Copy Number Variations in 375 patients with oesophageal atresia and/or tracheoesophageal fistula.

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ABSTRACT

Oesophageal Atresia (OA) with or without Tracheoesophageal Fistula (TOF) are rare anatomical congenital malformations whose cause is unknown in over 90% of patients. A genetic background is suggested and among the reported genetic defects are Copy Number Variations (CNVs). We hypothesized that CNVs contribute to OA/TOF development. Quantifying their prevalence could aid in genetic diagnosis and clinical care strategies. Therefore, we profiled 375 patients in a combined Dutch, American and German cohort via genomic micro-array and compared the CNV-profiles with their unaffected parents and published control cohorts. We identified 167 rare CNVs containing genes. (frequency < 0.0005 in our in-house cohort) Eight rare CNVs – in 6 patients - were *de novo,* including one CNV previously associated with oesophageal disease. (hg19 chr7:g.(143820444_143839360)_(159119486_159138663)del) 1.55% of isolated OA/TOF patients and 1.62% of patients with additional congenital anomalies had *de novo* CNVs. Furthermore, three (15q13.3, 16p13.3 and 22q11.2) susceptibility loci were identified based on their overlap with known OA/TOF associated CNV syndromes and overlap with loci in published CNV association case-control studies in developmental delay. Our study suggests that CNVs contribute to OA/TOF development. In addition to the identified likely deleterious *de novo* CNVs, we detected 167 rare CNVs. While not directly disease causing, these CNVs might be of interest, as they can act as a modifier in a multiple hit model, or as the second hit in a recessive condition.

INTRODUCTION

Oesophageal Atresia (OA) with or without Tracheoesophageal fistula (TOF) are anatomical congenital malformations believed to be caused by multiple genetic and environmental factors.[1] With a prevalence of 2-3 in 10,000 live births, OA/TOF is a rare foregut-related

anomaly.[2] Around 50% of affected individuals present with additional congenital anatomical malformations.[3] Often -but not exclusively- these belong to the VATER/VACTERL association spectrum of vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with or without oesophageal atresia (TE), renal anomalies (R) and radial limb defects (L).[4,5]

A confirmed genetic syndrome or a chromosomal anomaly -including aneuploidies as trisomy 13, 18 and 21- can be identified in 6-10% of patients[6] and there is a strong suspicion that genetic factors are involved in the remainder. A genetic background is further suggested by reports of families with multiple affected individuals, higher concordance rates in monozygotic twins compared to dizygotic twins[7], higher recurrence risk for siblings and children of affected individuals, and OA/TOF as a component features in numerous known chromosomal aberrations and monogenic syndromes.[8] Reports describing disease causing Copy Number Variations (CNVs) in patients with OA/TOF are rare.[9,10] In addition to their well-established role in the development of congenital anatomical malformations in general[11], CNVs contribute to disease aetiology in several genetic syndromes. These include those having OA/TOF as part of their phenotypic spectrum such as Feingold syndrome[12], 22q11 deletion syndrome[13], CHARGE syndrome[14] and mandibulofacial dysostosis.[15] Furthermore, *de novo* disease causing CNVs have been described in patients with non-syndromic OA/TOF and the VACTERL association.[16]

To determine the contribution of CNVs in OA/TOF aetiology, we profiled 375 Dutch, German and American OA/TOF patients in a comprehensive multiplatform array. We suggest that genomic *de novo* and rare overlapping CNVs contribute to isolated and non-isolated OA/TOF. These CNVs would harbour one or more disease-related genes or phenotype-modifying factors. We describe the variation detected in our large cohort. This study enabled

us to identify several rare overlapping CNVs and non-overlapping *de novo* CNVs which potentially provide new insights into the biological pathways and disease mechanisms involved in the development of OA/TOF.

METHODS

Study design

We assessed the CNVs according to the consensus statement for chromosomal micro-array analysis described by Miller and co-workers.[17] Our study design was based on the assumptions that CNVs are most likely to contributte to the abnormal phenotype in congenital anomalies if (I) a CNV is absent in large cohorts of unaffected individuals, (II) is absent in the unaffected parents of the affected individual and/or (III) is absent or has a population frequency below or comparable to the disease frequency and (IV) if it targets relevant genes or non-coding RNAs. Recurrence of loci affected by *de novo* CNVs in single cases could indicate loci harbouring genes mutated or otherwise affected in larger disease cohorts. A detailed description of the study design is given in the supplementary methods.

Patient cohort

This study was approved by the institutional ethics committee of each participating centre, and was conducted in accordance with the principles of the Declaration of Helsinki. Patients with OA/TOF (isolated or non-isolated) were identified from the medical records. All patient records were reviewed by the treating physicians or geneticists of each participating centre. After retrieval of parental informed consent, blood was drawn from a total of 375 patients and their parents, comprising 239 patients from the Erasmus MC- Sophia, 28 from the Baylor College of Medicine, and 108 from a German multi-centre study regarding the genetic and

environmental cause of OA/TOF ("The genetic risk for oesophageal atresia consortium [GREAT-consortium]").

Micro-array analysis

High-resolution analyses were performed using single-nucleotide polymorphism (SNP) microarrays (Illumina Inc., San Diego, CA, USA and Affymetrix Inc. Santa Clara, CA, USA) and CGH oligonucleotide-based arrays (Agilent Inc., San Diego, CA, USA) using standard protocols. SNP data (Log-R ratio, B-Allele Frequency) were visualized to identify potential CNVs via Biodiscovery Nexus CN7.5. (Biodiscovery Inc., Hawthorne, CA, USA) and the GenomeStudio genotyping module (v1.9.4, www.illumnia.com). A detailed description of chip types, normalized output generation and analysis settings is provided in the supplementary methods. CNVs were - prior to validation studies- first filtered and prioritized based on size, probe content, quality, frequency in reference cohorts, gene content and frequency in our OA cohorts. All CNVs passing the filter criteria were evaluated manually in modified version (i.e. excluding BAC arrays and small InDels) of the database of genomic variation (DGV, http://dgv.tcag.ca/dgv/app/home), ISCA (http://dbsearch.clinicalgenome.org/search/), ClinGen (https://www.clinicalgenome.org/data-sharing/clinvar/) and DECIPHER. (http://decipher.sanger.ac.uk) We classified CNVs to be rare if they were absent or present once in our in-house cohort of unaffected individuals. (n=3235 individuals) We searched for overlap in large CNV cohorts of control individuals published by Cooper *et al*.[18], Coe *et al*.[19] and Kaminsky et al.[20] We also evaluated the CNVs significantly different in these studies between patients and controls. To confirm the putative *de novo* and putative deleterious CNVs, patient and parental DNA were tested with either additional SNP array, Real Time Quantitative PCR, Fluorescence In Situ Hybridization (FISH) and/or Multiplex Amplicon Quantification (MAQ) (Multiplicon N.V., Gent, Belgium). A detailed description of these methods is given in the supplementary methods. All

rare CNVs are listed in supplementary table 3 and are deposited in the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/) using the submission name "CNV study in EA/TEF" and using the exact identifiers as described in this manuscript.

RESULTS

Patient cohort

In this study a total of 375 OA/TOF patients were screened for their respective CNV profile. 129 of these patients presented with OA/TOF as an isolated defect (34.4%). Of the non-isolated patients with OA/TOF, 142 met the afore mentioned criteria for the clinical diagnosis of VACTERL (37.8%).

Micro-array analysis

Screening the respective cohorts (see figure 1) with high-resolution oligonucleotide and SNP microarrays led to the identification of 169 CNV. (gene-rich –containing genes- (n=167) and gene-poor (n=2)) These, will be addressed as rare CNVs in the remainder of the manuscript. Their size distributions are depicted in figure 2, genomic locations, evaluation of presence in control databases and classifications are given in supplementary table 2. Almost all of the rare CNVs were widely distributed over the genome. However, our analysis yielded a total of 12 loci which were affected by a rare CNV more than once and were present in more than one patient (see supplementary table 1 for the regions and phenotypes of patients with rare CNVs and overlapping loci). Inheritance was determined using secondary technology as MAQ-assay or qPCR in 17 out of 74 CNVs either suspected to be *de novo* CNVs after trio analysis using micro-array or based on suspected deleteriousness in single patient micro-array analysis. (see supplementary figure 1)

Eight out of these 74 rare CNVs selected for further investigation (10.8 %) – in 6 patients (1.6%) - were confirmed to be *de novo*. (see table 1, and figure 3 and 4 for examples) Additionally, one locus harboured a 15q11 *de novo* CNV deletion (hg19 chr15:g.(?_19339852-20216728_?), common in the database of genomic variants. (see supplementary figure 1) All but one *de novo* CNVs were non-recurrent and non-overlapping in our cohort. For four patients DNA of only one parent was available, thus preventing determination of inheritance of the rare CNV in the missing parent. Haplotype analysis of the locus could confirm that the haplotype present in the patient was not the haplotype of the available parent in three out of four CNVs. In table 1 the phenotypes of patients with confirmed *de novo* CNVs detected in this study and in table 2 the *de novo* CNVs described in literature are shown. Most *de novo* CNVs described here and in literature are non-recurrent i.e. there are no overlapping loci. The only recurrent affected *de novo* locus is 7q35q36 (see figure 3). One *de novo* CNV (16p13.3 duplication, see table 1) overlapped two inherited 16p13.3 duplications. (see table 3) We classified the rare CNVs as benign (45), uncertain-likely benign (106) and 7 as uncertain. Interestingly, we could classify nine CNVs as uncertain-likely pathogenic and two as pathogenic. These putative deleterious CNVs seen in 10 patients (2,6%) are depicted in table 3. Two of these were confirmed to be *de novo*, four were inherited from parents without oesophageal atresia and for four CNVs the inheritance pattern is not known.

Discussion

We hypothesized that both *de novo* and rare overlapping CNVs could predispose to -or modify the phenotype of- OA/TOF patients. These disease associated CNVs should be below or in the same frequency range as OA/TOF disease prevalence. We identified 169 of these rare CNVs including eight *de novo* CNVs (non-overlapping) and twelve loci with overlapping

rare gene-rich CNVs. Six patients in our cohort had rare CNVs confirmed to be *de novo*. The distribution of these *de novo* CNVs is comparable between isolated and non-isolated OA/TOF patients: two patients with isolated OA/TOF had one *de novo* CNV each. (0.53% of total patient cohort; 1.55% of patients with isolated OA/TOF) Two patients with non-isolated OA/TOF had one *de novo* CNV each and two had two *de novo* CNVs. (1.06% in total cohort; 1.62 % of non-isolated OA patients) All *de novo* CNVs were non-recurrent in our cohort. However, there is overlap with structural chromosomal anomalies previously described in OA/TOF.[29] For instance, the chromosomal anomaly described by Jackson and co-workers (46,XX,-13,+der(18)t(13;18)(q12;p11.2)[30] overlaps with the 13q12 deletion detected in patient SKZ_1662. Genes in the deleted region may contribute to the OA/TOF aetiology. Unfortunately, little is known about the genes within the region of overlap.

Based on the assumption that a CNV has a high likelihood of being pathogenic if it is not present in cohorts of unaffected individuals as well as absent in both unaffected parents -we could classify two out of eight *de novo* CNVs as (likely-) pathogenic i.e. those at the loci 16p13.11 and at 7q35q36. No tracheoesophageal mouse phenotypes are described for any of the genes affected by *de novo* CNVs except for two genes in the 7q35q36 locus - *Shh* and *Slc4a2*. *Shh* knockout mice have numerous malformations including tracheoesophageal fistula, a small stomach, reduction of oesophageal tissue fused with the trachea, anal atresia and duodenal stenosis.[31] *Slc4a2* knockout mice develop hyperkeratosis in oesophageal and stomach epithelium.[32] However, the 7q35q36 deletion is very large – containing many genes – other genes could also contribute to the abnormal phenotype seen in this patient. The remaining six remaining *de novo* CNVs affected loci present in large CNV control cohorts. (see supplementary table 2) The total number of patients with rare *de novo* CNVs is six out of 375 (1.6%). The *de novo* rate of 1.6% is slightly elevated compared to the *de novo* rate per genome/generation described by Itsara and co-workers.[33,34] They estimate a *de novo* rate of

large CNVs to be 1.2%. However, these include more prevalent CNVs and not a selected subset. In other diseases, *de novo* CNVs have a much higher impact e.g. Congenital Diaphragmatic Hernia[35] or intellectual disability.[36] The *de novo* CNVs seen in this study are non-overlapping and some of them do not affect genes with clear association to the abnormalities seen in patients. Therefore, the significance of some of these *de novo* CNVs to the disease remain uncertain.

For instance, one *de novo* 13q11.2 deletion involves a female patient (SKZ_1662) born out of a twin pregnancy. Her twin died in utero. Unfortunately, there was neither patient material nor information regarding the observed congenital anomalies or zygosity status of this foetus available. The female index patient had OA/TOF, tracheal stenosis, a sacral abnormality and her left kidney was abnormally positioned in the midline. Within the deleted 13q12.11 region one transcribed mRNA (*AK054845*) and one lncRNA (*LINC00540*) are located. No biological role or putative function has been described for these RNA's, so far. However, one family – with congenital fibrosis of extra-ocular muscles-was reported having a translocation breakpoint (t(2;13)(q37.3;q12.11) in this region. Mice fgf9 knockouts showed a wide variety of abnormalities, including developmental problems of the skeletal, respiratory and the gastrointestinal system.[37] The 13q12.11 *de novo* deletion observed in our patient is approximately 500kb of *FGF9* suggesting that a regulatory region of *FGF9* might be affected by the deletion. Female patient SKZ_1307 has a *de novo* duplication affecting a long non-coding RNA, *LINC00114*, on chromosome 21 (chr21:40100880-40154748) confirmed with MAQ assay. *LINC00114* is located between V-Ets Avian Erythroblastosis Virus E26 Oncogene Homolog (*ERG*) and V-Ets Avian Erythroblastosis Virus E26 Oncogene Homolog 2 (*ETS2*) within the Down syndrome critical region.[19] The girl has OA/TOF and anal stenosis as main additional feature. She does not have distinct Down syndrome facial features or mental retardation. The ERG and ETS2 transcription factors might be regulated by the

LINC00114. Unfortunately, no mouse orthologue for this region exists.[38] *ERG* and *ETS2* are implicated as secondary hits -after an initial truncating *GATA1* mutation- in the development of neonatal transient myeloproliferative disease preceding myeloid leukaemia seen Down syndrome patients.[39] Patients with Down syndrome have a higher prevalence of several gastro-intestinal defects, including OA/TOF.[40] This is the first *de novo* duplication involving only one gene or long non-coding RNA in a patient with OA. Further investigation of the role of *LINC00114* in OA/TOF and Down syndrome patients with intestinal atresia is warranted. The identified *de novo* duplication on chromosome 3p26.1 in female patient DE12OSOUKBD100206 with OA and tracheomalacia comprises *LMCD1* encoding LIM and cysteine-rich domains protein 1, which acts as a transcriptional cofactor restricting the function of *GATA6*[41], a protein playing an important role in endodermal differentiation.[42] Moreover, *GATA6* expression has previously been reported to be elevated during the development and progression of Barrett's oesophagus in squamous epithelial cells.[43] Hence, the present finding of a *de novo* duplication comprising *LMCD1* in a patient with OA/TOF is suggestive of its pathogenic involvement in the development of OA/TOF. The importance and biological impact of the other *de novo* deletions/duplications is uncertain.

Of note, one *de novo* loss - a common polymorphism- was detected: hg19 chr15:g.(?_19339852)_(20216728_?)del. This CNV was detected during visual inspection of patient and parental SNP-arrays for inheritance of other CNVs. This 15q11.2 polymorphism overlapped with a previously described genetic loss implicated in patients with congenital anatomical malformations - including OA/TOF.[44] This region is deleted in three more oesophageal atresia patients in our cohort.[45] However, its` high frequency in unaffected individuals and repetitive nature (e.g. many LINE, SINE and other repetitive elements) hampers interpretation and classification of this CNV.

