European Journal of Medical Genetics 58 (2015) 129–133

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Clinical research

No evidence for mosaic pathogenic copy number variations in cardiac tissue from patients with congenital heart malformations

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ARTICLE INFO

Article history:
Received 16 November 2014
Accepted 10 January 2015
Available online 31 January 2015

Keywords:
Array CGH
Congenital heart malformations
Copy number variation
Mosaicism

ABSTRACT

The aim of this study was to investigate if pathogenic copy number variations (CNVs) are present in mosaic form in patients with congenital heart malformations. We have collected cardiac tissue and blood samples from 23 patients with congenital heart malformations that underwent cardiac surgery and screened for mosaic gene dose alterations restricted to cardiac tissue using array comparative genomic hybridization (array CGH). We did not find evidence of CNVs in mosaic form after array CGH analysis. Pathogenic CNVs that were present in both cardiac tissue and blood were detected in 2/23 patients (9%), and in addition we found several constitutional CNVs of unclear clinical significance. This is the first study investigating mosaicism for CNVs in heart tissue compared to peripheral blood and the results do not indicate that pathogenic mosaic copy number changes are common in patients with heart malformations. Importantly, in line with previous studies, our results show that constitutional pathogenic CNVs are important factors contributing to congenital heart malformations.

1. Introduction

Congenital heart malformations are the most commonly diagnosed birth defects, with potential severe effects on morbidity and mortality and a live birth incidence of around 0.8% [Khoshnood et al., 2012]. Substantial advances in cardiac surgery procedures and pediatric intensive care have increased short- and long-term survival for patients with congenital heart malformations as well as the demand for knowledge on molecular diagnosis and recurrence risk from patients planning to start their own families. The possibilities for genetic diagnostics have improved with the development of chromosomal microarrays and massive parallel sequencing and genetic alterations such as chromosomal aberrations, recurrent and rare copy number variants (CNVs) and single gene mutations are known causative factors [Glessner et al., 2014; Greenway et al., 2009; Lalani et al., 2013; Soemedi et al., 2012; Thienpont et al., 2007]. Despite recent technological advances a majority of patients does not receive an etiologic diagnosis.

One hypothesis that could explain the low diagnostic yield of clinical genetic investigations in patients with congenital heart malformations is somatic mosaicism for causative genetic aberrations. Somatic mosaicism describes the situation with a genetic abnormality being present in a subset of cells in an individual who has developed from a single fertilized egg, and it is the result of a post-zygotic genetic event. The low proportion of cells carrying the mutation in combination with the possible tissue-restriction requires sensitive screening methods as well as collection of appropriate material for DNA isolation in order to identify this type of causative aberration. With increasing resolution in analysis methods, there is emerging evidence that mosaic forms of different types of genetic aberrations are more common than previously believed [Campbell et al., 2014; Pham et al., 2014]. Specifically, mosaicism for CNVs has been found in multiple tissues including

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http://dx.doi.org/10.1016/j.ejmg.2015.01.003
1769-7212/

heart, and detected in patients with developmental disorders [Burrage et al., 2013; O'Huallachain et al., 2012; Palka et al., 2012; Piotrowski et al., 2008].

Somatic mutations in single genes restricted to cardiac tissue have been reported in patients with septal defects as well as in patients with hypoplastic heart phenotypes, where mutation screening of DNA from formalin-fixed cardiac tissue showed a high proportion of point mutations in cardiac disease genes [Reamon-Buettner et al., 2004; Reamon-Buettner et al., 2008], while these findings have not been confirmed by other studies when fresh frozen cardiac tissue was used for analysis [Draus et al., 2009; Esposito et al., 2011]. Notably, mutations in *GATA6* were recently reported to be detected in DNA isolated from freshly frozen cardiac tissue of 2/52 patients with sporadic tetralogy of Fallot, while no mutations were found in DNA from blood in the same individuals or in cardiac tissue of patients undergoing cardiac valve replacement due to rheumatic heart disease [Huang et al., 2013].

Since whole genome screening for copy number variants in cardiac tissue using array CGH has not previously been performed, the main objective of this study was to investigate if pathogenic somatic CNVs are present in cardiac tissue from patients with congenital heart malformations.

