

## ORIGINAL ARTICLE

# Pathogenic copy number variants are detected in a subset of patients with gastrointestinal malformations

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## Abstract

**Background:** Gastrointestinal atresias and urological defects are main causes of pediatric surgery in infants. As copy number variants (CNVs) have been shown to be involved in the development of congenital malformations, the aim of our study was to investigate the presence of CNVs in patients with gastrointestinal and urological malformations as well as the possibility of tissue-specific mosaicism for CNVs in the cohort.

**Methods:** We have collected tissue and/or blood samples from 25 patients with anorectal malformations, esophageal atresia, or hydronephrosis, and screened for pathogenic CNVs using array comparative genomic hybridization (array-CGH).

**Results:** We detected pathogenic aberrations in 2/25 patients (8%) and report a novel possible susceptibility region for esophageal atresia on 15q26.3. CNV analysis in different tissues from the same patients did not reveal evidence of tissue-specific mosaicism.

**Conclusion:** Our study shows that it is important to perform clinical genetic investigations, including CNV analysis, in patients with congenital gastrointestinal malformations since this leads to improved information to families as well as an increased understanding of the pathogenesis.

## KEYWORDS

anorectal malformations, array-CGH, copy number variation, esophageal atresia, hydronephrosis, mosaicism

## 1 | INTRODUCTION

Congenital malformations, such as gastrointestinal atresia and urological defects, are main causes of pediatric surgery in infants. Improvements in surgical procedures and technical advances in pediatric intensive care have increased survival rates and lowered morbidity for individuals born with congenital malformations, enabling a higher degree of reproduction in this

group. Identification of underlying causes and the possibility to perform accurate recurrence risk evaluations and offer prenatal diagnostics are accordingly becoming more important. Most congenital malformations are considered to be of multifactorial origin, and it is often difficult to determine the causative factor/factors in clinical investigations. Known genetic factors behind the development of malformations include chromosomal aberrations, microdeletions and -duplications and single gene

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defects. Copy number variation represents a relatively recently discovered level of genetic variation and around 5%–12% of the human genome has been shown to be covered by copy number variants (CNVs) (McCarroll et al., 2008; Redon et al., 2006). CNVs have traditionally been defined as segments of DNA that are at least 1,000 base pairs in size and differ in copy number when compared to a relevant reference genome (Feuk, Carson, & Scherer, 2006). According to the new definition from 2014, they can be as small as 50 bp (MacDonald, Ziman, Yuen, Feuk, & Scherer, 2014). Depending on their location in the genome and copy number status (gain/loss), CNVs can influence gene expression and thereby phenotypic variation, including development of disease. Factors that increase the likelihood of a detected CNV to be the cause of the patient phenotype include overlap with a known syndrome region, known disease-causing genes within the region, size of the region, high gene content, rare frequency in the general population, and de novo inheritance pattern (Kearney, 2011). The vast majority of CNVs are however considered to be benign normal variants that are found in healthy individuals. Since the introduction of chromosomal microarray analysis, using oligo or SNP-based technology, screening for CNVs has become widely used in clinical genetic investigations of patients with developmental delay, autism, and congenital malformations.

Pathogenic copy number variants have been identified in studies of various congenital malformations (Flottmann et al., 2018; Kruszka et al., 2017; Li et al., 2019; Salehi Karlslett et al., 2019; Zhu et al., 2018). Rare inherited and de novo CNVs have been implicated in patients with esophageal atresia (EA) (Brosens et al., 2016; Ferreira de Almeida & Bertola, 2013; Hilger et al., 2013; van Binsbergen et al., 2014). In patients with anorectal malformations (ARM), rare CNVs have been reported to be enriched in syndromic and non-syndromic patients compared to controls (Wong et al., 2013). Probable disease-causing rare CNVs were also present in 15% of patients with anorectal in combination with central nervous system malformations (Dworschak et al., 2015). In patients with congenital abnormalities of the kidneys or urinary tract (CAKUT), microarray studies have revealed rare CNVs in 10%–14% of patients (Caruana et al., 2015; Westland et al., 2015).

The advances in analysis techniques, with increasing resolution as the result, have enabled detection of increasingly lower levels of genetically aberrant cells. A concept emerging as more common than previously believed in patients with congenital disease is genetic mosaicism, referring to the presence of distinct cell lines differing in genetic content within an individual due to a genetic event after fertilization (Biesecker & Spinner, 2013; Campbell et al., 2014). While detection of mosaic forms of chromosomal disorders such as Down syndrome has long been feasible through chromosome analysis and fluorescent in situ hybridization (FISH), more recently developed techniques with high resolution and sensitivity such as array comparative genomic hybridization

(array-CGH), digital PCR, and massive parallel sequencing enable the detection of CNVs and single gene mutations in low-grade mosaic form (Biesecker & Spinner, 2013; Piotrowski et al., 2008).

In the present study, we systematically studied pathogenic CNVs in DNA isolated from the malformed tissue in patients with congenital gastrointestinal or urological malformations. In a subgroup of patients, we also compared the presence of CNVs in genomic DNA from tissue and blood from the same individuals to look for somatic copy number mosaicism in the malformed tissue specifically.

## 2 | MATERIALS AND METHODS

### 2.1 | Editorial policies and ethical considerations

Ethical approval for the study was obtained from the regional ethics committee at Karolinska Institutet, Stockholm, Sweden.

