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Genome-wide rare copy number variations contribute to genetic risk for transposition of the great arteries☆,☆☆

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ABSTRACT

Background: Transposition of the great arteries (TGA) is an uncommon but severe congenital heart malformation of unknown etiology. Rare copy number variations (CNVs) have been implicated in other, more common conotruncal heart defects like tetralogy of Fallot (TOF), but there are as yet no CNV studies dedicated to TGA.
Methods: Using high-resolution genome-wide microarrays and rigorous methods, we investigated CNVs in a group of prospectively recruited adults with TGA (n = 101) from a single center. We compared rare CNV burden to well-matched cohorts of controls and TOF cases, adjudicating rarity using 10,113 independent population-based controls and excluding all subjects with 22q11.2 deletions. We identified candidate genes for TGA based on rare CNVs that overlapped the same gene in unrelated individuals, and pre-existing evidence suggesting a role in cardiac development.
Results: The TGA group was significantly enriched for large rare CNVs (2.3-fold increase, p = 0.04) relative to controls, to a degree comparable with the TOF group. Extra-cardiac features were not reliable predictors of rare CNV burden. Smaller rare CNVs helped to narrow critical regions for conotruncal defects at chromosomes 10q26 and 13q13. Established and novel candidate susceptibility genes identified included *ACKR3*, *IFT57*, *ITGB8*, *KL*, *NF1*, *NKX1-2*, *RERE*, *SLC8A1*, *SOX18*, and *ULK1*.
Conclusions: These data demonstrate a genome-wide role for rare CNVs in genetic risk for TGA. The findings provide further support for a genetically-related spectrum of congenital heart disease that includes TGA and TOF.
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1. Introduction

Congenital heart disease (CHD) is the most common class of major malformations in humans [1,2]. The availability of karyotyping over the past few decades has allowed for the detection of gross chromosomal anomalies in a small minority of patients with CHD [3]. Later, several specific causal mutations, including chromosome 22q11.2 deletions, became discoverable using targeted testing [3]. With further advances in genetic technology, genome-wide submicroscopic structural variations in chromosomes (copy number variations; CNVs) are now emerging as collectively important contributors to tetralogy of Fallot (TOF) and other forms of CHD [4–18].

Transposition of the great arteries (TGA) is a rare life-threatening form of CHD of unknown etiology [19–22]. Shared underlying genetic susceptibility to clinically distinct forms of CHD like TOF is suggested by variable expression both within families [23–27], and of specific mutations like *NKX2-5* mutations [28,29] and 22q11.2 deletions [30,31]. Sporadic reports of chromosomal anomalies in subjects with TGA indicate that structural variation may be important [7,13,32,33], but there are no studies dedicated to genome-wide rare CNV in subjects with TGA.

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We showed previously that large rare CNVs – especially those overlapping exons of protein-coding genes – are enriched in adults with TOF relative to controls [4]. We hypothesized that a similar pattern and degree of enrichment would be observed in adults with TGA. We also anticipated finding evidence of shared genetic liability with TOF at the individual gene and pathway level. We used high-resolution microarrays and our established methods to characterize genome-wide rare CNV in a large group of well-phenotyped adults with TGA from a single center. The primary goal was to discover candidate loci and susceptibility genes for TGA and related CHD. A secondary goal was to explore potential phenotypic predictors and consequences of rare CNV burden across the lifespan.

2. Methods

2.1. Subjects

We prospectively recruited 101 unrelated adults [61 (60.4%) males] with TGA from a single clinic (Toronto Congenital Cardiac Centre for Adults; TCCCA). This included n = 83 [52 (62.7%) males] with complete TGA (dTGA) and n = 18 [9 (50.0%) males] with congenitally corrected TGA (ccTGA). TGA diagnosis was confirmed using an echocardiogram and/or cardiac catheterization together with review of other imaging and surgical data. Of the 83 with dTGA, 63 underwent a Mustard repair, 11 a Jatene repair, 5 a Rastelli repair, and 4 were either not repaired (n = 1) or had atypical repairs or palliation in the setting of more complex cardiac defects (n = 3). Ten of the 18 ccTGA defects were also repaired. Mean age at time of last contact with TCCCA or death (n = 10) for the entire cohort was 36.9 (SD 10.4) years.

All subjects underwent direct clinical screening for potential syndromic and extra-cardiac features, and available lifetime medical records were reviewed [4,34]. Subjects with documented syndromes, including 22q11.2 deletion syndrome, were excluded. Family and reproductive history data were collected as described elsewhere [23]. All phenotyping was done blind to genotype. Informed consent was obtained from each patient. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, and the study was approved by local institutional research ethics boards.

