

SHORT REPORT

5q11.2 deletion in a patient with tracheal agenesis

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Tracheal agenesis (TA) is a rare congenital anomaly of the respiratory tract. Many patients have associated anomalies, suggesting a syndromal phenotype. In a cohort of 12 patients, we aimed to detect copy number variations. In addition to routine cytogenetic analysis, we applied oligonucleotide array comparative genomic hybridization. Our patient cohort showed various copy number variations, of which many were parentally inherited variants. One patient had, in addition to an inherited 16p12.1 deletion, a 3.6 Mb deletion on chromosomal locus 5q11.2. This patient had a syndromic phenotype, including vertebral, anal, cardiovascular and tracheo-oesophageal associated anomalies, and other foregut-related anomalies, such as cartilage rings in the oesophagus and an aberrant right bronchus. No common deletions or duplications are found in our cohort, suggesting that TA is a genetically heterogeneous disorder.

European Journal of Human Genetics (2010) 18, 1265–1268; doi:10.1038/ejhg.2010.84; published online 16 June 2010

Keywords: tracheal agenesis; array comparative genomic hybridization; 5q11; deletion; VACTERL; TACRD

INTRODUCTION

Tracheal agenesis (TA) is a rare congenital anomaly in which the trachea is fully or partially absent. Ventilatory support may be successful occasionally, but TA typically has fatal consequences. Efforts have been directed at clarifying the anatomy of TA and at improving treatment.^{1,2} TA may be part of a complex of malformations and it is often associated with cardiovascular anomalies.^{3,4} The complex phenotypes show an overlap with the association of vertebral, anal, cardiovascular, tracheo-oesophageal, renal and limb (VACTERL) anomalies,^{5,6} or with an association including TA, cardiac anomalies, radial ray defects and duodenal atresia.^{6,7} TA can also be a part of Fraser syndrome, an autosomal recessive malformation disorder characterized by cryptophthalmos, syndactyly and abnormalities of the respiratory and urogenital tract, which is caused by mutations in *FRAS1* and *FREM2* genes.^{8,9}

Little is known about the aetiology of TA, apart from the fact that environmental factors might have a role.³ Animal models of different genetic defects, such as (conditional) inactivation of *Gli2*, *Gli3*, *Shh*, *Foxf1* and β -*catenin*, show agenesis of the trachea or incorrect septation of the foregut.^{10–14} To date, however, no mutations or chromosomal anomalies of any of the genes or pathways implicated in these animal models have been described in human patients with TA. In addition, there are no published reports on systematic screening of TA patients for copy number variations. To determine whether microdeletions or duplications could explain the TA phenotype, we conducted genome-wide assays in 12 TA patients.

MATERIALS AND METHODS

Cases

Ten cases were retrieved from the medical records of the Erasmus MC–Sophia Children's Hospital, Rotterdam, from 1988 onwards. Two cases had been seen

and included at the department of Pathology, University Medical Centre Utrecht. The medical records and pathology records were reviewed. Fibroblast cell lines and paraffin blocks had been stored.

Cytogenetic analysis

Karyotyping was performed according to standard analysis methods. DNA for genomic analysis was extracted from fibroblast cells by the Puregene DNA purification kit (Gentra Systems Inc., Minneapolis, MN, USA). Regarding six patients, only formalin-fixed paraffin-embedded (FFPE) samples from thymus tissue were available. DNA was extracted according to protocols provided by the supplier of microarrays.

Array comparative genomic hybridization (Array CGH)

Array CGH was performed using the 105 K oligonucleotide-based array CGH (Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's protocol. For analysis and visualization, Feature Extraction software (version 9.1; Agilent Technologies, Santa Clara, CA, USA) and CGH analytics software (version 3.3.28) were used. Aberrations were detected using the ADM2 algorithm and filtering options of a minimum of three probes and $\text{abs}(\log_2 \text{ratio}) > 0.3$. Aberration segments were manually reviewed.

Fluorescence *In Situ* hybridization analysis

To confirm results, BAC clones were selected from the University of Santa Cruz genome browser and ordered from BACPAC Resources. After isolation of BAC DNA, probes were labelled and used for FISH on chromosome preparations from patients and parents, according to standard protocols.¹⁵

Real-time quantitative PCR

Quantification was carried out by real-time quantitative PCR. Primer pairs were designed with Primer Express V2.0 (Applied Biosystems, Branchburg, NJ, USA) and tested for validity. DNA was processed using IQ SYBR green master mix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and analysed on the AB7300 (Applied Biosystems).

