The Clinical Spectrum of Adults with Tetralogy of Fallot: Cardiac and Extra-cardiac Features and Late Outcomes

by

Sara Piran

A thesis submitted in conformity with the requirements for the degree of Master’s of Science

Institute of Medical Science
University of Toronto

© Copyright by Sara Piran 2010
Abstract

Tetralogy of Fallot (TOF) is a form of complex congenital heart disease (CHD) with clinical and genetic heterogeneity. Of the few known causes, 22q11.2 deletion syndrome is most common. We sought to define other clinical subgroups by focusing on congenital cardiac and extra-cardiac features, and cardiac outcome. Patients were prospectively categorized as “syndromic” if they had at least two of three features: dysmorphic facies, learning difficulties or voice abnormalities. We compared cardiac and extra-cardiac characteristics, and late cardiac outcomes between the syndromic group and a nonsyndromic control group. The syndromic group had a more complex cardiac disease, was at elevated risk for developing later onset conditions including neuropsychiatric disorders, endocrine disorders, and hearing deficits, and had a higher mortality rate compared to the nonsyndromic group. Increased awareness of this subgroup with a multisystem condition may be helpful for optimizing management and identifying individuals for referral to medical genetics.
Acknowledgments

First and foremost I would like to thank my kind and amazing supervisor, Dr. Candice Silversides, for all her support, guidance, wisdom, and invaluable learning experience throughout my graduate studies.

I would also like to thank my exceptional advisory committee, Drs. Anne Bassett and Peter Liu, for their continuing support and contribution.

Very special thanks to Dr. Jasmine Grewal for her limitless support and help.

Many thanks to the members of the CGRP for their friendship, help, and all the fun moments we shared! To Dr. Jodi-Ann Swaby for her invaluable friendship, support, and all the fun talks and times we had.

To my dearest friends, Zeynep and Shahad, for always being there for me no matter what; I will always cherish our wonderful memories!

To all the staff and fellows at the TCCCA, thank you for your advice and help; working with all of you was very enjoyable!

Last but not least, I would like to sincerely thank my beautiful family for all their support and endless love, and for always believing in me!
# Table of Contents

Acknowledgments .................................................................................................................................. iii

Table of Contents ................................................................................................................................ iv

List of Figures .................................................................................................................................. viii

List of abbreviations ........................................................................................................................ ix

Overview ........................................................................................................................................ 1

Introduction ..................................................................................................................................... 5

1.1 Tetralogy of fallot ...................................................................................................................... 5

   1.1.1 Overview ............................................................................................................................ 5

   1.1.2 Basic TOF cardiac anatomy ............................................................................................. 5

   1.1.3 Anatomic variations of TOF ............................................................................................ 6

   1.1.4 Diagnosis .......................................................................................................................... 8

1.2 TOF in childhood ....................................................................................................................... 9

   1.2.1 Surgical management ......................................................................................................... 10

   1.2.2 Palliative surgery ............................................................................................................... 10

   1.2.3 Intracardiac repair of TOF .............................................................................................. 10

1.3 TOF in adulthood ...................................................................................................................... 12

   1.3.1 Cardiac features in adulthood late after repair ................................................................. 12

   1.3.2 Exercise Limitations .......................................................................................................... 13

   1.3.3 Reoperation ....................................................................................................................... 20

1.4 The non-cardiac phenotype in adults with TOF ................................................................... 22

   1.4.1 Congenital (structural) extra-cardiac anomalies ............................................................... 22

   1.4.2 Later onset manifestations ............................................................................................... 25

   1.4.3 Neurodevelopmental outcomes ....................................................................................... 26
1.5 Genetics of TOF ................................................................................................................ 27
    1.5.1 Heritability ............................................................................................................ 27
    1.5.2 Genetic causes of TOF .......................................................................................... 28
1.6 Genetic determinants of cardiac outcome .............................................................................. 31
1.7 22q11.2 deletion syndrome (reference group for Study 1 and Study 2) ................... 33
    1.7.1 Characteristic features of 22q11DS ....................................................................... 34
    1.7.2 Cardiovascular malformations in 22q11DS .......................................................... 34
    1.7.3 Ear, nose and throat in 22q11DS ........................................................................... 35
    1.7.4 Immune system in 22q11DS ................................................................................. 35
    1.7.5 Endocrine system in 22q11DS .............................................................................. 36
    1.7.6 Cognitive function, neuropsychiatric and central nervous system abnormalities in 22q11DS ............................................................................................................ 37
    1.7.7 Musculoskeletal system in 22q11DS .................................................................... 38
    1.7.8 Genitourinary system in 22q11DS ........................................................................ 39
2 Aims/Hypotheses ...................................................................................................................... 41
3 Study 1 ...................................................................................................................................... 44
    3.1 Objectives .................................................................................................................. 45
    3.2 Methods ...................................................................................................................... 46
    3.3 Results ......................................................................................................................... 49
    3.3.1 Congenital cardiovascular anomalies .................................................................... 50
    3.3.2 Congenital extra-cardiac anomalies ...................................................................... 51
    3.3.3 Late-onset manifestations ...................................................................................... 52
    3.4 Discussion ................................................................................................................... 54
    3.5 Clinical Implications ................................................................................................... 58
    3.6 Study limitations and advantages ............................................................................... 59
    3.7 Conclusion .................................................................................................................... 60
4 Study 2 .................................................................................................................................. 66
  4.1 Objectives .......................................................................................................................... 67
  4.2 Methods ............................................................................................................................. 68
  4.3 Primary outcomes .............................................................................................................. 70
  4.4 Secondary outcomes ......................................................................................................... 70
  4.5 Statistical analysis ........................................................................................................... 71
  4.6 Results .............................................................................................................................. 72
  4.7 Screening characteristics ............................................................................................... 72
    4.7.1 Baseline characteristics in childhood .................................................................... 72
  4.8 Primary outcomes .............................................................................................................. 73
    4.8.1 All-cause mortality ................................................................................................. 73
  4.9 Secondary outcomes ........................................................................................................ 75
    4.9.1 Adverse Nonfatal Cardiac Events ......................................................................... 75
    4.9.2 Cardiac interventions ............................................................................................. 76
  4.10 Discussion ...................................................................................................................... 77
  4.11 Clinical Implications ...................................................................................................... 80
  4.12 Study limitations and advantages ............................................................................... 80
  4.13 Conclusion .................................................................................................................... 81
5 Summary of Thesis and Future Directions ...................................................................... 91
  5.1 Future directions ............................................................................................................. 93
6 Appendices ......................................................................................................................... 95
7 References ............................................................................................................................ 101
List of Tables

Table I. Late postoperative complications in patients with TOF
Table II. Potential causes of death in patients with TOF
Table III. Congenital extra-cardiac anomalies associated with TOF
Table IV. Extra-cardiac abnormalities associated with 22q11.2 deletions
Table V. Baseline characteristics in 207 adults with TOF
Table VI. Congenital cardiovascular anomalies in adults with TOF
Table VII. Congenital extra-cardiac anomalies in adults with TOF
Table VIII. Late-onset extra-cardiac conditions in adults with TOF
Table IX. Childhood cardiac and extra-cardiac characteristics in adults with TOF
Table X. Pediatric cardiac interventions and adverse cardiac events
Table XI. Extra-cardiac characteristics in the five deceased subjects in the syndromic group
Table XII. Cardiac characteristics in the five deceased subjects in the syndromic group
Table XIII. Adverse nonfatal cardiac events in adulthood
Table XIV. Cardiovascular interventions in adulthood
List of Figures

**Figure 1.** Intracardiac anatomy of tetralogy of Fallot

**Figure 2.** Intracardiac repair of TOF

**Figure 3.** Example of a subject from the syndromic group

**Figure 4.** Categorization of patients into the nonsyndromic group, syndromic group, and 22q11DS reference group.

**Figure 5.** Proportion of adult TOF subjects with one or more congenital extra-cardiac anomaly

**Figure 6.** Differences in survival in adults with TOF

**Figure 7.** Freedom from adverse cardiac events in adults with TOF

**Figure 8.** Freedom from cardiac intervention in adults with TOF
List of abbreviations

22q11DS  22q11.2 deletion syndrome
ADD/ADHD  attention deficit disorder/attention deficit hyperactivity disorder
APVS  absent pulmonary valve syndrome
ASD  atrial septal defect
AICD  automatic implantable cardioverter defibrillator
BT  Blalock-Taussig
CAFS  conotruncal anomaly face syndrome
CHD  congenital heart disease
CHF  congestive heart failure
CNV  copy number variation
DGS  DiGeorge syndrome
ECG  electrocardiogram
MAPCA  major aorto-pulmonary collateral arteries
MSK  musculoskeletal
PVR  pulmonary valve replacement
RVOTO  right ventricular outflow tract obstruction
RV-PA  right ventricle-pulmonary artery
SVC  superior vena cava
TOF  tetralogy of Fallot
VCFS  velocardiofacial syndrome
VSD  ventricular septal defect
Overview

Tetralogy of Fallot (TOF) is the most common cyanotic congenital heart disease (Hoffman 1995) with both clinical and genetic heterogeneity. The most common underlying genetic anomaly in patients with TOF is 22q11.2 deletion syndrome (22q11DS), occurring in 10-16% of cases (Goldmuntz, Clark et al. 1998, Botto, May et al. 2003). This syndrome is associated with auxiliary cardiac and extra-cardiac anomalies and phenotype and genotype characteristics have been extensively studied. In most cases, however, the genetic etiology of TOF is unknown.

Brief clinical genetic screening can help to identify adults with 22q11DS and other genetic syndromes (Fung, Chow et al. 2008). Identification of these patients is important because there are important genetic and clinical issues in this patient population. Among patients with TOF, there is substantial phenotypic variability and there likely exist other, not yet identified syndromes, with unique clinical features. Understanding the clinical phenotypes and genetic variants in patients with TOF is important for both clinical management and genetic counseling.

The overriding goal of this thesis, presented in two parts (STUDY 1 and STUDY 2), was to attempt to identify clinical features of a subgroup of adults with TOF, identified on clinical genetic screening, who had features suggestive of a genetic syndrome. Ultimately, clustering patients into meaningful categories based on phenotypic features may help identify homogenous groups of patients who may have relevance to the pathogenesis of TOF.


**STUDY 1.**

TOF is a complex cardiac disease with a number of potential cardiac variations including the most severe form, pulmonary atresia/ventricular septal defect, to a milder form with minimal outflow tract obstruction. Patients may also have venous and arterial vascular anomalies. Extra-cardiac defects are also common with prevalence rates varying widely between 6-39% (Francannet, Lancaster et al. 1993, Gucer, Ince et al. 2005, Song, Hu et al. 2009, Lurie, Kappetein et al. 1995)(Ferencz, Rubin et al. 1987, Karr, Brenner et al. 1992). These extra-cardiac features may be particularly important, influencing health status and quality of life. Details pertaining to extra-cardiac features are less studied when compared to the cardiac manifestations of the disease, especially in the adult. Earlier studies on extra-cardiac features included patients with 22q11DS and other genetic syndromes, which are frequently associated with extra-cardiac anomalies thus biasing the prevalence estimates. In addition, previous studies have not focused on extra-cardiac characteristics that manifest later in life in TOF patients who do not have known genetic syndromes. Therefore, our first objective (STUDY 1) was to examine, in detail, variability in auxiliary cardiac and extra-cardiac features, including later onset extra-cardiac conditions, in adults with TOF without 22q11.2 deletions or other known generic syndromes. We compared these clinical characteristics in two groups of adults with TOF; those meeting criteria suggestive of a possible genetic syndrome, identified by clinical genetic screening, and those who do not meet such criteria (Fung, Chow et al. 2008). Cardiac and extra-cardiac features of adults with 22q11DS, a group already extensively studied by our group and others, was used as a reference (Driscoll, Spinner et al. 1992, McDonald-McGinn, Kirschner et al. 1999, Bassett, Chow et al. 2005). As the most common genetic disorder associated with TOF, 22q11DS provides a basis for understanding the relation between a genetic syndrome and TOF, and for
increasing awareness of some of the most common non-cardiac abnormalities in patients with TOF.

**STUDY 2.**

Cardiac complications in adults with TOF late after repair are well described with significant morbidity and mortality. While there have been multiple studies examining cardiac predictors of late outcomes, (Norgaard, Lauridsen et al. 1999, Nollert, Dabritz et al. 2003, Murphy, Gersh et al. 1993, Hickey, Veldtman et al. 2009), very few studies have focused on the impact of genetic syndromes on cardiac outcomes (Kyburz, Bauersfeld et al. 2008, Michielon, Marino et al. 2006) (Bassett, Chow et al. 2009). Although data is limited, outcome is worse in patients with genetic syndromes (Kyburz, Bauersfeld et al. 2008)(Anaclerio, Di Ciommo et al. 2004b)(Michielon, Marino et al. 2006)(Bassett, Chow et al. 2009). One pediatric study demonstrated that genetic syndromes such as VACTERL were associated with poor early surgical outcomes in children with TOF (Michielon, Marino et al. 2006). Other studies which have found high mortality and morbidity (i.e. cardiac reinterventions) used a mixed population of patients with congenital heart defects, many of whom had extra-cardiac features (Kyburz, Bauersfeld et al. 2008, Anaclerio, Di Ciommo et al. 2004b).