Overlapping rare CNVs

Rare CNVs are proposed to arise after replication errors [11] and have such a low population frequency that either they have arisen recently and have no biological meaning or -are somehow detrimental and are virtually extinct from the population. Interpretation of these CNVs is difficult. For instance, they can be ancestry specific.[46] Inheritance of a single CNV from a healthy parent is generally a characteristic of a benign CNV. However, absence of distinct abnormalities in parents carrying the same rare CNV could for instance be explained by a subclinical phenotype in these parents, variable gene expressivity, incomplete penetrance, skewed X-inactivation and/or mutations elsewhere in the genome.[11] Reduced penetrance or variable expressivity of CNVs has been described in patients with OA/TOF. For instance, Faguer described differences in expression of a microduplication[47] in patients with the same microduplication, a father with bilateral vesico-ureteric reflux and renal hypodysplasia and his child with left multicystic dysplastic kidney with megaureter, vesico-ureteric reflux, bladder diverticulae and OA/TOF. Both patients have the same duplication on chromosomal locus 17q12 which includes *HNF1B,* a gene mutated in one fifth of patients with dysplastic kidneys.[47]

The best way to see if a CNV is associated to a disease is to do a formal burden test.[48] We are not able to do this test due to the limited number of patients in a rare disease, and due to technical limitations (use of different array chips). More details are given in the supplementary discussion. However, we can look for overlap with CNVs described previously in CNV burden studies and inspect if OA/TOF has been described in patients with such a CNV. Therefore, we used the CNV burden studies published by Cooper *et al*.[18], Coe *et al*.[19] and Kaminsky et al.[20] as a proxy (Developmental delay vs controls) after filtering all common CNVs. Here, they did use sufficient numbers of patients and controls and find an enrichment of a small number of loci in this heterogeneous patient population of

developmental delay and/or congenital anomalies. Only the 16p13.3 duplication enriched in patients in these studies was recurrent in our cohort. The largest of the three duplications – seen in patient SKZ_2111 - was *de novo*. The two other paternally inherited 16p13.3 duplications were present in patient SKZ_1988 and SKZ_1150. Duplications of this region- between the *NOMO1* and *XYLT1* gene- have been described previously in patients with various phenotypical anomalies, including the OA/TOF associated congenital anomalies anal and cardiac malformations.[49] None of the other overlapping rare CNVs found in our cohort (see supplementary table 1) was enriched in the developmental delay study.

Non-recurrent CNVs seen in our cohort did overlap enriched CNVs in these burden studies or with CNVs published in patient databases. For instance, the 15q13.3 deletion seen in male patient SKZ_0856 overlaps with a known deleterious CNV[18] seen in patients with a highly variable phenotype which include mild to moderate intellectual disability and variable dysmorphic features.[50] Other CNVs with overlap in our study are the gain involving FAT1 on 4q35.2 in patient SKZ_1248, the 6p22 deletion in patient SKZ_1856[18] , the 2q13 duplication seen in patient DE61OSOUKBD100197[19] and 22q11 gain[18] seen in female patient SKZ_1780 Interestingly, two additional published EA/TEF patients have a 22q11 duplication overlapping the one seen in patient SKZ_1780. The DECIPHER database contains an inherited gain (chr22:19095778-19928090) described in patient 3771 – with TOF, upper respiratory tract abnormality, coloboma, hearing impairment, horseshoe kidney and a right aortic arch with mirror image branching. The second is a paternally inherited duplication in a patient with OA/TOF and ventricular septal defect.[51]

Rare CNVs could be determinants in secondary phenotypical anomalies and/or serve as a second 'hit' tilting the balance from normal to abnormal development. Duplications might be rescue mechanisms in which a normal copy is duplicated to balance out a copy affected by a

mutation, resulting in increased gene expression or deletions might worsen an otherwise less severe condition. OA/TOF is a variable feature in several single gene disorders. Perhaps the presence of these disorders is higher than currently diagnosed. Recognizing the phenotypical spectra might be hampered by uncharacteristic phenotypical features in patients carrying both a modifying rare CNV and a gene mutation. It might be worthwhile to screen large OA/TOF patient cohorts retrospectively for mutations in known disease genes. Unfortunately, due to the large number of genes and non-recurrence of *de novo* CNVs, it is not feasible to establish their contribution to OA/TOF disease etiology. Moreover, the lack of availability of OA/TOF patient samples and heterogeneity of the rare CNVs hamper formal burden analysis to prove association. However, the *de novo* nature of CNVs in patients and absence of overlapping CNVs in a large control cohorts is interesting. Perhaps future CNV profiling or sequencing studies will detect deleterious variation in overlapping genes paving the way for further single gene based functional studies.

Concluding remarks

We hypothesized that *de novo* and overlapping rare recurrent CNVs could contribute to the disturbed development of the oesophagus. Quantifying CNV prevalence and identity could aid in genetic diagnosis and clinical care selection. We found several *de novo* and rare overlapping CNVs. Our screening indicated that the prevalence of *de novo* CNVs on OA/TOF patient population is 1.6%. Based on their function, overlap with loci in published case-control studies, known CNV syndromes and foregut phenotypes in animal models we suggest *SHH* and *SLC4A2* as contributing factors in a contiguous gene deletion to OA/TOF disease aetiology and 15q13.3, 16p13.3 and 22q11.2 as candidate susceptibility loci. With aneuploidy and structural chromosomal anomalies (~4%) and single base pair mutations (~6 %) now CNVs (~1-2 %) totals the genetic contribution of OA/TOF disease aetiology to about ~11-12%. Mutation screening using candidate gene approaches, whole exome or whole genome

sequencing as well as sequencing large patient-parent cohorts – both prospectively and retrospectively- will likely reveal known and new pathogenic DNA variations, increasing the contribution of genetics and our knowledge of OA/TOF disease aetiology.

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Conflict of Interest

Authors do not have any potential conflicts (financial, professional, or personal) relevant to the manuscript to disclose.

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Figure and table legends

Figure 1. Filtering and prioritizing CNVs

After quality control and manual evaluation of CNVs 374 CNVs larger than 30kb, either absent or rare in the modified of the Database of Genomic Variants incorporated in the Nexus software remained. 123 out of 374 did not contain genes. 257 were absent and 5 were present once in our in-house control database. These 262 CNVs were either gene-rich –containing genes- (n=167) or gene-poor. (n=95) Two gene-poor CNVs were suspected of being de novo in micro-array trio analysis. Eight out of 74 evaluated CNVs were *de novo*. Almost all of the rare CNVs (140) were widely distributed over the genome. However, our analysis yielded a total of 12 loci –containing 29 CNVs- which were affected by a rare CNV more than once and were present in more than one patient.

Figure 2. Size and type distribution of rare CNV

Total number of rare CNVs in the Erasmus MC-Sophia, Baylor College of Medicine and University of Bonn OA/TOF cohort (=375) Homozygous loss is counted as loss. Bins represent size ranges e.g. the 50-100kb bin contains all CNVs within the size range of 50 to 100 kb.

Figure 3. *De novo* deletion ranging from chromosomal band 7q35 to 7q36.3.

Note the loss (red) in the patients logR track and the loss of Heterozygosity (yellow) in the patients B-allele frequency (BAF) plot. qPCR/FISH/MAQ assay validation results in supplementary figure 1.

Figure 4. *De novo* duplication on chromosome 8p22.

Note the gain (blue dots/arrow) in the patients' logR track and allelic imbalance (purple dots/arrow) in the patients B-allele frequency (BAF) plot. qPCR/FISH/MAQ assay validation results in supplementary figure 1.

Table 1 *de novo* CNVs in this cohort

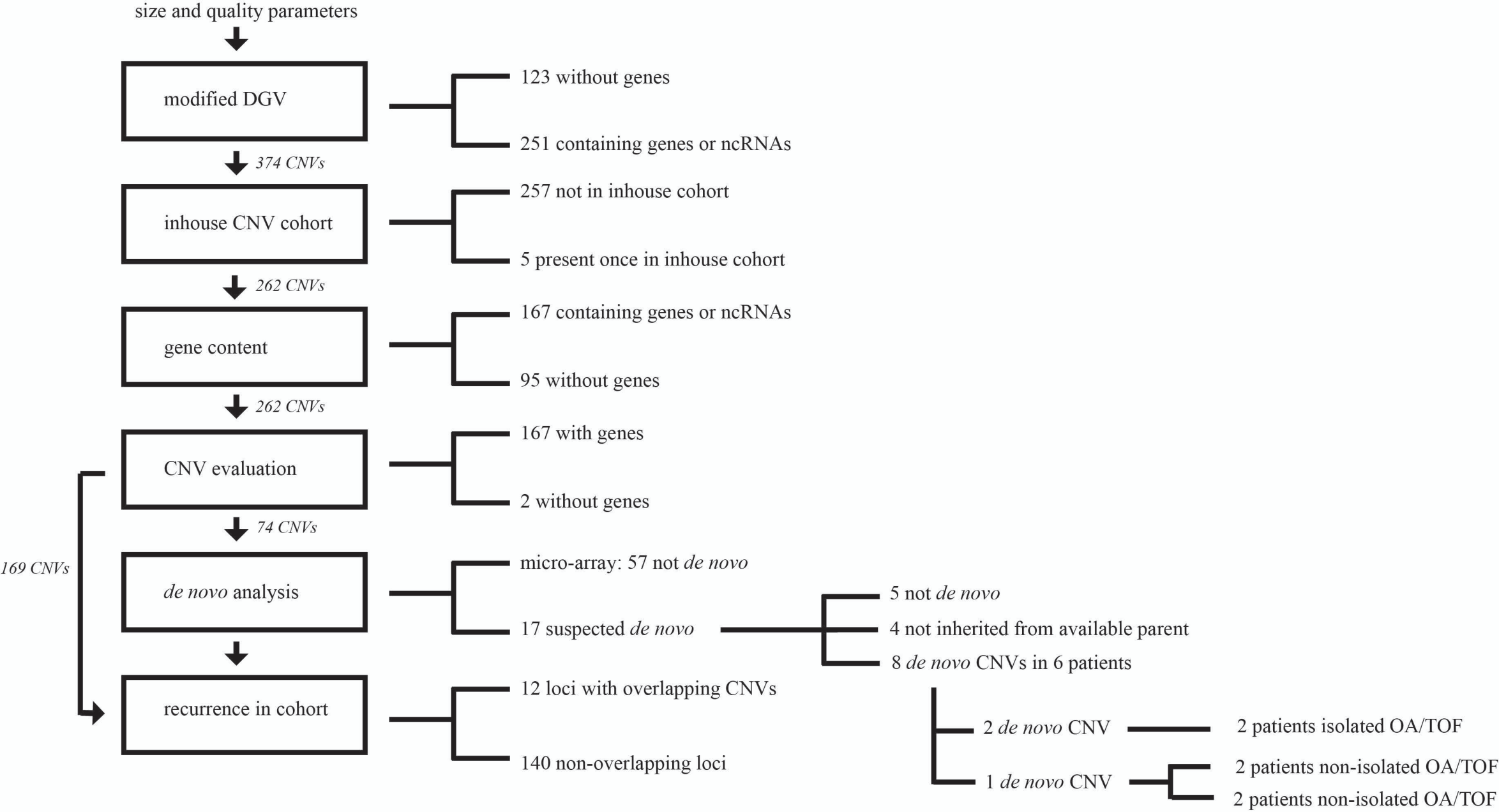
None of the parents had oesophageal atresia or trachea-oesophageal fistula. Therefore, main focus was on *de novo* CNVs. Depicted are the eight confirmed (with either qPCR or MAQ assay) *de novo* CNV out of 74 evaluated rare CNVs from the Erasmus MC-Sophia, Baylor College of Medicine and University of Bonn cohorts. qPCR/FISH/MAQ assay validation results in supplementary figure 1. Chromosome region according to build hg19.

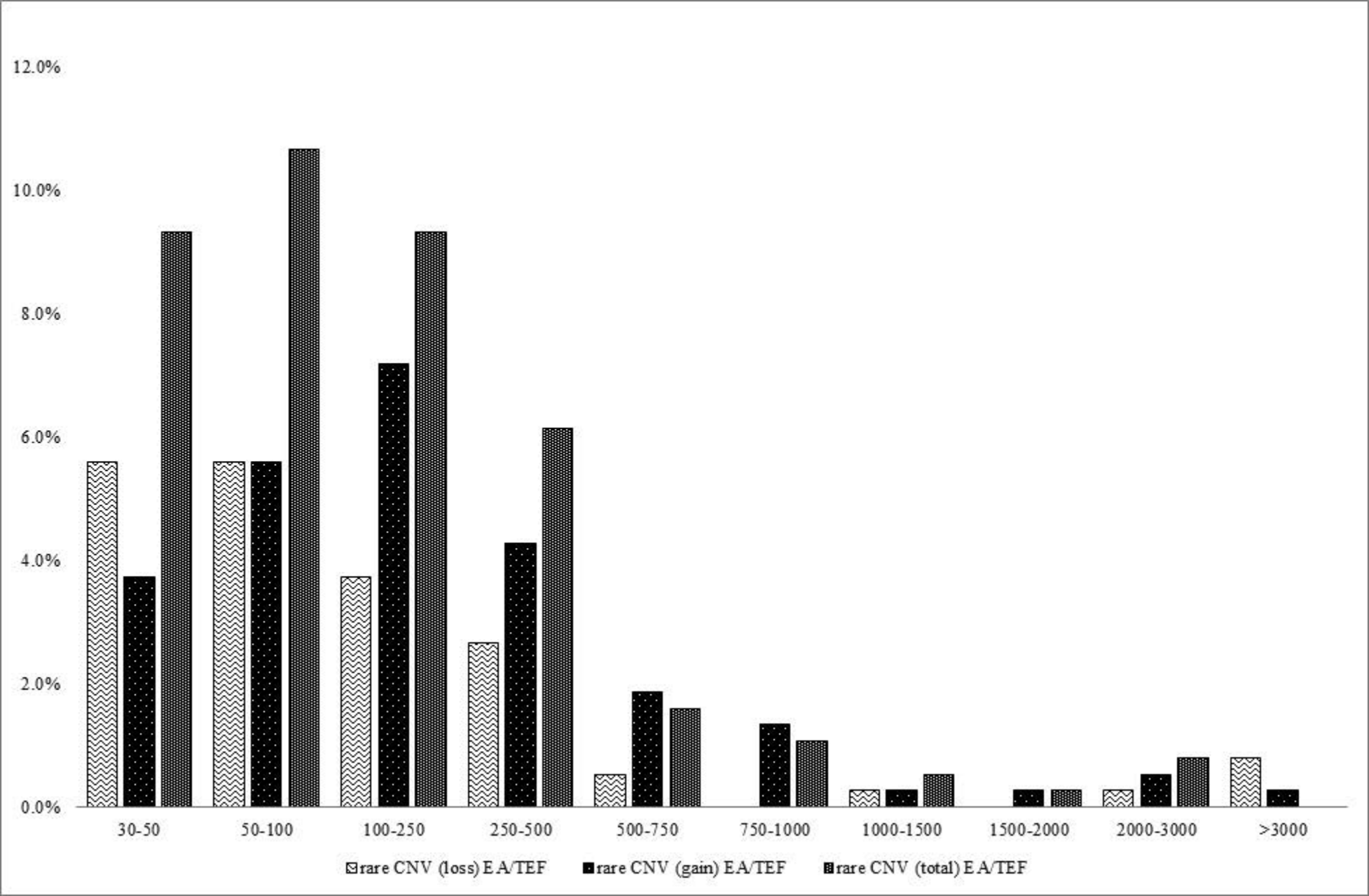
Table 2. *De novo* CNVs in OA/TOF patients described in literature