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Received 2 March 2010; revised 26 April 2010; accepted 28 April 2010; published online 16 June 2010

Prioritization

We used the Endeavour prioritization program¹⁶ to prioritize genes in the 5q11.2 chromosomal region. Reference genes were selected on the basis of recently reviewed genes on foregut-related anomalies in human and murine models: *SHH*, *GLI2*, *GLI3*, *FOXF1*, *TTF1*, *NOG*, *FGF2*, *HOXA5*, *RAR α* , *RAR β* , *BMP4*, *FOXF1*, *SOX2*, *FRAS1* and *FREM2*. In relation to cell cycle control and cell death processes, we performed an additional prioritization with selected reference genes: cell-division cycle two-like genes, proteinphosphatases, cyclin-dependent kinases, interleukins, apolipoprotein B, P53, *TNF* genes, *PDCD* genes and *CIDE* genes. The program is based on the hypothesis that novel candidate genes have roles similar to those of the genes known to be associated with the anomaly, or share biological processes with these genes.

RESULTS

Clinical data are summarized in Table 1. Conventional G-banding karyotyping was apparently normal in all patients. The quality of the array was poor in three patients from whom only FFPE material was available. The array CGH analysis revealed copy number variations in all patients. Most of these were known polymorphisms, described in

the Toronto Database of Genomic variants (DGV), the Copy Number Variation project at the Children's Hospital of Philadelphia (CHOP) or seen in our in-house control cohort.^{17,18} In two patients (nos. 3 and 10) these were deletions of loci not known as polymorphic variants: a 57.9 kb deletion on chromosome 15q25.1, containing exons 12–21 of the *BLM* gene, and a 488 kb deletion on chromosome 16p12.1, respectively. The deletions were confirmed with FISH and real-time quantitative PCR. However, they were also found in their unaffected mothers, hence we concluded that they were likely not causal.

We found an additional 3.6 Mb deletion on chromosomal locus 5q11.2 in patient 10 (Figure 1a), who presented with TA, anal atresia, persisting hemiazygos, cryptorchid testes, fork rib and mild dysmorphic features, including low-set posterior rotated ears, mild hypertelorism and a unilateral simian crease. The pregnancy was achieved after *in vitro* fertilization with an unknown sperm donor, and had been complicated by polyhydramnios. In addition, absence of a stomach bubble was seen on ultrasound examination. Prenatal cytogenetic analysis was normal. As labour started prematurely, antenatal corticosteroids were given twice before delivery to induce lung

Table 1 Patient characteristics and associated anomalies in the TA cohort

Case no.	Gender	Type TA	Gestational characteristics	Associated anomalies											
				V	A	C	TE	R	L	U	LA	D	Other		
1 ^a	F	Floyd III	Polyhydramnios	–	–	–	–	–	–	–	–	–	+	–	–
2	M	Floyd III	Prolonged ROM Maternal fever Anhydramnios Breech presentation	+	–	+	–	+	–	–	+	+	+	+	Galbladder agenesis Meckel's diverticulum
3	F	Floyd II with TEF	Polyhydramnios Breech presentation Cesarean section	–	–	+	+	–	–	–	–	+	+	+	Thymus atrophy Extra spleen Hypertelorism Dysmorphic right ear Single umbilical artery
4	M	Floyd III	Polyhydramnios	–	+	+	–	–	–	–	–	–	+	–	Hypertelorism Immature brain
5	M	Floyd II	Prenatal diagnosis of multiple anomalies IUGR; induction of labour	–	–	–	–	–	–	+	+	+	+	–	Low-set ears Plagiocephaly Ventriculomegaly and asymmetric skull Clubfoot Undescended testis
6 ^a	F	Floyd I with TEF	Polyhydramnios Breech Presentation Second of twins, other child healthy	–	–	–	+	–	–	+	–	–	–	–	Small left ear
7 ^a	M	Floyd I	IUGR	–	–	+	–	–	–	+	+	–	–	+	–
8	M	Floyd II with two TEF	–	–	+	+	+	–	–	–	+	–	–	–	–
9	M	Floyd I with TEF	Polyhydramnios Vacuum extraction	–	+	+	+	+	–	–	–	–	+	–	Heterotaxy Hypoplastic lungs
10	M	Floyd III	Polyhydramnios Induction of labour IVF pregnancy	+	+	+	–	–	–	–	–	–	–	–	Aberrant right bronchus
11	F	Floyd I	Termination of pregnancy	–	–	–	–	–	–	+	–	–	–	–	Low-set ears Hypertelorism Hyperplastic lungs
12	M	Floyd II	Polyhydramnios	–	–	–	–	+	–	–	–	–	–	–	Single umbilical artery

