ORIGINAL RESEARCH

Is Transposition of the Great Arteries Associated With Shortening of the Intrapericardial Portions of the Great Arterial Trunks? An Echocardiographic Analysis on Newborn Infants With Simple Transposition of the Great Arteries to Explore an Animal Model-Based Hypothesis on Human Beings

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BACKGROUND: The pathogenesis of transposition of the great arteries (TGA) as a congenital heart defect of the outflow tract with discordant ventriculoarterial connections remains an enigma. TGA usually have parallel great arteries suggesting that deficient torsion of the embryonic arterial heart pole might cause discordant ventriculoarterial connections. It has been speculated that deficient elongation of the embryonic outflow tract might prevent its normal torsion resulting in TGA. The aim of our study was to clarify whether the intrapericardial portions of the great arteries in human patients with TGA might be indeed shorter than in normal hearts.

METHODS AND RESULTS: Thirty-four newborns with simple TGA and 35 newborns with normal hearts were analyzed by using images of the outflow tract in their echocardiograms and the following defined lengths of the great arteries were measured: aortic length 1 (AoL-1) and aortic length 2 (AoL-2) = distance between left and right aortic valve level and origin of the brachiocephalic artery, respectively. Pulmonary trunk length 1 (PTL-1) and pulmonary trunk length 2 (PTL-2) = distance between left and right pulmonary valve level and origin of left and right pulmonary artery, respectively. All measurements of the AoL were significantly shorter in TGA compared to normal hearts (AoL-1: 1.6±0.2 versus 2.05±0.1; P<0.0001; AoL-2: 1.55±0.2 versus 2.13±0.1; P<0.0001). With regard to the pulmonary trunk (PT), PTL-1 and PTL-2 were found to be shorter and longer, respectively, in TGA compared with normal hearts, reflecting the differences in the spatial arrangement of the PT between the 2 groups as in TGA the PT is showing a mirror image of the normal anatomy. However, the overall length of the PT between the 2 groups did not differ.

CONCLUSIONS: Our data demonstrate that, compared with normal newborns, the ascending aorta is significantly shorter in newborns with TGA whereas the overall length of the PT does not differ between the 2 groups. This finding is in accord with the animal model-based hypothesis that TGA may result from a growth deficit at the arterial pole of the embryonic heart.

Key Words: echocardiography ■ infants ■ outflow tract ■ pathogenesis ■ transposition of the great arteries
What Is New?

- The pathogenesis of transposition of the great arteries as a congenital heart defect of the outflow tract with discordant ventriculo-arterial connections remains unknown.
- We have shown in our study that, compared with normal newborns, the ascending aorta is significantly shorter in newborns with transposition of the great arteries whereas the overall length of the pulmonary trunk does not differ between the 2 groups.
- To the best of our knowledge, these data are the first data from human beings supporting the animal model-based hypothesis that transposition of the great arteries may result from deficient elongation of the outflow tract of the embryonic heart.

What Are the Clinical Implications?

- Improvements in the diagnosis and treatment of congenital heart defects may arise from advancements in the understanding of the functional morphology of normal and malformed hearts.
- Clinical care for patients with congenital heart defects may profit from new mechanistic insights into the pathogenesis of congenital heart defects.
- These new insights may allow future development of new strategies to prevent congenital heart defects or to correct or palliate them by mimicking the physiological heart condition as close as possible.

Nonstandard Abbreviations and Acronyms

- AoL: aortic length
- BCA: brachiocephalic artery
- KACC: King Abdulaziz Cardiac Center
- OFT: outflow tract
- PTL: pulmonary trunk length
- SHF: secondary heart field
- TGA: transposition of the great arteries

Thus, hearts with TGA are usually assigned to 1 of 2 different diagnostic groups: (1) the group of “noncorrected” variants, which are characterized by discordant ventriculoarterial connections in combination with discordant atroioventricular connections; or (2) the group of congenitally corrected variants in which the discordant ventriculoarterial connections is congenitally “corrected” by the presence of discordant atroioventricular connections. Noncorrected variants belong to the most common and severe congenital malformations of the heart, accounting for 5% to 7% of all CHD.2–5 They represent the second most frequent cyanotic CHD but the most common cyanotic CHD diagnosed within the neonatal period.3

TGA is traditionally considered to belong to the pathogenetic group of the so-called conotruncal heart defects. CHDs assigned to this pathogenetic group are characterized by abnormal ventriculoarterial connections combined with abnormal positioning of the great arterial trunks. In classical cases of TGA, for example, the discordant ventriculoarterial connection is associated with parallel (nonspiralling) great arteries.6,7 Only in a very few cases worldwide, TGA was found in association with normally related (spiralling) great arteries.8 The term conotruncal heart defects reflects the hypothesis that CHDs assigned to this pathogenetic group may result from problems in the development of the outflow tract (OFT) of the embryonic heart, which was traditionally divided in a proximal segment, called the conus, and a distal segment, called the truncus. Today, it is recommended to avoid usage of the classical terms conus and truncus. Instead, the embryonic OFT is subdivided into a proximal, intermediate, and distal portion, which provide the future ventricular outlets, the arterial roots, and the intrapericardial arterial trunks, respectively.9 Although it has been hypothesized for a long time that TGA may result from abnormal development of the embryonic OFT, its etiology and formal pathogenesis is still an enigma.6,10,11 This may be explained in part by the fact that, in contrast to other CHDs (eg, atrial septal defect, ventricular septal defect, double outlet right ventricle), TGA does not have a precedent in phylogeny or ontogeny. Another reason is the lack of animal models with a high incidence of TGA, facilitating documentation of the pathogenesis of the defect from early to late stages of embryonic development.12