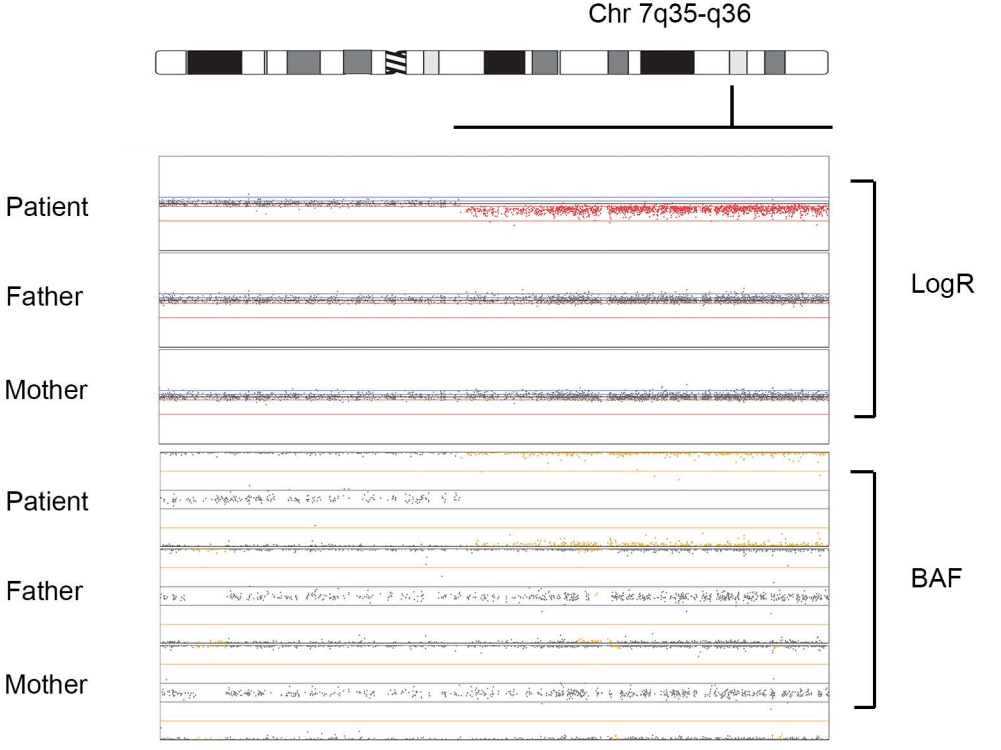
Depicted are the few published *de novo* CNVs in patients with nonsyndromic OA/TOF. Chromosome region according to build hg19.

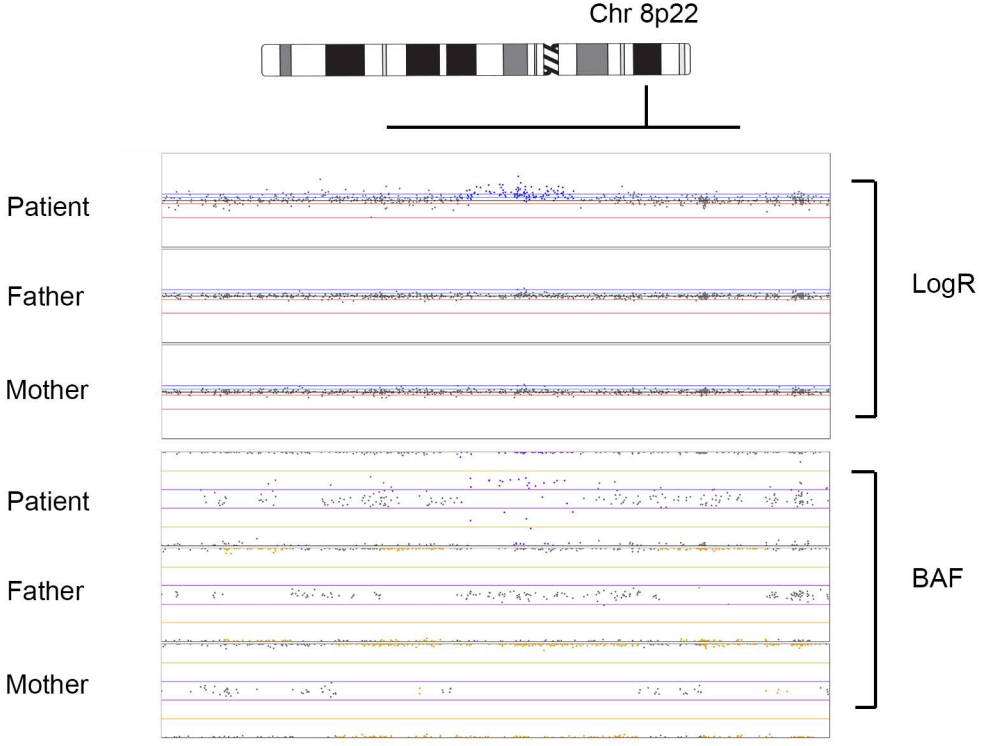
Table 3: Putative deleterious rare CNVs in this cohort

Total number of putative deleterious CNVs in the Erasmus MC-Sophia, Baylor College of Medicine and University of Bonn OA/TOF cohort (=375) Chromosome region according to build hg19. All CNVs were absent from our in-house control cohort. U; Inheritance – Undetermined, M; Inheritance – Maternal, P; Inheritance – Paternal; NP; No parental DNA available, NZ; Nullizygous.


size and quality parameters
modified DGV
123 without genes
251 containing genes or ncRNAs
374 CNVs
inhouse CNV cohort
257 not in inhouse cohort
5 present once in inhouse cohort
262 CNVs
gene content
167 containing genes or ncRNAs
95 without genes
262 CNVs
CNV evaluation
167 with genes
2 without genes
74 CNVs
169 CNVs
de novo analysis
micro-array: 57 not de novo
17 suspected de novo
5 not de novo
4 not inherited from available parent
8 de novo CNVs in 6 patients
recurrence in cohort
12 loci with overlapping CNVs
140 non-overlapping loci
2 de novo CNV
2 patients isolated OA/TOF
1 de novo CNV
2 patients non-isolated OA/TOF
2 patients non-isolated OA/TOF


12.0%
10.0%
8.0%
6.0%
4.0%
2.0%
0.0%
30-50
50-100
100-250
250-500
500-750
750-1000
1000-1500
1500-2000
2000-3000
>3000
rare CNV (loss) EA/TEF
rare CNV (gain) EA/TEF
rare CNV (total) EA/TEF


Chr 7q35-q36
Patient
Father
Mother
LogR
Patient
Father
Mother
BAF


Chr 8p22
Patient
Father
Mother
LogR
Patient
Father
Mother
BAF

Supplementary discussion of article entitled:

Copy Number Variations in 375 patients with oesophageal atresia and/or tracheoesophageal fistula.

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Evaluation of impact of rare inherited CNVs; burden test

The best way to see if a rare CNV is associated to a disease is to do a formal burden test.[1] We are not able to do this test for two reasons: (1) the limited number of available patients (375) and controls (3235) and (2) the use of different DNA-micro-array technologies (CGH-array, SNP-array) and different chip-types (e.g. different probes, probe distributions and probe spacing) in patients and control samples. We categorized a CNV in the same bin as it had an overlap of more than 70%, was comparable in size and was of the same CNV type (loss or gain). Such a CNV can be heterozygous or homozygous and we can use the gene counting method and χ^2 test to estimate power and sample size at a significance level of $P<0.05$. Doing so, we concluded that we do not have the power to detect significant differences assuming absence in controls and a presence of one (3.4%), two (9.4%), three (12.1%) or four (14.6%) times of an overlapping CNV. Assuming a presence of 4 times (the maximum of overlapping CNVs found in this study) and an 1:10 distribution of patients and controls, we would need a tenfold increase in number of patients (n=3508) and controls (n= 35075) to detect a difference with an eighty percent power. These patient numbers required for rare CNV enrichment analysis are not feasible for a rare disorder as esophageal atresia.

Evaluation of impact of rare inherited CNVs; overlap with previously published studies

Non-recurrent CNVs seen in our cohort did have overlap with enriched CNVs in these burden studies or with CNVs in published in patient databases. For instance, the 15q13.3 deletion seen in male patient SKZ_0856 overlaps with a known deleterious CNV[2] seen in patients with a highly variable phenotype which include mild to moderate intellectual disability and variable dysmorphic features.[3] The 15q13.3 paternally inherited loss (chr15:32457092-32771537) seen in patient SKZ_0856 -with OA/TOF, Anal atresia, bifid/fused ribs, aortic coarctation, abnormal arterial supply right lung and an abnormal sacrum- had overlap with a maternally inherited gain (chr15:32021733-32510863) seen in female patient 280592 – published in the DECIPHER database- with OA/TOF, laryngeal stenosis, polycystic kidney dysplasia and ventricular septal defect. Other CNVs with overlap in our study are the gain involving FAT1 on 4q35.2 in patient SKZ_1248, the 6p22 deletion in patient SKZ_1856[2] and the 2q13 duplication seen in patient DE61OSOUKBD100197.[4] Two other rare CNVs containing genes in this study had overlap with OA/TOF patients published by the DECIPHER community (http://decipher.sanger.ac.uk). The first one is a 11p15.4 gain (chr11:4371631-5253127) seen in patient SKZ_1855 -with isolated OA/TOF- which has overlap with the 11p15.4 loss (chr11:4815122-4901807) of unknown inheritance seen in male patient 288096 with TOF, cardiac anomalies, kidney anomalies, vertebral anomalies and anal atresia. No parental DNA was available for patient SKZ_1855, hampering classification of this CNV.

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4. Coe, B.P., et al., *Refining analyses of copy number variation identifies specific genes associated with developmental delay*. (1546-1718 (Electronic)).

Supplementary Figure 1. SNP-array, qPCR and MAQ assay results putative *de novo* CNV

of article entitled:

Copy Number Variations in 375 patients with oesophageal atresia and/or tracheoesophageal fistula.

Authors and affiliations

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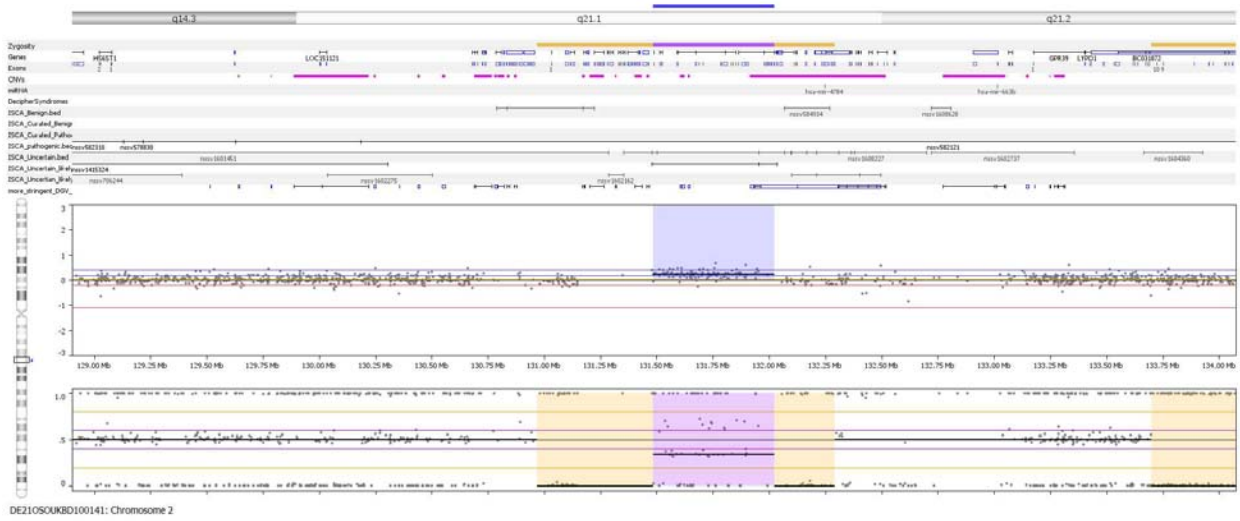
DE21OSOUKBD100141; Gain 2q21.1 (Chr2:131349899-131991166)



	Forward primer	Reverse primer	Size	Chromosome	Start	Stop
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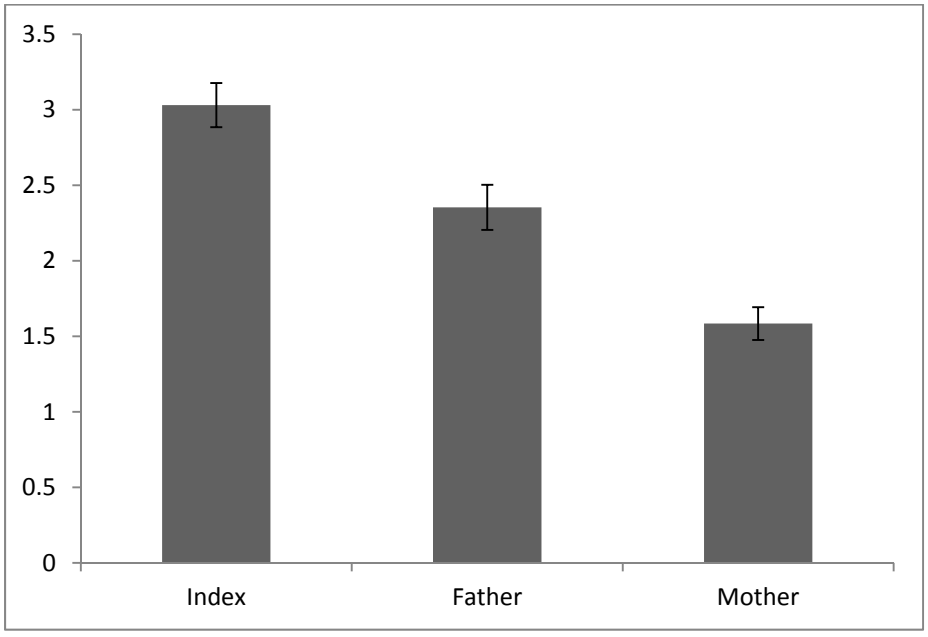
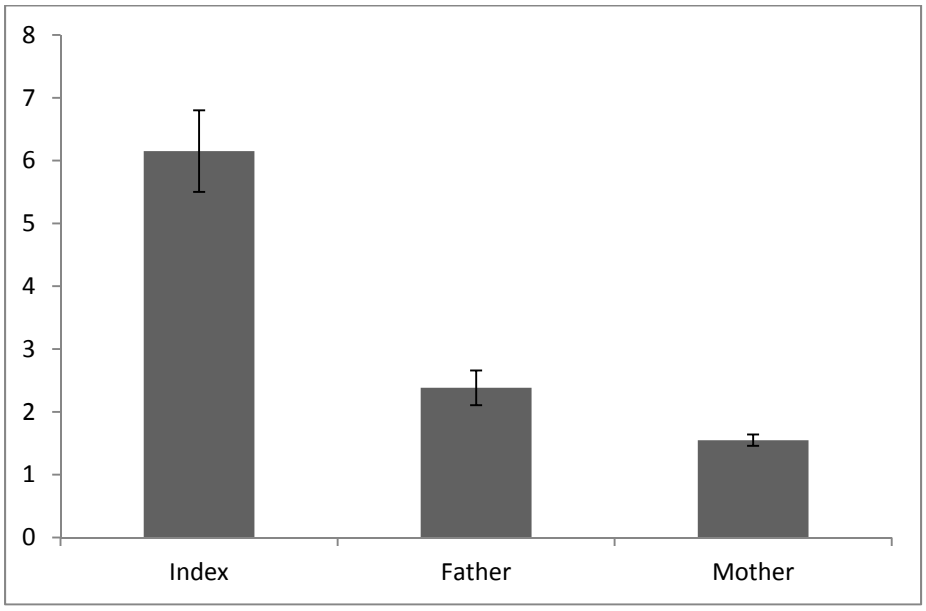
QPCR_2_2q21.1	AGAGGAAGGACAGCGTGTTC	TGACTGTGAGAGCAACTGGG	131	Chr2	131688450	131688580
QPCR_3_2q21.1	GACTGCATCAACATCCGCAC	GGGAAGGAAGAGGACCAAGC	115	Chr2	131883442	131883556



qPCR cutoff for duplication 2.5 and at least two out of three primer pairs matching the criteria.