The few available studies examining outcomes as they relate to genetic syndromes have focused on pediatric populations (Kyburz, Bauersfeld et al. 2008)(Anaclerio, Di Ciommo et al. 2004b)(Michielon, Marino et al. 2006). However, late-onset features of disease are not seen in pediatric studies and these may impact outcome. Indeed, our group has recently reported on premature death in adults with 22q11DS (Bassett, Chow et al. 2009). Therefore, our second objective (STUDY 2) was to determine the impact of phenotypic variation on late outcomes by
comparing late outcomes in our previously defined adult syndromic group (enriched for extra-cardiac characteristics) to those without syndromic features and a reference 22q11DS group.
Introduction

1.1 Tetralogy of fallot

1.1.1 Overview

Congenital heart disease (CHD) is the most common congenital birth defect and the leading cause of death in infants and children (Boneva, Botto et al. 2001). TOF is the most common of the complex cyanotic heart defects with a prevalence of 2.2 per 10,000 live births, and is a major cause of death in infants (Francannet, Lancaster et al. 1993, Marelli, Mackie et al. 2007, Thom, Haase et al. 2006). Although Niels Stensen first described an abnormal heart, Etienne-Louis Arthur Fallot is credited for this disease, based on his description in 1888 of a lesion with pulmonary artery stenosis, ventricular septal communication, rightward deviation of the aorta’s origin, and right ventricular hypertrophy (Evans 2008). Subsequently, TOF was described by many other clinicians and pathologists. In 1924 the eponym tetralogy of Fallot was coined by Maude Abbott. It was Maude Abbott’s 1936 Atlas that widely spread the use of this term and increasing recognition of TOF, which led to improvements in diagnosis and treatment of this condition (Evans 2008).

1.1.2 Basic TOF cardiac anatomy

TOF is characterized by four anatomic abnormalities, which result from the anterior and cephalad deviation of the infundibular septum: ventricular septal defect (VSD), right ventricular outflow tract obstruction (RVOTO), overriding aorta (deviation of the aorta to the right), and secondary right ventricular hypertrophy (Figure 1). The VSD usually occurs in the perimembranous region; however, in some cases it can also extend to the muscular septum. As a
result of the septation defect between the two ventricles, most infants born with TOF appear blue due to the mixing of oxygenated and deoxygenated blood.

**Figure 1. Intracardiac anatomy of Tetralogy of Fallot (TOF).** a) Normal heart anatomy. b) TOF is characterized by four anatomic abnormalities which arise from the anterior and cephalad deviation of the infundibular septum: overriding aorta (*), ventricular septal defect, right ventricular outflow tract obstruction and secondary right ventricular hypertrophy. RA: right atrium; RV: right ventricle; LA: left atrium; LV: left ventricle; RVOT: right ventricular outflow tract; VSD: ventricular septal defect; RVH: right ventricular hypertrophy. Adapted from Greenway et al., *Nat Genet*; 41: 931-935.

1.1.3 Anatomic variations of TOF

Although the hallmark of TOF is the four anatomic abnormalities previously described, there can also be variations in the cardiac features within TOF (Rao, Anderson et al. 1971). The anatomic variations in TOF may involve differences at the level of RVOTO, complexity, the
branching of the aortic arch, pulmonary artery anatomy as well as other associated cardiac anomalies (Dabizzi, Teodori et al. 1990, Marino, Digilio et al. 1996).

The RVOTO can occur at multiple levels. Most frequently, the pulmonary valve in TOF is stenotic and bicuspid and usually hypoplastic, all of which contribute to the mechanical obstruction to pulmonary blood flow (Perloff, 1987). However, RVOTO can also occur at the subvalvar and supravalvar levels as a result of infundibular stenosis and hypoplasia or stenosis of the pulmonary arteries, respectively.

Some patients with TOF have pulmonary atresia with ventricular septal defect, a more severe variant of TOF. This may be characterized by valvar pulmonary atresia with normal pulmonary arteries and duct dependent pulmonary circulation, or others may have diminutive pulmonary arteries with dependence on major aortopulmonary collateral arteries (MAPCAs) for blood supply to the lungs (Rome, Mayer et al. 1993).

Another uncommon yet severe condition associated with TOF is absent pulmonary valve syndrome (APVS) occurring in about 3-5% of cases of TOF (Perloff, 1987). Patients with APVS have variable degrees of RVOTO, dysplastic or entirely absent pulmonary valve leaflets, as well as aneurysmal (dilated) pulmonary arteries (Kirshbom, Jaggers et al. 1999). The variation in anatomy and complexity of TOF is important when these patients are undergoing reparative cardiac surgery.

Associated congenital cardiac anomalies are common in TOF, with an estimated prevalence of approximately 40%. Aortic arch anomalies are the most common cardiac anomaly associated with TOF, occurring in about 25% of cases (Tozzi, Hernanz-Schulman et al. 1989, Zidere, Tsapakis et al. 2006). In TOF, aberrant right and left subclavian arteries are typically found in 8% and 5% of cases, respectively (Rao, Anderson et al. 1971).
Atrial septal defects (ASD) exists in approximately 15% of cases of TOF (Lev, Eckner 1964) (Perloff), although patent foramen ovale is the most common form of interatrial communication, typically occurring in half the cases of TOF (Lev, Eckner 1964). Less common is atrioventricular septal defect, present in <5% of cases of TOF (Lev, Eckner 1964).

Approximately 5-9% of patients with TOF have coronary anomalies (Dabizzi, Caprioli et al. 1980). Coronary anomalies may include anterior descending artery from right coronary artery/sinus and crossing the right ventricular outflow tract, accessory anterior descending artery from right coronary artery/sinus, single coronary artery from left/right coronary sinus or less common anomalies (Gupta, Saxena et al. 2001).

A left superior vena cava is present in approximately 11% of cases of TOF, almost always draining into the coronary sinus (Rao, Anderson et al. 1971). Other associated anomalies that have been described include anomalies of the mitral valve (hooding or clefting of one of the leaflets), tricuspid valve (clefting in one of the leaflets and an accessory pouch), prolapse of the right aortic cusp with aortic insufficiency, subaortic stenosis, accessory orifice of the mitral valve, dextroversion, septal hypertrophy causing left ventricular outflow tract obstruction, left umbilical vein draining directly into the coronary sinus, and anomalous muscle bundle of the right ventricle causing subpulmonary stenosis (Rao, Anderson et al. 1971).

1.1.4 Diagnosis

TOF is frequently diagnosed during fetal life by the detection of the above described symptoms in association with a systolic ejection murmur, and from the turbulent blood flow across the narrowed right ventricular outflow tract (Apitz, Webb et al. 2009) (Gersony, 2002). The intensity of the murmur decreases with increasing severity of RVOTO.
The chest x-ray of a patient with TOF shows a heart of normal size, but with a boot-shaped appearance due to right ventricular hypertrophy and the diminished size of the main pulmonary artery shadow (Gersony, 2002). In patients with TOF, the electrocardiogram (ECG) usually displays sinus rhythm with right axis deviation and right ventricular hypertrophy. In postoperative adults, however, QRS prolongation of the right bundle branch block type is expected (Perloff, 1987). Echocardiography allows for the visualization of the distinctive anatomic features of TOF, most notably details of the anteriorly displaced infundibular septum, the malaligned VSD and the outflow tract obstruction of the right ventricular outflow tract (Perloff, 1987).

1.2 TOF in childhood

The VSD in TOF is almost always single, large and non-restrictive (Apitz, Webb et al. 2009). The direction and magnitude of blood flow through the defect depends on the severity of the RVOTO. In cases when the RVOTO is severe or in pulmonary atresia (i.e. complete absence of the pulmonary valve), a right-to-left shunt occurs, lowering arterial oxygen saturations thus causing these infants to present with cyanosis (Apitz, Webb et al. 2009). Infants with more mild forms of pulmonary stenosis may initially present with congestive heart failure secondary to left-to-right shunting through the VSD (Gersony, 2002). However, infants with minimal or no cyanosis at birth often develop cyanosis during the first few weeks to months of life, when the RVOTO increases (Apitz, Webb et al. 2009, Starr 2010). In addition to cyanosis, infants with TOF can also present with exertional dyspnea and hypercyanotic spells, known as “Tet spells” (Gersony, 2002).
1.2.1 Surgical management

Prior to the advent of open-heart surgery, TOF was managed by palliation with a shunt from the subclavian artery or aorta to the pulmonary artery. However, the evolution of cardiopulmonary bypass technique opened the era to open-heart surgery, which made possible the definitive repair of TOF by closing the VSD and relieving RVOTO. In older cohorts, infants were initially palliated with shunts for many years, before the transition to early repair was made (Fraser, McKenzie et al. 2001). The age of corrective surgery has gradually deceased, and in the current era it is between 3-6 months (Apitz, Webb et al. 2009)(Van Arsdell, Maharaj et al. 2000).

1.2.2 Palliative surgery

In 1944, the first palliative treatment for TOF was introduced by Drs. Blalock and Taussig, known as the Blalock-Taussig (BT) shunt (Harlan, 1995). During the BT shunt, the subclavian artery is anastomosed to the pulmonary artery, which increases pulmonary blood flow, improves exercise capacity and reduces cyanosis (Gersony, 2002).

The Potts and Waterston shunts consist of an anastomosis between the descending and ascending thoracic aorta and pulmonary artery, respectively. This procedure, however; is rarely used today because it results in excessive pulmonary blood flow and development of pulmonary vascular disease (Kirklin, 1993).

1.2.3 Intracardiac repair of TOF

In 1954, the first open heart surgery using cardiopulmonary bypass to repair TOF was performed by Dr. C Walton Lillehei and colleagues (Neill, Clark 1994). The repair of TOF, which is typically performed in childhood, involves closing the VSD and relieving RVOTO.
Surgical interventions performed to relieve the RVOTO may include pulmonary valvotomy, resection of the infundibular muscle, right ventricular outflow tract patch, transannular patch (Figure 2), pulmonary valve implantation, an extracardiac conduit placed between the right ventricle and pulmonary artery, and angioplasty or patch augmentation of central pulmonary arteries (Gatzoulis, 2003). In the right ventricular outflow tract patch approach, a patch is placed across the right ventricular outflow tract (over the infundibulum) without disrupting the integrity of the pulmonary valve annulus. Transannular patching involves placing a patch across the pulmonary valve annulus (Harlan, 1995)(Gatzoulis, 2003). However, a transannular patch renders the pulmonary valve incompetent and creates potential for hemodynamic abnormalities including pulmonary regurgitation (Gatzoulis, 2003). An alternative procedure to transannular patching (when the pulmonary valve annulus is restrictive) is an extracardiac conduit from the right ventricle to the distal main pulmonary artery. However, this approach is more frequently used in patients with TOF-pulmonary atresia, and there is still the possibility of stenosis/calcification of the conduit (Gatzoulis, 2003). In patients with restrictive supravalvar anatomy (hypoplastic main pulmonary trunk and/or stenosis of the central pulmonary arteries), angioplasty (dilation) or patch augmentation (aterioplasty) can be preformed (Gatzoulis, 2003).
1.3 TOF in adulthood

1.3.1 Cardiac features in adulthood late after repair

Prior to the advent of cardiac surgery, roughly half of the patients with TOF died within the first year of life (Apitz, Webb et al. 2009). Without surgical intervention, 40% die by three years of age, 70% by ten years of age and 95% by forty years of age (Starr 2010). The evolution of congenital heart disease surgery has made it possible for most children born with TOF to live up to adulthood (Karamlou, McCrindle et al. 2006). Nevertheless, late morbidity and mortality are not uncommon. Although the anatomic and physiologic abnormalities in TOF can be
surgically repaired with excellent early outcomes in survival, residual problems and sequelae still persist in the majority of patients. Residual cardiac problems after repair of TOF include exercise limitations, pulmonary regurgitation/RVOTO, tricuspid regurgitation, right ventricular dysfunction/heart failure, arrhythmias, left ventricular dysfunction/heart failure, residual VSD, aortic insufficiency and conduction abnormalities (Table I).

Table I. Late postoperative complications in patients with TOF

<table>
<thead>
<tr>
<th>Complications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise capacity</strong></td>
<td>Exercise limitations</td>
</tr>
<tr>
<td><strong>Hemodynamic lesions</strong></td>
<td>Pulmonary regurgitation, tricuspid regurgitation, aortic insufficiency</td>
</tr>
<tr>
<td><strong>Ventricular dysfunction</strong></td>
<td>Right ventricular dysfunction, left ventricular dysfunction, heart failure</td>
</tr>
<tr>
<td><strong>Arrhythmias</strong></td>
<td>Ventricular tachyarrhythmias, atrial tachyarrhythmias, bradyarrhythmia</td>
</tr>
<tr>
<td><strong>Residual cardiac defects</strong></td>
<td>Residual ventricular septal defect, right ventricular outflow tract obstruction</td>
</tr>
<tr>
<td><strong>Conduction abnormalities</strong></td>
<td>Right bundle branch block</td>
</tr>
</tbody>
</table>

1.3.2 Exercise Limitations

Several studies have reported impaired exercise capacity in TOF patients after repair (Wessel, Cunningham et al. 1980, Sarubbi, Pacileo et al. 2000, Yetman, Lee et al. 2001, Rowe, Zahka et al. 1991). It has been previously shown that residual pulmonary regurgitation

Impairments in exercise capacity post repair are independent of age at operation, as children who had surgery as infants (<18 months of age) still presented with impairments in aerobic capacity (Yetman, Lee et al. 2001). Furthermore, in the same study by Yetman et al, peak VO₂ (measure of oxygen consumption) was significantly less in patients with right ventricular dysfunction. Nevertheless, the cause of impaired exercise capacity in postoperative TOF patients is likely multifactorial and is of great value for the long-term assessment of these patients (Yetman, Lee et al. 2001).

1.3.2.1 Residual RVOTO/pulmonary regurgitation

Residual RVOTO in postoperative TOF patients may occur at the infundibular, valvar and/or supravalvar levels (Karamlou, McCrindle et al. 2006) (Gersony, 2002). Reintervention following repair is required if there is residual pulmonary stenosis with right ventricular pressure two-thirds or more than systemic pressure (Gatzoulis, 2003). In cases where there is mechanical obstruction of the pulmonary arteries (such as that created from a previous Potts shunt or Waterston shunt), the most widely used procedure is stenting to relieve the obstruction (Gersony, 2002).