### 2.2 | Patients

Children with congenital malformations undergoing surgery at the department of Pediatric Surgery at Astrid Lindgren Children's Hospital, Stockholm, were recruited to the study after fulfilling three criteria; (a) informed consent was given from the parents (b) tissue from the malformation was discarded as part of the surgical procedure (c) the discarded tissue was collected and stored by the operating surgeon. The collected tissue originated from the malformation itself; in the case of EA, tissue was taken from the esophageal fistula, in the case of ARM the tissue was taken from the atretic and discarded intestine and in the case of hydronephrosis the discarded tissue originated from the renal pelvis. After surgical removal, the tissue was directly placed in sodium chloride solution (9 g/L) and in addition, blood samples from the patients were collected when possible. For two patients (P4 and P25), tissue was not available while blood samples were obtained for analysis. In total, samples from 25 patients were collected and analyzed.

### 2.3 | DNA extraction

To isolate genomic DNA from patient tissue, the Gentra Puregene Blood kit (QIAGEN) was used in combination with Proteinase K (Finnzymes) with minor modifications to the manufacturer's recommendations. Genomic DNA was isolated from peripheral blood from patients and parents according to the standard procedures.

## 2.4 | Array-CGH

Array-CGH was performed using a 180K whole genome coverage oligonucleotide array from Oxford Gene Technology as described previously (Winberg et al., 2013). In initial analyses, genomic tissue-derived DNA was hybridized against sex-matched, pooled reference DNA derived from peripheral blood of 10 healthy controls. DNA labeling, hybridization, scanning, and data analysis was performed principally according to the manufacturer's recommendations as reported (Winberg et al., 2014). In the data analysis of pathogenic CNVs, we used previously applied criteria of three consecutive probes with deviating log<sub>2</sub>-ratios, using two different log<sub>2</sub>-ratio cutoff levels for germline (>0.35 for duplications and <-0.65 for deletions) and somatic (>0.10 for duplications and <-0.10 for deletions) CNVs, allowing detection of a mosaicism level of approximately 15%. For detection of mosaic CNVs, a size limit of 5 Mb was used, due to a high false positive rate for small aberrations. We also chose to visually inspect every chromosome for mosaic aberrations smaller than 5 Mb. Evaluation and classification of identified CNVs was performed manually as previously reported by us, including comparison to gene dose alterations reported in public databases and the clinical database at the Department of Clinical Genetics, Karolinska University Hospital, comprising more than 8,100 patient samples analyzed by array-CGH (Winberg et al., 2015). Pathogenicity classification was performed following published guidelines for remaining CNVs (Kearney et al., 2011).

Array-CGH was performed using DNA isolated from malformed tissue in all patients except patients P4 and P25, from whom only blood samples were available. In addition, to investigate the occurrence of somatic mosaicism, array-CGH analysis was performed using DNA isolated from peripheral blood from the seven patients from whom both tissue and blood samples were available (P1, P6, P9, P10, P11, P15, and P21). Detected CNVs not classified as common benign variants were compared between results from tissue and blood for each patient. For patient P24, mosaicism was investigated using FISH and chromosome analysis as described under Cytogenetic studies.

Parental sample analysis was performed by hybridizing Cy3-labeled maternal samples against Cy5-labeled paternal samples.

## 2.5 | Cytogenetic studies

Chromosome analysis and fluorescent in situ hybridization (FISH) analysis for patient P24 and her parents was performed as described previously (Winberg et al., 2010). Metaphase spreads and interphase nuclei were prepared from peripheral blood. For FISH analysis two commercially available probe sets; LSI SNRPN/CEP15/PML (Vysis/Abbott) and subtelomere 15qter (blue) (Kreatech) as well as an inhouse

α-satellite (D15Z) probe (15c pTRA) and one BAC chromosome 15q26.3-specific probe (RP11-66B24) were used.

## 2.6 | Methylation-specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA)

DNA from patient P24 was analyzed with methylation-specific MLPA using the SALSA MS-MLPA probemix ME028 Prader-Willi/Angelman kit from MRC-Holland, including five methylation-specific probes in the *SNRPN* and *NDN* loci to determine the methylation pattern in the Prader-Willi/Angelman syndrome region. The reactions were carried out according to the recommendations by the manufacturer and analyzed on an ABI 3500×1 genetic analyzer (Applied Biosystems) followed by software analysis using the GeneMapper software (Applied Biosystems).

## 2.7 | Microsatellite marker analysis

In order to determine the parental origin of the extra chromosome 15 material in patient P24, DNA isolated from peripheral blood from the patient, mother and father was analyzed using polymorphic short tandem repeat markers (STRs) located on chromosome 15. The polymorphic markers were amplified in a PCR-reaction using fluorescent primers from panels 21 and 22 of the ABI PRISM<sup>®</sup> Linkage Mapping Sets v 2.5 for chromosome 15 and 16 (Applied Biosystems). Amplified products were separated on an ABI 3500×1 genetic analyzer (Applied Biosystems) and analyzed with the GeneMapper software (Applied Biosystems).

# 3 | RESULTS

## 3.1 | Patients

The clinical features of included patients are summarized in Table 1. In total five patients with esophageal atresia (EA), 14 patients with anorectal malformations (ARM) and six patients with hydronephrosis (HN) were analyzed using array-CGH. In total, 12 of 25 patients (48%) had isolated malformations (Table 1).