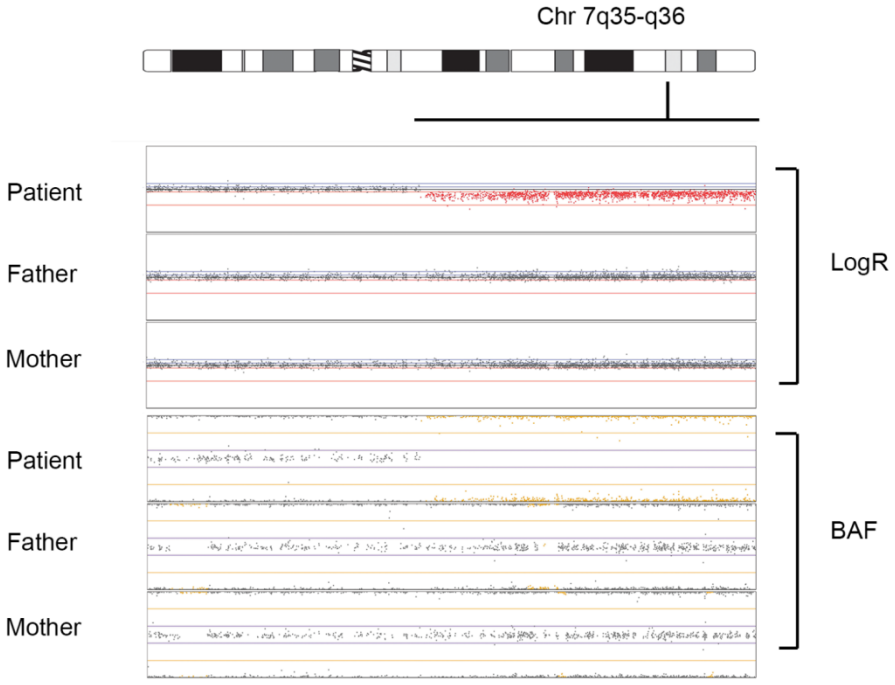
DE12OSOUKBD100206; Gain 3p26.1(Chr3:8519329-8551649)

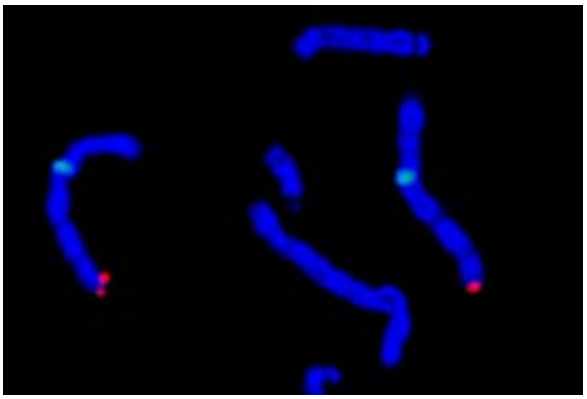
Amplicon	Forward primer	Reverse primer	Size	Chromosome	Start	Stop
QPCR_1_3p26.1	TGACAACGACATCTGCAATCG	TCCAGGGCCACAGTTAACAC	136	Chr3	8523922	8524057
QPCR_2_3p26.1	AGGTGTCCGGTTAAGTCTCTG	TGTAGGCAGAGAGGGGATCC	139	Chr3	8540503	8540641
QPCR_3_3p26.1	GGTGGCTAAGGACCTCAACC	AGTTTCCGCGAACAGATGGG	109	Chr3	8543633	8543741



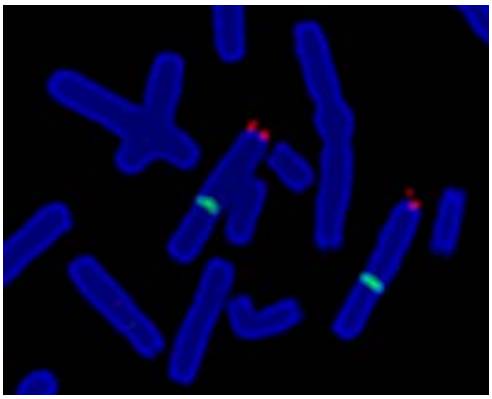
qPCR cutoff for duplication 2.5 and at least two out of three primer pairs matching the criteria.

SKZ_2111; loss 7q35q36 (chr7:143,839,360-159,138,663)





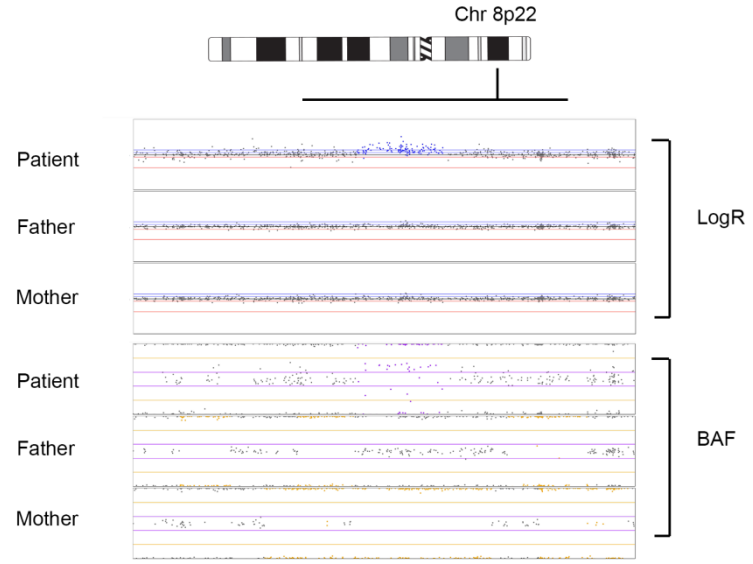
BAC clone 7qter (red+ BAC clone RP1-3K23 (green) in father

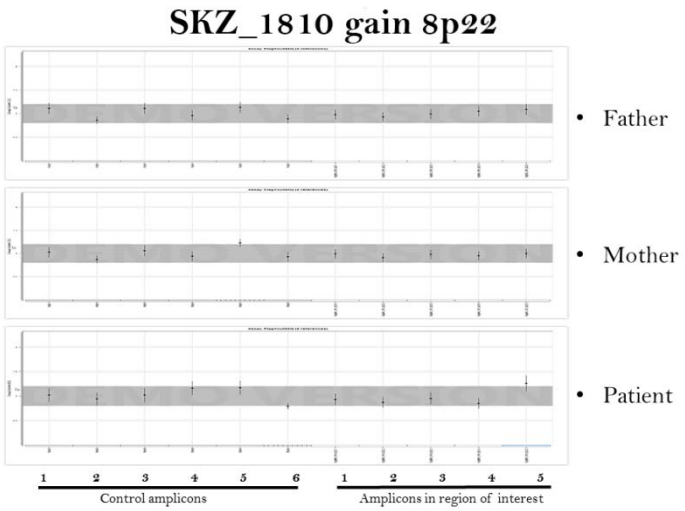


BAC clone 7qter (red+ BAC clone RP1-3K23 (green) in mother

The 7q35q36 loss is not the result of a balanced translocation in one of the parents

SKZ_1810; Gain 8p22 (chr8:17625479-17813225)





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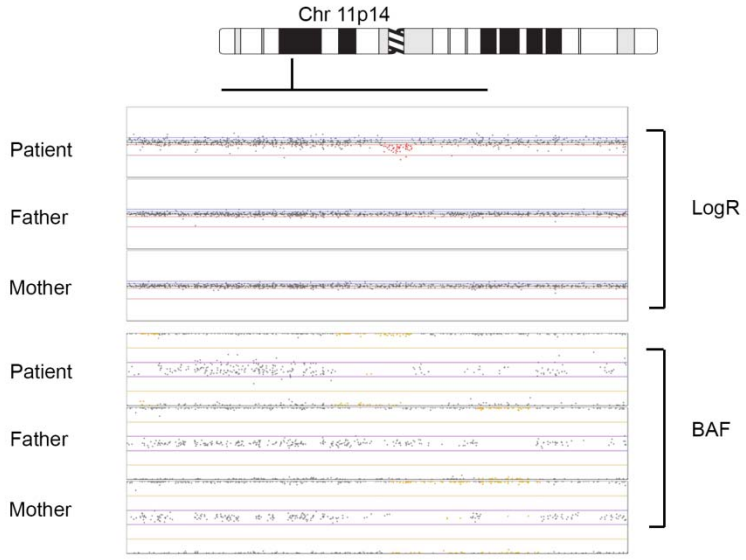
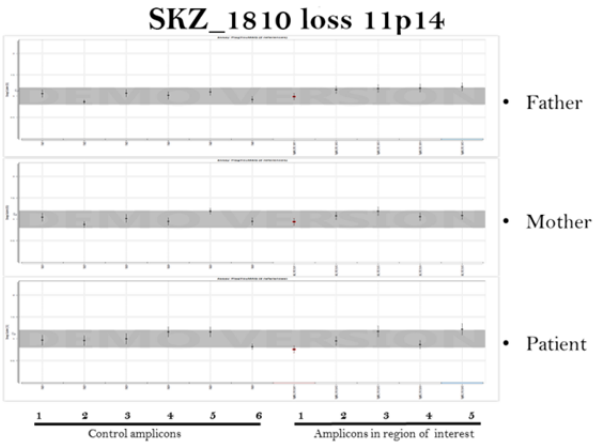
FlagMAQ_1810_8p22.2	5'AGCGGATAACAATTTCACACAGGAGCCATGTGCTGCAAACC-3'	5'-GTTTCTTTGAAGCCTCAAGTGTGAAAGC-3'	169	chr8	17600090	17600228
FlagMAQ_1810_8p22.3	5'AGCGGATAACAATTTCACACAGGCCAAGTGGAAC CTCCAAGC-3'	5'-GTTTCTTGATGAAGTGTCTCCACGAACC-3'	214	chr8	17669963	17670146
FlagMAQ_1810_8p22.4	5'AGCGGATAACAATTTCACACAGGGCACAACTGCACCAAAGCTA-3'	5'-GTTTCTTTGGCAGGCACAATACAGG-3'	295	chr8	17754984	17755248
FlagMAQ_1810_8p22.5	5'AGCGGATAACAATTTCACACAGGAGCTAACATGGCAACAGAATAATG-3'	5'-GTTTCTTGGAAAGAGAATGTCCAAATATGC-3'	180	chr8	17823952	17824101

SKZ_1810; loss 11p14.3 (chr11:21853276-22016176)





Amplicon	Forward primer	Reverse primer	Size	Chr	Start	Stop
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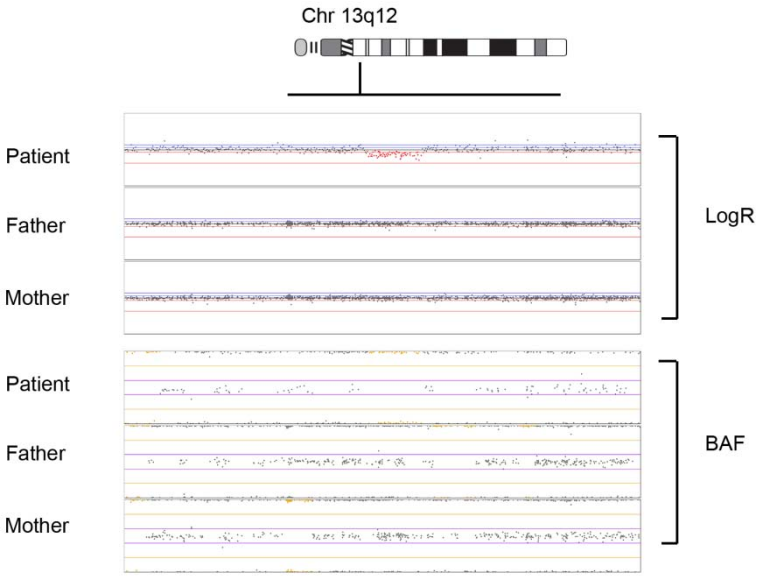
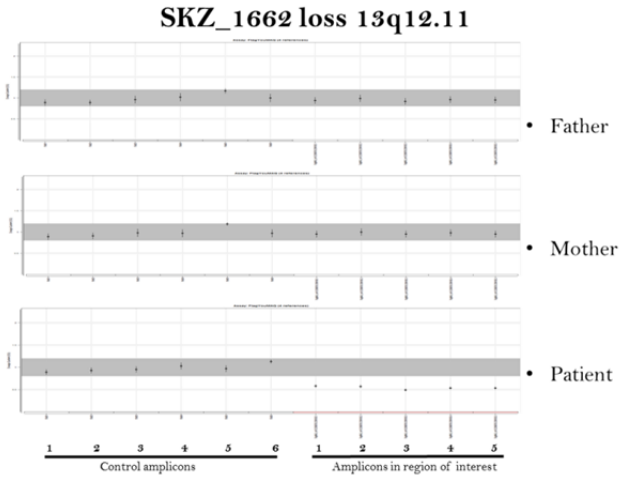
FlagMAQ_1810_11p14.2	5'AGCGGATAACAATTTCACACAGGTGAGTATTCAACAGGAACTAAACTGG-3'	5'-GTTTCTTGGTGCAGGCTTTGAGTCC-3'	197	chr11	21849190	21849356
FlagMAQ_1810_11p14.3	5'AGCGGATAACAATTTCACACAGGTGTTGAGCAACCA AGAGAAATTAAG-3'	5'-GTTTCTTCAACAACTAGAATGGTCCACTGA-3'	184	chr11	21918986	21919139
FlagMAQ_1810_11p14.4	5'AGCGGATAACAATTTCACACAGGAGTTGGAGTTATTAAAGGCTGAGGA-3'	5'-GTTTCTTTGTGGCAGGGAGTGAATG-3'	177	chr11	21996140	21996286
FlagMAQ_1810_11p14.5	5'AGCGGATAACAATTTCACACAGGCAGAGCCTCACTACCCACAGA-3'	5'-GTTTCTTTTTAATATTCGCTCTCAATAATGTCC-3'	136	chr11	22067921	22068026

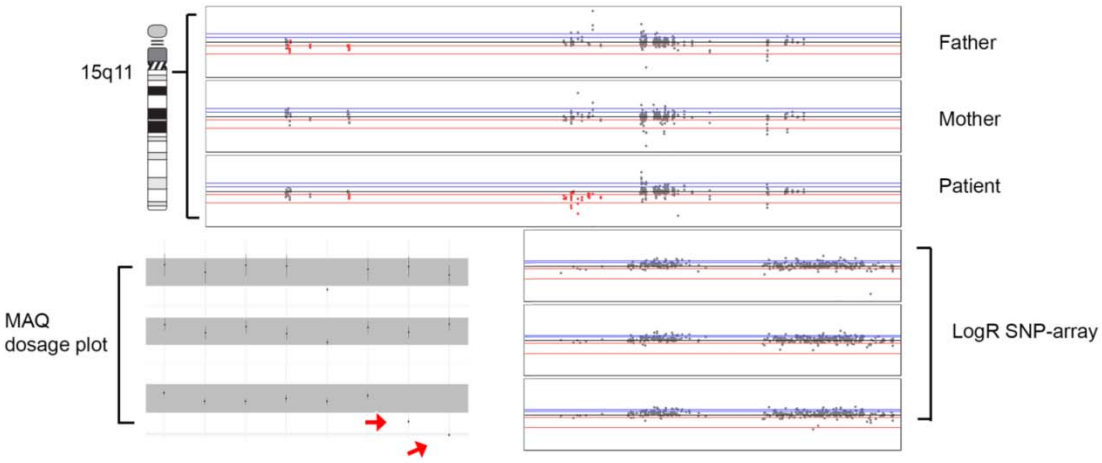
SKZ_1662; loss 13q12.11 (chr13:22688792-22981935)