The use of transannular patch and intracardiac repair via a ventriculotomy approach may contribute to pulmonary valve regurgitation, which in the long term can have devastating consequences such as progressive right ventricular dilatation and failure, tricuspid valve regurgitation, exercise intolerance, arrhythmia, and sudden death (Gatzoulis, 2003). Atrial arrhythmias may also ensue in patients with pulmonary regurgitation (Gatzoulis, 2003). Pulmonary regurgitation can be well tolerated in severe cases; however, right ventricular
volume-overload results in right ventricular dilatation and dysfunction, which worsens with secondary tricuspid regurgitation (Hickey, Veldtman et al. 2009). A dilated right ventricle creates potential for electrical instability, as Gatzoulis et al have shown that risk of symptomatic arrhythmias is greater in patients with right ventricular dilation and QRS prolongation (Gatzoulis, Balaji et al. 2000, Gatzoulis, Till et al. 1995). Management of pulmonary regurgitation in patients late after repair may include pulmonary valve replacement, often in combination with atrial or ventricular cryoablation. However, further clarification is needed regarding the optimal timing of pulmonary valve replacement (Hickey, Veldtman et al. 2009).

### 1.3.2.2 Tricuspid regurgitation

Tricuspid valve regurgitation occurs frequently in postoperative TOF patients with right ventricular enlargement (Uretzky, Puga et al. 1982). In 2003, Mahle et al investigated the factors contributing to tricuspid regurgitation and the relation between tricuspid regurgitation and right ventricular dilation (Mahle, Parks et al. 2003). They found that not only is tricuspid regurgitation common (present in 32% of patients), but it is related to structural valve abnormalities and dilatation of the tricuspid annulus (most likely related to previous surgery). Furthermore, tricuspid regurgitation was significantly correlated with right ventricular volume, suggesting that tricuspid regurgitation may also contribute significantly to right ventricular dilatation in postoperative TOF patients. These results are in agreement with the study by Kobayashi et al who found that tricuspid regurgitation is significantly associated with pulmonary regurgitation, residual VSD, and right ventricular end-systolic and end-diastolic pressures (Kobayashi, Kawashima et al. 1991).
1.3.2.3 Right ventricular dysfunction and right heart failure

Right ventricular function has been extensively assessed in the pre- and postoperative patient with TOF and is influenced by many factors including age of the patient, the size of right ventriculotomy and the extent of muscle resection, adequacy of myocardial protection (and thus likely the era in which repair was carried out), and severity of pulmonary regurgitation (Freedom, 2004). In addition to residual right ventricular outflow tract obstruction and pulmonary regurgitation, Davlouros et al reported a negative influence of RVOT aneurysms and/or RVOT akinesia on right ventricular dysfunction (Davlouros, Kilner et al. 2002). Furthermore, infundibular resection and ischemic insult have been hypothesized to worsen right ventricular function. This is supported by the work of Atallah et al, who reported less right ventricular dilation and preserved right ventricular systolic function late after repair of TOF by using a modified approach for relieving RVOT obstruction and avoiding extensive myectomy (Atallah-Yunes, Kavey et al. 1996). Preservation of the pulmonary valve, less employment of RVOT or transannular patching and avoidance of extensive myectomy would likely help in preserving long-term right ventricular systolic function (Davlouros, Kilner et al. 2002).

1.3.2.4 Left ventricular dysfunction and left heart failure

Although most of the focus has been on right ventricular dysfunction as a late complication after repair of TOF, there is evidence of biventricular dysfunction and it has been shown that left ventricular dysfunction is a risk factor for sudden death late after repair (Ghai, Silversides et al. 2002). Davlourost et al have previously identified the length of time a patient remains palliated, aortic regurgitant fraction, and right ventricular ejection fraction as independent predictors of left ventricular ejection fraction (Davlouros, Kilner et al. 2002). The results from this study and others suggest right ventricular-to-left ventricular interaction,
highlighting the importance of preserving right ventricular function for multiple long-term benefits (Davlouros, Kilner et al. 2002).

Symptomatic TOF patients post repair often present with congestive heart failure (CHF) (Lillehei, Levy et al. 1964). In the study by Rocchini et al determining the causes of CHF in postoperative TOF patients, CHF was reported in 35% (36/102) of subjects who were followed up for ten years (Rocchini, Rosenthal et al. 1977). The major factor associated with CHF in this study was a large residual VSD. However, RVOTO alone was not identified as a common cause of CHF in these patients. Other major causes identified included tricuspid regurgitation, pulmonary stenosis, and persistent systemic to pulmonary artery shunts when there was a large residual VSD present. In the study by Nollert et al, CHF was identified as the second most common cause of death in TOF patients post repair (Nollert, Fischlein et al. 1997b), thus making it important to carry out thorough invasive investigations to identify additional muscular VSDs or residual VSDs prior to (re)intervention (Rocchini, Rosenthal et al. 1977).

1.3.2.5 Aortic insufficiency

Symptomatic aortic insufficiency is uncommon in TOF patients after repair; however, some of the causes include dilation of the aortic root, bacterial endocarditis, or injury to the aortic valve at the time of repair (Gersony, 2002). Patients with aortic regurgitation resulting from progressive aortic root dilation may eventually require aortic valve replacement (Niwa, Siu et al. 2002).

1.3.2.6 Residual intracardiac shunts

A residual VSD is usually related to an inadequate VSD patch, but may also be related to additional VSDs (muscular) that were not initially diagnosed during surgery (Gersony, 2002).
1.3.2.7 Supraventricular arrhythmias

Atrial flutter and atrial fibrillation are relatively common in postoperative patients with TOF, with a reported prevalence between 12% and as high as 34% (Harrison, Siu et al. 2001)(Gatzoulis)(Roos-Hesselink, Perrhoth et al. 1995). The reported prevalence is likely a factor of the age of the cohort studied. There is, however, a relationship between hemodynamic abnormalities and atrial arrhythmias (Gatzoulis, Balaji et al. 2000, Harrison, Siu et al. 2001, Karamlou, Silber et al. 2006). In a multicentre study assessing risk factors for arrhythmias, Gatzoulis et al identified tricuspid regurgitation as the predominant hemodynamic lesion in patients with atrial fibrillation/flutter (Gatzoulis, Balaji et al. 2000). Harrison et al identified structural abnormalities, specifically a larger mean right atrial volume and a higher frequency of significant pulmonary regurgitation in patients with atrial arrhythmias (Harrison, Siu et al. 2001). Hemodynamic lesions (i.e. tricuspid regurgitation and pulmonary regurgitation) can result in right atrial dilation and increase predisposition to atrial arrhythmogenesis. It has also been reported that the QRS threshold for atrial arrhythmias is 160 msecs (Karamlou, Silber et al. 2006). Gatzoulis et al have found that patients with atrial flutter/fibrillation have an early increase in QRS duration, followed by a slower QRS rate of change late after repair (Gatzoulis, Balaji et al. 2000). This suggests that monitoring the rate of QRS change is of greater prognostic value than reporting absolute QRS duration values at any given time (Gatzoulis, Balaji et al. 2000).

1.3.2.8 Conduction Abnormalities

Right bundle branch block is a very common phenomenon after surgical repair of TOF. Late onset of complete heart block after repair of TOF, however, is rare. The incidence of
complete heart block has been reported as 0.6% up to 1.3% in patients with TOF post repair (Freedom, 2004). In cases of late onset complete heart block or transient complete heart block, pacemaker implantation becomes mandatory (Freedom, 2004)(Gatzoulis, 2003).

1.3.2.9 Ventricular Arrhythmias and Sudden Death

Ventricular arrhythmia is an adverse sequelae in postoperative patients with TOF, which may serve as a substrate for sudden cardiac death (Yap, Harris 2009). Nonsustained ventricular arrhythmia (lasting <30 seconds) is very common, occurring in up to 60% of patients with TOF following repair (Gatzoulis, 2003). Sustained ventricular arrhythmias (lasting ≥30 seconds), however, are uncommon (Gatzoulis 2003). It has been reported that prolonged QRS duration (≥180 msecs) serves as a risk factor for ventricular arrhythmias (Gatzoulis, Balaji et al. 2000, Gatzoulis, Till et al. 1995). Furthermore, pulmonary regurgitation has been identified as the predominant hemodynamic lesion predicting ventricular arrhythmias and sudden death. It has also been shown that the mechanical and electrical properties of the right ventricle are interrelated; ventricular dilation and slowed conduction (demonstrated by a QRS≥180 msecs) after repair of TOF poses severe risk to ventricular arrhythmias and sudden cardiac death (Gatzoulis, Balaji et al. 2000).

Usually, the focus where the arrhythmia originates is in the right ventricular outflow tract, in the area of previous infundibulectomy or VSD closure (Gatzoulis, 2003). However, in 20% of the time the reentry focus may be multiple, involving the entire body of the right ventricle (Gatzoulis, 2003).

Table II demonstrates some of the most common causes of death in patients with TOF. The most common cause of death after repair of TOF is sudden cardiac death caused by ventricular tachycardia and fibrillation (Bricker 1995). Although sudden death is uncommon post
repair of TOF (Gernosy, 2002), the incidence of arrhythmic sudden death in late follow-up series is reported to be between 0.5-6%, accounting for roughly one-third to one-half of late deaths in this patient population (Gatzoulis, 2003). Indeed, in a study by Nollert et al, the risk of sudden cardiac death was found to increase after 10 years from 0.06%/year to 0.20%/year (Nollert, Fischlein et al. 1997b). The risk factors associated with late cardiac events include older age at repair, a high mean ratio of peak systolic right-to-left ventricular pressures immediately after repair, and the presence of a Potts anastomosis (Murphy, Gersh et al. 1993, Hickey, Veldtman et al. 2009, Karamlou, McCrindle et al. 2006). In a study by Hickey et al, pulmonary branch stenosis, arioven tricular septal defect and pulmonary atresia variants were reported as risk factors influencing late hazard for death, with a three-fold higher late risk for death in the pulmonary atresia variant than classic TOF (Hickey, Veldtman et al. 2009). The rate of increase of sudden cardiac death in TOF patients after repair warrants long-term follow-up by cardiologists to identify possible risk factors and improve overall management.

1.3.3 Reoperation

Although most patients have favourable long-term outcomes after repair of TOF (Hickey, Veldtman et al. 2009, Karamlou, McCrindle et al. 2006), roughly 5-7% of patients require reoperation (Norgaard, Lauridsen et al. 1999, Nollert, Fischlein et al. 1997a, Oechslin, Harrison et al. 1999). Indications for reoperation may include pulmonary insufficiency, pulmonary stenosis, residual RVOTO, residual VSD, aortic valve regurgitation, aortic valve stenosis, conduit degeneration, and tricuspid regurgitation (Gatzoulis, 2003). Reoperation procedures, which commonly include pulmonary valve replacement, tricuspid valve replacement/repair and pulmonary arterioplasty, are usually done in adulthood. In a study by Oechslin et al, the mean age at reoperation was 33 years and Karamlou et al reported a median age of 23 years in TOF.
patients undergoing reoperation after initial repair of TOF (Karamlou, Silber et al. 2006, Oechslin, Harrison et al. 1999). Mid-term survival after reoperation of TOF is excellent, where the ten-year actuarial survival has been reported as 92-93% (Karamlou, Silber et al. 2006, Oechslin, Harrison et al. 1999). However, there is currently limited knowledge about the timing of reoperation to increase long-term survival, especially relating to pulmonary valve replacement (PVR) in order to avoid irreversible right ventricle damage from pulmonary insufficiency (Uretzky, Puga et al. 1982).

**Table II. Potential causes of death in patients with TOF**

<table>
<thead>
<tr>
<th>Cardiac causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operative mortality</td>
</tr>
<tr>
<td>Reoperation or interventions</td>
</tr>
<tr>
<td>Arrhythmias – ventricular tachycardia and ventricular fibrillation</td>
</tr>
<tr>
<td>Heart failure – related to ventricular dysfunction, valve lesions or residual shunts</td>
</tr>
<tr>
<td>Thromboembolic complications – strokes</td>
</tr>
<tr>
<td>Myocardial infarction – secondary to coronary anomalies</td>
</tr>
<tr>
<td>Aortic root dilation and dissection or rupture</td>
</tr>
<tr>
<td>Acquired heart disease – coronary artery disease</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-cardiac causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-cardiac surgery</td>
</tr>
<tr>
<td>Other diseases – asthma, diabetes</td>
</tr>
<tr>
<td>Secondary to congenital extra-cardiac anomalies - i.e. renal failure</td>
</tr>
<tr>
<td>Infection/sepsis</td>
</tr>
<tr>
<td>Thromboembolic complications – stroke or pneumonia</td>
</tr>
<tr>
<td>Suicide</td>
</tr>
<tr>
<td>Accidents</td>
</tr>
<tr>
<td>Side effects of medications</td>
</tr>
</tbody>
</table>
1.4 The non-cardiac phenotype in adults with TOF

1.4.1 Congenital (structural) extra-cardiac anomalies

While there have been extensive studies examining the cardiac features of TOF, much less has been published on the extra-cardiac features of this condition, except in the case of a few specific conditions such as trisomy 21 or 22q11DS (McDonald-McGinn, Kirschner et al. 1999, Bassett, Chow et al. 2005, McDonald-McGinn, Gripp et al. 2005, Driscoll, Salvin et al. 1993) (Jones). The prevalence of congenital extra-cardiac anomalies in TOF has been estimated to range from 6 to 39% (Francannet, Lancaster et al. 1993, Gucer, Ince et al. 2005, Song, Hu et al. 2009, Lurie, Kappetein et al. 1995, Ferencz, Rubin et al. 1987, Karr, Brenner et al. 1992, Calzolari, Garani et al. 2003, Meberg, Hals et al. 2007, Marden, Smith et al. 1964). However, the reported prevalence depends on the inclusion criteria specific to each study, as some studies consider a genetic syndrome as an extra-cardiac anomaly (Eskedal, Hagemo et al. 2004) and others have a different classification of what constitutes a major and minor anomaly and to which organ system an anomaly belongs to (Francannet, Lancaster et al. 1993, Song, Hu et al. 2009, Marden, Smith et al. 1964). The congenital extra-cardiac anomalies associated with TOF include malformations affecting any organ system, and may occur as part of a genetic or non-genetic syndrome (Table III). Depending on the severity of the anomaly, surgery may be required.