Studies on animal models for CHDs with abnormal ventriculoarterial connections have shown that a morphogenetic process called cardiac looping is most critical for the determination of atrioventricular as well as ventriculoarterial connections in the mature heart. Pexieder and coworkers reported that abnormal looping of the embryonic heart tube was the first sign of abnormal cardiogenesis in a mouse model for TGA.13 They speculated that
abnormal ventriculoarterial connections were the consequence of abnormal cardiac looping.\textsuperscript{13} Experimental studies on animal models have shown that abnormalities in cardiac looping can result from a failure of normal lengthening of the cardiac OFT due to reduced addition of myocardial cells from the so-called secondary heart fields (SHF).\textsuperscript{14} Other studies have demonstrated that cardiac neural crest cells are necessary for the normal addition of SHF-derived myocardium to the arterial pole of the developing heart loop.\textsuperscript{15,16} Altered cardiac looping, resulting from problems in the addition of second heart field-derived myocardium to the cardiac OFT, can lead to CHDs with abnormal ventriculoarterial connections, such as tetralogy of Fallot, double outlet right ventricle, and TGA.\textsuperscript{14,17} Human CHDs with juxtaposition of the atrial appendages are frequently associated with TGA. In teratogen-induced (Trypanblue, Suramin) animal models for juxtaposition of the atrial appendages, Männer and coworkers have noted that TGA was associated with a significant growth deficit of the great arterial trunks.\textsuperscript{18} It appears that, based on data from animal models, defective heart looping, caused by failure in lengthening of the OFT of the embryonic heart, may be one of the most attractive pathogenetic explanations for TGA.

If TGA would result from deficient elongation of the OFT of the embryonic heart, we would expect that, in classical cases of TGA, the length of the intrapericardial portions of the great arterial trunks should be significantly shorter than in normal hearts. The aim of our present study was to test the validity of this prediction on a population of human patients born with TGA. We demonstrate, to the best of our knowledge, for the first time that the ascending aorta of newborns with TGA is significantly shorter than the ascending aorta of newborns with normal hearts, supporting the hypothesis that TGA may result from deficient elongation of the OFT of the embryonic heart.

\section*{METHODS}

The data that support the findings of this study are available from the corresponding author upon reasonable request.

\section*{Patient Population}

Our study was conducted in a specialized cardiovascular center (King Abdulaziz Cardiac Center [KACC]) located at King Abdulaziz Medical City in Riyadh, Saudi Arabia. We looked through echocardiographic images for all cases of TGA that were diagnosed in KACC from November 1999 until April 2018. Cases were assigned to 3 different diagnostic subgroups:

1. Simple TGA (“noncorrected transposition”): This diagnostic subgroup comprised all cases of TGA without additional complicating cardiovascular lesions (e.g., pulmonary stenosis, coarctation of the aorta, etc.). Cases of TGA with ventricular septal defects that did not affect their clinical presentation or did not lead to a different initial surgical approach (such as pulmonary artery banding) were included in this subgroup as well.

2. TGA with additional complicating heart lesions: This diagnostic subgroup comprised all cases of TGA with additional complicating heart lesions, such as multiple ventricular septal defects, pulmonary stenosis, straddling tricuspid valve, coarctation of the aorta.

3. TGA in the setting of complex heart defects: This diagnostic subgroup comprised all cases of TGA found in the setting of complex cardiac anomalies (i.e., atrioventricular septal defects, common atrium, pulmonary atresia, tricuspid atresia, and mitral atresia). In this group, TGA was not the clinically leading defect but rather an additional defect.

After the aforementioned collection of TGA cases, we extracted cases further according to the following inclusion and exclusion criteria:

\subsection*{Inclusion Criteria}

TGA study group (retrospective cross-sectional study group):

- All cases of simple TGA that were diagnosed in newborn patients younger than 14 days, with good quality images of the outflow tract in their echocardiograms (n=34).

\subsection*{Exclusion Criteria}

- Cases of “complex” TGA (TGA with additional complicating heart lesions; TGA in the setting of complex heart defects) as defined previously.
- Cases of simple TGA that were diagnosed in patients older than 14 days.
- Cases of simple TGA with unclear echocardiographic images of the outflow tract in their echocardiograms.

\section*{Control Group (Prospective Cohort Study Group)}

All newborn patients who were born at King Abdulaziz Medical City, during the period September 2016 till May 2018, and referred to KACC to rule out any CHD and who were found to have a structurally normal heart in their echocardiograms (n=35).

\section*{Variables}

The following outflow tract parameters were measured on recorded images acquired by echocardiography in all patients from the TGA study group (n=34) and from the control group (n=35); (Figures 1 and 2).
Measurements of the Length of the Intrapericardial Portions of the Great Arterial Trunks

**Measurement #1** (from suprasternal long axis): Aortic length 1 (AoL-1): distance between left aortic valve level (ie, from the hinge of the leaflet at the left side of aortic wall) and origin of brachiocephalic artery (BCA) (ie, at the base of BCA on lateral side), as shown in Figures 1 and 2 with solid blue and dashed purple lines.