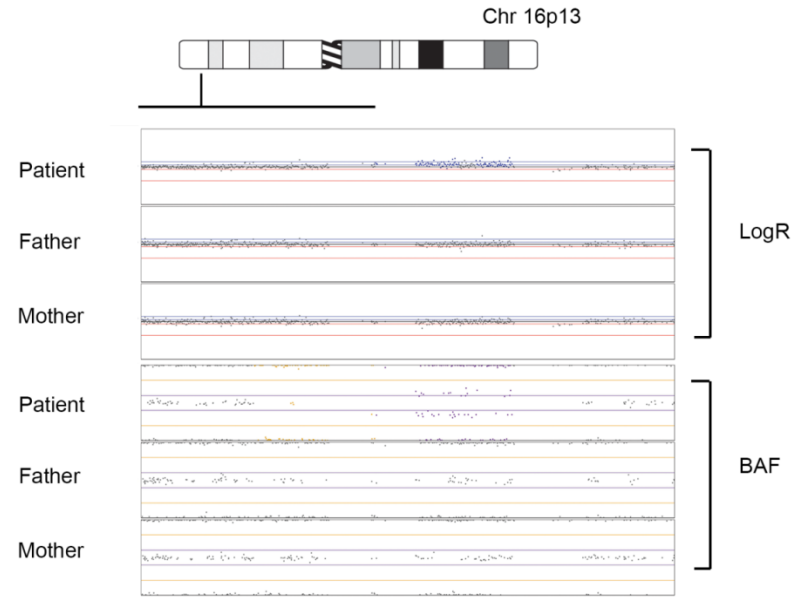
Amplicon	Forward primer	Reverse primer	Size	Chr.	Start	Stop
FlagMAQ_chr13:22688792-22981935.1	5'AGCGGATAACAATTTCACACAGGGGTGTAGTTTGCTTAATTCACAGG-3'	5'-GTTTCTTAAAGATACCATCGAAACCACTGA-3'	339	chr13	22696898	22697206
FlagMAQ_chr13:22688792-22981935.2	5'AGCGGATAACAATTTCACACAGGAGGACGAGGAAGGGCAGA-3'	5'-GTTTCTTGAAAGGACAGGCTGGAAATG-3'	218	chr13	22761030	22761217
FlagMAQ_chr13:22688792-22981935.3	5' AGCGGATAACAATTTCACACAGGCTTGTTTCGGGAGTCATAAGTCA-3'	5'-GTTTCTTCCCTTTCAATTGTCAACAGCTA-3'	168	chr13	22817621	22817758
FlagMAQ_chr13:22688792-22981935.4	5'AGCGGATAACAATTTCACACAGGTGCTTATCACTGAACCACAACC-3'	5'-GTTTCTTAATCACAAAGGAGCTAGAGGAATG-3'	237	chr13	22897279	22897485
FlagMAQ_chr13:22688792-22981935.5	5'AGCGGATAACAATTTCACACAGGGACTTCCAACACCACAAACTAATG-3'	5'-GTTTCTTTCCTAGACACAGCTACCAATGC-3'	287	chr13	22965582	22965838

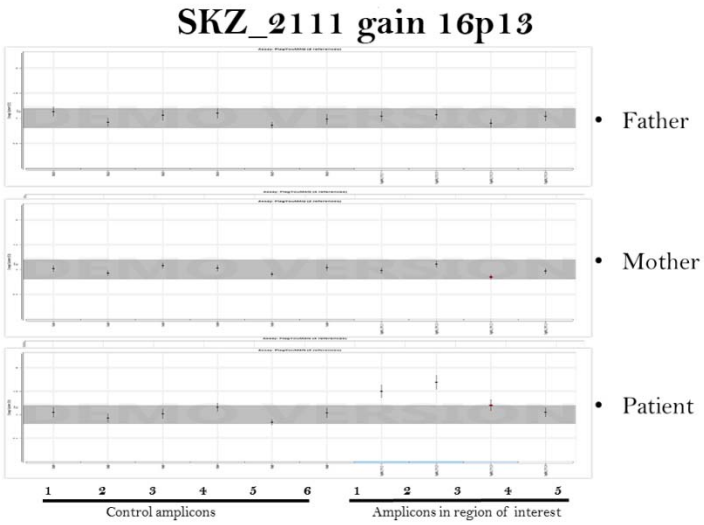
SKZ_0092; loss 15q11



One polymorphism , in the 15q11 region was detected with WES-CN. In the upper panel red dots indicate loss, two MAQ assay probes (red arrow) inside the deleted region, confirm this loss. SNP-array resolution was not enough to call this loss. This is not one of the 167 rare CNVs.

SKZ_2111; Gain 16p13.3(chr16:14,985,615-17,000,304)

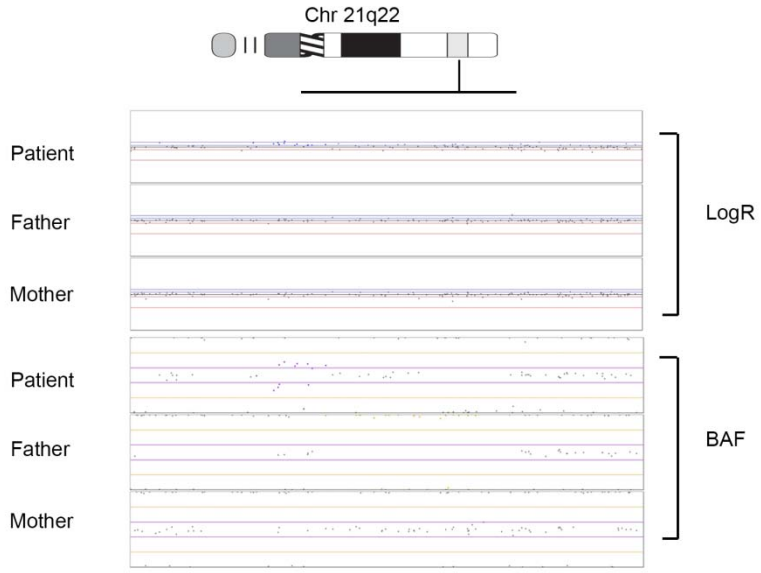


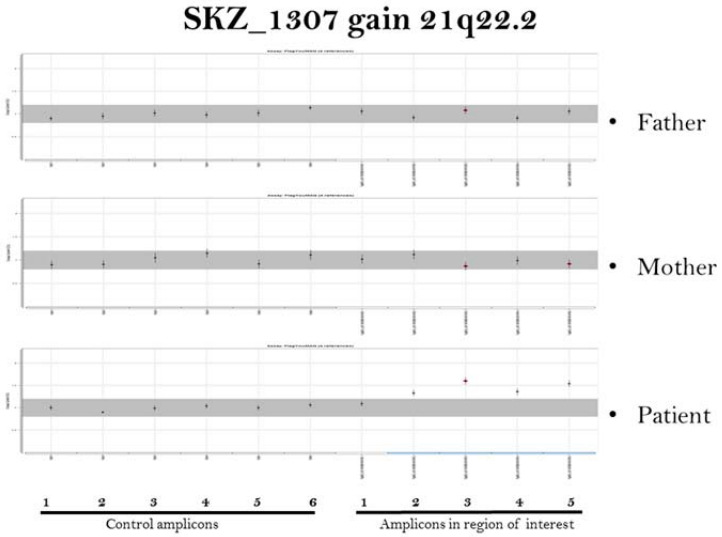


Gain in HG19:

Amplicon	Forward primer	Reverse primer	Size	Chr	Start	Stop
FlagMAQ_2111_2.1	5'AGCGGATAACAATTTCACACAGGCAGGGAGACACCATGCAGA-3'	5'-GTTTCTTGGTATGATGAGAGAGGTGAAGGA-3'	173	chr16	15141570	15141712
FlagMAQ_2111_2.2	5'AGCGGATAACAATTTCACACAGGGTCGACCCTTCCCAAACC-3'	5'-GTTTCTTTGCAATGGAAGATGAAGAGGA-3'	126	chr16	15586155	15586250
FlagMAQ_2111_2.3	5'AGCGGATAACAATTTCACACAGGCTGGGTGAAGAGGCCAAGT-3'	5'-GTTTCTTTGGCCACCCTACAGACAGA-3'	134	chr16	16061100	16061203
FlagMAQ_2111_2.4	5'AGCGGATAACAATTTCACACAGGTGGATGTGAGAAATGCCAAGT-3'	5'-GTTTCTTGGAGGATGAGGAGCAAACC-3'	216	chr16	16965277	16965462

SKZ_21q22.2; Gain 21q22.2 (chr21:40,095,493-40,160,135)





Amplicon	Forward primer	Reverse primer	Size	Chr.	Start	Stop
FlagMAQ_chr21:40100880-40154748.1	5'AGCGGATAACAATTTCACACAGGAACTTCGCCTCTTGGAACC-3'	5'-GTTTCTTAATTCCTCATCACATGGGTCA-3'	131	chr21	40102211	40102311
FlagMAQ_chr21:40100880-40154748.2	5'AGCGGATAACAATTTCACACAGGCTCCCAAATGCACTGAAATG-3'	5'-GTTTCTTAGGTGATAGAATGGGTGCTCTTA-3'	271	chr21	40117025	40117265
FlagMAQ_chr21:40100880-40154748.3	5'AG CGGATAACAATTTCACACAGGCAGGCAGACAGCTCACGTT-3'	5'-GTTTCTTAGAGAGGCACAGGCACAGA-3'	100	chr21	40125236	40125305
FlagMAQ_chr21:40100880-40154748.4	5'AGCGGATAACAATTTCACACAGGCTGCAAATACAGTGCAAACTGA-3'	5'-GTTTCTTTTCCTCCATCACTTTCTTTAACTTC-3'	122	chr21	40144161	40144252
FlagMAQ_chr21:40100880-40154748.5	5'AGCGGATAACAATTTCACACAGGCAGAGAGAGCAACAAACAATGC-3'	5'-GTTTCTTGCTGTGGTGATGAGGGCTA-3'	110	chr21	40154178	40154257

Supplementary methods of article entitled:

Copy Number Variations in 375 patients with oesophageal atresia and/or tracheoesophageal fistula.

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Study design

We assessed the CNVs according to the consensus statement for chromosomal micro-array analysis described by Miller and co-workers.[17] Our study design was based on the assumptions that CNVs are most likely to be causal for the abnormal phenotype in congenital anomalies if (I) a CNV is absent in large cohorts of unaffected individuals, (II) is absent in the unaffected parents of the affected individual and (III) and if it targets relevant genes or non-coding RNAs. Recurrence of loci affected by *de novo* CNVs in single cases could indicate loci harbouring genes mutated or otherwise affected in larger disease cohorts.

As OA/TOF disease frequency ranges between 2-3 in 10,000 live births (0.0002 to 0.0003), we classified CNVs to be rare if they were absent or present once in our in-house cohort of unaffected individuals. This cohort contains high-quality data of CNVs in autosomes (n=3,235 individuals) and sex chromosomes (n= 1,859 individuals). Overlap with one CNV in our in-house cohort reflects a population frequency of 0.0003 for autosomes and of 0.00054 for sex chromosomes. Inherited CNVs affect exactly the same locus as in their unaffected parent. As a single entity, these CNVs are therefore assumed not to be sufficient to give a severe phenotype. However, these CNVs might be of interest if present in more patients that share phenotypical characteristics, as they can act as a modifier in a multiple hit model, or as the second hit in a recessive condition. Special interest is given to shared anatomical malformations in addition to OA/TOF as these could hint at a specific sub-population in the cohort. Therefore, we also evaluated rare CNVs if they had overlapping loci in multiple patients with shared phenotypical characteristics.

Patient cohort

This study was approved by the institutional ethics committee of each participating centre, and was conducted in accordance with the principles of the Declaration of Helsinki. Patients with OA/TOF (isolated or non-isolated) were identified from the medical records. All patient records were reviewed by the treating physicians or geneticists of each participating centre. After retrieval of parental informed consent, blood was drawn from a total of 375 patients and their parents, comprising 239 patients from the Erasmus MC- Sophia, 28 from the Baylor College of Medicine, and 108 from a German multi-centre study regarding the genetic and environmental cause of OA/TOF ("The genetic risk for oesophageal atresia consortium [GREAT-consortium]"). Contact with patients and their families was established through their treating physicians or the German patient-support organization for patients with disorders of the oesophagus (KEKS e.V.; http://www.keks.org/). Previously, 68 patients of the Erasmus MC-Sophia and Baylor College of Medicine cohorts were evaluated in a study describing CNVs in VACTERL association.[16]

GTG-banded karyotyping and multiplex ligation-dependent probe amplification (MRC Holland, Amsterdam, the Netherlands) have ruled out the presence of large structural rearrangements

or aneuploidies in the majority of enrolled patients from the Erasmus MC- Sophia and the Baylor College of Medicine.[18] The German cohort was exclusively screened with micro-array. From 2009 onwards micro-array was the first tier-diagnostic tool used in the clinic, replacing GTG-banded karyotyping as a screening tool for large CNVs and aneuploidies. Patients born before 2009 were almost exclusively screened in a research setting and were enriched for the more complex non-isolated OA/TOF phenotypes. This causes over-representation for non-isolated OA/TOF patients in our cohort. Hence, over-representation of complex cases in this study is the result of selection bias and is not indicative for an actual deviance from the general isolated OA prevalence.

Literature search

We reviewed the literature (PubMed) in order to identify previously described disease-causing CNVs described in individuals with OA and / or TOF. Search terms included CNV, CNA (Copy Number Alteration), micro-array analysis, oesophageal atresia, tracheoesophageal fistula and VACTERL association.

DNA isolation

For the Dutch and American cohorts DNA for genomic analysis was extracted from peripheral blood, saliva and fibroblast cells with the Puregene DNA purification kit (Gentra Systems, USA) or the QIAamp DNA Blood Midi Kit (Qiagen, Inc., Hilden, Germany) or the Oragene DNA Kit. (DNA Genotek Inc., Ontario, Canada) DNA quality was evaluated with the Thermo Scientific NanoDrop 2000 (Thermo Fisher Scientific Inc., Waltham, USA), and dsDNA quantity with the Quant-iT™ PicoGreen ® dsDNA kit. (Invitrogen, Carlsbad, CA, USA). In the German Cohort DNA for genomic analysis was either extracted from peripheral blood or saliva, using the Chemagic Magnetic Separation Module I (ParkinElmer chemagen, Baesweiler, Germany) or the Oragene DNA Kit respectively.

Array-based molecular karyotyping

In the Dutch and American cohorts karyotyping was performed according to standard analytical methods. All DNA samples were tested for subtelomeric aberrations with Multiplex ligation-dependent probe amplification analysis, using the P036E1 and P070A2 Salsa telomere kits (MRC Holland, Amsterdam, and the Netherlands) as published previously. [1] High-resolution analyses for genomic imbalances present in the DNA of these patients were performed using single-nucleotide polymorphism (SNP) microarrays (Illumina Inc., San Diego, CA, USA and Affymetrix Inc. Santa Clara, CA, USA) and CGH oligonucleotide-based arrays (Agilent Inc., San Diego, CA, USA) using standard protocols. Micro-array analysis in the American and Dutch cohort was initially performed using three types of array chips (GeneChip Human Mapping 250K Nsp, 12-HumanCytoSNP DNA Analysis BeadChips v1-v2.1, and Agilent Human Genome CGH 105K and 244K), later these were replaced by chips with a higher resolution (Illumina Human 610-Quad Beadchip and Illumina HumanOmniExpress BeadChip, Illumina Infinium CytoSNP-850k BeadChip

and Agilent SurePrint G3 Human CGH 1M Oligo Microarray Kits G4411B/G4447). The majority of the Dutch cohort cases were processed on more than one array chip type. Material from all German patients enrolled in this study was screened using the HumanOmniExpress 12 v1.1 chip (Illumina Inc., San Diego, CA, USA).