The most commonly reported extra-cardiac anomalies in patients with TOF include musculoskeletal, gastrointestinal, and genitourinary anomalies (Francannet, Lancaster et al. 1993, Song, Hu et al. 2009). However, these studies included individuals with 22q11DS and other genetic syndromes. In the study by Francannett et al, patients with TOF had the highest prevalence of extra-cardiac anomalies compared to two other CHDs, hypoplastic left heart
syndrome and transposition of the great vessels. In the same study, gastrointestinal anomaly was most common, with esophageal atresia and anal atresia predominating. Genitourinary anomalies were the second most common anomaly, with renal agenesis as the most common defect (Francannet, Lancaster et al. 1993).

More recently, Rauch et al studied genotype-phenotype relations in 230 children (103 female, 127 male, median age 9.9 years) with TOF (Rauch, Hofbeck et al. 2010). They reported genetic aberrations in 18% (42/230) of these patients who also had associated extra-cardiac abnormalities. These genetic aberrations included 22q11.2 deletion, trisomy 21, single gene disorders (involving \textit{JAG1}, \textit{NKX2.5}, and \textit{TBX1}), and other chromosomal aberrations ((Rauch, Hofbeck et al. 2010). For example, one patient with chromosome 21q22.3 deletion had cleft palate, inguinal hernias, and hip dysplasia. Another patient with chromosome 1p32.2p31.1 deletion (14 Mb in size) had hydrocephaly, club foot, renal dysplasia, and agenesis of corpus callosum. Even in those patients with no identified genetic aberration, extra-cardiac anomalies were common, and in some cases similar compared to those with 22q11DS (Rauch, Hofbeck et al. 2010).

Results from these studies highlight the clinical and genetic heterogeneity that exists within TOF. However, most of these studies have not comprehensively reviewed phenotypic variability in adult patients with TOF in the absence of major genetic anomalies. Identifying extra-cardiac features is important as multiple organ malformations often form recognizable patterns that may be indicative of a particular genetic syndrome (Jones, 2006). Furthermore, the number and pattern of extra-cardiac features can help in identifying possible subgroups of TOF that may have increased homogeneity relevant to the genetic pathogenesis of TOF (Scutt, Chow et al. 2001).
### Table III. Congenital extra-cardiac anomalies associated with TOF

<table>
<thead>
<tr>
<th>Organ system</th>
<th>Examples of extra-cardiac anomalies</th>
<th>Examples of co-existing genetic syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central nervous system</td>
<td>Hydrocephaly, microcephaly, neural tube defects (e.g. spina bifida)</td>
<td>22q11DS, Alagille syndrome</td>
</tr>
<tr>
<td>Pharyngeal arch related anomalies</td>
<td>Cleft lip/palate, thyroid agenesis, thymus anomalies, laryngeal stenosis</td>
<td>22q11DS, CHARGE</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Tracheoesophageal fistula, esophageal atresia, omphalocele, imperforate anus, duodenal atresia, biliary duct atresia</td>
<td>22q11DS, VACTERL, Alagille syndrome, trisomy 21</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Anomalous thumb, hemivertebrae, polydactyly, syndactyly, hip dislocation, talipes</td>
<td>22q11DS, Holt-Oram syndrome, Alagille syndrome, VACTERL, Klippel-Feil syndrome, Cornelia de Lange syndrome</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>Duplex kidney, horseshoe kidney, renal ectopy, hydronephrosis, ureter agenesis/stenosis, hypoplastic ovaries, hypospadias</td>
<td>22q11DS, VACTERL, Klinefelter syndrome</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Agenesis of lung, congenital diaphragmatic hernia, bronchial malformation</td>
<td>22q11DS</td>
</tr>
<tr>
<td>Other</td>
<td>Agenesis of ears, craniofacial abnormalities, situs inversus</td>
<td>22q11DS, Goldenhar anomaly, Distichiasis-lymphedema syndrome, Williams syndrome, Treacher Collins syndrome, Alagille syndrome</td>
</tr>
</tbody>
</table>

1.4.2 Later onset manifestations

One of the advantages to studying adults is that it provides information on lifetime features that manifest later in life, such as psychiatric illnesses (Bassett, Chow et al. 2005). Apart from 22q11DS and many other known genetic syndromes, there is limiting data on late manifesting extra-cardiac conditions in adults with TOF. Most studies to date have looked at neurodevelopmental outcomes in children after repair of TOF (Hovels-Gurich, Konrad et al. 2006) (Zeltser, Jarvik et al. 2008a). However, there are some reports of late manifesting extra-cardiac conditions in children with TOF both in the presence and absence of specific genetic aberrations (Momma, Takao et al. 2001). 22q11DS, an extensively studied and well recognized genetic syndrome, provides an example of a syndrome co-existing with TOF, and serves as a template for studying extra-cardiac abnormalities in TOF patients without 22q11.2 deletions. In patients with TOF and 22q11DS, hypoparathyroidism, schizophrenia, major depression, anxiety, and hearing loss are well documented (Bassett, Chow et al. 2005, Bassett, Chow et al. 2005, Momma, Takao et al. 2001, Bassett, Hodgkinson et al. 1998). In 2005, Bassett et al reported 58.1% prevalence for psychiatric disorders (including schizophrenia, major depression, anxiety disorders, attention deficit hyperactivity disorder, impulse control disorders, and substance use disorders) in an ascertainment subgroup of adults with TOF (Bassett, Chow et al. 2005). Similarly, other late-onset conditions reported in TOF patients with or without genetic anomalies include seizures, mental retardation, frequent infections, impaired hearing, and hypernasality (commonly associated with velopharyngeal insufficiency) (Bassett, Chow et al. 2005, Rauch, Hofbeck et al. 2010). Velopharyngeal insufficiency, impaired hearing, seizures, and frequent infections are less prevalent in TOF patients with no genetic aberration and are higher in those with 22q11DS and other chromosomal aberrations (Rauch, Hofbeck et al. 2010). In 2009,
Kovacs et al interviewed 58 adult CHD patients (including a subset with TOF) and reported 50% (29/58) prevalence for lifetime mood or anxiety disorder (Kovacs, Saidi et al. 2009). Although the CHD population in this study was not homogenous, the results highlight the need for the development of psychosocial interventions and the need to raise awareness of the cardiologist to the high prevalence of psychiatric disorders in adult CHD patients (Kovacs, Saidi et al. 2009). Despite the above mentioned studies, there is currently limited data on the prevalence of late-onset extra-cardiac conditions in adult patients with TOF (with no identified genetic anomalies). As well, most studies have used a pediatric cohort, which makes it limiting to study neuropsychiatric disorders such as schizophrenia that manifest in adulthood.

1.4.3 Neurodevelopmental outcomes

Neurodevelopmental outcomes in CHD have been extensively studied; however, most of these studies used patients with hypoplastic left heart syndrome and transposition of the great arteries (Hovels-Gurich, Konrad et al. 2006, Zeltser, Jarvik et al. 2008a)(Wernovsky 2006, Wernovsky, Shillingford et al. 2005, Licht, Wang et al. 2004). In a study by Hovels-Gurich et al assessing neurodevelopmental outcome in 40 school-age children (with normal chromosomal status and without 22q11.2 deletions) following repair of TOF or VSD, perioperative hypoxemia was identified as a significant risk factor for neurodevelopmental abnormalities, including mild cognitive impairment with additional deficits in language and motor function (Hovels-Gurich, Konrad et al. 2006). In 2008, Zeltser et al studied neurodevelopmental outcomes in sixty 1-year old children with classic TOF and TOF-pulmonary atresia (excluding patients with major recognizable genetic defects and those with genetic and phenotypic syndromes, except for 22q11DS) and found an association between genetic variants and worse neurodevelopmental outcomes (Zeltser, Jarvik et al. 2008a). Although initially not detected, 18.3% of patients were
diagnosed with a genetic syndrome later in the study. Zeltser et al found that in addition to genetic variants, children with TOF and co-existing genetic syndromes had poor neurodevelopmental outcomes. In a study by DeMaso et al, 22% of children with TOF (most of whom had undergone reparative cardiac surgery) were reported to have scored less than 1.5 standard deviations on standardized intelligence quotient testing (although no reference was made as to whether patients had normal chromosomal status) (DeMaso, Campis et al. 1991). The results from these studies highlight the multifactorial influence of both genetic factors and the environment in determining neurodevelopmental outcomes in patients with TOF.

1.5 Genetics of TOF

1.5.1 Heritability

TOF is considered to be a complex disease with a multifactorial mode of inheritance. Familial cases have been described, where a Mendelian inheritance pattern may be present (Eldadah, Hamosh et al. 2001, Pitt 1962). In the majority of cases, however, TOF occurs sporadically (i.e. non-familial). In cases with 2 or 3 affected siblings, autosomal recessive transmission has been hypothesized (Cassidy, Allen 1991). Although there is a tendency for CHD (and TOF) to run in families, transmission does not follow a Mendelian mode of inheritance in the majority of cases. Therefore, a multifactorial model of inheritance, i.e. the interaction of several genetic loci with environmental factors, has been proposed as the most likely mechanism (Cassidy, Allen 1991, Nora, Nora 1978)(Nora 1968). Reported recurrence risks vary from 0.5% to as high as 16% for first degree relatives of individuals with TOF (Nora, Nora 1978, Nora 1968, Digilio, Marino et al. 1997, Burn, Brennan et al. 1998, Whittemore, Wells et al. 1994). However, most of these studies did not exclude probands with 22q11.2 deletions and other anomalies; thus, recurrence risks reported from these studies are biased by
the inclusion of such patients. In 1997, Digilio et al reported a recurrence estimate of 3% in siblings, 0.5% in parents, and 0.2% in aunts or uncles in families of 102 nonsyndromic probands with TOF, but without 22q11DS and other anomalies (Digilio, Marino et al. 1997). Further studies, after excluding probands with genetic anomalies, would be helpful in identifying unknown genetic aberrations such as copy number variations (CNVs) that could potentially segregate within families of probands with TOF.

1.5.2 Genetic causes of TOF

In most instances, the exact molecular mechanisms responsible for the formation of TOF are unknown. There is, however, evidence suggesting that genetic factors and to some extent environmental factors are important determinants of this disease. Environmental factors account for only 2% of all congenital heart defects (Kuciene, Dulskiene 2008). Examples of potential environmental causes of congenital heart malformations, including TOF, are teratogens such as rubella (Gibson, Lewis 1952), phenylketonuria (Jenkins, Correa et al. 2007), and maternal insulin-dependent diabetes mellitus (Abu-Sulaiman, Subaih 2004). In approximately one fifth of patients with CHD, there is a co-existing syndrome or chromosomal aberration (Perloff, 1991). 22q11DS, the most common genetic disorder associated with TOF (occurring in up to 16% of cases) will be discussed further below (Goldmuntz, Clark et al. 1998, Botto, May et al. 2003). The second most common chromosomal anomaly associated with TOF is trisomy 21, occurring in up to 8% of patients with TOF (Ferencz, Neill et al. 1989, Wells, Barker et al. 1994). Other genetic syndromes co-occurring with TOF are rare, and include Alagille syndrome, Treacher Collins syndrome, Klippel-Feil syndrome, Cornelia de Lange syndrome, trisomy 13, trisomy 18, Holt-Oram syndrome, Okihiro syndrome and Townes-Brocks syndrome (Lammer, Chak et al. 2009, Ferencz, Neill et al. 1989, Weismann, Gelb 2007).
In addition to genetic and chromosomal anomalies, haploinsufficiency of transcription factors \((NKX2.5, TBX1, TBX5, GATA4)\), transmembrane receptors \(NOTCH1\) and \(NOTCH2\) and their ligand \(JAG1\) altering gene dosage have been implicated in TOF (Eldadah, Hamosh et al. 2001, Greenway, Pereira et al. 2009, Goldmuntz, Geiger et al. 2001). Structural genomic changes or CNVs have also been implicated in CHD (Greenway, Pereira et al. 2009, Costain, Silversides et al. 2010). Recently, Greenway et al showed that approximately 10% of nonsyndromic cases of TOF may result from \textit{de novo} CNVs (Greenway, Pereira et al. 2009).

In addition to those with identified genetic or chromosomal abnormalities, there may be important subgroups of TOF that could potentially have relevance to a genetic pathogenesis of this disease (Scutt, Chow et al. 2001). Determining the genetic etiology of TOF will not only help in understanding the mechanism of this disease but also in identifying genotype-phenotype correlations.

1.5.2.1 Single gene disorders

Recurrence of CHD in families of patients with TOF suggests that single gene mutations may play a role in disease pathogenesis. In 2001, Goldmuntz et al identified heterozygous mutations in the \(NKX2.5\) gene in 6/114 (5%) nonsyndromic TOF patients without 22q11DS (Goldmuntz, Geiger et al. 2001). Five of the six patients with \(NKX2.5\) mutations had right aortic arch, and none had any extra-cardiac anomaly.

In a single large kindred with autosomal dominant TOF and reduced penetrance, Eldadah et al identified missense mutations in the \(JAG1\) gene without features of Alagille syndrome (Eldadah, Hamosh et al. 2001). Nine of the eleven (82%) mutation carriers manifested cardiac disease, including TOF, ventricular septal defect with aortic dextroposition, and isolated peripheral pulmonic stenosis. Furthermore, all mutation carriers had characteristic but variable
facial features distinct from Alagille syndrome, and had no pulmonary, skeletal, or gastrointestinal abnormalities as is commonly present in this syndrome.