**Measurement #2** (from suprasternal long axis): Aortic length 2 (AoL-2): distance between right aortic valve level (ie, from the hinge of the leaflet at the right side of aortic wall) and origin of BCA (ie, at the base of BCA on lateral side), as shown in Figures 1 and 2 with solid blue and dashed purple lines.

**Measurement #3** (from subcostal coronal view): Pulmonary trunk length 1 (PTL-1): distance between left pulmonary valve level (ie, from the hinge of the leaflet at the left side of pulmonary artery [PA] wall) and origin of left PA (ie, starting site of branching into the left PA), as shown in Figures 1 and 2 with solid red and dashed purple lines.

**Measurement #4** (from subcostal coronal view): Pulmonary trunk length 2 (PTL-2): distance between right pulmonary valve level (ie, from the hinge of the leaflet at the right side of PA wall) and origin of right PA (ie, starting site of branching into right PA), as shown in Figures 1 and 2 with solid red and dashed purple lines.

**Study Design and Data Collection**

This study was conducted at a single center and comprised (1) a retrospective cross-sectional investigation on newborn patients with simple TGA; and (2) a prospective cohort study on a control group of newborns with morphologically normal hearts. Data were collected according to the previously defined inclusion and exclusion criteria for patients with TGA (retrospective cross-sectional study group) as well as the patients in the control group (prospective cohort study group). Demographic data such as sex, age, and weight at diagnosis and date of birth were also collected. The data were extracted by our clinical pediatric research technician in the cardiac center and given to us in an Excel spreadsheet. The principal investigator (S.O.O.) reviewed all available digital medical charts and records (such as Best Care, Xcelera R4.1L14.1.1.1133-2013) to identify all patients who were diagnosed at KACC with TGA and established with 1 of the coinvestigators (T.M.Y.) our study group of patients with simple TGA following the inclusion and exclusion criteria as defined. After identifying...
all patients with simple TGA, all recorded echocardiograms for this group of patients were reviewed. Only the patients with simple TGA under the age of 14 days with good quality images for the outflow tract in their echocardiograms were selected. Once the relevant patient population with simple TGA under the age of 14 days was identified, a Data Master-file in Excel (Microsoft Office, Version 2010) was generated, including screenshots of good quality images for the outflow tract in their echocardiograms and all defined variables as listed above were added to this file (ie, AoL-1, AoL-2, PTL-1, and PTL-2). With regard to the control group, the coinvestigators (N.C.C. and C.E.) selected prospectively the patients who were born at King Abdulaziz Medical City and referred to KACC to rule out any CHD; they acquired the required proper views for the outflow tract for these patients during the same echocardiography examination that was requested to rule out CHD in our main echocardiography laboratory. Then the principal investigator...
performed the required measurements and generated another Data Master-file in Excel (Microsoft Office, Version 2010) for the control group with same defined variables as mentioned previously. The designated measurements in all echocardiograms for the patients with simple TGA and for the control group were reviewed by 1 in a second run together with a senior pediatric cardiologist (1 of the coinvestigators, T.M.Y.) to confirm that these measurements were correct and accurate. Finally, during a third run all echocardiograms of all patients with simple TGA and the control group with the aforementioned measurements were reviewed one more time independently by another senior cardiologist (F.H.) who verified the accuracy of these measurements. After the collection of all the data from included patients with simple TGA and the control group within the study period we analyzed our results in the master data file to find out the individual length of outflow tract components (as defined) in patients with simple TGA and in the control group (see Figures 1 and 2). At the end we compared the difference in the measurements between the 2 groups and calculated if it is statistically significant or not.

Statistical Analysis

Data are presented as mean±SD for continuous variables together with corresponding 95% CIs. Independent sample t-test was applied to compare means and a \( P \) value≤0.05 was considered significant.

Ethical Considerations

This research was approved by the ethical research committee within the institutional review board at the center’s medical research center with the research protocol number RC18/088/R. There was no need for a consent form because our data collection method consisted mainly of a chart review. Patients’ confidentiality was maintained at all levels as only the principal investigator and coinvestigators had access to the data and were able to collect it. In addition, the data collected were kept in a secure place, using computerized methods in 5 specific password-protected computers, and by using coded serial numbers, we insured that the data did not contain any identification of the patients included in the research. None of the patient’s medical record numbers were sent with the data for analysis as well.

RESULTS

Demographic Data

Our study population comprised 34 newborn patients (diagnostic age ≤14 days) with simple TGA and the control group of 35 newborn patients (diagnostic age ≤3 days) with a structurally normal heart (Table 1).

Age at Diagnosis

In patients with simple TGA, the mean age at diagnosis was 3.5±2.9 days. At this time point, most of the patients had an age <1 week (total 30 patients) whereas the age of the remaining patients ranged from 8 to 10 days. In patients with a structurally normal heart (control group), the mean age was 1.7±0.7 days and their age ranged from 1 to 3 days. Although there was a statistical difference in the diagnostic age between simple TGA (3.5±2.9 days) and control group (1.7±0.7 days), this difference was not reflected in the body weight and body surface area (discussed later).