## 3.2 | Array-CGH, cytogenetic studies, and MS-MLPA

In the patients with EA, results from array-CGH screening for gene dose alterations revealed one clearly pathogenic aberration

**TABLE 1** Clinical features and detected CNVs of pathogenic or uncertain clinical significance using array-CGH in patients with gastrointestinal and urological malformations

Patients	Sex	Malformations	Detected variant	CNV size and affected genes <sup>a</sup>	Analyzed tissues	Classification
P1	M	EA, vertebral defects, three small VSDs	–	–	Esophageal tissue, blood	–
P4	F	EA, omphalocele	arr[hg19]15q26.3q26.3(100168075_100632516)x3	CNV1 (0.5 Mb): <i>MEF2A</i> , <i>LYSMD4</i> , <i>ADAMTS17</i>	Blood	VUS
P6	F	EA, ASD, VSD, persisting left v. cava sup.	–	–	Esophageal tissue, blood	–
P24	F	EA, VSD	arr[hg19]15q11.2q11.2(22676913_23864240)x3, 15q11.2q12(23685952_27460180)x4, 15q12q13.1(27462869_30137106)x3, 15q26.3q26.3(100923767_101626187)x1 dn	CNV1 (1.0 Mb): <i>GOLGA6L1</i> , <i>TUBGCP5</i> , <i>ZYFIP1</i> , <i>NIPA2</i> , <i>NIPA1</i> , <i>LOC283683</i> , <i>WHAMMP3</i> , <i>HERC2P2</i> , <i>LOC102723534</i> , <i>HERC2P7</i> , <i>GOLGA8EP</i> , <i>GOLGA8S</i> , <i>GOLGA6L2</i> CNV2 (3.7 Mb): <i>MKRN3</i> , <i>MAGEL2</i> , <i>NDN</i> , <i>PWRN4</i> , <i>PWRN1</i> , <i>NPAP1</i> , <i>SNURF</i> , <i>SNRPN</i> , <i>PWARSN</i> , <i>SNORD107</i> , <i>PWARS5</i> , <i>SNORD64</i> , <i>SNORD108</i> , <i>SNORD109A</i> , <i>SNORD109B</i> , <i>SNORD116–19</i> , <i>IPW</i> , <i>PWARI</i> , <i>SNORD115–1</i> , <i>PWAR4</i> , <i>UBE3A</i> , <i>ATP10A</i> , <i>LINC00929</i> , <i>GABRB3</i> , <i>GABRA5</i> , <i>GABRG3</i> CNV3 (2.7 Mb): <i>GABRG3</i> , <i>GABRG3-AS1</i> , <i>OCA2</i> , <i>HERC2</i> , <i>GOLGA8G</i> , <i>GOLGA8F</i> , <i>GOLGA8M</i> , <i>LOC100289656</i> , <i>GOLGA6L7P</i> , <i>APBA2</i> , <i>FAM189A1</i> , <i>NDNL2</i> , <i>TJPI</i> CNV4 (0.7 Mb): <i>CERS3-AS1</i> , <i>CERS3</i> , <i>PRKXP1</i> , <i>LINS1</i> , <i>ASB7</i> , <i>ALDH1A3</i> , <i>LRRK1</i>	Esophageal tissue, blood <sup>b</sup>	CNV1–3:P CNV4: VUS
P25	F	EA, duodenal atresia, pelvic kidney and aortic arch anomalies	arr[hg19]15q26.3q26.3(100179453_100623895)x4	CNV1 (0.5 Mb): <i>MEF2A</i> , <i>LYSMD4</i> , <i>ADAMTS17</i>	Blood	VUS
P2	M	ARM <sup>c</sup>	–	–	Anal tissue	–
P3	F	ARM, caudal regression syndrome, unilateral renal agenesis	–	–	Anal tissue	–
P5	M	ARM	–	–	Anal tissue	–
P7	F	ARM, bilateral duplex renal anomalies	–	–	Anal tissue	–
P8	F	ARM	–	–	Anal tissue	–
P9	M	ARM	–	–	Anal tissue, blood	–
P12	M	ARM, vertebral defects, small VSD	arr[hg19]22q11.22q11.23(22971867_23643138)x1 pat <sup>d</sup>	CNV1 (0.67 Mb): <i>GGTLC2</i> , <i>MIR650</i> , <i>MIR571</i> , <i>IGLL5</i> , <i>RSPH14</i> , <i>GNAZ</i> , <i>RAB36</i> , <i>BCR</i> , <i>FBXW4P1</i>	Anal tissue	P
P13	M	ARM, Arnold-Chiari malformation	–	–	Anal tissue	–
P15	F	ARM	–	–	Anal tissue, blood	–
P17	M	ARM	arr[hg19]2q12.1q12.1(103090517_103148433)x1	CNV1 (0.06 Mb): <i>SLC9A4</i>	Anal tissue	VUS

(Continues)

**TABLE 1** (Continued)

Patients	Sex	Malformations	Detected variant	CNV size and affected genes <sup>a</sup>	Analyzed tissues	Classification
P20	M	ARM	–		Anal tissue	–
P21	M	ARM, two small VSDs, PFO	–		Anal tissue, blood	–
P22	M	ARM, vertebral defects, urethral stricture, mild hypospadias, small PFO, PDA	arr[hg19]7q34q34(139478979_139550968)x3, 15q12q12(27462869_27544157)x3	CNV1 (0.07 Mb): <i>TBXAS1</i> CNV2 (0.08 Mb): <i>GABRG3</i>	Anal tissue	CNV1-2:VUS
P23	F	ARM <sup>c</sup>	–		Anal tissue	–
P10	M	HN	–		Tissue from renal pelvis, blood	–
P11	F	HN	–		Tissue from renal pelvis, blood	–
P14	F	HN	–		Tissue from renal pelvis	–
P16	F	HN	–	–	Tissue from renal pelvis	–
P18	M	HN	–	–	Tissue from renal pelvis	–
P19	F	HN	–	–	Tissue from renal pelvis	–

Abbreviations: ASD, atrial septal defect; CNV, copy number variant; na, not analysed; P, pathogenic; PDA, patent ductus arteriosus; PFO, patent foramen ovale; VSD, ventricular septal defect; VUS, variant of uncertain clinical significance.

