatal diagnosis because of advanced maternal age and a two vessel-cord on level II sonogram, baby normal at birth and 3 months	Fetal MRI: Normal	2q37.2-q37.3 13q32.2-q32.2	384 kb Gain 564 kb Gain
2 ^A	CDO	46,XX,dup(13)(q32-q34)	Unilateral Postaxial polydactyly, ASD/VSD, macrocephaly, dilatation of the renal pelvis	MRI: Poorly developed white matter, temporal lobe hypoplasia	13q31.2q33.2	18.7 Mb Gain
3	CDO	46,XY,add(13)(q34)	Developmental delay	MRI: Anterior temporal arachnoid cyst	13q22.1q33.1	Non continuous gain, total of 27.09 Mb Gain
4	Warburton et al. [2000]	47,XY,-inv dup(13)(q21qter)	Hypotonia, cleft lip, postaxial polydactyly, ASD, VSD, PDA, died at 13 days of age	CT scan: Within normal limits	13q21.33-q34	45.90 Mb Gain (four copies)
5	CDO	47,XXY,der(2)t(2;13)(p25;q22)mat	Coloboma, hearing loss, bilateral club foot, seizures	MRI: Mid brain hypoplasia, no focal mass or gliosis; slight narrowing of foramen magnum	2p25.3-p25.3 13q22.1-q34	909 kb Loss 41.92 Mb Gain
6	Warburton et al. [2000]	47,XX,-neo(13)(qter-r-q21:)	Developmental delay, bilateral postaxial polydactyly, microphthalmia	N/A	13q21.2-q34	61.57 Mb Gain (four copies)
7	CDO	46,XX,-13,+t(13;13)(p13;q21.1)	50 yrs old, frontal bossing, cataract, limited speech, profound mental retardation	MRI: Moderate periventricular and subcortical white matter abnormality. Mild enlargement of ventricle communication without ventriculomegaly	13q14.3-q34	63.5 Mb Gain
8	Warburton et al. [2000]	47,XY,-neo(13)(qter-r-q14:)	Left plagiocephaly, bilateral retinoblastoma, hypospadias	CT scan: Abnormal brain density	13q13.2-q14.2 13q14.2-q34	13.63 Mb Loss 64.78 Mb Gain

CUMC – Columbia University medical Center, CDO – Chromosome Disorder Outreach family support group. N/A – Not Available

CNS - Central Nervous System, MRI – Magnetic Resonance Imaging, ASD – Atrial Septal Defect, VSD – Ventricular Septal Defect, CT – Computed Tomography, PDA – Patent Ductus Arteriosus.

^A Microarray on this patient was performed by a clinical laboratory offering diagnostic microarray test.

Table II

Genes in the duplicated regions at the translocation breakpoints [UCSC 2006 (NCBI36/hg18) Assembly]

Chromosome Region	Gene	Description	Function
2q37.2q37.3	<i>IQCA1</i>	IQ motif containing with AAA domain 1	Function not known
2q37.3	<i>CXCR7</i>	Chemokine orphan receptor 1	Involved in cell growth, survival, adhesion and tumor growth
13q32.3	<i>CLYBL</i>	Citrate lyase beta like	Function not known
13q32.3	<i>ZIC2</i>	Zinc finger protein of the cerebellum 2	Developmental transcription factor
13q32.3	<i>ZIC5</i>	Zinc finger protein of the cerebellum 5	Encodes a member of the ZIC family of C2H2-type zinc finger proteins, closely linked to ZIC2
13q32.3	<i>PCCA</i>	Propionyl-Coenzyme A carboxylase, alpha	Amino acid metabolism