Sex Distribution

In both study groups, there was a slight preponderance of male patients. There were 18 boys (52.9%) in the TGA group and 20 boys (57.1%) in the control group. The preponderance of boys was not significant statistically (\( P = 0.81 \)).

Body Weight, Body Length, and Body Surface Area

In the group of patients with simple TGA, the mean body weight was 3.1±0.4 kg ranging from 2 to 4 kg. In the control group, the mean body weight was 3.2±0.5 kg ranging from 2 to 4.2 kg. Statistical analysis disclosed that the mean body weight did not differ significantly between the 2 study groups (\( P=0.204 \); 95% CI; −0.08 to 0.35). The mean body length in the group of patients with TGA was 49.8±3.3 cm and 50.8±2.3 cm in the control group. Corresponding to the mean body

Table 1. Demographic Data

| Variable                  | Normal (n=35) | TGA (n=34) | 95% CI of the Difference | \( P \) Value |
|---------------------------|---------------|------------|--------------------------|---------------|
| Sex (male)                | 20 (57.1%)    | 18 (52.9%) | ...                      | 0.81          |
| Age, d                    | 1.7±0.7       | 3.5±2.9    | −2.79 to −0.78           | 0.001         |
| Weight, kg                | 3.2±0.5       | 3.1±0.4    | −0.08 to 0.35            | 0.204         |
| Body surface area, m²     | 0.21±0.02     | 0.21±0.02  | −0.002 to 0.02           | 0.136         |
| Height, cm                | 50.8±2.3      | 49.8±3.3   | −0.34 to 2.35            | 0.141         |

TGA indicates transposition of the great arteries.
weight, the mean body length did not differ significantly between the 2 study groups \( (P=0.141; \ 95\% \ CI, \ -0.34 \ to \ 2.35) \). Finally, we found that the mean body surface area was \( 0.21\pm0.02 \ m^2 \) for both groups and that this value also did not differ significantly between the 2 study groups \( (P=0.136; \ 95\% \ CI, \ -0.002 \ to \ 0.02) \).

**Length of the Intrapericardial Portions of the Great Arterial Trunks**

The lengths of the great arterial trunks (measured on suprasternal and subcostal views as shown in Figures 1 and 2) were as follows:

1. **Length of the ascending aorta as defined by the distance between the left-sided aortic valve level and the origin of the BCA (AoL-1):** In patients with simple TGA, the mean length was \( 1.6\pm0.2 \ cm \). This value was significantly shorter than the value in patients with a structurally normal heart \( (2.05\pm0.1 \ cm; \ P<0.0001; \ 95\% \ CI, \ 0.38 \ to \ 0.51) \).

2. **Length of the ascending aorta as defined by the distance between the right-sided aortic valve level and the origin of the BCA (AoL-2):** In patients with simple TGA, the mean length was \( 1.55\pm0.2 \ cm \), which was significantly shorter than in patients with a normal structured heart \( (2.13\pm0.1 \ cm; \ P<0.0001; \ 95\% \ CI, \ 0.51 \ to \ 0.64) \).

3. **Length of the pulmonary trunk as defined by the distance between the left-sided pulmonic valve level and the origin of the left pulmonary artery (PTL-1):** In patients with simple TGA, the mean length was \( 1.19\pm0.1 \ cm \) and significantly shorter than in patients with a normal structured heart \( (1.37\pm0.1 \ cm; \ P<0.0001; \ 95\% \ CI, \ 0.12 \ to \ 0.24) \).

4. **Length of the pulmonary trunk as defined by the distance between the right-sided pulmonic valve level and the origin of the right pulmonary artery (PTL-2):** In patients with simple TGA, the mean length was \( 1.36\pm0.1 \ cm \). This was significantly longer compared with patients with a normal structured heart \( (1.2\pm0.1 \ cm; \ P<0.0001; \ 95\% \ CI, \ -0.29 \ to \ -0.19) \).

When we looked at the data, we noticed that the 2 measurements used for the definition of the length of the ascending aorta \( (AoL-1, \ AoL-2) \) provided consistent values suggesting that, in our newborns with TGA, the ascending aorta indeed was shorter than in normal hearts (Table 2). With respect to the measurements used for the definition of the length of the pulmonary trunk \( (PTL-1, \ PTL-2) \), however, we noticed that the measurements provided inconsistent values, which seemed to complicate the interpretation of our data. This was because 1 parameter \( (PTL-1) \) suggested that patients with TGA showed an abnormal shortening of the pulmonary trunk, whereas the other parameter \( (PTL-2) \) suggested that these patients had an abnormal elongation of the pulmonary trunk (Table 2). We then speculated that the inconsistency in the measured values for the length of the pulmonary trunk simply reflected differences in the spatial arrangement of the pulmonary trunk between the 2 study groups. In patients with TGA, the pulmonary trunk was thought to show a mirror image of the normal situation. To test this idea, we looked at whether there might be significant differences in the mean length between the shorter vessel walls of the pulmonary trunk of normal patients \( (PTL-2=1.12\pm0.09 \ cm) \) and patients with TGA \( (PTL-1=1.19\pm0.13 \ cm) \), and between the longer vessel walls of the pulmonary trunk of normal patients \( (PTL-1=1.37\pm0.12 \ cm) \) and patients with TGA \( (PTL-2=1.36\pm0.12 \ cm) \) (Table 3). Statistical analyses \( (t\text{-test}) \) did not disclose a significant difference between the measured values for the longer vessel walls (normal PTL-1 versus TGA PTL-2, \( P=0.669; \ 95\% \ CI, \ -0.05 \ to \ 0.07) \). With respect to the shorter vessel walls (normal PTL-2 versus TGA PTL-1), however, we found that the mean length was significantly longer than normal in the TGA.