<sup>a</sup>CNV numbering refers to the order of reported CNVs for each patient in the “Detected variant” column.

<sup>b</sup>FISH analysis was performed to confirm presence of the CNV in peripheral blood.

<sup>c</sup>Cloacal malformation.

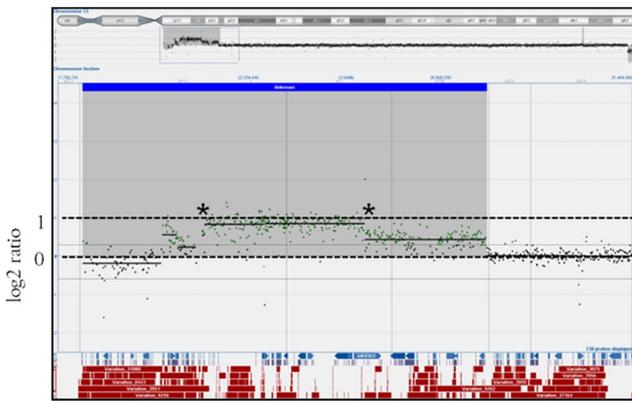
<sup>d</sup>Deletion previously published as disease-associated by Mikhail et al. (Mikhail et al., 2013).

in patient P24 detected in both esophageal tissue and peripheral blood. The patient had additional clinical manifestations including an atrial septal defect (ASD) and two minor ventricular septal defects (VSDs), developmental delay and seizure episodes. In her second and third year of life she was hospitalized for long periods due to severe respiratory tract infections that required intensive care treatment with tracheostomy. Array-CGH analysis showed an aberrant pattern in a 7.5 Mb region on the proximal q-arm of chromosome 15 (Prader–Willi/Angelman syndrome region), with deviating log<sub>2</sub>-ratios indicating duplication of the 15q11.2q12 and 15q13.1q13.3 regions and triplication of the intermediary 15q11.2q12 region (Figure 1a, Table 1). The observed log<sub>2</sub>-ratios indicate triplication for a large part of the region (3.7 Mb) between the 15q segmental duplications commonly referred to as BP2 and BP3 (the triplicated region reaching as far as the *GABRG3* gene), followed by duplication of the region from *GABRG3* to BP4. In addition, the patient had a 0.7 Mb deletion on 15q26.3 of unknown clinical significance (Figure 1b, Table 1).

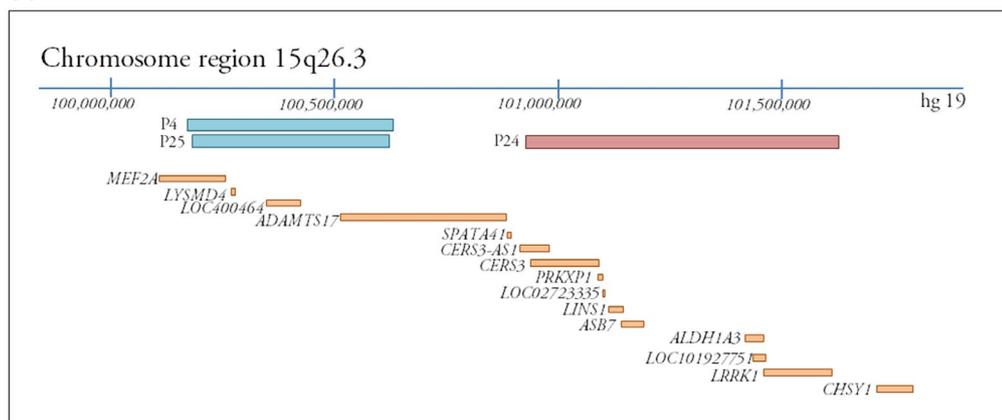
Chromosome analysis in lymphocytes from patient P24 revealed three different cell populations with

the karyotype: 46,XX,add(15)(p10)[12]/47,XX,15ps-,+mar[8]/46,XX,15ps-[2] (Figure 1c). FISH analysis identified the extra material on 15p as comprising an additional centromere, two additional copies of proximal 15q and one additional copy of 15p (Figure 1c). The marker chromosome had one centromere, two copies of proximal 15q and one copy of 15p. The two most abundant aberrant cell lines result in the same net gain of chromosome 15q-material, equaling partial tetrasomy 15 or idic(15), in 20/23 cells (87%). In the two cell populations that did not carry the add(15)(p10) rearrangement, one of the chromosome 15 homologs lacked p-arm (15ps-). FISH analysis with a specific probe located to the 15q26.3 deletion region showed that both the triplication and the deletion in 15q26.3 affected the same chromosome 15 homolog (data not shown). Parental sample chromosome and FISH analyses showed normal patterns, indicating that both aberrations had occurred de novo. Furthermore, results from MS-MLPA showed a methylation ratio of 3:1 in the Prader–Willi syndrome region, and informative microsatellite markers (D15S1002 and D15S128) with location