Normalized output was generated using Feature Extraction software (v.9.1), alongside CGH Analytics software (v.3.3.28), with Affymetrix GTYPE (v.4.1, Affymetrix, Santa Clara, CA) or Illumina Genomestudio (v.2011.1, Illumina, San Diego, CA, USA), depending on chip type. CNVs in patient samples were visualized as log2-Ratios (Log2R) detected through comparison of patient probe intensity data with those of a virtual reference set of 270 HapMap samples of various ancestries (Illumina manifest file) or by comparison with sex matched controls of unaffected, unrelated individuals or reference DNA (Promega Corporation, Madison, WI). Since the German cohort was coherently processed on one chip type only, normalized output could be generated using the Genomestudio (v. 2011.1) genotyping module (v.1.9.4, Illumina, San Diego, CA, USA).

CNV filtering

For SNP-arrays, potential Copy Number Variants (CNVs) were estimated using a combination of allelic ratio (B-Allele Frequency, BAF) and SNP array copy number state (Log2R), utilizing Hidden-Markov-Model based algorithms. For this purpose Biodiscovery Nexus CN7.5 (Biodiscovery Inc, Hawthorne, CA, USA) was used for both cohorts. In the Nexus software, segmentation significance threshold was set at 5.0E-7 with a minimum of 5 probes per segment and a maximum probe spacing of 1,000 kb. The log2R-ratio thresholds were set at +0.18 (single copy gain), -0.18 (single copy loss), 0.4 (gain of two or more copies) and -1.1 (homozygous loss). The Homozygous Frequency/ Homozygous Value/ Heterozygous Imbalance Threshold were set at 0.95/0.8/0.4. The minimum LOH length was set at 100 kb and minimum SNP probe density, at 10 probes/Mb. Gender correction was used with a 3:1 sex chromosome gain threshold of 1.2 and a 4:1 sex chromosome gain threshold of 1.7. Log2R ratios of CGH-array results were determined with the ADM2 algorithm with filtering options of a minimum of 3 probes. (log2Ratio) >0.3. Each segment deviating from the normal situation was reviewed by visual inspection in Nexus CN 7.5. During the course of this study, the genome build switched from build hg18 to hg19. Not all arrays-chips could be re-processed or re-analyzed in the new genome build. For these array chips, the detected CNV regions were transformed using the UCSC lift-over tool and re-evaluated in the hg19 build for overlap with known polymorphisms (see below) and overlap with our in-house cohort (see below). Re-analysis of SNP-array in the new genome build, together with improved segmentation and waving correction algorithms resulted in the loss of several low confidence CNV calls.

Each observed CNV was compared to the frequency of that CNV at the particular locus in a modified version (i.e. excluding BAC arrays and small InDels) of the Database of Genomic Variants

(DGV, http://dgvbeta.tcag.ca/dgv/app/home?ref=NCBI36/hg19) incorporated in the Biodiscovery Nexus CN7.5 analysis package. Only those CNVs with a physical overlap with a DGV locus less than 70% and which occurred ≤5 times in the DGV were compared to an in-house control database of unrelated and unaffected parental samples and healthy control individuals. This cohort contains high-quality data of CNVs in autosomes (n=3235 individuals) and sex chromosomes (n= 1859 individuals). Overlap with one CNV in our in-house cohort reflects a population frequency of 0.0003 for autosomes and of 0.00054 for sex-chromosomes. We only considered CNVs which were either absent or present once for further analysis. All CNVs passing the filter criteria were evaluated manually by comparing the CNV to its presence in the most current versions of the database of genomic variation (DGV, http://dgv.tcag.ca/dgv/app/home), ISCA (http://dbsearch.clinicalgenome.org/search/), ClinGen (https://www.clinicalgenome.org/data-sharing/clinvar/) and DECIPHER. (http://decipher.sanger.ac.uk)

We searched for overlap in large CNV cohorts of control individuals published by Cooper *et al*.[2], Coe *et al*.[3] and Kaminsky et al.[4] We also evaluated the CNVs significantly different in these studies between patients and controls. As we used different array chips in this experiment, each with different marker spacing, distribution and content, we set a size CNV restriction threshold of minimum 30kb for single events, except when evaluating parental and patient SNP-arrays (trio analysis) . In trio analysis, all CNVs containing more than five probes were visually inspected using Nexus CN 7.5. We confirmed Copy Number (CN) state and inheritance pattern of private and rare CNVs if their size exceeded the restrictions thresholds and if the CNV contained genes or micro RNA's. All patient CNVs were visually re-evaluated using the Nexus software package e.g. we evaluated if CN state matched the allelic state of a CNV type.

Validation of microarray results

In the Dutch and American cohort, putative *de novo* events were confirmed via patient and parental copy number quantification using either additional SNP array, Fluorescence In Situ Hybridization (FISH) and/or Multiplex Amplicon Quantification (MAQ)(MultiplicoM N.V., Niel, Belgium) as well as real time quantitative PCR, the procedure also used for confirmation in the German cohort. At least two primer pairs for qPCR were designed within the putative CNVs using Primer Express Software v2.0 (Applied Biosystems, Foster City, CA, USA) for the Dutch cohort and Primer3web (v4.0.0.; http://primer3.ut.ee/)[5-7] for the German cohort. For the Dutch cohort the absence of SNPs in primer sequences was confirmed in dbSNP (build 135) and specificity of amplified region determined with the University of Santa Cruz (UCSC) *in silico* PCR and melting-curve analysis. Primer pairs with repeats in their resulting amplicon, according to CENSOR repeat masker[8] were excluded. Sequences for the German cohort were checked for SNPs and specificity using the UCSC human genome browser build 19 (http://genome.ucsc.edu).[9] QPCR experiments for the Dutch cohort were performed using a LightCycler 1.5 instrument and LightCycler FastStart DNA

Master SYBER Green I kit with C14ORF145 as a control locus. (Roche Molecular Diagnostics, Indianapolis, IN, USA) Locus exon 4 of the KIAA1279-gene was used as a control.[10] Cut-off values of <0.7 were used for deletions and values of >1.3 for duplications. For the German cohort QPCR experiments were performed on a LightCycler 480 II (Roche Molecular Diagnostics Indianapolis, IN, USA) in combination with Power SYBR Green Master Mix (Applied Biosystems, Foster City, USA). In order to normalize threshold cycle (Ct) values, each samples Ct value was compared to those of three reference genes (BCN1, CFTR, RNAseP subunit p38). Analysis was done as described by Scott *et al*.[11] In both cohorts each sample, including the no-template control (NTC) and control DNA, was run in triplicate.

For FISH confirmation, BAC-clones were selected from the UCSC genome browser and ordered from BACPAC Resources. After isolation of the BAC-DNA, the probes were amplified, labeled and used for FISH, according to standard protocols as described earlier.[12] The MAQ assay is a PCR-based-amplification method which uses 6 primer pairs on different loci for sample-internal copy number normalization, maximal 5 CNV specific primer pairs developed with the manufacturers' software package, and one type of FAM labeled primer specific to the sequence-tagged forward primers to amplify 20ng of dsDNA input. The DNA of 4 unaffected, unrelated individuals was used as sample-external copy number normalization. We amplified the DNA according to the manufacturer's protocol in a thermocycler with a heated lid, and analyzed the resulting FAM-labelled amplicons of patient, parental and controls using an automated sequencer (ABI 3730XL, Applied Biosystems, Foster City, CA, USA). This capillary electrophoresis step separates fragments on the basis of their length, these differences in amplicon length make multiplexing possible. The differences in fluorescence intensity reflect copy number state and were visualized in the MAQ-S analysis tool (Multiplicom Inc., Niel, Belgium) which compares amplicon size to the Genescan LIZ 500 size standard and normalizes copy number state to the internal amplicons and 4 external controls.

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Supplementary Table 1 Genomic coordinates and patient phenotypes of rare overlapping inherited CNVs

Internal	Type	Chr	Cytoband	Chromosome region HG19	Length	Genes affected	Inheritance	Classification
SKZ_1112	Gain	3	p14.1	chr3:64992802-65051455	58653	*ADAMTS9-AS2, BC040632*	no parental DNA	Uncertain
SKZ_0999	Gain	3	p14.1	chr3:64993928-65024494	30566	*ADAMTS9-AS2*	maternal	Uncertain : likely benign
DE82OSOUKBD100013	Loss	8	p23.2	chr8:3619348-3650542	31195	*CSMD1*	undetermined	Benign
DE27OSOUKBD100324	Loss	8	p23.2	chr8:4323965-4359306	35342	*CSMD1*	undetermined	Benign
DE37OSOUKBD100144	Loss	9	p24.1	chr9:8802640-8836439	33800	*PTPRD*	undetermined	Uncertain- likely benign
DE60OSOUKBD100312	Loss	9	p23	chr9:9919638-9990269	70632	*PTPRD*	undetermined	Uncertain- likely benign
DE63OSOUKBD100161	Gain	9	q34.3	chr9:138147997-138238503	90507	*C9orf62*	undetermined	Benign
DE63OSOUKBD100161	Gain	9	q34.3	chr9:138147997-138238503	90507	*C9orf62*	undetermined	Benign
DE14OSOUKBD100461	Loss	10	p12.33	chr10:18550958-18592873	41916	*CACNB2*	undetermined	Uncertain
DE37OSOUKBD100047	Loss	10	p12.33	chr10:18550958-18583866	32909	*CACNB2*	undetermined	Uncertain
SKZ_0773	Loss	11	q22.1	chr11:99497530-99583304	85774	*CNTN5*	undetermined	Benign
DE43OSOUKBD100133	Loss	11	q22.1	chr11:99519242-99568648	49407	*CNTN5*	undetermined	Benign
DE76OSOUKBD100315	Loss	11	q22.1	chr11:99519242-99568648	49407	*CNTN5*	undetermined	Benign
SKZ_1432	Loss	11	q22.1	chr11:99519242-99568648	49406	*CNTN5*	no parental DNA	Benign
DE35OSOUKBD100471	Gain	12	q24.12 - q24.13	chr12:112183225-112327166	143942	*ACAD10- MAPKAPK5*	undetermined	Benign
SKZ_1516	Gain	12	q24.12	chr12:112183225-112327166	143941	*ACAD10- MAPKAPK5*	maternal	Benign
148-04-01	Gain	12	q24.12	chr12:112183921-112318246	134325	*ACAD10- MAPKAPK5*	maternal	Benign
SKZ_1976	Gain	13	q31.3	chr13:92958981-93035128	76147	*GPC5*	maternal	Benign
SKZ_1746	Gain	13	q31.3	chr13:92979291-93049171	69880	*GPC5*	maternal	Benign
DE44OSOUKBD100309	Gain	15	q26.3	chr15:102325461-102373183	47723	*OR4F6, OR4F15*	undetermined	Benign
DE76OSOUKBD100315	Gain	15	q26.3	chr15:102332446-102368280	35835	*OR4F6, OR4F15*	undetermined	Benign
SKZ_2111	Gain	16	p13.11	chr16:14985615-17000304	2014689	*NOMO1-NPIP*	*de novo*	Uncertain : likely pathogenic
SKZ_1988	Gain	16	p13.11	chr16:15034035-15998820	964785	*NPIP-FOPNL*	paternal	Uncertain
SKZ_1150	Gain	16	p13.11	chr16:15539023-16291541	752518	*C16ORF45-ABCC6*	paternal	Uncertain
DE45OSOUKBD100291	Loss	16	q24.1	chr16:84429584-84490643	61060	*ATP2C2*	undetermined	Benign
SKZ_1500	Loss	16	q24.1	chr16:84435058-84470476	35418	*ATP2C2, KIAA0703*	maternal	Benign
DE94OSOUKBD100282	Gain	X	p22.33	chrX:370599-612414	241816	*SHOX*	undetermined	Uncertain- likely benign
SKZ_1508	Gain	X	p22.33	chrX:405941-596245	218468	*SHOX*	maternal	Uncertain- likely benign
SKZ_0374	Gain	X	p22.33	chrX:407015-1008051	788690	*SHOX*	maternal	Uncertain- likely benign

Casenr	Gender	Major anomalies
SKZ_1112	M	EA
SKZ_0999	M	EA/TEF
DE82OSOUKBD100013	F	EA, developmental delay
DE27OSOUKBD100324	F	EA, Atrial septum defect I, tracheomalacia
DE37OSOUKBD100144	M	EA
DE60OSOUKBD100312	F	EA, alopecia, Cafe-au-lait spots, eye anomalies
DE63OSOUKBD100161	F	EA, vesicourethral reflux, PUV
DE14OSOUKBD100461	F	EA
DE37OSOUKBD100047	M	EA, tracheomalacia
SKZ_0773	F	EA/TEF, vertebral anomalies, anal , genital and ear anomalies, renal anomalies, upper limb anomalies, cleft lip+jaw+palate, duodenal atresia
DE43OSOUKBD100133	F	EA, brochial anomalies
DE76OSOUKBD100315	F	EA, lung anomalies
SKZ_1432	M	EA/TEF, anal anomalies, renal anomalies, urethral fistula and atresia, genital anomalies
DE35OSOUKBD100471	M	Esophageal stenosis, achalasia
SKZ_1516	F	EA/TEF, ventricular septal defect, right sided aortic arch
148-04-01	F	TEF, ventricular septal defect, anal atresia and vertebral anomalies
SKZ_1976	M	EA/TEF, tracheo-laryngomalacia
SKZ_1746	M	EA/TEF
DE44OSOUKBD100309	F	EA, tracheomalacia
DE76OSOUKBD100315	F	EA, lung anomalies
SKZ_2111	M	TEF, anal anomaly, multiple VSDs, hypospadia
SKZ_1988	F	EA/TEF, anal anomalies,
SKZ_1150	F	EA/TEF + Atrio-ventricular septal defect
DE45OSOUKBD100291	F	EA, hypoplastic thumb, Atrial septum defect II, kidney anomalies, tracheomalacia
SKZ_1500	F	EA/TEF, anorectal anomaly,
DE94OSOUKBD100282	M	EA, tracheomalacia, scoliosis, undescended testicle
SKZ_1508	M	EA/TEF, upper limb anomalies Atrial septum defect, dysmorphisms