### Table 2. Defined Measurements of Outflow Tract Parameters (in cm)

| Variable | Normal (n=35) | TGA (n=34) | 95% CI of the Difference | \( P \) Value |
|----------|---------------|------------|-------------------------|---------------|
| AoL-1    | 2.05±0.1      | 1.6±0.2    | 0.38 to 0.51            | <0.0001       |
| AoL-2    | 2.13±0.1      | 1.55±0.2   | 0.51 to 0.64            | <0.0001       |
| PTL-1    | 1.37±0.1      | 1.19±0.1   | 0.12 to 0.24            | <0.0001       |
| PTL-2    | 1.12±0.1      | 1.36±0.1   | -0.29 to -0.19          | <0.0001       |

AoL-1 indicates aortic length 1; AoL-2, aortic length 2; PTL-1, pulmonary trunk length 1; PTL-2, pulmonary trunk length 2; and TGA, transposition of the great arteries.

### Table 3. Comparison of the Short and Long Side of Pulmonary Trunk Length Between Normal Patients and Patients with TGA

| Variables                     | Length (cm) | 95% CI of the Difference | \( P \) Value |
|-------------------------------|-------------|-------------------------|---------------|
| The short side of pulmonary trunk length |             |                         |               |
| PTL-2 (Normal) n=35           | 1.12±0.09   | -0.13 to -0.02           | 0.008         |
| PTL-1 (TGA) n=34              | 1.19±0.13   |                         |               |
| The long side of pulmonary trunk length |             |                         |               |
| PTL-1 (Normal) n=35           | 1.37±0.12   | -0.05 to 0.07            | 0.669         |
| PTL-2 (TGA) n=34              | 1.36±0.12   |                         |               |

PTL-1 indicates pulmonary trunk length 1; PTL-2, pulmonary trunk length 2; and TGA, transposition of the great arteries.
group (P=0.008; 95% CI, −0.13 to 0.02). These data suggest that, in our group of newborns with TGA, the mean length of the pulmonary trunk is slightly longer than normal whereas the overall length of the pulmonary trunk does not differ between the 2 groups.

DISCUSSION

Key Concepts for the Pathogenesis of TGA

Although CHDs with TGA have fascinated physicians since the first description by Matthew Baillie in 1797,\textsuperscript{19} its pathogenesis remains an enigma. During the past 150 years, a remarkable number of diverse mechanistic concepts have been proposed to explain the development of normal spiraling of the great arterial trunks as well as their abnormal parallel arrangement typically found in TGA. Among these diverse concepts a tendency toward 2 extreme views can be noted: (1) "rotation/torsion first" concepts and (2) "septation first" concepts.

"Rotation/torsion first concepts" are based on the assumption that the materials forming the future great arterial trunks occupy distinct genetically predetermined subdomains within the wall of the undivided OFT of the embryonic heart. These subdomains are said to run initially in a parallel fashion with the aortic subdomain to be ventral (superior) and the pulmonary subdomain to be dorsal (inferior). Spiraling of the great arterial trunks is explained by rotation/torsion of the embryonic OFT because of cardiac looping and differences in the growth behavior between the aortic and pulmonary subdomains of the OFT (for examples, see the now historical concepts of differential conal growth/absorption).\textsuperscript{20–22} The parallel arrangement of the great arteries, typically found in TGA, is regarded chiefly as a consequence of deficient rotation/torsion of the developing OFT owing to abnormal changes in the size of the proximal OFT.

"Septation first concepts" are based on the assumption that the wall of the common embryonic OFT does not possess a significant genetic pre patterning into aortic and pulmonary subdomains. It is hypothesized that the identity, spatial arrangement, and ventricular connections of the great arterial trunks are chiefly determined by the process of structural division of the common embryonic OFT into aortic and pulmonary flow pathways. Proponents of "septation first" concepts attribute the spiral course of the great arterial trunks chiefly to a primary spiral arrangement and spiral fusion of the septal anlagen (major outflow cushions) within the embryonic OFT.\textsuperscript{23,24} Oblique ingrowth of the aorticopulmonary septum from the aortic sac into the distal portion of the embryonic OFT also seems to contribute to the emergence of the normal spiraling course of the great arterial trunks.\textsuperscript{9} The parallel course of the arterial trunks, found in TGA, is attributed to an abnormal septation of the embryonic OFT owing to a primary straight arrangement and fusion of the major outflow cushions, possibly combined with a horizontal ingrowth of the aorticopulmonary septum.