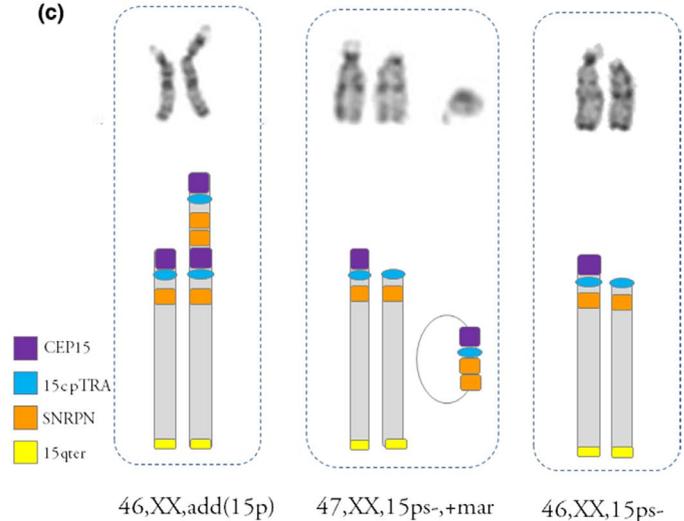
## (a) Chromosome 15



## (b)



## (c)



**FIGURE 1** Results from array-CGH analysis for chromosome 15 in patient P24, showing a proximal copy number gain in the region 15q11.2q13.1. The log<sub>2</sub>-ratio pattern seen indicates a rearrangement of proximal 15q, with the log<sub>2</sub>-ratio close to 0 seen most proximally likely reflecting the combination of benign CNVs on the different chromosome 15 homologues in the patient. The mean log<sub>2</sub>-ratio of probes located in the region marked by asterisks indicates tetrasomy (triplication) in this region (chr15:23,685,952–27,460,180 (hg19)) while the flanking regions have a mean log<sub>2</sub>-ratio consistent with trisomy (duplication). Y-axis showing the log<sub>2</sub>-fluorescence signal ratio and X-axis showing chromosomal location for each probe. Figure showing an overview of the genomic region 15q26.3 with CNVs detected by array-CGH in patients P4, P24 and P25 represented by light blue (duplication), dark blue (triplication) and red (deletion) bars. Below, RefSeq genes in the region are represented by orange bars. Results from chromosome analysis in patient P24 showing the different chromosome 15 rearrangements found in the three cell populations in the patient. Below, schematic images derived from the combined results of FISH analysis are shown, including FISH probes used in analyses. FISH probe locations: CEP15 (15p11.2), 15c pTRA (15q α-satellite/centromere), SNRPN (15q11) and 15qter

within the same region showed a skewed pattern with identical maternal alleles with increased peak heights (data not shown). The findings are consistent with inheritance of a normal chromosome 15 homolog from the father while the homolog involved in the rearrangement is of maternal origin. The presence of a normal paternal homolog precludes that the patient should have Prader–Willi syndrome.

In the group with ARMs, a 0.67 Mb deletion on 22q11.2, overlapping the most telomeric part of the 22q11.2 distal deletion region, was detected in patient P12 who showed additional clinical features of vertebral defects and a VSD (Figure 2, Table 1). The deletion was inherited from a healthy father. Furthermore, variants of uncertain clinical significance

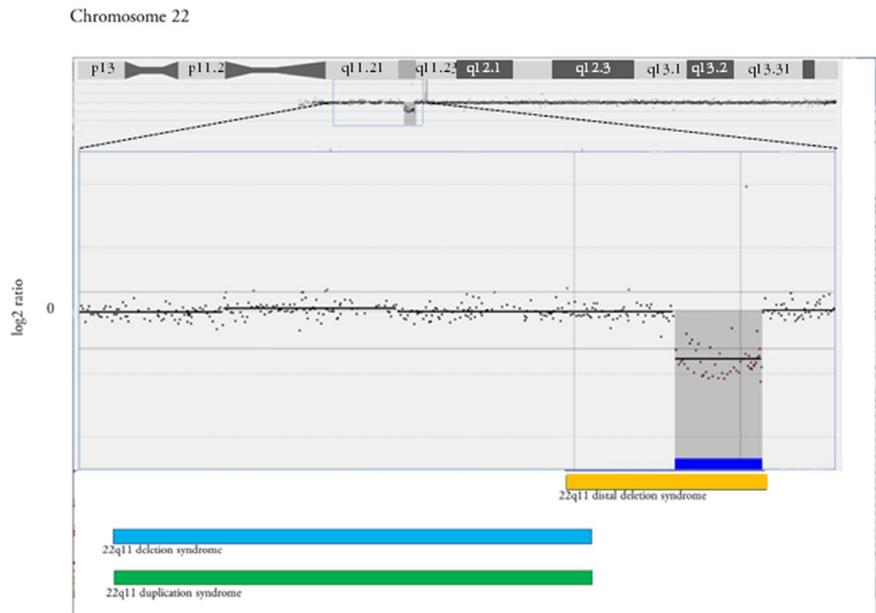
(VUS) were detected in two patients with ARM (Table 1). Parental samples were not available.

No pathogenic aberrations or VUS were detected in the patients with HN (Table 1).

Comparison of gene dose alterations detected by array-CGH in separately analysed tissue and blood (in patient P24 array-CGH and FISH, respectively) from eight patients did not reveal discrepancies indicative of somatic mosaicism.

Detected CNVs listed in Table 1 and submitted to ClinVar have the following accession numbers: SCV000930653, SCV000930654, SVC000930655, SCV000930656, SCV000930657, and SCV000930658 (<https://www.ncbi.nlm.nih.gov/clinvar/>).

**FIGURE 2** Results from array-CGH showing a 0.67 Mb deletion in the 22q11.22q11.23 affecting the distal part of the 22q11 distal deletion region, detected in patient P12. Bars below the array showing syndromes located in the 22q11 region. Y-axis showing the log<sub>2</sub>-fluorescence signal ratio and X-axis showing chromosomal location for each probe



## 4 | DISCUSSION

In this study, we screened 25 patients with gastrointestinal and urological congenital malformations using array-CGH analysis for detection of causative CNVs and detected pathogenic aberrations in 2 of 25 patients (8%) and VUS in 5 of 25 patients (20%). Notably, we did not find evidence for somatic copy number mosaicism in a subgroup analysis of eight patients where we compared CNV findings in tissue and peripheral blood from the same individuals.