VEGF, an endothelial cell-specific mitogen and angiogenic inducer, has been proposed as a possible modifier gene for TOF that in addition to other factors could increase risk of CHD. In 2005, Lambrechts et al showed that a 3-SNP haplotype, known to lower VEGF levels, was associated with an increased risk of TOF (Lambrechts, Devriendt et al. 2005). They found that the low-VEGF haplotype (AAG) was overtransmitted to affected children (p=0.008). Thus, VEGF is the first modifier gene identified in TOF. However, these results have not been replicated (Griffin, Hall et al. 2009).

In a study using 47 subjects (ranging in age from birth to 16.5 years) with sporadic TOF, 2 patients were found to have missense mutations in the ZFPM2/FOG2 gene, suggesting that ZFPM2/FOG2 mutations may contribute to TOF (Pizzuti, Sarkozy et al. 2003). However, no extra-cardiac features were reported in this study. Furthermore, of the two patients with the hemizygous mutations, one had TOF-pulmonary atresia with MAPCAs and the other had classic TOF.

In 2007, Karkera et al demonstrated that loss of function mutations in the Growth Differentiation Factor-1 (GDF1) is associated with congenital heart lesions ranging from TOF to transposition of the great arteries (Karkera, Lee et al. 2007). Three out of eight subjects with GDF1 mutations had TOF, where one had additional VSD, aortic root dilation, and bicuspid stenotic pulmonary valve stenosis. However, there were no reports on extra-cardiac findings in patients with the mutations.

In a study investigating genotype-phenotype relations in 230 children with syndromic and non-syndromic cases of TOF, Rauch et al reported a polyalanine stretch expansion within a
region in the TBX1 gene. This mutation resulted in the loss of transcriptional activity due to cytoplasmatic aggregation of the mutated protein (Rauch, Hofbeck et al. 2010). Cardiac and extra-cardiac features in this patient included scoliosis, facial asymmetry, upslanting palpebral fissures, absent pulmonary vein, and isolated left pulmonary artery. The results from this study suggest that intragenic mutations in the TBX1 gene (resulting in cytoplasmic aggregation of the mutant protein) may cause nonsyndromic cases of TOF.

1.5.2.2 Copy Number Variants

There has only been one study to investigate high resolution genome-wide CNVs in patients with TOF. By studying 114 TOF trios (TOF proband and unaffected parents), Greenway et al identified 11 de novo CNVs at 10 unique loci that were absent or rare in a control sample of 98 trios (Greenway, Pereira et al. 2009). Seven loci identified in this study were new, and were found to increase risk for sporadic nonsyndromic TOF by about 9-fold. However, in study subjects with 22q11.2 deletions, no extra-cardiac features were found, as are typically found in 22q11DS. Furthermore, one control subject had a large 22q11.2 deletion. In one subject with gain of function CNV on chromosome 3p25.1, extra-cardiac features including subtle craniofacial abnormalities and hyperactivity were reported. Together, the identified de novo CNVs accounted for 10% of cases of TOF in this study.

1.6 Genetic determinants of cardiac outcome

Few studies have previously examined the effects of genetic syndromes on immediate surgical or long term outcomes in patients with CHD (Lin, Basson et al. 2008). In 2006, Michielon et al retrospectively examined the impact of genetic syndromes and congenital extra-cardiac anomalies on surgical outcomes in pediatric TOF patients without pulmonary atresia
(Michielon, Marino et al. 2006). They identified hypoplastic pulmonary arteries and intervention for extra-cardiac anomalies as independent predictors of mortality. Furthermore, the ten-year actuarial survival was worse in syndromic patients (i.e. those with a confirmed diagnosed genetic syndrome) compared to nonsyndromic patients. Poor outcome in pediatric CHD patients with 22q11.2DS has been reported by Kyburz et al (Kyburz, Bauersfeld et al. 2008). In this study, just under half of the causes of death were non-cardiac related (either due to multiorgan failure, immunologic causes, pulmonary complications and multiple malformations), suggesting that extra-cardiac anomalies associated with 22q11.2DS can affect morbidity and mortality in CHD patients. However, while morbidity was elevated, none of the deaths occurred in TOF patients. Anacleterio et al have previously shown that in 350 children with conotruncal anomalies, mortality was higher in syndromic patients than in nonsyndromic patients, although this was at the trend level (p=0.06) (Anacleterio, Di Ciommo et al. 2004a). Immediate surgical mortality was significantly higher in 22q11.2 deleted patients with TOF-pulmonary atresia and 22q11.2 deleted patients with interrupted aortic arch.

In a study investigating mortality in adults with 22q11DS, Bassett et al showed that patients with 22q11DS (with or without CHD) have significantly diminished life expectancy (deaths in the 22q11.2 deleted group occurred at 47.5 years vs. 80.4 years in the Canadian general population) and are at increased risk of sudden death compared to their unaffected siblings (Bassett, Chow et al. 2009). Of the 12 deceased patients, four had TOF (two with TOF-pulmonary atresia, one with TOF-absent pulmonary valve syndrome, and one with classic TOF). Only one patient with TOF had history of arrhythmia (atrial arrhythmia). Two deaths were reported as sudden, presumably from arrhythmia. Two patients with non-sudden related death had clinical heart failure. Although no unifying factor could be attributed to the premature
deaths, this study emphasizes the importance of genetic background in determining (cardiac) outcome.

1.7 22q11.2 deletion syndrome (reference group for Study 1 and Study 2)

The most common underlying genetic anomaly in patients with TOF is 22q11DS, occurring in 10-16% of cases (Goldmuntz, Clark et al. 1998, Botto, May et al. 2003). We used patients with 22q11DS as a reference group because extra-cardiac features in this group of patients are well characterized (McDonald-McGinn, Kirschner et al. 1999, Bassett, Chow et al. 2005). 22q11DS is a multisystem disorder associated with chromosome 22q11.2 interstitial deletions (Bassett, Chow et al. 2005) with a prevalence of 1 in 4,000 live births (Goodship, Cross et al. 1998, Liling, Cross et al. 1999). About 90% of deletions are de novo; however, the mode of inheritance is autosomal dominant so that an offspring of an affected parent has a 50% chance of inheriting the deletion (Swillen, Vogels et al. 2000). 22q11DS is phenotypically variable and includes velocardiofacial syndrome (VCFS), DiGeorge syndrome (DGS), and conotruncal anomaly face syndrome (CTAFS) (Cohen, Chow et al. 1999). 22q11DS is known to affect organs derived from the third and fourth pharyngeal pouches, namely, the heart, great vessels, the thymus gland, parathyroid gland, face and branchial arch arteries (Scambler 2010). In addition to these pharyngeal pouch-related abnormalities, patients with 22q11DS may also develop many other anomalies and neurobehavioural problems, many of which may manifest in childhood and adulthood (Bassett, Chow et al. 2005, Cuneo 2001). There is phenotypic variability even within a family or between identical twins (Yamagishi, Ishii et al. 1998, Goodship, Cross et al. 1995). This has also been observed consistently in experimental mouse
models of the deletion 22q11.2, even when background strain and environment are strictly controlled (Merscher, Funke et al. 2001).

1.7.1 Characteristic features of 22q11DS

Common characteristic features in patients with 22q11DS include congenital heart defects, hypocalcemia, palatal anomalies (e.g. cleft palate), renal anomalies (e.g. absent kidney), neurological conditions e.g. (seizure disorder), lower limb anomalies and neuropsychiatric conditions (Table IV) (Bassett, Chow et al. 2005, Lindsay 2001). Phenotypic features in patients with 22q11DS vary by age group, as some characteristics become more noticeable or manifest later in life. Neonates with 22q11DS can present with feeding difficulties (occurring in 40-90% of cases) and seizures (possibly secondary to hypocalcemia) (Cuneo 2001). Many children with 22q11DS experience recurrent ear and upper respiratory infections, velopharyngeal insufficiency (which can manifest as hypernasal speech), developmental delay, learning difficulties and behavioural problems (Bassett, Chow et al. 2005, Cuneo 2001). In contrast, psychiatric features usually manifest in later in life ((Bassett, Chow et al. 2005).

1.7.2 Cardiovascular malformations in 22q11DS

Cardiovascular malformations are very common in patients with 22q11DS, with an estimated prevalence of 80% (Momma 2010). Several multi-center studies have identified TOF as the most common CHD in patients with 22q11DS, with prevalence varying between 13-39% (McDonald-McGinn, Kirschner et al. 1999, Ryan, Goodship et al. 1997, Park, Ko et al. 2007, Matsuoka, Kimura et al. 1998, Oskarsdottir, Persson et al. 2005). TOF-pulmonary atresia with MAPCAs has been documented in as many as 25% of patients with 22q11DS (Matsuoka, Kimura et al. 1998). Although most congenital heart defects in 22q11DS are associated with
right-sided heart lesions such as conotruncal and aortic arch anomalies, left-sided cardiac defects have also been observed, although rarely (Goldmuntz, Clark et al. 1998).

1.7.3 Ear, nose and throat in 22q11DS

Palatal abnormalities are a very common finding in 22q11DS (Bassett, Chow et al. 2005, Cuneo 2001), where the prevalence of velopharyngeal incompetence has been reported in as many as 70% of patients. (McDonald-McGinn, Kirschner et al. 1999). Submucous and overt cleft palate are frequently associated with 22q11.2 deletions with prevalence of 16% and 11%, respectively (McDonald-McGinn, Kirschner et al. 1999).

Craniofacial findings in patients with 22q11.2 deletions include auricular abnormalities, nasal abnormalities (such as protruding or microtic ears with over-folded or squared helixes), ptosis (hooded eyelids), ocular hypertelorism (widely spaced out eyes), bulbous tip nose, narrow palpebral fissures, small mouth, as well as some other variable features including long, narrow face and malar flatness (Fung, Chow et al. 2008, McDonald-McGinn, Gripp et al. 2005, Cuneo 2001).

1.7.4 Immune system in 22q11DS

The prevalence of immunodeficiency in patients (which are usually present from infancy) with 22q11DS has been reported in 40-93% of cases (McDonald-McGinn, Kirschner et al. 1999, Smith, Driscoll et al. 1998). Immunodeficiency is a risk factor for recurrent infections, which are seldom life-threatening but common in young children with 22q11DS (Ryan, Goodship et al. 1997, Jawad, McDonald-Mcginn et al. 2001). In cases where immunodeficiency is severe (i.e. in patients with thymic aplasia or absent T-cells), aggressive immunodeficiency treatment and possibly thymic transplantation may be required (Cuneo 2001). Autoimmune disorders are also
very common in 22q11D, some of which include juvenile rheumatoid arthritis, idiopathic thrombocytopenia purpura, hyperthyroidism, hypothyroidism, and hemolytic anemia (Jawad, McDonald-McGinn et al. 2001, Kawame, Adachi et al. 2001, Keenan, Sullivan et al. 1997).

1.7.5 Endocrine system in 22q11DS

Hypocalcemic hypoparathyroidism, caused by absent or small parathyroid glands is found in 40-60% of patients with 22q11.2 deletions and is typically most serious in the neonatal period (McDonald-McGinn, Kirschner et al. 1999, Bassett, Chow et al. 2005, Ryan, Goodship et al. 1997). Neonatal hypocalcemia can either resolve spontaneously and completely or recur later in life (Greig, Paul et al. 1996). Hypocalcemia requires treatment as untreated patients may develop secondary seizures, and is clinically significant in the case of the adult or adolescent with a repaired conotruncal defect who is already at an increased risk of arrhythmias, syncope and sudden death (Cuneo 2001, Kao, Mariani et al. 2004).

Another endocrine abnormality associated with the 22q11.2 deletion is hypothyroidism. In a study by Driscoll et al, hypothyroidism was noted in 7% of subjects with VCFS, and Wilson et al reported a prevalence of 5% in patients with DGS (Driscoll, Budarf et al. 1992, Wilson, Burn et al. 1993). In 2005, Bassett et al studied cardiac and extra-cardiac features in 78 adults with 22q11DS, including late-onset features, and documented a prevalence of 20.5% for hypothyroidism (Bassett, Chow et al. 2005).

Short stature, typically in part due to growth hormone insufficiency, is also very common in the 22q11.2 deletion, reported in 10-40% of patients (Cuneo 2001).
1.7.6 Cognitive function, neuropsychiatric and central nervous system abnormalities in 22q11DS

Learning disability and psychiatric problems are common in patients diagnosed with 22q11.2DS (Goldberg, Motzkin et al. 1993, Shprintzen, Goldberg et al. 1992, Shprintzen, Goldberg et al. 1978). Cognitive disabilities are documented in 40-60% of subjects who have 22q11.2 deletions (Cuneo 2001). Cognitive functioning of children with 22q11.2DS represents a spectrum: intelligence may vary from moderate mental retardation to average intelligence, with an average IQ of about 70 (Bassett, Chow et al. 2005, Swillen, Devriendt et al. 1997, Moss, Batshaw et al. 1999). Patients in early childhood typically present with impairments of language and motor development (Swillen, Devriendt et al. 1997, Gerdes, Solot et al. 1999) and verbal IQ scores are typically higher than nonverbal or performance IQ scores (Cuneo 2001, McDonald-McGinn, LaRossa et al. 1997, Swillen, Devriendt et al. 1999). Autism/autistic spectrum disorder is also common in children with 22q11.2 deletions, with a reported prevalence of 20% (Fine, Weissman et al. 2005).

In the study by Kao et al, the incidence of unprovoked seizures (precipitating events such as hypocalcemia, fever and recent surgery) was reported as 7%, suggesting that seizure is a primary manifestation of the 22q11.2DS and not a secondary result of other features seen in this syndrome (Kao, Mariani et al. 2004).