It should be noted that the emergence of a spiral or parallel course of the great arterial trunks does not necessarily result in concordant or discordant ventriculoarterial connections, respectively. This is because the proximal roots of both great arteries are originally committed only to the embryonic right ventricle. It is the fusion of the proximal outflow cushions that forms a shelf in the roof of the right ventricle that normally commits the subaortic component of the OFT to the developing left ventricle.\textsuperscript{25} Therefore, a recent concept about the pathogenesis of TGA emphasizes the fact that TGA can result only when an arterial nonspiraling combines with an abnormal transfer of the subpulmonary component of the proximal OFT to the developing left ventricle, possibly because of the abnormal orientation of the proximal outflow cushions.\textsuperscript{26}

Is Deficient Elongation of the Embryonic OFT Involved in TGA Pathogenesis?

Despite the fact that TGA can be formally explained by deviations from the normal spatial orientation of embryonic OFT components, the mechanistic base of such deviations is still unclear at the present time. Current data from embryological studies on animal models suggest that such deviations may result from deficient elongation of the OFT of the embryonic heart owing to defective addition of SHF-derived progenitor cells to the arterial heart pole. To the best of our knowledge, our present study is the first to test the plausibility of this animal model-based hypothesis on human beings. For this purpose, we have measured the length of the intrapericardial portions of the great arterial trunks in newborns with simple TGA ("noncorrected transposition without complicating cardiovascular lesions") and in newborns with a morphologically normal heart. We found that the overall length of the pulmonary trunk did not really seem to differ between newborns with simple TGA and those with a morphologically normal heart. The ascending aorta of our patients with simple TGA, on the other hand, was significantly shorter than the ascending aorta of newborns with normal hearts. Thus, our data seem to confirm the presence of a significant growth defect at the level of the arterial segment of the heart in newborn patients with simple TGA. To the best of our knowledge, these data are the first data from human beings supporting the animal model-based hypothesis that TGA may result from deficient elongation of the OFT of the embryonic heart.

We should emphasize that our data not only show that neonatal TGA is associated with a significant
portions of the OFT are formed by SHF-derived pro-
transition/intermediate zone between its proximal and
bular blood vessel that shows a prominent kink at the
of the embryonic heart has the shape of a solitary tu-
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great arterial trunks derive from the intermediate (roots)
and distal (arterial trunks) portions of the originally un-
divided OFT of the embryonic heart. The common OFT
of the embryonic heart has the shape of a solitary tu-
bular blood vessel that shows a prominent kink at the
transition/intermediate zone between its proximal and
distal portions. The proximal, intermediate, and distal
portions of the OFT are formed by SHF-derived pro-
genitor cells that become successively added to the
arterial heart pole during the phase of cardiac looping.
Before the first appearance of the septal anlagen (major
outflow cushions, aorticopulmonary septum) that nor-
ally leads to the structural division of the OFT into
aortic and pulmonary flow pathways, the OFT seems
to lack any morphologically visible sign for a patterning
into aortic and pulmonary subdomains. In view of this
finding, it is tempting to speculate that the identity of
the 2 arterial trunks as well as their connections to the
ventricles, their spatial arrangement, and lengths, are
chiefly determined by the process of septation of the
embryonic OFT (septation first concept), rather than by
a genetic aorticopulmonary prepatterning combined
with positional and morphological changes driven by
changes in length of the embryonic OFT (rotation/tor-
sion first concept). Septation first concept attributes
the spiral course of the great arterial trunks to a pri-
mary spiral arrangement and fusion of the major out-
flow cushions. The parallel course of the arterial trunks,
frequently found in TGA, is attributed to a primarily
straight arrangement of the major outflow cushions
discussed previously). According to this concept, the
abnormal shortness of the ascending aorta, found in
our patients with TGA, can be simply explained by an
abnormal septation of the embryonic OFT and not by
a locally confined growth defect of a genetically prede-
termined aortic subdomain of the common OFT. Thus,
if we consider classical morphological data alone, it
seems to be highly questionable that the abnormal
shortness of the ascending aorta found in our patients
with TGA may indicate a defect in elongation of a pre-
sumed aortic subdomain of the embryonic OFT.
However, if we consider contemporary data from
developmental biology of the heart, the situation looks
different. This is because recent data from fate map-
ing studies, gene expression analyses, and experi-
mental studies done on mouse embryos have shown
that the wall of the intermediate portion of the morpho-
logically common OFT of the embryonic heart is formed
by 2 genetically distinct progenitor cell populations that,
first, derive from different subregions of the SHF and,
second, occupy 2 distinct subdomains of the OFT that
were found to provide the base of the ascending aorta
and pulmonary trunk, respectively.27,28 The aortic sub-
domain was found to form the originally superior wall
of the OFT, whereas the pulmonary subdomain formed
the originally inferior wall of the OFT. Other studies have
shown that the SHF not only contributes progenitor
cells to the heart and base of the great arteries but
also to the walls of the ascending aorta and pulmo-
nary trunk.29 Furthermore, a recent study on mouse
embryos has shown that the addition of “arterial” pro-
genitor cells from the SHF to the OFT of the embryonic
heart proceeds in 2 waves. The first of these waves
(at embryonic day 7.5) provides cells that preferentially
contribute to the wall of the ascending aorta, whereas
the second wave (embryonic days 8.5 to 11.5) provides
cells that preferentially contribute to the wall of the pul-
monary trunk.30 Thus, it seems that, during a specific
time window in embryonic development, the elonga-
tion of the cardiac OFT is chiefly driven by the addition
of SHF-derived “aortic” progenitor cells to the arterial
heart pole. In light of these findings, we think that a de-
fective addition of “aortic” progenitor cells to the OFT of
the embryonic heart is a plausible mechanistic explana-
tion for the shortening of the ascending aorta found in
our population of neonates with TGA.