2. Materials and methods

2.1. Patients

Patients with congenital heart malformations that underwent cardiac surgery between 2008 and 2010 were recruited at the two centers performing pediatric cardiac surgery in Sweden; the Unit of Pediatric Cardiac Surgery at Queen Silvia Children's Hospital, Gothenburg, and the Pediatric Cardiac Surgery Unit, Children's Hospital at the University Hospital in Lund. Patients were included after informed parental consent in cases when the planned surgical procedure involved removal of cardiac tissue. During the surgical procedure, discarded tissue was directly placed in sodium chloride solution (9 g/L) and in addition, blood samples from the patients were collected. Altogether, samples from 28 patients were collected. After excluding five patients due to insufficient amounts of starting material (tissue/DNA) analysis was performed on 23 samples. Ethical approval for the study was obtained from the regional ethics committee at Karolinska Institutet, Stockholm, Sweden.

2.2. DNA extraction

Genomic DNA was isolated from heart tissue using the Gentra Puregene Blood Kit (QIAGEN Sciences, Maryland, USA) in combination with Proteinase K (Finnzymes, Espoo, Finland) with minor modifications to the manufacturer's protocol. Genomic DNA was isolated from peripheral blood samples and in one case from saliva (parents of patient P5) from patients and parents according to standard procedures.

2.3. Array CGH

Array CGH was performed using a whole genome coverage 180K oligonucleotide array from Oxford Gene Technology as described previously [Winberg et al., 2014]. DNA isolated from cardiac tissue was hybridized against sex-matched, pooled reference DNA derived from peripheral blood from 10 healthy controls.

For an overall screening analysis, CNVs with a minimum of three consecutive probes with deviating log2-ratios were identified. For detection of CNVs present in all cardiac cells, standard probe cut off levels used in routine diagnostics at the Department of Clinical Genetics, Karolinska University Hospital (log2-ratios above 0.35 for duplications and below −0.65 for deletions) were applied. To enable detection of putative mosaic CNVs in cardiac tissue, we used the circular binary segmentation algorithm of CytoSure Interpret software with the possibility to vary the thresholds for log2-ratios and aberration size. We used cutoff levels for the log2-ratio of >0.10 and <-0.10 (corresponding to a mosaicism level of approximately 15%) for duplications and deletions respectively, and a size limit of 5 Mb due to a high false positive rate for small aberrations. We also chose to visually inspect every chromosome for mosaic aberrations smaller than 5 Mb.

CNV calls were compared to variants in the general population from the Database of Genomic Variants (http://projects.tcag.ca/variation/) and patient data in DECIPHER (http://decipher.sanger.ac.uk/), ISCA (http://www.iscaconsortium.org), ECARUCA (http://www.ecaruca.net), OMIM (http://www.omim.org), publications indexed in PubMed and the array CGH database at the Department of Clinical Genetics, Karolinska University Hospital, comprising 3400 analyzed patient samples (3116 clinical samples and 284 research project samples). Detected CNVs overlapping with variants reported in several studies and individuals in the Database of Genomic Variants or frequently observed ($>0.5\%$) in the clinical database were considered likely to be benign. For the remaining CNVs pathogenicity classification was performed in line with published guidelines [Kearney et al., 2011].

To investigate the occurrence of genetic mosaicism in heart tissue, array CGH analyses using patient DNA isolated from blood were performed for patients in whom pathogenic CNVs or CNVs of unclear clinical significance >50 kb in size were detected in the initial tissue analysis. For analysis of parental samples, Cy3-labelled maternal samples were hybridized against Cy5-labelled paternal samples.

2.4. Fluorescent in situ hybridization (FISH)

Metaphase chromosome spreads and interphase nuclei were prepared from peripheral blood. FISH was performed on DAPI-stained chromosomes as described previously [Winberg et al., 2010]. For patient P4 an 18p-specific BAC clone, RP11-778P8 (Childrens Hospital Oakland Research Institute), was used in combination with a centromeric chromosome 18 specific probe and a subtelomeric probe specific to 22q (Aquaris probes).

3. Results

3.1. Patients

Clinical features of patients included in this study are presented in Table 1. Of 23 included patients, seven had congenital heart malformations and additional malformations while 16 patients had isolated heart malformations. A brother of the maternal grandfather of patient P16 had a heart transplant, although the diagnosis causing the transplant is unknown. Patient P20 and her mother both displayed bilateral congenital cataracts, but the mother did not have any known heart malformation.

3.2. Array-CGH and FISH analysis

Screening cardiac tissue-derived DNA identified pathogenic CNVs in two patients and variants of unclear significance (VOUS) in nine patients (Table 1).

In one boy (P15) a 4.3 Mb terminal deletion on 5p, del(5)(p15.33p15.33), and a 51 Mb terminal duplication of 8q, dup(8)(q22.1q24.3), were detected. The boy presented with ventricular septal defect (VSD) and pulmonary valve stenosis in combination with additional symptoms such as small penis, cryptorchidism,