Supplementary discussion of article entitled:

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

Authors and affiliations

Erwin Brosens[1,2]*,& Florian Marsch[3]*, Elisabeth M. de Jong[1,2], Hitisha P. Zaveri[4], Alina C. Hilger[3], Vera Gisela Choinitzki[3], Alice Hölscher[5], Per Hoffmann[3, 6], Stefan Herms[3, 6], Thomas M. Boemer[5], Benno M. Ure[7], Martin Lacher[7], Michael Ludwig[8], Bert H. Eussen[1], Robert M. van der Helm[1], Hannie Douben[1], Diane van Opstal[1], Rene M.H. Wijnen[2], H. Berna Beverloo[1], Yolande van Bever[1], Alice S. Brooks[1], Hanneke IJsselstijn[2], Daryl A. Scott[4], Johannes Schumacher[3], Dick Tibboel[2], Heiko Reutter[3,*], Annelies de Klein[1*]

*[1]Department of Clinical Genetics, Erasmus Medical Centre – Sophia Children's Hospital, Rotterdam, the Netherlands. [2]Department of Pediatric Surgery, Erasmus Medical Centre – Sophia Children's Hospital, Rotterdam, the Netherlands. [3]Institute of Human Genetics, University of Bonn, Bonn, Germany. [4]Department of Molecular and Human Genetics and [5]Departments of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas, USA. [5]Department of Pediatric Surgery and Urology, Children's Hospital of Cologne, Cologne, Germany [6]Human Genomics Research Group, Department of Biomedicine, University Hospital and University of Basel, Basel, Switzerland. [7]Center of Pediatric Surgery Hannover, Hannover Medical School and Bult Children's Hospital, Hannover, Germany. [8]Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Bonn, Germany. [9]Department of Neonatology and Pediatric Intensive Care, University of Bonn, Bonn, Germany. *authors contributed equally to the manuscript*

***authors contributed equally to the manuscript**

Corresponding Author:

#E. Brosens PhD

Erasmus Medical Centre – Sophia Children's Hospital

Room Ee971

P.O. Box 2040

3000 CA Rotterdam

Tel +31 10 70 37643

Fax: +31 10 70 44736

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.

References

1. Barnes, C., et al., *A robust statistical method for case-control association testing with copy number variation.* Nat Genet, 2008. **40**(10): p. 1245-52.
2. Cooper, G.M., et al., *A copy number variation morbidity map of developmental delay*. Nat Genet, 2011. **43**(9): p. 838-46.
3. van Bon, B.W., et al., *Further delineation of the 15q13 microdeletion and duplication syndromes: a clinical spectrum varying from non-pathogenic to a severe outcome.* (1468-6244 (Electronic)).
4. Coe, B.P., et al., *Refining analyses of copy number variation identifies specific genes associated with developmental delay*. (1546-1718 (Electronic)).