2.2. Comparison samples for formal analyses

To optimize our analyses, we used an independent Canadian control sample from the Ontario Population Genomics Platform (OPGP) genetic epidemiologic project that comprised 415 unrelated adults of European ancestry [207 (49.9%) males; mean age 44.9 (SD 12.0) years] [4,35,36]. We also used a Canadian sample of 433 unrelated adults with idiopathic TOF [239 (55.2%) males; mean age 32.6 (SD 12.3) years; 340 of European ancestry] [4]. These subjects were recruited in parallel with the TGA subjects through the TCCCA using an identical phenotyping approach, including exclusion of those with documented genetic syndromes [4,23,34]. All TOF and OPGP control samples were handled and experiments performed by the same laboratory using identical array methods and protocols, including CNV analyses and rarity assignation using separate large control cohorts, as for the TGA cases (see below).

2.3. Genotyping

We used our proven CNV pipeline to interrogate and adjudicate genome-wide CNV [4,35]. High quality genomic DNA derived from blood samples was genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0, which contains 1.8 million markers for CNV interrogation. CNV analysis and adjudication of all samples were performed at The Centre for Applied Genomics (Toronto, Canada). Arrays meeting Affymetrix-recommended quality control guidelines of contrast QC > 0.4 were used for further analysis, as outlined below.

2.4. Ancestry

In addition to self-reported ethnicities, genotypes of the TGA cases from 1120 genome-wide unlinked SNPs were clustered by the program STRUCTURE together with those from 270 HapMap samples, which were used as references of known ancestry during clustering. Ancestries were assigned with a threshold of coefficient of ancestry >0.9. Of the 101 TGA subjects, there were 89 of European, 11 of Admixed, and 1 of East Asian ancestry.

2.5. CNV determination, adjudication, and prioritization

Genome-wide CNVs were determined using a multiple-algorithm approach to maximize sensitivity and specificity of CNV calling [4,35]. For each subject we defined "stringent" CNV calls as those detected by at least two of three different CNV calling algorithms: Birdsuite [37], iPattern [38], and Affymetrix Genotyping Console, and spanning at least 10 kb in length and five or more consecutive array probes. Overlapping calls at the sample level from Birdsuite and iPattern were merged with the outside probe boundaries. Singleton calls from either iPattern or Birdsuite were included if they overlapped with an Affymetrix Genotyping Console call from the same sample. CNVs with ≥75% of their genomic extent consisting of segmental duplications were excluded. All subsequent analyses focused on the stringent CNVs, which in our experience have very high positive validation rates (>95%) by independent methods [4,35,39].

Each stringently defined CNV was then adjudicated for rarity by comparison to those CNVs identified in independent, population-based controls (Table S1) [40–46]. We adopted a conservative definition of rare CNVs, retaining only those CNVs present in <0.1% of these 10,113 population controls using 50% reciprocal overlap criteria [4,35,39]. All rare CNVs in the n = 101 subjects with TGA are reported in Table S2. Genomic coordinates in the text and supplemental files refer to GRCh37/hg19. Because of between-group differences in demographic features, for major analyses we used only autosomal CNVs in individuals of European ancestry.

Large CNVs were defined as those >500 kb in size [4,35]. In addition to our TGA and TOF CNV datasets, we systematically reviewed and considered other published sources of CNV data in CHD. We then prioritized smaller rare CNVs meeting the following criteria for more detailed examination: (i) those that overlapped the same heart-related gene (i.e., one associated with any cardiac phenotype in human or model organism) in unrelated individuals with CHD, at least one having TGA, and (ii) those that overlapped a gene with pre-existing evidence suggesting a role in cardiac development.

2.6. Statistical methods

Statistical analysis and plotting were performed using the software R (http://www.r-project.org/). The main analyses compared rare autosomal CNV burden in the 89 TGA cases of European ancestry with those in the 340 TOF cases of European ancestry and 415 OPGP controls. Secondary analyses explored potential phenotypic predictors and consequences of rare CNV burden, including learning difficulties/special education, global dysmorphic facial features, family history of CHD in a first-to-third degree relative, sex-corrected growth parameters in adulthood, and reproductive fitness. Chi-square tests or Fisher's exact tests were used to compare categorical variables, and Student's t tests or Mann–Whitney U tests for continuous variables, as appropriate. All tests were two-sided, with statistical significance defined as $p < 0.05$.

3. Results

3.1. Rare CNV burden in TGA

We first compared the burden of large (>500 kb) rare CNVs in the 89 TGA cases of European ancestry and the OPGP controls. A significantly

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