A New Mechanistic Concept for the
Morphogenesis of TGA
The question now is, how can a defective addition of
“aortic” progenitor cells to the OFT of the embryonic
heart lead to a nonspiralizing and abnormal positioning
of the great arterial trunks? To answer this question, we
should have a look on the dynamic form changes of the
embryonic heart during the phase of cardiac looping.
The human embryonic heart arises from the fusion of
bilaterally paired heart fields along the ventral midline of
the foregut by the end of the third week of embryonic
development (fifth gestational week). Initially, it is a short,
tubular blood vessel that pumps blood by a valveless
pumping mechanism.31 Subsequent to its establish-
ment, the heart tube elongates by continuous addition of

J Am Heart Assoc. 2021;10:e019334. DOI: 10.1161/JAHA.120.019334
new heart field-derived material to its venous and arterial poles. The initially straight heart tube thereby becomes deformed into a helically coiled heart loop (Figure 3).

Measurements on vertebrate embryos and simulation experiments suggest that growth differences between the heart and pericardial cavity may generate the main driving forces for the helical deformation of the embryonic heart loop. At the beginning of cardiac looping, the axial length of the straight heart tube corresponds to the craniocaudal length of the pericardial cavity (see Figure 3A). During cardiac looping, however, the tubular heart undergoes a 3- to 4-fold increase in length, whereas the pericardial cavity does not significantly change its dimensions (see Figure 3B). The continuously elongating heart tube, thus, becomes compressed within the pericardial cavity and undergoes a series of characteristic deformations. This deformation process can be subdivided into 2 phases. During the first phase of cardiac looping, excessive elongation of the heart tube forces its proximal limb (future left ventricle, atrioventricular canal, and common atrium) to acquire a helical configuration. The handedness of this helix normally is determined by the prelooping position of the venous end of the heart. A prelooping position to the left of the body midline favors the emergence of a left-handed helix, the greater curvature of which points toward the right of the body. This loop-configuration is traditionally called ventricular D (dextral)-loop. It is the normal and phylogenetically highly conserved heart loop configuration among vertebrates (see Figure 3C). The emergence of a mirror-imaged loop (Figure 4), traditionally called ventricular L (levo)-loop, is an extremely rare event, mostly induced by genetic defects or teratogens (eg, maternal diabetes mellitus). During the second phase of cardiac looping, continuing addition of new material to the arterial heart pole not only leads to an elongation of the distal limb of the heart loop (future right ventricle and embryonic OFT) but also forces this segment to acquire a helical configuration, the handedness of which is opposite to that of the proximal limb of the heart loop. This means that, at the end of cardiac looping, the embryonic heart loop normally has the configuration of a 2-handed helix (see Figure 3D). This helix consists of 2 limbs of opposite helical handedness: a proximal limb, which normally has an anticlockwise (left-handed) helical winding, and a distal limb, which normally has a clockwise (right-handed) helical winding. The latter feature may be of special interest because the intrapericardial portions of the great arterial trunks arise from the distal limb of the heart loop and normally spiral in a clockwise helical winding.

Based on the preceding description of cardiac looping, it is tempting to speculate that growth problems, suspected to cause TGA, may become apparent during the second phase of cardiac looping. At this stage, a standstill in elongation of the heart loop, due to the absence of the first (aortic) wave of SHF-derived arterial progenitor cells, would block the emergence of the helical coiling of its distal limb and thereby may prevent clockwise "spiraling of the OFT." This, in turn, might predispose for the development of discordant ventriculoarterial connections.

Figure 3. Schematic drawing illustrating the sequence of idealized geometrical form changes characterizing the looping morphogenesis of the tubular heart of higher vertebrate embryos. During the initial phase of looping, the straight tube (A) starts bending along the midsagittal plane toward the ventral body wall (B). Rightward torsion deforms the bending tube (C) into a helically coiled loop with a counterclockwise winding (“C-shaped” loop). The loop finally acquires the complex helical configuration of a 2-handed helix (D), which consists of a caudal limb with a counterclockwise winding and a cranial limb with a clockwise winding (“S-shaped” loop). A indicates atrium; AV, atrioventricular canal; dO, distal outflow tract; LV, embryonic left ventricle; pO, proximal outflow tract; RV, embryonic right ventricle; and SV, sinus venosus. From Bayraktar and Manner.
In summary: we understand TGAs as CHDs resulting basically from impaired cardiac looping. The abnormal persistence of a single-handed helical heart loop configuration (lack of helical deformation of the distal limb), owing to deficient elongation of the OFT, may predispose for the development of discordant ventriculoarterial connections. The handedness of the heart loop configuration (ventricular dextral loop or ventricular levo loop), on the other hand, may determine the atrioventricular connections (Figure 4).33 In the setting of viscerocentral situs solitus, for example, ventricular dextral loop may predispose for development of discordant atrioventricular connections, whereas ventricular levo loop is thought to predispose for development of discordant atrioventricular connections. This would mean that, if there is a genetic disposition for persistence of a simple helical heart loop configuration, ventricular dextral loop topology is expected to result in (noncorrected) TGA whereas ventricular levo loop topology is expected to result in congenitally corrected TGA.