Attention deficit with hyperactivity disorder, attention deficit disorder, emotional instability and anxiety have also been reported during childhood (Goldberg, Motzkin et al. 1993). Obsessive compulsive disorder has also been described, ranging from 8-33% (Gothelf, Presburger et al. 2004, Papulos, Faedda et al. 1996). Schizophrenia, one of the most common
psychiatric abnormalities diagnosed in 22q11.2DS, is reported in roughly one fifth of patients (Murphy, Jones et al. 1999, Chow, Zipursky et al. 2002).

Magnetic resonance imaging studies have been conducted to shed light on the neurobiological basis of 22q11DS and to find a relationship between neuroanatomic abnormalities and the cognitive/psychiatric anomalies that commonly afflict patients with this syndrome (Swillen, Vogels et al. 2000). Specifically, structural abnormalities in the central nervous system have been identified and related to cognitive and neuropsychiatric conditions in patients with 22q11DS (Eliez, Schmitt et al. 2000, Kates, Burnette et al. 2001, Eliez, Antonarakis et al. 2001, Campbell, Daly et al. 2006, Eliez, Barnea-Goraly et al. 2002, Kates, Miller et al. 2006, Simon, Ding et al. 2005)(Chow, Zipursky et al. 2002, Bearden, van Erp et al. 2004). For example, in the study by Chow et al, 22q11.2 deleted patients with schizophrenia were reported to have significantly smaller total gray matter volume and larger lateral ventricles than controls (Chow, Zipursky et al. 2002). Given that approximately 25% of patients with 22q11.2DS develop schizophrenia, these findings suggest that structural brain differences may predispose some 22q11.2 deleted patients to psychotic disorders (Chow, Zipursky et al. 2002).

**1.7.7 Musculoskeletal system in 22q11DS**

Musculoskeletal (MSK) anomalies are also very common in patients with 22q11DS, with a reported prevalence varying from 36% to 48% (Bassett, Chow et al. 2005, Ming, McDonald-McGinn et al. 1997). Ming et al found upper limb anomalies (e.g. preaxial polydactyly) in 6% and lower limb anomalies (e.g. club foot) in 15% of subjects with 22q11.2DS. Other MSK anomalies identified include vertebral (e.g. butterfly vertebrae), and rib anomalies (e.g. rib fusion), the former of which is highly associated with conotruncal heart defects (Ming, McDonald-McGinn et al. 1997). In the study by Bassett and colleagues, approximately 48% of
adults with 22q11DS were reported to have scoliosis, of which 6.4% required surgical treatment (Bassett, Chow et al. 2005). The pathogenesis of MSK anomalies in this syndrome remains to be identified (Ming, McDonald-McGinn et al. 1997).

1.7.8 Genitourinary system in 22q11DS

In 1996, Devriendt et al reported renal anomalies in 4 out of 39 (10%) cases with 22q11DS, which included bilateral obstructive megaureter, 2 cases of unilateral renal agenesis, and a right multicystic kidney. In 2002, Wu and colleagues identified a structural urinary tract anomaly in 31% of patients with 22q11.2DS, which included duplex kidney, unilateral hypertrophied left kidney, multicystic left kidney, echogenic kidney, bilateral small kidneys, renal cysts, nephrolithiasis, as well as ureteral and bladder anomalies (Wu, Rusnack et al. 2002). Of 149 male patients, 6% and 8.1% had an undescended testis and hypospadias, respectively. In the study by Bassett et al, renal agenesis and hydronephrosis/hydroureter were reported in roughly 6% and 8% of adults with 22q11DS, respectively (Bassett, Chow et al. 2005). The exact molecular mechanism of genitourinary anomalies in patients with 22q11.2DS has yet to be elucidated, although it is suspected that a gene(s) in the deleted region may play a role in ureter bud development during embryogenesis (Devriendt, Swillen et al. 1996).
Table IV. Extra-cardiac abnormalities associated with 22q11.2 deletions.

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Frequency of finding</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiac anomalies</strong></td>
<td></td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>75%</td>
</tr>
<tr>
<td>Interrupted aortic arch</td>
<td>17-22%</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>14-15%</td>
</tr>
<tr>
<td>Truncus arteriosus</td>
<td>13-14%</td>
</tr>
<tr>
<td><strong>Hypocalcemia</strong></td>
<td>64%</td>
</tr>
<tr>
<td><strong>Hypothyroidism</strong></td>
<td>21%</td>
</tr>
<tr>
<td><strong>Palatal abnormalities</strong></td>
<td>69%</td>
</tr>
<tr>
<td>Submucosal and overt cleft palate</td>
<td>11-16%</td>
</tr>
<tr>
<td>Bifid uvula</td>
<td>5%</td>
</tr>
<tr>
<td>Velopharyngeal insufficiency</td>
<td>27%</td>
</tr>
<tr>
<td><strong>Renal anomalies</strong></td>
<td>36-37%</td>
</tr>
<tr>
<td>Agenesis</td>
<td>6.4%</td>
</tr>
<tr>
<td>Hydronephrosis/hydroureter</td>
<td>7.7%</td>
</tr>
<tr>
<td><strong>Recurrent seizures</strong></td>
<td>40%</td>
</tr>
<tr>
<td><strong>Lower limb anomalies</strong></td>
<td>15%</td>
</tr>
<tr>
<td><strong>Intellectual disability</strong></td>
<td>92%</td>
</tr>
<tr>
<td><strong>Attention Deficit Hyperactivity Disorder</strong></td>
<td>35-46%</td>
</tr>
<tr>
<td><strong>Schizophrenia</strong></td>
<td>20-30%</td>
</tr>
</tbody>
</table>

2 Aims/Hypotheses

Chromosomal aberrations, single gene disorders, and structural genomic or copy number variations (mostly large) changes have been implicated in the genetic etiology of TOF. However, these account for only a minority of cases and in most cases the genetic etiology of TOF is unknown. In addition to genetic heterogeneity, there is substantial phenotypic variability in patients with TOF. Phenotypic variability may relate to genetic causation and outcome, and may be represented by clinical characteristics such as auxiliary cardiac and extra-cardiac abnormalities (i.e. congenital structural defects present from birth or conditions that manifest or become detectable later in life). For example, atrioventricular septal defects are commonly associated with trisomy 21 in patients with TOF (Goldmuntz, Clark et al. 1998, Botto, May et al. 2003)(Rauch, Hofbeck et al. 2010). Anomalies of the aortic arch laterality or branching are a typical finding in TOF patients with 22q11DS (Rauch, Hofbeck et al. 2010) (Perloff, 1991). Similarly, extra-cardiac anomalies have been reported in association with TOF in the presence of specific genetic syndromes. Patients with TOF and Holt-Oram syndrome often have anomalies affecting the limb, and TOF patients with Alagille syndrome consistently present with hepatic anomalies. However, these only account for a minority of cases. In the vast majority with no identified genetic cause, there is inconsistency of extra-cardiac features from patient to patient. Clustering patients into meaningful categories based on phenotypic features may help identify a homogenous group of patients who may have relevance to the pathogenesis of TOF and late outcomes.

Most studies that have examined extra-cardiac features associated with TOF have focused on infants and children (Kyburz, Bauersfeld et al., 2008, Michielon, Marino et al. 2006). Except in a few select genetic conditions, there are no studies of later onset extra-cardiac features
in adults and these are important as they may aid in pattern recognition of specific genetic syndromes associated with specific anomalies. While the pediatric studies have been important, more information on the extra-cardiac features and late-onset disease in the adult population is needed, as these features may impact outcome. Furthermore, the pattern of congenital and later onset extra-cardiac features (i.e. phenotypic variability) may help in identifying possible subgroups of TOF that may be enriched for genetic cause(s) of TOF. Emerging genomic disorders and future genetic discoveries will help identify more molecular genetic causes of TOF, and recognition of phenotypic features can help.

Although cardiac morbidity and mortality in TOF are well described, there is less information available examining the relationship between genetic variability and cardiac outcomes. Available data suggests that patients with genetic syndromes have worse outcomes (Kyburz, Bauersfeld et al. 2008, Michielon, Marino et al. 2006, Bassett, Chow et al. 2009, Anaclerio, Di Ciommo et al. 2004a). For example, substantial morbidity (e.g. the need for cardiac reinterventions, multiorgan failure, and prolonged hospital stay), mortality and premature death have been reported in patients with 22q11DS, with or without CHD (Kyburz, Bauersfeld et al. 2008, Bassett, Chow et al. 2009). However, similar to studies on extra-cardiac features in TOF, most studies on the genetic determinants of outcome have been done in children or have not specifically focused on TOF (i.e. studies included mixed population of patients with CHD) ((Kyburz, Bauersfeld et al. 2008, Anaclerio, Di Ciommo et al. 2004a). In adults, apart from studies on 22q11DS or trisomy 21, the relation between genetic variability and late outcomes have not been well studied (Bassett, Chow et al. 2009, Bell, Rankin et al. 2003). Differences in cardiac outcomes between “syndromic” and nonsyndromic subjects may relate to underlying genetic factors and help with identifying non-cardiac risk factors of cardiac outcome.
We therefore sought to investigate phenotypic variability in adults with TOF by identifying a “syndromic group” of adults with TOF, but without 22q11.2 deletions or other major known genetic syndromes. In STUDY 1, we compared the lifetime prevalence of congenital cardiac and extra-cardiac anomalies, and later onset extra-cardiac conditions between the syndromic group and a nonsyndromic control group. We further sought to determine if the syndromic group has worse adverse outcomes compared to the nonsyndromic comparison group (STUDY 2). As reference, we also recorded information on patients with TOF and 22q11DS, as this group is well characterized (Bassett, Chow et al. 2005)(McDonald-McGinn, Kirschner et al. 1999, Driscoll, Salvin et al. 1993)

We hypothesized that the syndromic group, who may represent a more homogenous group of patients enriched for genetic causes of TOF, would have a greater prevalence of congenital cardiac and extra-cardiac anomalies, later onset manifestations, and worse adverse outcomes compared to the nonsyndromic comparison group. Identifying this group of patients using brief clinical genetic screening would have great relevance to clinical care. In addition, this subgroup of adults with TOF may have relevance to a genetic pathogenesis of TOF.
3 Study 1

Differentiating Patterns of Cardiac and Extra-cardiac Anomalies in Adults with Tetralogy of Fallot
3.1 Objectives

It is known that approximately 10-16% of patients with TOF have 22q11DS (Goldmuntz, Clark et al. 1998, Botto, May et al. 2003). However, in most cases, the genetic basis is unknown and among these patients there is substantial phenotypic variability. Using a previously established screening criterion to select for patients with 22q11DS (Fung, Chow et al. 2008) we have noted a subgroup of individuals without 22q11DS, but with features suggestive of a genetic syndrome. We aimed to determine if TOF patients with syndromic features have a different clinical presentation compared to those without syndromic features with specific focus on the following characteristics:

1. Congenital cardiovascular anomalies
2. Congenital extra-cardiac anomalies
3. Late-onset manifestations

Clinical features of a reference population of patients with TOF and 22q11DS were also collected.
3.2 Methods

This study was approved by the institutional ethics boards. Adults (≥18 years) with TOF seen at the Toronto Congenital Cardiac Centre for Adults and who had undergone prospective clinical screening (Fung, Chow et al. 2008) from 1998 to 2008 [n=447, mean age 39±13 years, 239 males (53%)] comprised the sample population. All patients with TOF seen at the clinic and who consented were screened. Individuals with major genetic anomalies such as Down syndrome were not included in the initial screening study. Of these 447 patients, 88% (n=395, 12%) had TOF and the remainder (n=52) had TOF-pulmonary atresia.

Subjects were categorized into three groups: syndromic, nonsyndromic, and 22q11DS. Subjects were classified as syndromic if they had at least two of the three criteria previously established to identify adults with 22q11DS: an overall impression of dysmorphic facial features (e.g. narrow palpebral fissures), cognitive impairment (e.g. self-reported childhood learning difficulties), and speech problems (predominantly hypernasal speech) (Fung, Chow et al. 2008, Bassett, Chow et al. 2005). Figure 3 represents an example of a syndromic patient in the study with dysmorphic facial features, learning difficulties, and hypernasal speech. Subjects with 22q11DS who met the syndromic criteria and had a confirmed 22q11.2 deletion (n=39) were used as a reference group (Fung, Chow et al. 2008, Fung, Chow et al. 2008, Bassett, Chow et al. 2005)(Fung, Chow et al. 2008). Of the initial 447 subjects, 15% (n=66) were classified as syndromic, 9% (n=39) had 22q11DS and the remainder (n=342) formed the total nonsyndromic group, not meeting syndromic screening criteria on clinical genetic screening (Figure 4). Excluded from the syndromic group were five subjects with other known genetic syndromes (CHARGE, Kartagener, Klippel-Feil, Klinefelter’s and distichiasis-lymphedema syndromes) and five patients with a history of stroke and acquired speech and/or learning difficulties. Subjects in
the remaining syndromic group (n=56) were matched randomly by age (within 5 years) and gender in a 1:2 ratio with subjects who did not meet the syndromic criteria. The matched nonsyndromic comparison group thus comprised 112 of 342 available subjects (Figure 4). Most (n=50, 89%) of the subjects in the syndromic group had clinical genetic testing to rule out 22q11DS and other major genetic anomalies.