Internal	Type	Chr	Cytoband	Chromosome region HG19	Length	Inheritance	CNV-frequency cohort	multiple	ISCA	DGV database web	ClinGen CNV (likely) benign	Control group developmental delay	Authors classification
148-13-01	Loss	1	q24.2	chr1:168441289-168697617	256328	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1545	Loss	1	q25.2	chr1:178806664-181082264	2275600	Inheritance - Paternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE84OSOUKBD100074	Gain	1	q43	chr1:241155581-241186738	31158	Inheritance - Undetermined	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE83OSOUKBD100480	Gain	1	q44	chr1:246256444-246700236	443793	Inheritance - Undetermined	0.000	-	Benign	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1840	Loss	1	q32.3	chr1:213092201-213176992	84791	No parental DNA available	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain
DE21OSOUKBD100141	Gain	2	q21.1	chr2:131485402-132024166	538765	Inheritance - de novo	0.000	-	Uncertain	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign
SKZ_0708	Loss	2	q22.1	chr2:137827978-138371164	543186	Inheritance - Maternal	0.000	-	Uncertain- likely benign	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE82OSOUKBD100013	Gain	2	q14.2	chr2:121588391-121754337	165947	Inheritance - Undetermined	0.000	-	Uncertain- likely pathogenic	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_2051	Loss	2	q23.1	chr2:149089645-149192958	103313	Inheritance - Paternal	0.000	-	no comparable CNVs	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE69OSOUKBD100150	Loss	2	q21.2	chr2:133927604-133963658	36055	Inheritance - Undetermined	0.000	-	no comparable CNVs	Benign	no comparable CNVs	Uncertain- likely benign	Benign
SKZ_0739	Loss	2	p22.1	chr2:39982433-40037717	55284	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1307	Gain	2	p16.1	chr2:56277274-56381718	104444	Inheritance - Paternal	0.000	-	Uncertain	Benign	Benign	Uncertain- likely benign	Benign
DE54OSOUKBD100323	Gain	2	q37.1	chr2:233181492-233325849	144358	Inheritance - Undetermined	0.000	-	Benign	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_0832	Gain	2	p16.1	chr2:61271844-61368778	96934	Inheritance - Paternal	0.000	-	no comparable CNVs	no comparable CNVs	Benign	Uncertain- likely benign	Benign
SKZ_1381	Gain	2	p12	chr2:77199019-77249536	50517	Inheritance - Maternal	0.000	-	no comparable CNVs	Uncertain- likely benign	Benign	no comparable CNVs	Uncertain- likely benign
SKZ_1780	Loss	2	p12	chr2:78306103-78697833	391730	Inheritance - Maternal	0.000	-	Uncertain- likely benign	Benign	Uncertain- likely benign	Uncertain- likely benign	Benign
SKZ_0887	Loss	2	q37.1	chr2:231012029-231089979	77950	Inheritance - Paternal	0.000	-	Uncertain	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_2051	Gain	2	q35	chr2:218647714-219053933	406219	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain
DE61OSOUKBD100197	Gain	2	q13	chr2:111186302-113273657	2087356	Inheritance - Undetermined	0.000	-	Uncertain- likely benign	Uncertain	Benign	Uncertain- likely pathogenic	Uncertain- likely pathogenic
SKZ_0999	Gain	3	p14.1	chr3:64993928-65024494	30566	Inheritance - Maternal	0.000	1	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE62OSOUKBD100179	Loss	3	q22.2	chr3:134978716-135053549	74834	Inheritance - Undetermined	0.000	-	no comparable CNVs	Benign	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
DE26OSOUKBD100342	Loss	3	p14.2	chr3:62955270-62988884	43615	Inheritance - Undetermined	0.000	-	no comparable CNVs	Benign	no comparable CNVs	Benign	Benign
SKZ_1112	Gain	3	p14.1	chr3:64992802-65051455	58653	No parental DNA available	0.000	1	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain
DE07OSOUKBD100005	Loss	3	p12.3	chr3:76848167-76951689	103523	Inheritance - Undetermined	0.000	-	Uncertain	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_0374	Gain	3	q13.13	chr3:108616806-109014594	397788	Inheritance - Paternal	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE92OSOUKBD100221	Loss	3	p12.1	chr3:85561190-85612793	51604	Inheritance - Undetermined	0.000	-	Benign	Benign	Benign	Uncertain- likely benign	Benign
SKZ_1416	Gain	3	p12.3 - p12.2	chr3:80453558-83463345	3009787	Inheritance - Maternal	0.000	-	Uncertain	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign	Benign
DE12OSOUKBD100206	Gain	3	p26.1	chr3:8520585-8552850	32266	Inheritance - de novo	0.000	-	Uncertain	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain
SKZ_2032	Gain	3	p12.1	chr3:86387381-86615770	228389	Inheritance - Maternal	0.000	-	Uncertain	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_0367	Gain	3	q22.1	chr3:133034333-133089757	55424	No parental DNA available	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain
DE62OSOUKBD100179	Gain	4	q32.1	chr4:156745682-157287454	541773	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign
SKZ_1523	Gain	4	p15.32	chr4:16922258-17800667	878409	No parental DNA available	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE53OSOUKBD100147	Loss	4	q13.2	chr4:69668636-69713319	44684	Inheritance - Undetermined	0.000	-	no comparable CNVs	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1002	Loss	4	p16.1	chr4:7696394-7760459	64065	Inheritance - Paternal	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1248	Gain	4	q35.2	chr4:187540292-187849681	309389	Inheritance - Maternal	0.000	-	Uncertain	Uncertain	Uncertain- likely benign	Uncertain	Uncertain- likely pathogenic
DE05OSOUKBD100138	Loss	4	q28.1	chr4:125251081-128286629	3035549	Inheritance - Undetermined	0.000	-	Pathogenic	Pathogenic	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE69OSOUKBD100150	Gain	5	q35.1	chr5:171395554-171508587	113034	Inheritance - Undetermined	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE11OSOUKBD100224	Gain	5	q23.1	chr5:120513455-120921346	407892	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_0255	Loss	5	q31.1	chr5:131145181-131266601	121420	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1925	Gain	5	p14.1	chr5:25017057-25426009	408952	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1248	Gain	5	p15.1	chr5:16652934-16771046	118112	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1453	Gain	5	q12.1	chr5:59718323-59752640	34317	Inheritance - Maternal	0.000	-	no comparable CNVs	Benign	Uncertain- likely benign	Benign	Benign
SKZ_1800	Gain	5	p15.31 - p15.2	chr5:7198626-8305678	1107052	Inheritance - Maternal	0.000	-	Uncertain	Uncertain	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1032	Loss	5	q11.2	chr5:53784524-54985838	1201314	Inheritance - Paternal	0.000	-	Uncertain	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE85OSOUKBD100056	Gain	6	q16.3	chr6:101950970-102062025	111056	Inheritance - Undetermined	0.000	-	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
DE53OSOUKBD100050	Gain	6	q26	chr6:162201050-162260159	59110	Inheritance - Undetermined	0.000	-	no comparable CNVs	Benign	no comparable CNVs	Uncertain- likely benign	Benign
DE61OSOUKBD100294	Loss	6	p21.33	chr6:31969589-32007227	37639	Inheritance - Undetermined	0.000	-	Uncertain- likely benign	Benign	Benign	Benign	Benign
DE11OSOUKBD100127	Gain	6	p21.31	Chr6:35285720-35434273	148554	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1506	Loss	6	q23.2	chr6:134730991-135020544	289553	Inheritance - Paternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1856	Loss	6	p22.3	chr6:20884837-21082258	197421	No parental DNA available	0.000	-	no comparable CNVs	Uncertain- likely pathogenic	no comparable CNVs	no comparable CNVs	Uncertain- likely pathogenic
148-16-01	Loss	7	q34	chr7:142825643-142890809	65166	Inheritance - Maternal	0.000	-	no comparable CNVs	Benign	no comparable CNVs	Benign	Uncertain- likely benign
SKZ_1174	Gain	7	q35	chr7:143342375-143913713	571338	Inheritance - Maternal	0.000	-	Benign	Benign	Benign	Uncertain- likely benign	Benign
SKZ_1662	Gain	7	q36.3	chr7:157730598-157826278	95680	Inheritance - Paternal	0.000	-	Uncertain- likely benign	Benign	Uncertain- likely benign	Uncertain- likely benign	Benign
148-17-01	Loss	7	p22	chr7:6123758-6139997	16239	Inheritance - not Maternal	0.000	-	Benign	Benign	Benign	no comparable CNVs	Uncertain- likely benign
DE20OSOUKBD100450	Loss	7	q21.13	chr7:88645832-89092714	446883	Inheritance - Undetermined	0.000	-	Uncertain	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1035	Gain	7	p21.1	chr7:17243791-17590700	346909	Inheritance - Paternal	0.000	-	Uncertain	Benign	Benign	Benign	Benign
SKZ_1035	Gain	7	p14.1	chr7:40156332-40204960	48628	Inheritance - Paternal	0.000	-	no comparable CNVs	Benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_2111	Loss	7	q35q36.3	chr7:143839360-159138663	15299303	Inheritance - de novo	0.000	-	Pathogenic	no comparable CNVs	no comparable CNVs	no comparable CNVs	Pathogenic
DE82OSOUKBD100013	Loss	8	p23.2	chr8:3619348-3650542	31195	Inheritance - Undetermined	0.000	2	no comparable CNVs	Uncertain- likely benign	Benign	no comparable CNVs	Benign
DE27OSOUKBD100324	Loss	8	p23.2	chr8:4323965-4359306	35342	Inheritance - Undetermined	0.000	2	Uncertain- likely benign	Uncertain- likely benign	Benign	no comparable CNVs	Benign
DE18OSOUKBD100098	Loss	8	p23.1	chr8:10447116-10504819	57704	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign
DE92OSOUKBD100221	Loss	8	q13.3	chr8:71608781-71646728	37948	Inheritance - Undetermined	0.000	-	Uncertain	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_0426	Gain	8	q24.3	chr8:142438569-142482615	44046	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign	Benign
SKZ_1923	Gain	8	p23.3	chr8:1445011-1797072	352061	Inheritance - Paternal	0.000	-	no comparable CNVs	Benign	Benign	Uncertain- likely benign	Benign
SKZ_1810	Gain	8	p22	chr8:17592647-17859657	267010	Inheritance - de novo	0.000	-	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign
DE63OSOUKBD100161	Gain	9	q34.3	chr9:138147997-138238503	90507	Inheritance - Undetermined	0.000	4	Uncertain- likely benign	Benign	Benign	Uncertain- likely benign	Benign
DE63OSOUKBD100161	Gain	9	q34.3	chr9:138147997-138238503	90507	Inheritance - Undetermined	0.000	4	Uncertain- likely benign	Benign	Benign	Uncertain- likely benign	Benign
DE12OSOUKBD100303	Gain	9	q34.2	chr9:136850292-137153876	303585	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
DE37OSOUKBD100144	Loss	9	p24.1	chr9:8802640-8836439	33800	Inheritance - Undetermined	0.000	3	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE60OSOUKBD100312	Loss	9	p23	chr9:9919638-9990269	70632	Inheritance - Undetermined	0.000	3	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1825	Loss	9	p22.3	chr9:15300542-15381614	81072	Inheritance - Maternal	0.000	-	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign	Benign
DE37OSOUKBD100047	Loss	10	p12.33	chr10:18550958-18583866	32909	Inheritance - Undetermined	0.000	5	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE14OSOUKBD100461	Loss	10	p12.33	chr10:18550958-18592873	41916	Inheritance - Undetermined	0.000	5	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign

Internal	Type	Chr	Cytoband	Chromosome region HG19	Length	Inheritance	CNV-frequency cohort	multiple	ISCA	DGV database web	ClinGen CNV (likely) benign	Control group developmental delay	Authors classification
DE60OSOUKBD100118	Gain	11	q22.1	chr11:101397368-101472351	74984	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_2039	Gain	11	q22.3	chr11:103971318-104028957	57639	No parental DNA available	0.000	-	Uncertain	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_2026	Loss	11	p15.4	chr11:1115899-1261163	145264	No parental DNA available	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE63OSOUKBD100355	Gain	11	p15.2	chr11:14865608-14906548	40941	Inheritance - Undetermined	0.000	-	Uncertain	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1441	Loss	11	p12	chr11:40567422-40660470	93048	Inheritance - Undetermined	0.000	-	Benign	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1810	Loss	11	p14.3	chr11:21836986-22032466	195480	Inheritance - de novo	0.000	-	Uncertain	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_0773	Loss	11	q22.1	chr11:99497530-99583304	85774	Inheritance - Undetermined	0.001	6	no comparable CNVs	Benign	Benign	Uncertain- likely benign	Benign
DE43OSOUKBD100133	Loss	11	q22.1	chr11:99519242-99568648	49407	Inheritance - Undetermined	0.001	6	no comparable CNVs	Benign	Benign	Uncertain- likely benign	Benign
DE76OSOUKBD100315	Loss	11	q22.1	chr11:99519242-99568648	49407	Inheritance - Undetermined	0.001	6	no comparable CNVs	Benign	Benign	Uncertain- likely benign	Benign
SKZ_1432	Loss	11	q22.1	chr11:99519242-99568648	49406	No parental DNA available	0.001	6	no comparable CNVs	Benign	Benign	Uncertain- likely benign	Benign
SKZ_0400	Gain	11	q13.5	chr11:76268060-76417696	149636	Inheritance - Paternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1415	Loss	11	q14.2 - q14.3	chr11:87099219-91921385	4822166	Inheritance - Maternal	0.000	-	Uncertain	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1855	Gain	11	p15.4	chr11:4371631-5253127	881496	No parental DNA available	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely pathogenic
DE04OSOUKBD100156	Gain	12	q24.11	chr12:109549884-109667024	117141	Inheritance - Undetermined	0.000	-	Benign	Uncertain- likely benign	Benign	Uncertain- likely benign	Benign
DE35OSOUKBD100471	Gain	12	q24.12 - q24.13	chr12:112183225-112327166	143942	Inheritance - Undetermined	0.000	7	Benign	Uncertain- likely benign	Benign	Uncertain- likely benign	Benign
SKZ_0999	Gain	12	p13.2	chr12:10423943-10552714	128771	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1845	Gain	12	q24.31	chr12:123596909-123989546	392637	No parental DNA available	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_0406	Gain	12	q24.32	chr12:127488730-127837052	348322	Inheritance - Undetermined	0.000	-	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign
SKZ_0703	Gain	12	q24.33	chr12:133199968-133337730	137762	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1506	Gain	12	p13.33	chr12:706022-1125143	419121	Inheritance - Undetermined	0.000	-	Uncertain	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1516	Gain	12	q24.12	chr12:112183225-112327166	143941	Inheritance - Maternal	0.000	7	Benign	Benign	Benign	Uncertain- likely benign	Benign
148-04-01	Gain	12	q24.12	chr12:112183921-112318246	134325	Inheritance - Maternal	0.000	7	Benign	Benign	Benign	Uncertain- likely benign	Benign
DE76OSOUKBD100315	Gain	12	q22	chr12:95530434-95627379	96946	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1301	Gain	12	p13.31	chr12:8069848-8101856	32008	Inheritance - Maternal	0.000	-	Benign	Benign	Benign	Uncertain- likely benign	Benign
SKZ_1790	Loss	12	q21.31	chr12:83765377-84013252	247875	Inheritance - Maternal	0.000	-	no comparable CNVs	Uncertain	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE93OSOUKBD100203	Loss	13	q12.12	chr13:23803922-23853233	49312	Inheritance - Undetermined	0.000	-	Uncertain	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1497	Loss	13	q12.11	chr13:19612306-19679872	67566	Inheritance - Paternal	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE35OSOUKBD100471	Gain	13	q14.11	chr13:43146433-43187994	41562	Inheritance - Undetermined	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE67OSOUKBD100477	Gain	13	q14.11	chr13:44032193-44171911	139719	Inheritance - Undetermined	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_0876	Loss	13	q12.3	chr13:29714684-29828112	113428	Inheritance - Paternal	0.000	-	no comparable CNVs	Benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1840	Loss	13	q31.1	chr13:85909019-86064348	155329	No parental DNA available	0.000	-	Uncertain	Benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign
SKZ_1746	Loss	13	q21.31	chr13:64295459-64400173	104714	Inheritance - Paternal	0.000	-	Uncertain- likely benign	Benign	Uncertain- likely benign	Uncertain- likely benign	Benign
SKZ_1976	Gain	13	q31.3	chr13:92958981-93035128	76147	Inheritance - Maternal	0.000	8	no comparable CNVs	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1746	Gain	13	q31.3	chr13:92979291-93049171	69880	Inheritance - Maternal	0.000	8	no comparable CNVs	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1662	Loss	13	q12.11	chr13:22688792-22981935	293143	Inheritance - de novo	0.000	-	no comparable CNVs	no comparable CNVs	Benign	Uncertain- likely benign	Uncertain
DE91OSOUKBD100336	Gain	14	q11.2	chr14:21353205-21421877	68673	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE86OSOUKBD100232	Gain	14	q12	chr14:24947323-24986166	38844	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE92OSOUKBD100221	Loss	14	q21.2	chr14:44928342-45378475	450134	Inheritance - Undetermined	0.000	-	Uncertain	Uncertain	Benign	Uncertain- likely benign	Benign
DE14OSOUKBD100461	Loss	15	q21.3	chr15:57711740-57749246	37507	Inheritance - Undetermined	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE44OSOUKBD100309	Gain	15	q26.3	chr15:102325461-102373183	47723	Inheritance - Undetermined	0.000	9	Uncertain- likely benign	Uncertain- likely benign	Benign	Uncertain- likely benign	Benign
DE76OSOUKBD100315	Gain	15	q26.3	chr15:102332446-102368280	35835	Inheritance - Undetermined	0.000	9	Uncertain- likely benign	Uncertain- likely benign	Benign	Uncertain- likely benign	Benign
SKZ_2032	Loss	15	q11.2	chr15:22665220-23147419	482199	Inheritance - not Maternal	0.000	-	Uncertain	Benign	Benign	Uncertain- likely benign	Benign
SKZ_0845	Gain	15	q21.3	chr15:55354493-55956320	601827	Inheritance - Maternal	0.000	-	Uncertain	Uncertain	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1500	Loss	15	q24.2 - q24.3	chr15:76524284-76625437	101153	Inheritance - Maternal	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_0856	Loss	15	q13.3	chr15:32457092-32771537	314445	Inheritance - Paternal	0.000	-	Uncertain- likely pathogenic	Pathogenic	no comparable CNVs	Uncertain- likely benign	Uncertain- likely pathogenic
DE62OSOUKBD100179	Gain	16	q12.1 - q12.2	chr16:52464934-52676323	211390	Inheritance - Undetermined	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1150	Gain	16	p13.11	chr16:15539023-16291541	752518	Inheritance - Paternal	0.000	10	Uncertain	Uncertain	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely pathogenic
SKZ_1988	Gain	16	p13.11	chr16:15034035-15998820	964785	Inheritance - Paternal	0.000	10	Uncertain	Uncertain	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely pathogenic
SKZ_1381	Gain	16	p13.2	chr16:7303708-7357294	53586	Inheritance - Paternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_0680	Loss	16	q23.2	chr16:81234781-81287239	52458	Inheritance - Maternal	0.000	-	Uncertain- likely benign	Benign	Uncertain- likely benign	Uncertain- likely benign	Benign
DE45OSOUKBD100291	Loss	16	q24.1	chr16:84429584-84490643	61060	Inheritance - Undetermined	0.000	11	no comparable CNVs	Benign	no comparable CNVs	Uncertain- likely benign	Benign
SKZ_1500	Loss	16	q24.1	chr16:84435058-84470476	35418	Inheritance - Maternal	0.000	11	no comparable CNVs	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
DE52OSOUKBD100262	Gain	16	q24.2	chr16:87738873-87812644	73772	Inheritance - Undetermined	0.000	-	Uncertain	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_2111	Gain	16	p13.11	chr16:14985615-17000304	2014689	Inheritance - de novo	0.000	10	Uncertain	Uncertain- likely pathogenic	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely pathogenic
DE08OSOUKBD100084	Loss	17	p11.2	chr17:17316541-17349835	33295	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	Benign	Uncertain- likely benign	Benign
SKZ_2032	Loss	17	p11.2	chr17:19417785-19579313	161528	Inheritance - not Maternal	0.000	-	Benign	Benign	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
DE13OSOUKBD100285	Loss	17	p11.2	chr17:20051012-20592172	541161	Inheritance - Undetermined	0.000	-	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign
DE89OSOUKBD100081	Loss	17	p13.2	chr17:3505638-3559746	54109	Inheritance - Undetermined	0.000	-	Benign	Benign	no comparable CNVs	Benign	Benign