**Notes on the Genetics of TGA**

Studies on human patients have shown that TGA and congenitally corrected TGA both can occur in the same family, suggesting that there might be a common genetic basis for the development of anomalies in the determination of visceral asymmetries (eg, situs inversus, ventricular levo loop) and TGA (reviewed by Unolt et al6). In view of this finding and our present findings, the question arises as to whether basic research on animal models may have disclosed candidate genes that are involved in the elongation of the embryonic cardiac outflow tract as well as in the determination of the left-right body axis. In our opinion, one of the most promising candidates is the planar cell polarity signaling pathway. Studies on animal models have shown that polarity signaling pathway signaling is not only involved in the control of early and late steps in the development of left-right body asymmetry (reviewed by Axelrod34). It is, additionally, required for proper elongation of the embryonic cardiac outflow tract and cardiac looping (reviewed by Li and Wang35). Future genetic studies on human patients are needed to clarify as to whether defects in signaling pathways involved in the determination of visceral asymmetries as well as in the elongation of the embryonic cardiac outflow tract may play important roles in the pathogenesis of TGA in human beings or whether they may play only a minor role. Clinical data from our institution suggest that such defects seem to play only a minor role in the pathogenesis of human TGA.36

**Is There a Potential Clinical Relevance of Our Data?**

Improvements in the diagnosis and treatment of CHDs not only arose from the invention of new technical devices
or new surgical procedures, but, additionally, from advancements in the understanding of the functional morphology of normal and malformed hearts (eg, abnormal course of the conduction system in congenitally corrected TGA) as well as from advancements in knowledge about the developmental/genetic background of the normal and malformed cardiovascular system (eg, prevention of CHD by folate supplementation and avoidance of teratogens such as retinoic acid). Thus, the clinical disciplines caring for patients with CHDs may profit from the discovery of hitherto neglected morphological peculiarities of CHDs as well as from new mechanistic insight into the pathogenesis of CHD. Such new insights may allow development of new strategies in future to prevent CHDs and, if they had occurred, to correct or palliate them by mimicking the physiological heart condition as close as possible. A nice example in this regard is the modified surgical approach proposed by Chiu and his colleagues for the arterial switch operation by employing a spiral reconstruction of the great arteries in newborns with TGA, so that the spiral relationship of the normally related great arteries can be resumed.37,38

This study may also have implications for the surgical and postsurgical treatment and management of patients with TGA because our data suggest that, in CHDs with TGA, it is mainly the ascending aorta, which suffers from a prenatally reduced growth capacity, whereas the pulmonary trunk may not be affected. Thus, it is conceivable that a reduced biological capacity of the ascending aorta for longitudinal growth can lead to late postoperative problems after spiral reconstruction of the great arteries in TGA.

Limitations

The key measurements on which our study is based were done on echocardiographic images acquired from suprasternal long axis and subcostal coronal views in newborns with normal hearts and newborns with TGA. Although the accuracy of this well-established imaging method is, as we believe, very good, it would have been very useful to employ another imaging modality, such as computed cardiac tomography, to do the same defined measurements in a sample group of patients <2 weeks with normal intracardiac anatomy referred to computed cardiac tomography for various reasons to cross-check the accuracy and reproducibility of our measurements in echocardiography. Despite our efforts we failed to recruit or identify such patients for logistic and ethical reasons. To compensate for this limitation we tried over long periods to obtain, in another sample group of patients, morphological photographs of the outflow tracts in newborns with TGA in the operating theater during surgical repair to conduct defined measurements of the OFT on scaled images, again, to cross-check the accuracy and reproducibility of our measurements in echocardiography versus morphological data. Even this relatively simple appearing task proved itself to be practically impossible given the many logistic disturbances and deficiencies. These unsuccessful efforts caused, unfortunately, a significant delay for submission of our study for publication. A further limitation of our study is the fact that we have measured the length of the great arterial trunks only on 2-dimensional images. This approach is in line with the clinical practice of sonography-based measurements but, unfortunately, neglects the 3-dimensional configurations of the 2 arteries. Measurements on 2-dimensional images cannot disclose the true length of a helically wound vessel such as the normal aorta. Thus our measurements can provide only approximations to the true values. Future studies are needed to uncover the true length of the great arteries in TGA by 3-dimensional approaches.

CONCLUSIONS

Our data demonstrate that, compared with normal newborns, the ascending aorta is significantly shorter in newborns with TGA while the overall length of the pulmonary trunk does not differ between the 2 groups. This finding is in accord with the hypothesis that TGA may result from a growth deficit at the arterial pole of the embryonic heart.

ARTICLE INFORMATION

Received November 19, 2020; accepted May 17, 2021.

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Acknowledgments

We thank Ms Audrey MacDonald, a clinical pediatric research technician at KACC, for her valuable help during data extraction from our large database. We also appreciate the help of Dr Mohammed H. Alghamdi, Consultant Pediatric Cardiologist at KACC, in obtaining the echocardiographic image used in Figure 2 to show the branching pulmonary arteries. Further, we are very grateful to Dr. Abdul Salam M.A. Alsaiad, Consultant Pediatric Cardiologist at KACC, for fruitful discussions and suggestions during the revisions of our article.

Sources of Funding

None.

Disclosures

None.

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