In addition to the prospectively collected screening data, comprehensive chart reviews using records from the adult hospital (Toronto General Hospital, Toronto, Canada) and the local referring pediatric hospital (Hospital for Sick Children, Toronto, Canada) provided lifetime information on cardiac and minor and major extra-cardiac features. Congenital cardiovascular abnormalities other than TOF were recorded (e.g. atrial septal defect). Greater emphasis was placed on major congenital extra-cardiac anomalies because they have the potential to affect the functioning of the affected individual (Marden, Smith et al. 1964). Based on previous definitions, major congenital extra-cardiac anomalies included any of the following: central nervous system (e.g. spina bifida), gastrointestinal (e.g. esophageal atresia), renal (e.g. renal agenesis), genital (e.g. bicornuate uterus), major ear deformities requiring surgery (e.g. absent left ear lobe), and major musculoskeletal (e.g. club foot) (Marden, Smith et al. 1964) (Jones, 2006). Minor congenital anomalies (e.g. syndactyly) were described separately. Short stature (<3rd percentile) was defined as a height at last follow up of less than 150 cm and 161 cm for females and males, respectively (http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/datafiles.htm).

We also recorded late-onset extra-cardiac conditions (including thyroid disorders, disorders of calcium metabolism, and neuropsychiatric disorders) commonly found in 22q11DS and other genetic syndromes (Jones, 2006). Neuropsychiatric disorders included seizures, Attention Deficit Disorder/Attention Deficit Hyperactivity Disorder (ADD/ADHD),
schizophrenia, and anxiety and/or depression. Treatments for these conditions were also recorded. Other late-onset conditions frequently found in the general population and/or that were infrequent in this study were not reported (e.g. gastroesophageal reflux).

Subjects with genetic testing had standard karyotype and fluorescence in-situ hybridization (FISH) testing for 22q11.2 deletions using a TUPLE 1 (Vysis) or N25 (ONCOR) probe (Fung, Chow et al. 2008, Bassett, Chow et al. 2005, Driscoll, Salvin et al. 1993).

**Statistical analysis**

The statistical analysis was performed using SPSS software (version 16, SPSS, Inc., Chicago, Illinois). Continuous data are presented as mean ± standard deviation. Differences between groups (i.e. syndromic and nonsyndromic groups) were determined using Chi-square, Fisher exact, Kruskal-Wallis or Student’s t tests as appropriate. A p values <0.05 (2-tailed) was considered statistically significant.

![Figure 3. Example of a subject from the syndromic group. This patient has dysmorphic facial features, learning difficulties, and hypernasal speech. Used with permission.](image-url)
Figure 4. Categorization of patients into the nonsyndromic group, syndromic group, and 22q11DS reference group. Patients with known syndromes (except for 22q11DS) and a history of stroke and acquired speech and/or learning difficulties were excluded.

### 3.3 Results

Table V presents the characteristics of the three TOF groups (n=207, 106 males (51%), mean age 36 ±10 years). Most subjects had intracardiac repair of TOF (nonsyndromic 98% vs. syndromic 91% vs. 22q11DS 92%, p=0.08). In the syndromic group (n=56), 62% (35/56) met two and 38% (21/56) met three of the predefined clinical screening criteria (Fung, Chow et al. 2008). Baseline clinical characteristics are shown in Table V. Results for all subjects in the syndromic and nonsyndromic groups who underwent genetic testing were negative (Table V). There were no significant differences between the syndromic and nonsyndromic groups in regards to age, sex, or family history of CHD (Table V). The 22q11DS reference group was younger (p=0.007) and had a higher proportion of subjects with a family history of CHD.
compared to the main study groups. As expected, given the screening criteria which emphasized hypernasality, the syndromic group had a significantly greater prevalence of overt and submucous cleft palate compared to the nonsyndromic group (11% vs. 0, p=0.001).

There was a trend for proportionately more subjects with short stature in the syndromic compared to the nonsyndromic group (n=8, 14% vs. n=7, 6%, p=0.09). Of the 15 subjects in the syndromic and nonsyndromic groups with short stature, 6 (40%) had scoliosis.

### 3.3.1 Congenital cardiovascular anomalies

Table VI demonstrates the prevalence of congenital cardiac and vascular anomalies in the study sample. The overall prevalence of individuals with cardiovascular anomalies was lowest in the nonsyndromic group (62%), followed by the syndromic group (70%) and the 22q11DS reference group (85%). Of the individual anomalies studied the syndromic group had a significantly higher proportion of subjects with pulmonary atresia and/or major aorto-pulmonary collateral arteries (MAPCAs) compared to the nonsyndromic group (Table VI). In the syndromic group, there were 7 subjects (13%) with MAPCAs and 11 subjects (20%) with pulmonary atresia. In the nonsyndromic group, 7 subjects (6%) had MAPCAs and 12 (11%) had pulmonary atresia.

The most common venous anomaly besides left superior vena cava draining into coronary sinus was bilateral/left superior vena cava. Coronary artery anomalies were the most common arterial anomaly besides aberrant subclavian artery. Aberrant subclavian artery was much more common in the 22q11DS reference group compared to the main study groups. Other uncommon cardiac findings included sub-aortic stenosis (n=2), cardiomyopathy (n=2), semilunar valve
stenosis (n=1), mitral valve prolapse (n=1), straddling of tricuspid valve (n=1), mesocardia (n=1), double chambered right ventricle (n=2), and cleft mitral valve leaflet (n=1).

3.3.2 Congenital extra-cardiac anomalies

3.3.2.1 Major anomalies

The overall prevalence of one or more congenital extra-cardiac anomaly in subjects in the syndromic and nonsyndromic groups was 17% (29/168). There was a trend toward a higher prevalence of subjects in the syndromic group with one or more major extra-cardiac anomaly compared to the nonsyndromic group (25% vs. 13%, \( p=0.06 \)) (Figure 5). Four percent (8/207) of subjects had two or more congenital extra-cardiac anomalies; 4 subjects in the syndromic group, 2 in the nonsyndromic group, and 2 in the 22q11DS reference group. Other anomalies were uncommon (Table VII).

The prevalence of congenital anomalies involving organ systems other than cardiovascular, was similar between the syndromic group and the 22q11DS group (25% vs. 36%, \( p=0.25 \)). Interestingly, we found that when the syndromic and nonsyndromic groups were combined, subjects with these congenital extra-cardiac anomalies were more common in the 28 subjects with pulmonary atresia and/or MAPCAs compared to the 140 without these cardiac features (\( n=9, 32\% \) vs. \( n=20, 14\%; \ p=0.02 \)), with musculoskeletal anomalies predominating in the former group, present in seven (78%) subjects.

In the syndromic and nonsyndromic groups combined, genitourinary anomalies were the most common extra-cardiac anomaly followed by musculoskeletal anomalies, with overall prevalence of 9% (15/168) and 8% (14/168), respectively. However, neither of these distinguished the syndromic from nonsyndromic groups (Table VII).
3.3.2.2 Minor anomalies

The syndromic group had a significantly greater prevalence of minor congenital extra-cardiac anomalies compared to the nonsyndromic group (23% vs. 9%, \(p=0.01\)), but lower than that in the 22q11DS group (23% vs. 62%). Musculoskeletal anomalies were the most common minor congenital extra-cardiac anomaly in this study group. These were mostly prevalent in the 22q11DS group (17/39, 44%), followed by the syndromic group (5/56, 9%) and the nonsyndromic group (4/112, 4%). Minor musculoskeletal anomalies were camptodactyly (n=9), syndactyly (n=3), torticollis (n=4), tapering fingers (n=8), hypoplastic nails/fingers (n=6), clindodactyly (n=2), cubitus valgus (n=1), and simian/palmar crease (n=2).

Hernias were the second most common minor congenital anomaly (n=24). There were nine cases of inguinal hernias, one hiatus, one diaphragmatic, and one umbilical; the remaining were unspecified. The prevalence of hernias was similar between the syndromic and nonsyndromic groups (5% vs. 13%, \(p=0.10\)); however, it was significantly more prevalent in the 22q11DS group (\(p=0.001\)).

Strabismus was the most common ocular anomaly in all three groups. The 22q11DS had the highest prevalence of ocular abnormalities (\(p=0.001\)), which included strabismus in every case (one subject also had Duane syndrome). One subject in the syndromic group and one in the nonsyndromic group had strabismus and anisocoria, respectively. There was one case of congenital lower limb lymphedema in the syndromic group.

3.3.3 Late-onset manifestations

Overall, the prevalence of subjects with any neuropsychiatric disorder was high in the syndromic and nonsyndromic groups combined (22%) and was significantly greater in the
syndromic group compared to the nonsyndromic group (32% vs. 17%, p=0.03) (Table VIII). This appeared to be attributable to seizures, conditions with neurodevelopmental origins including schizophrenia, and ADD/ADHD (Table VIII). ADD/ADHD, first onset of seizure activity, recurrent otitis media, and in some cases hearing deficits developed in childhood, while thyroid dysfunction (except for one case in the syndromic group with congenital hypothyroidism), schizophrenia, anxiety and depression were diagnosed in adulthood. Not surprisingly, 14 of the 15 subjects in the syndromic group and 2 of the 19 subjects in the nonsyndromic group with ADD/ADHD, schizophrenia, and/or seizures had learning difficulties. In the 22q11DS reference group, all but one subject with ADD/ADHD, schizophrenia, and/or seizures had learning difficulties. Although the prevalence of reported anxiety and/or depression was similar between the two groups, anxiolytic medications were more commonly used in the syndromic than in the nonsyndromic group (9% vs. 1%, p=0.02).

The high prevalence of endocrine disorders in the syndromic group was mainly related to thyroid dysfunction (Table VIII). Of the 23 subjects in the sample with hypothyroidism, 70% were female. Two subjects in the 22q11DS reference group had hypogonadotropic hypogonadism in addition to hypocalcemia; one also had a hypoplastic pituitary gland. The high prevalence of hypocalcemia in the 22q11DS group was likely related to our routine practice to check ionized calcium levels in this group of patients (Bassett, Chow et al. 2005).

Although there was a greater proportion of subjects with hearing deficits in the syndromic group compared to the nonsyndromic group, this appeared to be at least partly accounted for by a history of recurrent otitis media (Table VIII). Myringotomy tube procedure was performed in 41% (17/41) of all subjects with a history of otitis media. Of the 21 subjects with hearing deficits, three subjects in the syndromic group (one with congenital deafness and
two with congenital ear deformities requiring surgery) and one subject in the 22q11DS group (with congenital ear deformity requiring surgery) had a congenital cause accounting for their hearing loss.

When we reanalyzed the data after excluding the six subjects from the syndromic group who have not yet had genetic testing, along with the corresponding 12 age and gender-matched subjects from the nonsyndromic group, the results showed little change. Compared to the nonsyndromic group, the syndromic group’s higher prevalence of subjects with seizures (n=8, 16% vs. n=6, 6%; p=0.047), schizophrenia (n=2, 4% vs. 0%; p=0.04), gastrointestinal anomalies (n=2, 4% vs. 0%; p=0.04) and ear deformities (m=2, 4% vs. 0%; p=0.04) reached significance, while pulmonary atresia and/or MAPCAs (n=12, 24% vs. n=12, 12%; p=0.059) were at the trend level.

3.4 Discussion

This is the first study to describe phenotypic variability in adults with TOF. The results from this study demonstrate that in a substantial proportion of subjects, TOF represents a multisystem disorder, even when those with 22q11DS and chromosomal anomalies are excluded. We previously used a brief screening protocol to delineate criteria that successfully identified adults with 22q11DS. Using this criteria, we have also identified a subgroup of patients without 22q11.2 deletions or other known genetic anomalies, but with syndromic features suggestive of a genetic syndrome. We found that this group of subjects had evidence of a more severe cardiac disease, and were at elevated risk for developing later onset neuropsychiatric and endocrine disorders compared to a gender and age-matched nonsyndromic group. These findings are novel and have potential implications for clinical management.
We found that one in four subjects in the syndromic group had complex cardiac disease (pulmonary atresia and/or MAPCAs), cardiac features that can be associated with worse outcomes (Hickey, Veldtman et al. 2009). In some instances, genetic variants have been related to these more complex cardiac lesions including mutations in \textit{NKX2.5} or 22q11.2 deletions (Goldmuntz, Geiger et al. 2001) (Chessa, Butera et al. 1998).

The overall prevalence of one or more major congenital extra-cardiac anomaly in our study sample, excluding the 22q11DS group, was 17%. Previous reports have documented prevalence varying from 6-39% (Francannet, Lancaster et al. 1993, Gucer, Ince et al. 2005, Lurie, Kappetein et al. 1995, Ferencz, Rubin et al. 1987, Karr, Brenner et al. 1992, Calzolari, Garani et al. 2003, Meberg, Hals et al. 2007). However, some samples used to determine previously reported prevalence of comparable anomalies for TOF would have included subjects with 22q11.2 deletions and other genetic syndromes. Furthermore, the proportion of extra-cardiac anomalies reported is dependent on the inclusion criteria, as we chose to focus mostly on major extra-cardiac anomalies. The most common major anomalies encountered in the present study were genitourinary and musculoskeletal, in keeping with some previous reports (Francannet, Lancaster et al. 1993, Song, Hu et al. 2009). However, we did not find a high proportion of TOF subjects with gastrointestinal anomalies, as previously documented (Francannet, Lancaster et al. 1993, Song, Hu et al. 2009). This may be due to differences in inclusion criteria and age of the cohort studied.

The overall prevalence of minor congenital extra-cardiac anomalies in both the syndromic and nonsyndromic groups was 14%, compared to 62% in the 22q11DS group. While minor anomalies may not be clinically significant, Marden et al have previously reported that minor anomalies are common in infants with major embryonic defects (Marden, Smith et al.
1964). This suggests that minor anomalies may be used as indicators of more severe embryonic
defects, possibly involving a major genetic anomaly (Marden, Smith et al. 1964).