Internal	Type	Chr	Cytoband	Chromosome region HG19	Length	Inheritance	CNV-frequency cohort	multiple	ISCA	DGV database web	ClinGen CNV (likely) benign	Control group developmental delay	Authors classification
SKZ_1489	Gain	17	p13.3	chr17:735198-797669	62471	No parental DNA available	0.000	-	no comparable CNVs	Uncertain- likely benign	Benign	Uncertain- likely benign	Benign
SKZ_1003	Loss	17	q11.2	chr17:25972552-26066964	94412	Inheritance - Maternal	0.000	-	no comparable CNVs	Uncertain	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1746	Gain	18	q11.2	chr18:22883781-23015036	131255	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
SKZ_0369	Loss	18	q12.2	chr18:34546490-34698627	152137	Inheritance - Paternal	0.000	-	Uncertain	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1130	Gain	19	q13.41	chr19:53647182-53693873	46691	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE61OSOUKBD100294	Loss	19	q13.42	chr19:55434213-55486661	52449	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE67OSOUKBD100477	Loss	20	p13	chr20:113651-184813	71163	Inheritance - Undetermined	0.000	-	no comparable CNVs	Benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_2032	Loss	20	p12.1	chr20:15062923-15103156	40233	Inheritance - Maternal	0.000	-	Uncertain- likely benign	Benign	Benign	Benign	Benign
SKZ_1679	Loss	20	p13	chr20:3857650-3900324	42674	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE92OSOUKBD100221	Loss	20	p13	chr20:4931970-4972088	40119	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign
SKZ_1977	Loss	20	q11.23	chr20:36905828-36972896	67068	Inheritance - Maternal	0.000	-	no comparable CNVs	Benign	Benign	Uncertain- likely benign	Benign
DE83OSOUKBD100480	Gain	21	q21.1	chr21:19622822-19810737	187916	Inheritance - Undetermined	0.000	-	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign
DE02OSOUKBD100095	Gain	21	q22.12	chr21:37487858-37598197	110340	Inheritance - Undetermined	0.000	-	Benign	Uncertain- likely benign	Benign	Benign	Benign
SKZ_1307	Gain	21	q22.2	chr21:40100880-40154748	53868	Inheritance - de novo	0.000	-	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely benign
SKZ_1825	Loss	22	q13.33	chr22:50645586-50949482	303896	Inheritance - not Maternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain
DE84OSOUKBD100074	Gain	22	q13.2	chr22:43090481-43161021	70541	Inheritance - Undetermined	0.000	-	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
DE37OSOUKBD100144	Loss	22	q12.1	chr22:26838242-26881031	42790	Inheritance - maternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Uncertain- likely benign
SKZ_1780	Gain	22	q11.21	chr22:18637139-20289862	1652723	Inheritance - Maternal	0.000	-	Pathogenic	Pathogenic	Uncertain- likely benign	Uncertain- likely benign	Uncertain- likely pathogenic
DE29OSOUKBD100191	Gain	X	q13.1	chrX:71537559-71609005	71447	Inheritance - Undetermined	0.000	-	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
SKZ_1743	Gain	X	p22.31	chrX:8075662-8148705	73043	Inheritance - Undetermined	0.000	-	Uncertain	Uncertain- likely benign	Benign	no comparable CNVs	Benign
DE86OSOUKBD100232	Gain	X	q28	chrX:154873222-155010594	137373	Inheritance - Undetermined	0.000	-	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
DE46OSOUKBD100176	Gain	X	p22.2	chrX:13354873-13493086	138214	Inheritance - Undetermined	0.000	-	Benign	Uncertain- likely benign	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
SKZ_2027	Gain	X	p22.33	chrX:1427577-1592679	165102	Inheritance - Maternal	0.000	-	Uncertain- likely benign	Uncertain	Benign	no comparable CNVs	Uncertain- likely benign
SKZ_0374	Gain	X	p22.33	chrX:636950-820594	183644	Inheritance - Paternal	0.000	-	Uncertain	Uncertain	Benign	no comparable CNVs	Uncertain- likely benign
SKZ_1508	Gain	X	p22.33	chrX:417624-636092	218468	Inheritance - Maternal	0.000	12	Uncertain	Uncertain- likely benign	Benign	no comparable CNVs	Uncertain- likely benign
DE94OSOUKBD100282	Gain	X	p22.33	chrX:370599-612414	241816	Inheritance - Undetermined	0.000	12	Uncertain	Uncertain- likely benign	Benign	no comparable CNVs	Uncertain- likely benign
SKZ_0887	Gain	X	p22.33	chrX:2059627-2314248	254621	Inheritance - Maternal	0.000	-	Uncertain- likely benign	Uncertain	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
DE27OSOUKBD100227	Gain	X	p22.31	chrX:8219053-8484026	264974	Inheritance - Undetermined	0.000	-	Uncertain	Uncertain- likely benign	Benign	no comparable CNVs	Benign
SKZ_0680	Nullizygous	X	p22.2	chrX:10299643-10638042	338399	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	no comparable CNVs	no comparable CNVs	Pathogenic
SKZ_1855	Gain	X	q22.3	chrX:105098359-105625090	526731	No parental DNA available	0.000	-	no comparable CNVs	Uncertain- likely benign	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
SKZ_0887	Gain	X	q26.1	chrX:129681013-130305495	624482	Inheritance - Maternal	0.000	-	no comparable CNVs	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
SKZ_1932	Gain	X	p11.3	chrX:44073185-44716100	642915	Inheritance - Undetermined	0.000	-	Uncertain	no comparable CNVs	Uncertain- likely benign	no comparable CNVs	Uncertain- likely benign
SKZ_0374	Gain	X	p22.33	chrX:426736-1215426	788690	Inheritance - Paternal	0.000	12	Uncertain	Uncertain- likely benign	Benign	no comparable CNVs	Uncertain- likely benign

Sample	Cytoband	CNV	(Candidate) genes	Phenotypes	Validation procedure	Classification
DE21OSOUKBD100141	2q21.1	hg19 chr2:g.(131349899_131486169)_(131991166_132057166)dup	Multiple incl. *POTEJ, GPR148, AMER3, ARHGEF4, FAM168B, PLEKHB2*	OA/TOF	qPCR	Uncertain-likely benign
DE12OSOUKBD100206	3p26.1	hg19 chr3:g.(8519329_8521841)_(8551649_8554052)dup	*LMCD1, LMCD1-AS1*	OA/TOF, tracheomalacia	qPCR	Uncertain
SKZ_2111	7q35q36.3	hg19 chr7:g.(143820444_143839360)_(159119486_159138663)del	Multiple incl. *SHH, MNX1, SLC4A2*	TOF, abnormally placed anus, hypospadias, ventricle septal defects	FISH	Pathogenic
SKZ_1810	8p22	hg19 chr8:g.(17613606_17625479)_(17613606 _17844353)dup	*FGL1, LFIRE1, MTUS1, PCM1*	OA/TOF, aplastic left lung, Anencephaly, craniorachyschisis, kyphosis, ventricular septal defect, pulmonary	MAQ-assay	Uncertain-likely benign
SKZ_1810	11p14.3	hg19 chr11:g.(21600567_21853276)_(22015682_22016670)del	-	stenosis, radial club hand, hypoplastic thumbs, horseshoe kidney, broad thorax	MAQ-assay	Uncertain-likely benign
SKZ_1662	13q12.11	hg19 chr13:g.(22685293_22688792)_(22978753_22981935)del	*AK054845, LINC00540*	OA/TOF, tracheal stenosis, abnormal sacrum and kidney abnormality	MAQ-assay	Uncertain
SKZ_2111	16p13.11	hg19 chr16:g.(14985615_15156180)_(16289532_17000304)dup	Multiple	TOF, abnormally placed anus, hypospadias, ventricle septal defects	MAQ-assay	Uncertain-likely pathogenic
SKZ_1307	21q22.2	hg19 chr21:g.(40099972_40100880)_(40152372_40154748)dup	*LINC00114*	OA/TOF, anal stenosis and long toes and fingers	MAQ-assay	Uncertain-likely benign

Cytoband	CNV	minimum Length (bp)	(Candidate) genes	Phenotypes	Reference
	hg19 chr1:g.(?_217879151)_(218010441_?)dup	131290	*SPATA17*	OA, butterfly vertebra, anal atresia without fistula, atrial and ventricular septal defects,	
1q41	hg19 chr8:g.(?_144940222)_(145060112_?)dup	119890	*PLEC-1, PARP10*	bilateral dystopic kidneys, left-sided renal dysplasia	21
2q33.2q35	hg19 chr2:g.(?_204,394,564)–(219,189,331_?)del	14794767	*MAP2*	OA, agenesis of umbilical artery, multiple brain abnormalities, tapered fingers , clinodactyly of the fifth fingers, deviated feet	22
3q28	hg19 chr3:g.(?_187913191)_(188468682_?)del	555491	*LPP*	OA/TOF, Tetralogy of Fallot, rib anomalies, hypospadias, small kidneys	23
7q35q36	hg19 chr7:g.(?_147683847)_(159088636_?)del	11404789	*SHH, MNX1*	oesophageal stenosis, microcephaly, facial dysmoprphism hypoplasia of the lower lumbar vertebrae, lumbosacral hyperlordosis, partial agenesis of sacrum, distended bladder, right ectopic kidney, developmental delay	24,25
11q13.1q13.2	hg19 chr11:g.(?_65508902)_(67473140_?)del	1964238	*GSTP1*	OA, facial dysmorphism, developmental delay	26
17q12	hg19 chr17:g.(?_34727386)_(36297053_?)dup	1569667	*AATF, TADA2L, HNF1B*	OA/TOF, anal atresia, sacral bone defect, cryptorchidism	27
20q13.33	hg19 chr20:g.(?_60238426)_(60895697_?)del	657271	*GTPBP5*	OA/TOF, ventricular septal defects, hypospadias, large unilateral hydrocele, large left cystic hygroma	28

Sample	Cytoband	CNV	Inheritance	Authors classification	Phenotype
DE61OSOUKBD100197	2q13	hg19 chr2:g.(111186302_111667198)_(113094793_113273657)dup	U	Uncertain- likely pathogenic	OA/TOF , sister as unilateral renal agenesis, both patient and sister are otherwise healthy
SKZ_1248	4q35.2	hg19 chr4:g.(187540292_187318091)_(187318091_187849681)dup	M	Uncertain- likely pathogenic	OA/TOF, pyloric stenosis; dysmorphisms, hearing loss
SKZ_1856	6p22.3	hg19 chr6:g.(20884837_20901311)_(20901267_21082258)del	NP	Uncertain- likely pathogenic	OA/TOF, septal defect, club foot
SKZ_1855	11p15.4	hg19 chr11:g.(4371631_4391231)_(5132119_5253127)dup	NP	Uncertain- likely pathogenic	OA/TOF
SKZ_0856	15q13.3	hg19 chr15:g.(32457092_32457092)_(32514341_32771537)del	P	Uncertain- likely pathogenic	OA/TOF, Anal atresia, bifid/fused ribs, coarctation, abnormal arterial supply right lung, abnormal sacrum
SKZ_1150	16p13.11	hg19 chr16:g.(15539023_15545022)_(16282307_16291541)dup	P	Uncertain- likely pathogenic	OA/TOF + Atrio-ventricular septal defect
SKZ_1988	16p13.11	hg19 chr16:g.(15034035_15092778)_(15998820_16106095)dup	P	Uncertain- likely pathogenic	OA/TOF, anal anomalies
SKZ_1780	22q11.21	hg19 chr22:g.(18637139_18640300)_(20286099_20289862)dup	M	Uncertain- likely pathogenic	OA/TOF, Anal atresia, ventricular septal defect
SKZ_0680	Xp22.2	hg19 chrX:g.(10299643_10302384)_(10637327_10638042)del	M	Pathogenic	Laryngo-tracheo-oesophageal-cleft, hypospadias, dysmorphims, hypotonia, pyloric stenosis; Opitz syndrome