In the patients with EA, one pathogenic CNV was detected. In patient P24, an atypical structural mosaicism with two different rearrangements of the extra genetic material was detected, with the net gain of chromosome material equaling that of partial tetrasomy 15q in 87% of peripheral blood cells. The aberration was found to have occurred de novo and since the 46,XX,add(15)(p10) unbalanced cell line was the most abundant, it is likely that this had been the primary cell population, that the marker chromosome formed secondarily in a subset of cells due to the instability of the aberration, and was subsequently lost in a small cell population. The array-CGH pattern seen in the patient with distinct mean log<sub>2</sub>-ratios for the distal parts of the aberration indicated that the formation mechanism included unequal crossing-over in meiosis, a known mechanism in formation of marker chromosomes such as idic(15) (Battaglia, 2008). While the clinical features of developmental delay, seizures and cardiac malformations in the patient can be explained by partial tetrasomy 15, EA has not previously been reported in this patient group (Battaglia, 2008).

In addition to the partial tetrasomy 15 finding, the patient had a heterozygous de novo 0.7 Mb deletion on 15q26.3. The deletion affects five genes and two non-coding RNAs

(Table 1). None of the genes have any known relation to EA. Three of the genes are indexed as disease causing for autosomal recessive disorders in OMIM; *CERS3* mutations cause autosomal recessive congenital ichthyosis-9 (OMIM#615023), *LINS1* mutations cause autosomal recessive mental retardation (OMIM#614340) and *ALDH1A3* mutations cause isolated microphthalmia (OMIM#615113). Little is known about specific functions of the proteins encoded by the other two genes, *LRRK1* (OMIM\*610986) and *ASB7* (OMIM\*615052) genes, expression of both genes can be found in small intestine and *ASB7* expression is also reported in esophagus although no fetal data are available (The human protein atlas, <http://www.proteinatlas.org/>) (Korr et al., 2006; Uhlen et al., 2015). There are no patients with overlapping deletions and EA reported in the DECIPHER database (<https://decipher.sanger.ac.uk>).

Interestingly, in two other patients with EA, a duplication (patient P4) and a triplication (patient P25) with strikingly similar breakpoints in 15q26.3 were detected. The distal breakpoints of the duplication and triplication were located approximately 0.3 Mb upstream of the proximal breakpoint of the 15q26.3 deletion found in patient P24 (Figure 1b, Table 1). The duplication was found in patient P4 who had omphalocele in addition to EA. The triplication was detected in patient P25, a female twin with EA, duodenal atresia, pelvic kidney and aortic arch anomalies and was also detected in her healthy monozygous twin. Unfortunately, parental samples were unavailable in both cases. The duplication and triplication overlap with duplications classified as likely benign in ClinVar and genes affected by the duplication/triplication include *MEF2A* (OMIM\*600660), *LYSMD4* (no OMIM accession number), and *ADAMTS17* (OMIM\*607511). *LYSMD4* encodes a protein of unknown function while *MEF2A* mutations

are implicated in an autosomal dominant form of coronary artery disease and homozygous mutations in *ADAMTS17* cause Weill–Marchesani-like syndrome (OMIM#613195) (Wang, Fan, Topol, Topol, & Wang, 2003). The clinical findings of patient P4, P24, and P25 do not overlap with published phenotypes associated with known disease-associated genes in the 15q26.3 region (none of these phenotypes include EA). With the identification of CNV breakpoint clustering in 15q26.3 in three patients with EA, it is possible that CNVs interrupt important regulatory regions of one or more genes involved in development of the esophagus and that 15q26.3 is a novel susceptibility region for EA.

In the group with ARMs, a 0.67 Mb deletion on 22q11.2 was detected in patient P12 (Figure 2). The affected region was distal to the 3 Mb region in which recurrent deletions cause the 22q11-deletion syndrome. Patients with deletions in the distal 22q11.2 region show dysmorphic features, global developmental delay, intellectual disability, language delay, growth restriction, and cardiac defects (OMIM#611867), thus representing a condition distinct from, but sharing some clinical features with, the 22q11-deletion syndrome (Mikhail et al., 2014). The deletion detected in our patient was similar to the smallest deletion reported in patients with the syndrome, with breakpoints flanked by low copy repeat regions LCR22-E and -F and associated with a relatively mild phenotype with predominantly developmental delay and intellectual disability as clinical manifestations, while heart defects are rare (Mikhail et al., 2014; Rauch et al., 2005). It has been noted that this deletion often occurs de novo (Mikhail et al., 2014). Patient P12 had a more severe malformation phenotype, fulfilling criteria for VACTERL association, while his neurodevelopmental status is unknown. This fact and the inheritance of the deletion from a healthy father indicates a reduced penetrance. The clinical manifestations in our patient may be caused by the distal 22q11 deletion in combination with other genetic or environmental factors, or have a different genetic cause altogether.

No disease-causing aberrations were detected in the patients with HN. Considering the number of patients in our study and the fact that they all had isolated malformations, it is not surprising that we did not identify pathogenic CNVs in this patient group.

We did not find evidence of somatic mosaicism for detected CNVs. The resolution of our array-CGH platform precludes detection of small CNVs and low-level mosaicism may be missed. Moreover single nucleotide variants have not been analysed in this study. Only the tissue discarded as part of the surgical procedure was used and it is not known whether this was the most appropriate tissue to analyse in each case.

In conclusion, our study shows that CNV analysis is important as part of clinical genetic investigations in patients with gastrointestinal and urological malformations, since pathogenic copy number variants were detected in 8% (2 of 25) of patients and a novel putative susceptibility region for EA was identified.