Abbreviations: A, anal atresia; C, cardiovascular malformations; D, duodenal atresia; F, female; IUGR, intra-uterine growth retardation defined as weigh for gestational age $\leq 2SD$; L, limb abnormalities including radial ray defects; LA, laryngeal defects; M, male; R, renal anomaly; ROM, rupture of membranes; TA, tracheal agenesis; TE, oesophageal atresia and/or tracheo-oesophageal fistula; TEF, tracheo-oesophageal fistula; U, urogenital anomaly; V, vertebral and/or rib abnormalities; +, present; –, absent.

^aArray inconclusive in paraffin-embedded tissue sample.

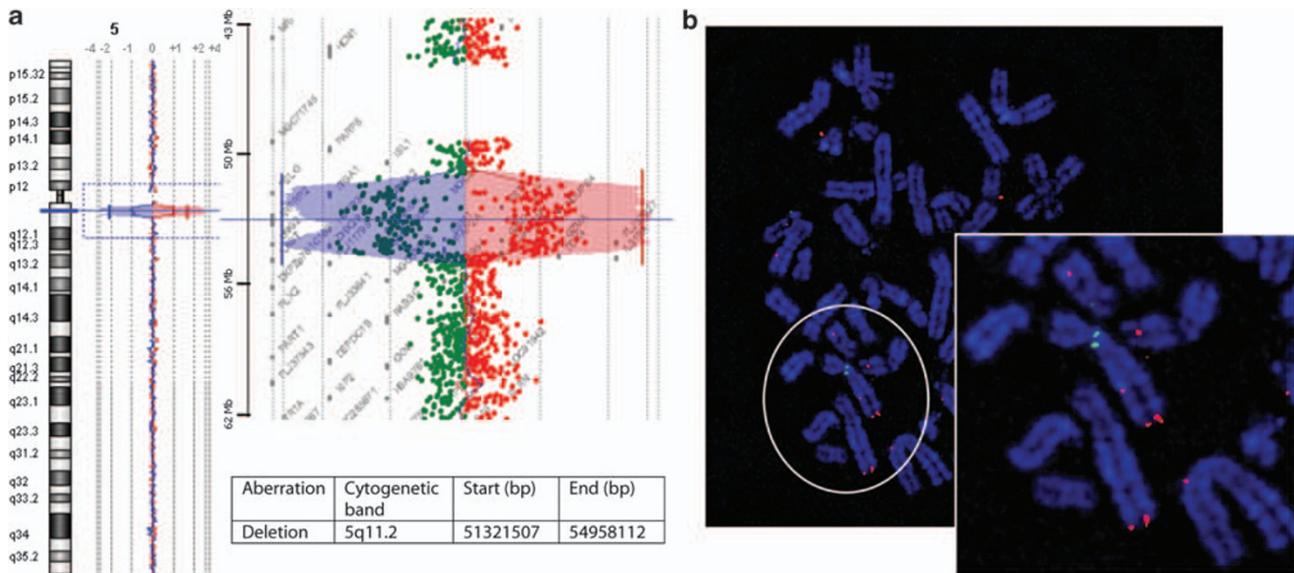


Figure 1 Visualization and confirmation of deletion 5q11.2. (a) 5q11.2 deletion visualized with Agilent CGH analytics software (version 3.3.28); deviation from 0 on a log₂ scale, experiment performed in duplicate. The maximum deleted region ranged from 51 079 864 to 55 001 348 Mb (NCBI Build 36.1). The minimum deleted region ranged from 51 321 507 to 54 958 112 Mb. (b) FISH to a metaphase spread, showing the absence of a green fluorescent signal (BAC RP11-160F8) at 5q11.2 on one chromosome 5. The red signal (BAC RP11-11K19; control probe) is present on both chromosomes on the 5q subtelomeric regions.

development. The male baby was spontaneously delivered at 29 $\frac{3}{7}$ weeks. Body weight was 1090 g (30th centile), length 38 cm (50th centile) and occipitofrontal circumference 28 cm (40th centile). Cyanosis, severe respiratory distress and the absence of an audible cry were noted immediately after birth. Intubation failed after multiple attempts. Apgar scores were 3, 0 and 0 after 1, 5 and 10 min, respectively. Autopsy confirmed multiple congenital anomalies, including TA, which was classified as Floyd III.¹⁹ In addition, cartilage rings in the oesophagus and an aberrant right bronchus were reported.