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## CONFLICTS OF INTEREST

None of the authors have any commercial or other associations that might pose a conflict of interest in connection with the submitted article.

## AUTHOR CONTRIBUTIONS

A. Nordgren, A. Nordenskjöld and PG conceived and designed the experiments. JW performed the experiments. JW, PG, and ES analyzed the data. A. Nordgren, MF, TW, ML, HE, and A. Nordenskjöld contributed reagents/materials/analysis tools. JW, PG, A. Nordgren, and A. Nordenskjöld wrote the paper.

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## REFERENCES

- Battaglia, A. (2008). The inv dup (15) or idic (15) syndrome (Tetrasomy 15q). *Orphanet Journal of Rare Diseases*, 3, 30, 3–30. <https://doi.org/10.1186/1750-1172-3-30>
- Biesecker, L. G., & Spinner, N. B. (2013). A genomic view of mosaicism and human disease. *Nature Reviews Genetics*, 14(5), 307–320. <https://doi.org/10.1038/nrg3424>
- Brosens, E., Marsch, F., de Jong, E. M., Zaveri, H. P., Hilger, A. C., Choinitzki, V. G., ... de Klein, A. (2016). Copy number variations in 375 patients with oesophageal atresia and/or tracheoesophageal fistula. *European Journal of Human Genetics*, 24(12), 1715–1723. <https://doi.org/10.1038/ejhg.2016.86>
- Campbell, I. M., Yuan, B. O., Robberecht, C., Pfundt, R., Szafranski, P., McEntagart, M. E., ... Stankiewicz, P. (2014). Parental somatic mosaicism is underrecognized and influences recurrence risk of genomic disorders. *American Journal of Human Genetics*, 95(2), 173–182. <https://doi.org/10.1016/j.ajhg.2014.07.003>
- Caruana, G., Wong, M. N., Walker, A., Heloury, Y., Webb, N., Johnstone, L., ... Bertram, J. F. (2015). Copy-number variation associated with congenital anomalies of the kidney and urinary tract.

- Pediatric Nephrology (Berlin, Germany)*, 30(3), 487–495. <https://doi.org/10.1007/s00467-014-2962-9>
- Dworschak, G. C., Draaken, M., Hilger, A. C., Schramm, C., Bartels, E., Schmiedeke, E., ... Reutter, H. (2015). Genome-wide mapping of copy number variations in patients with both anorectal malformations and central nervous system abnormalities. *Birth Defects Res A Clin Mol Teratol*, 103(4), 235–242. <https://doi.org/10.1002/bdra.23321>
- Ferreira de Almeida, T., & Bertola, D. R. (2013). Microdeletion 11q13.1q13.2 in a patient presenting with developmental delay, facial dysmorphism, and esophageal atresia: Possible role of the GSTP1 gene in esophagus malformation. *Birth Defects Research Part A: Clinical and Molecular Teratology*, 97(7), 463–466. <https://doi.org/10.1002/bdra.23115>
- Feuk, L., Carson, A. R., & Scherer, S. W. (2006). Structural variation in the human genome. *Nature Reviews Genetics*, 7(2), 85–97. <https://doi.org/10.1038/nrg1767>
- Flöttmann, R., Kragestein, B. K., Geuer, S., Socha, M., Allou, L., Sowińska-Seidler, A., ... Spielmann, M. (2018). Noncoding copy-number variations are associated with congenital limb malformation. *Genetics in Medicine*, 20(6), 599–607. <https://doi.org/10.1038/gim.2017.154>
- Hilger, A., Schramm, C., Pennimpede, T., Wittler, L., Dworschak, G. C., Bartels, E., ... Draaken, M. (2013). De novo microduplications at 1q41, 2q37.3, and 8q24.3 in patients with VATER/VACTERL association. *European Journal of Human Genetics*, 21(12), 1377–1382. <https://doi.org/10.1038/ejhg.2013.58>
- Kearney, H. M., Thorland, E. C., Brown, K. K., Quintero-Rivera, F., & South, S. T. (2011). American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genetics in Medicine*, 13(7), 680–685. <https://doi.org/10.1097/GIM.0b013e3182217a3a>
- Korr, D., Toschi, L., Donner, P., Pohlenz, H. D., Kreft, B., & Weiss, B. (2006). LRRK1 protein kinase activity is stimulated upon binding of GTP to its Roc domain. *Cellular Signalling*, 18(6), 910–920. <https://doi.org/10.1016/j.cellsig.2005.08.015>
- Kruszka, P., Tanpaiboon, P., Neas, K., Crosby, K., Berger, S. I., Martinez, A. F., ... Muenke, M. (2017). Loss of function in ROBO1 is associated with tetralogy of Fallot and septal defects. *Journal of Medical Genetics*, 54(12), 825–829. <https://doi.org/10.1136/jmedgenet-2017-104611>
- Li, Z., Huang, J., Liang, B., Zeng, D., Luo, S., Yan, T., Tang, N. (2019). Copy number variations in the GATA4, NKX2-5, TBX5, BMP4 CRELD1, and 22q11.2 gene regions in Chinese children with sporadic congenital heart disease. *Journal of Clinical Laboratory Analysis*, 33(2), e22660. doi: <https://doi.org/10.1002/jcla.22660>.
- MacDonald, J. R., Ziman, R., Yuen, R. K., Feuk, L., & Scherer, S. W. (2014). The database of genomic variants: A curated collection of structural variation in the human genome. *Nucleic Acids Research*, 42(D1), D986–992. <https://doi.org/10.1093/nar/gkt958>
- McCarroll, S. A., Kuruvilla, F. G., Korn, J. M., Cawley, S., Nemes, J., Wysoker, A., ... Altshuler, D. (2008). Integrated detection and population-genetic analysis of SNPs and copy number variation. *Nature Genetics*, 40(10), 1166–1174. <https://doi.org/10.1038/ng.238>
- Mikhail, F. M., Burnside, R. D., Rush, B., Ibrahim, J., Godshalk, R., Rutledge, S. L., ... Carroll, A. J. (2014). The recurrent distal 22q11.2 microdeletions are often de novo and do not represent a single clinical entity: A proposed categorization system. *Genetics in Medicine*, 16(1), 92–100. <https://doi.org/10.1038/gim.2013.79>
- Piotrowski, A., Bruder, C. E. G., Andersson, R., de Ståhl, T. D., Menzel, U., Sandgren, J., ... Dumanski, J. P. (2008). Somatic mosaicism for copy number variation in differentiated human tissues. *Human Mutation*, 29(9), 1118–1124. <https://doi.org/10.1002/humu.20815>
- Rauch, A., Zink, S., Zweier, C., Thiel, C. T., Koch, A., Rauch, R., ... Hofbeck, M. (2005). Systematic assessment of atypical deletions reveals genotype-phenotype correlation in 22q11.2. *Journal of Medical Genetics*, 42(11), 871–876. <https://doi.org/10.1136/jmg.2004.030619>
- Redon, R., Ishikawa, S., Fitch, K. R., Feuk, L., Perry, G. H., Andrews, T. D., ... Hurler, M. E. (2006). Global variation in copy number in the human genome. *Nature*, 444(7118), 444–454. <https://doi.org/10.1038/nature05329>
- Salehi Karlslätt, K., Pettersson, M., Jäntti, N., Szafranski, P., Wester, T., Husberg, B., ... Nordenskjöld, A. (2019). Rare copy number variants contribute pathogenic alleles in patients with intestinal malrotation. *Mol Genet Genomic Med*, 7(3), e549. <https://doi.org/10.1002/mgg3.549>
- Uhlen, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., ... Ponten, F. (2015). Proteomics. Tissue-based map of the human proteome. *Science*, 347(6220), 1260419. <https://doi.org/10.1126/science.1260419>
- van Binsbergen, E., Ellis, R. J., Abdelmalik, N., Jarvis, J., Randhawa, K., Wyatt-Ashmead, J., ... Van Haelst, M. M. (2014). A fetus with de novo 2q33.2q35 deletion including MAP2 with brain anomalies, esophageal atresia, and laryngeal stenosis. *American Journal of Medical Genetics. Part A*, 164A(1), 194–198. <https://doi.org/10.1002/ajmg.a.36202>
- Wang, L., Fan, C., Topol, S. E., Topol, E. J., & Wang, Q. (2003). Mutation of MEF2A in an inherited disorder with features of coronary artery disease. *Science*, 302(5650), 1578–1581. <https://doi.org/10.1126/science.1088477>
- Westland, R., Verbitsky, M., Vukojevic, K., Perry, B. J., Fasel, D. A., Zwijnenburg, P. J. G., ... Sanna-Cherchi, S. (2015). Copy number variation analysis identifies novel CAKUT candidate genes in children with a solitary functioning kidney. *Kidney International*, 88(6), 1402–1410. <https://doi.org/10.1038/ki.2015.239>
- Winberg, J., Berggren, H., Malm, T., Johansson, S., Johansson Ramgren, J., Nilsson, B., ... Nordgren, A. (2015). No evidence for mosaic pathogenic copy number variations in cardiac tissue from patients with congenital heart malformations. *European Journal of Medical Genetics*, 58(3), 129–133. <https://doi.org/10.1016/j.ejmg.2015.01.003>
- Winberg, J., Gustavsson, P., Lagerstedt-Robinson, K., Blennow, E., Lundin, J., Iwarsson, E., ... Nordgren, A. (2010). Chimerism resulting from parthenogenetic activation and dispermic fertilization. *American Journal of Medical Genetics. Part A*, 152A(9), 2277–2286. <https://doi.org/10.1002/ajmg.a.33594>
- Winberg, J., Gustavsson, P., Papadogiannakis, N., Sahlin, E., Bradley, F., Nordenskjöld, E., ... Nordenskjöld, A. (2014). Mutation screening and array comparative genomic hybridization using a 180K oligonucleotide array in VACTERL association. *PLoS ONE*, 9(1), e85313. <https://doi.org/10.1371/journal.pone.0085313>
- Winberg, J., Lagerstedt Robinson, K., Naess, K., Lesko, N., Wibom, R., Liedén, A., ... Gustavsson, P. (2013). Partial tetrasomy 14 associated with multiple malformations. *American Journal of Medical Genetics. Part A*, 161A(6), 1284–1290. <https://doi.org/10.1002/ajmg.a.35887>
- Wong, E. H. M., Cui, L., Ng, C.-L., Tang, C. S. M., Liu, X.-L., So, M.-T., ... Garcia-Barcelo, M.-M. (2013). Genome-wide copy number variation

study in anorectal malformations. *Human Molecular Genetics*, 22(3), 621–631. <https://doi.org/10.1093/hmg/dds451>

Zhu, Q., High, F. A., Zhang, C., Cerveira, E., Russell, M. K., Longoni, M., ... Lee, C. (2018). Systematic analysis of copy number variation associated with congenital diaphragmatic hernia. *Proceedings of the National Academy of Sciences of the United States of America*, 115(20), 5247–5252. <https://doi.org/10.1073/pnas.1714885115>

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