The deletion, ranging from 51.32 to 55.00 Mb (NCBI Build 36.1), was confirmed by FISH analysis (Figure 1b). Karyotyping and FISH analysis on lymphocytes of the mother were normal. Paternal material was not available because pregnancy has been achieved after *in vitro* fertilization; the sperm donor was unknown. Neither DGV, CHOP nor our control cohort describes this large deletion. This chromosomal region contains 12 protein-encoding genes.

DISCUSSION

As far as we know, one other patient with a deletion in the 5q11.2 region is described in literature.²⁰ This patient showed Tetralogy of Fallot, a bifid uvula and significant developmental delay. The 5 Mb deletion found in this patient includes the 3.6 Mb region found in our patient. Another case of overlapping deletion was retrieved from a search in two public online databases (<http://www.ecaruca.net/>;²¹ <https://decipher.sanger.ac.uk/>²²). The Decipher database reports a patient with a large deletion del(5)(q11.2;q13.2), with a 2 Mb overlap with our patient. This patient had significant developmental delay and hypochondroplasia. To the best of our knowledge, this deletion has not yet been described as a common variant. Owing to lack of paternal material, we could not verify the inheritance of the deletion, but we expect this deletion to be *de novo*.

We used the Endeavour prioritization program¹⁶ to prioritize genes in the 5q11.2 chromosomal region, the haploinsufficiency of which may have influenced the congenital TA phenotype in

our patient. Integrin α -1 (*ITGA1*) was ranked the highest priority, followed by follistatin (*FST*), endothelial cell-specific molecule-1 (*ESM1*) and integrin α -2 (*ITGA2*). Integrin α -1 is crucial in regulating mesenchymal stem cell proliferation and cartilage production.²³ *FST* is an activin-binding protein. *FST*-deficient mice are growth retarded, have decreased intercostal muscles mass, skeletal defects, and abnormal tooth development.²⁴ The protein also modulates the actions of several members of the TGF- β family, e.g., it inhibits *BMP-4*. Li *et al.*,²⁵ confirm that Bmp signalling is present in the anterior foregut, the site of origin of the tracheal primordium, and that targeted knockouts for *Bmp-4* at this site have no trachea development. Que *et al.*,²⁶ demonstrated that certain proteins that antagonize these BMPs, such as *Noggin* transcriptional processes, could explain the degree of tracheal development, and therefore could discriminate between subtypes of TA.

The variable phenotypes in patients described with 5q11.2 deletions can be potentially explained by different causes: environmental factors, incomplete penetrance, other genetic mutations elsewhere in the genome, compensation of other genes and/or epigenetic factors. This patient, in addition to the 5q11.2 deletion, also had an inherited 16p12.1 deletion that could possibly contribute to the phenotype. This deletion was recently described by Girirajan *et al.*²⁷ In addition to large *de novo* deletions, they identified a recurrent 520 kb microdeletion of 16p12.1, associated with developmental delay. They proposed a two-hit model: the inherited 16p12.1 deletion in addition to a large copy number variation indicates the variable expressivity of the developmental delay phenotype.²⁷ We were unable to ascertain the developmental status of the patient as he died. However, as the mother is of normal intelligence and carries the 16p12.1 deletion, the patient could fit the two-hit model; the additional large copy number variation could indicate the variable expressivity in our patient.

The presence of inherited and polymorphic variations in our TA cohort suggests that TA is genetically heterogeneous. The rare 5q11 deletion found in one of our patients includes several candidate genes.

However, the clinical significance of this large chromosomal aberration is hard to pinpoint, because of the variability in clinical presentation. We recommend standard detailed molecular cytogenetic analysis in patients with TA as a means to further unravel the genetic background of TA.

In conclusion, our findings support the hypothesis that TA is a disorder with a heterogeneous phenotype and genotype.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

E M de J was funded by the Sophia Foundations for Scientific Research, project No. 493. We thank J. Hagoort for editorial support.

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