The prevalence of congenital extra-cardiac anomalies reported in our study and previous
reports is substantially higher than general population expectations (Francannet, Lancaster et al.
the prevalence of genitourinary anomalies in the syndromic and nonsyndromic groups combined
(9%) is significantly greater than that reported in the general population (0.096%) (Jung, Kim et
al. 1999). Associated extra-cardiac anomalies with TOF encountered in subjects from the
syndromic and nonsyndromic groups may represent new syndromes, a random association, result
from independent disturbances, or occur secondary to the morphogenetic abnormality that caused
TOF (Lurie, Kappetein et al. 1995). For example, since musculoskeletal and cardiac
development occur at different periods of fetal development, one mechanism to explain co-
existing structural defects in both systems may relate to genetic factors regulating both heart and
limb development. TBX5 mutations in Holt-Oram syndrome (Basson, Bachinsky et al. 1997)
provide a model for such mechanisms. Similarly, the higher prevalence of extra-cardiac
anomalies (predominantly involving the musculoskeletal system) in TOF patients with
pulmonary atresia and/or MAPCAs compared to patients without more severe variant forms of
TOF may suggest a more pleiotropic and potentially earlier developmental genetic origin such as
transcription factors.

A novel finding of our study is the high prevalence of several late-onset conditions in the
syndromic group, comparable to those in 22q11DS. Our reported prevalence is greater than that
reported in the general population for seizures (4-10%), attention deficit disorder (5%) and
thyroid dysfunction (up to 1%) (Friedman, Sharieff 2006, Bilous, Tunbridge 1988, Polanczyk, de
Lima et al. 2007). Similar to 22q11DS, the syndromic group had a high prevalence of thyroid disorders. The etiology of thyroid dysfunction in our sample is unknown and may be related to several causes, including commonly encountered autoimmune causes, congenital hypothyroidism (which can be caused by missense mutations in the \textit{NKX2.5} gene) or faulty neural crest-derived thyroid glandular cells (Dentice, Cordeddu et al. 2006) (Burke, Johnson et al. 1987)(Weetman, McGregor 1994).

Almost all subjects in the syndromic and nonsyndromic groups had reparative cardiac surgery, performed at a similar age (i.e. at a similar era) in both groups. Although multifactorial in nature, the elevated prevalence of neuropsychiatric conditions observed in the syndromic and 22q11DS groups may be related to genetic and/or other prenatal influences than environmental factors related to open heart surgery (Fung, Chow et al. 2008, Bassett, Chow et al. 2005, Zeltser, Jarvik et al. 2008b). Patients with TOF secondary to Down syndrome and 22q11DS have been shown to have worse neurodevelopmental outcomes compared to those without genetic syndromes, suggesting that genetic factors, not simply environmental factors related to open heart surgery, are important (Atallah, Joffe, et al. 2007).

As in the 22q11DS group, we found a strong overlap between learning difficulties and neuropsychiatric disorders involving ADD/ADHD, seizures, and/or schizophrenia in the syndromic group, features that may be related to genetic variations. For example, copy number variations (CNV) on chromosome 1q21.1 have been associated with neurocognitive and neuropsychiatric disorders including schizophrenia, autism spectrum disorder and mental retardation, as well as CHD (Mefford, Sharp et al. 2008b, International Schizophrenia Consortium 2008)(Greenway, Pereira et al. 2009). CNV on chromosome 16p12.1 have also been associated with learning disability, craniofacial and skeletal abnormalities, psychiatric disorders,
growth retardation and congenital heart defects (Girirajan, Rosenfeld et al. 2010). We would predict that this patient population would be enriched for CNVs. In addition to high penetrant CNVs causing syndromal features, there still may be low penetrant CNVs in either the syndromic or nonsyndromic group or both (Bassett, Scherer et al. 2010).

The syndromic group identified represents an initial step towards delineating more homogenous populations with TOF that could help identify those enriched for specific genetic causes of this disease. The largest identifiable subgroup, with 22q11.2 deletions, suggests that other CNVs may play a role in the etiology of TOF, and this is supported by recent studies (Greenway, Pereira et al. 2009)(Costain, Silversides et al. 2010). The multisystem nature of the syndromic group would be consistent with CNVs. However, single gene mutations in signaling factors regulating transcription may also cause multisystem genetic anomalies such as Holt-Oram syndrome, which can be associated with TOF (Zhang, Sun et al. 1986b). Many other factors may contribute to the etiology of TOF, including epistatic interactions (Clark, Yutzey et al. 2006) and teratogenic factors (Jenkins, Correa et al. 2007). Delineating the genetic causes of TOF is important not only for understanding the disease mechanism but also for determining genotype phenotype relations.

3.5 Clinical Implications

The high prevalence of congenital extra-cardiac defects and late-onset conditions in adults with TOF should alert physicians to the potential multisystem nature of this disorder. An awareness of these phenotypes is important in the clinical setting; the common association of certain extra-cardiac defects with TOF provides patterns for diagnosis and allows for early detection and treatment (Greenwood, Rosenthal et al. 1975). Multidisciplinary management with the endocrinologist, orthopedic surgeon, psychiatrist, geneticists and other specialties may be
necessary. Consultation with a clinical geneticist may help identify recognizable genetic syndromes, allowing for appropriate genetic counseling and anticipatory guidance. Since certain extra-cardiac conditions are present from birth, some develop in childhood, and others develop later, more emphasis on extra-cardiac conditions should be made on transition from pediatric to adult care to ensure that care is being followed through from the pediatric to the adult setting.

3.6 Study limitations and advantages

This was largely a retrospective study that relied on chart review to identify features. The prevalence of cardiac and extra-cardiac anomalies reported may therefore be underestimated. To attempt to minimize this bias, we comprehensively reviewed records from both adult and paediatric clinics. Also, we chose to focus on extra-cardiac anomalies and late-onset conditions that are common in subjects with 22q11DS and that we have extensively studied and are well defined (Bassett, Chow et al. 2005). Most subjects in the syndromic group have not yet had a direct clinical genetics evaluation as an adult to attempt to identify an underlying genetic etiology. Many had previous genetic evaluation in childhood, when only limited genetic testing was available. The genetic testing we performed would only have missed copy number changes below the resolution of the karyotype (approximately 5-10 Mb) and FISH clinical probes used. These probes detect over 95% of 22q11.2 deletions. Although screening of patients was not done by a geneticist, the research personnel who screened the patients in clinic were trained at the same centre, thus minimizing inter-rater bias. As this study examined an adult cohort, paediatric subjects who did not survive to adulthood were not captured. This may have resulted in the underrepresentation of extra-cardiac conditions relevant to early mortality. While it is routine practice to check for other cardiac anomalies in TOF patients using magnetic resonance imaging, angiography, or catheter lab procedures, extra-cardiac conditions (especially non-overt) can be
under-diagnosed, thus the prevalence of some conditions may be underestimated. As our focus was on determining the prevalence of cardiac and extra-cardiac features, we did not record the frequency of imaging done for non-cardiac anomalies. It is therefore important for the clinician to be aware of the multisystem involvement in many patients with TOF in order to make timely diagnoses and appropriate referrals. For this study, our focus was on adult outcomes and thus we did not record pediatric pre and post operative courses. An important advantage of this study, however, was that our sample included adult subjects, which made it possible to study late-onset conditions with developmental origins, including neuropsychiatric and endocrine disorders.

3.7 Conclusion

Brief clinical screening in the clinical setting can identify a syndromic group of TOF subjects, in addition to those with 22q11DS (10), who have multisystem disease involving congenital extra-cardiac features, late-onset conditions, as well as elevated prevalence of severe cardiac disease. An awareness of these multisystem phenotypic variations is important for the clinician in cardiac clinics because of their potential impact on management. These extra-cardiac abnormalities warrant regular monitoring in patients with TOF in order to take timely preventative measures and improve management. These findings may also provide for a means of identifying a subset of patients enriched for certain genetic causes of TOF that warrant referral to medical genetics. Genome-wide microarrays and other specific genetic testing may delineate the underlying molecular anomalies in some, or eventually many cases.
Table V. Baseline Characteristics in 207 adults with TOF

<table>
<thead>
<tr>
<th></th>
<th>Nonsyndromic group (n=112)</th>
<th>Syndromic group (n=56)</th>
<th>22q11DS reference group (n=39)</th>
<th>P-value (Nonsyndromic vs. Syndromic groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>37 ± 10</td>
<td>37 ± 11</td>
<td>31 ± 10</td>
<td>0.71</td>
</tr>
<tr>
<td>Males</td>
<td>56 (50%)</td>
<td>28 (50%)</td>
<td>22 (56%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Family history of CHD</td>
<td>15 (13%)</td>
<td>6 (11%)</td>
<td>12 (31%)</td>
<td>0.62</td>
</tr>
<tr>
<td>First degree relatives</td>
<td>11 (10%)</td>
<td>5 (9%)</td>
<td>8 (21%)</td>
<td>0.85</td>
</tr>
<tr>
<td>Second degree relatives</td>
<td>6 (5%)</td>
<td>1 (2%)</td>
<td>6 (15%)</td>
<td>0.43</td>
</tr>
<tr>
<td>Ethnicity-Caucasian (n=203)</td>
<td>87/108 (81%)</td>
<td>48 (86%)</td>
<td>38 (97%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Genetic testing results</td>
<td>9 (8%)</td>
<td>50 (89%)</td>
<td>39 (100%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>available*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Clinical karyotype and fluorescence in-situ hybridization (FISH) testing using probes for common 22q11.2 deletions
Table VI. Congenital cardiovascular anomalies in adults with TOF

<table>
<thead>
<tr>
<th></th>
<th>Nonsyndromic group (n=112)</th>
<th>Syndromic group (n=56)</th>
<th>22q11DS reference group (n=39)</th>
<th>P-value (Nonsyndromic vs. Syndromic groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any cardiovascular anomaly</td>
<td>69 (62%)</td>
<td>39 (70%)</td>
<td>33 (85%)</td>
<td>0.31</td>
</tr>
<tr>
<td>Pulmonary atresia and/or MAPCA</td>
<td>14 (13%)</td>
<td>14 (25%)</td>
<td>10 (26%)</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>11 (10%)</td>
<td>11 (20%)</td>
<td>6 (15%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Right aortic arch</td>
<td>28 (25%)</td>
<td>21 (38%)</td>
<td>18 (46%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Left SVC draining into coronary sinus</td>
<td>5 (4%)</td>
<td>4 (7%)</td>
<td>2 (5%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Aberrant subclavian artery</td>
<td>9 (8%)</td>
<td>6 (11%)</td>
<td>14 (36%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Hypoplastic pulmonary artery</td>
<td>20 (18%)</td>
<td>12 (21%)</td>
<td>12 (31%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Other arterial anomalies*</td>
<td>17 (15%)</td>
<td>7 (13%)</td>
<td>6 (15%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Absent pulmonary valve</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
<td>2 (5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Other venous anomalies†</td>
<td>8 (7%)</td>
<td>4 (7%)</td>
<td>1 (3%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

MAPCA: Major aorto-pulmonary collateral arteries; SVC: Superior Vena Cava.

*Arterial anomalies included anomalous origin of coronary arteries, vertebral artery anomalies, innominate artery anomalies, double aortic arch, subclavian artery anomalies, aberrant coronary artery crossing over the right ventricular outflow tract or pulmonary valve, absent left coronary artery, hemitruncus arteriosus, and bitruncus brachiocephalic arteries.

†Venous anomalies included left sided SVC, bilateral SVC, innominate vein anomalies, hemitransection of the right upper pulmonary vein, anomalous right superior pulmonary vein draining into the right atrium, and partial anomalous pulmonary vein drainage to the SVC.
Table VII. Congenital extra-cardiac anomalies in adults with TOF

<table>
<thead>
<tr>
<th></th>
<th>Nonsyndromic group (n=112)</th>
<th>Syndromic group (n=56)</th>
<th>22q11DS reference group (n=39)</th>
<th>P-value (Nonsyndromic vs. Syndromic groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Musculoskeletal anomaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 (6%)</td>
<td>7 (13%)</td>
<td>6 (15%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Major musculoskeletal anomaly*</td>
<td>5 (4%)</td>
<td>5 (9%)</td>
<td>4 (10%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Scoliosis requiring surgery</td>
<td>2 (2%)</td>
<td>3 (5%)</td>
<td>2 (5%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Genitourinary anomaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal‡</td>
<td>7 (6%)</td>
<td>4 (7%)</td>
<td>3 (8%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Genital‡</td>
<td>2 (2%)</td>
<td>3 (5%)</td>
<td>2 (5%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Gastrointestinal anomaly§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear anomaly‖</td>
<td>0</td>
<td>2 (4%)</td>
<td>3 (8%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Central nervous system anomaly¶</td>
<td>1 (1%)</td>
<td>2 (4%)</td>
<td>3 (8%)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Major musculoskeletal anomalies were polydactyly, bifid terminal phalanx of thumb, digitalized thumb, club foot, hemi and butterfly vertebrae, and dysplastic ribs
†Renal anomalies were renal agenesis, horseshoe kidney, ectopic kidney, collecting duct anomaly, and congenital hydronephrosis
‡Genital anomalies were undescended testes, hypospadias, chordee, and bicornuate uterus
§Gastrointestinal anomalies included esophageal atresia, anal atresia/stenosis, pyloric stenosis and Meckel’s diverticulum
‖Major ear anomalies included absent left ear lobe, absent left external ear canal and congenital lop ear deformity
¶Central nervous system anomalies included spina bifida, hydrocephaly, hypoplastic pituitary gland, and diffuse cerebral and cerebellar atrophy
<table>
<thead>
<tr>
<th>Condition</th>
<th>Nonsyndromic group (n=112)</th>
<th>Syndromic group (n=56)</th>
<th>22q11DS reference group (n=39)</th>
<th>P-value (Nonsyndromic vs. Syndromic groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any neuropsychiatric disorder</td>
<td>19 (17%)</td>
<td>18 (32%)</td>
<td>20 (51%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Seizures</td>
<td>7 (6%)</td>
<td>8 (14%)</td>
<td>8 (21%)</td>
<td>0.09</td>
</tr>
<tr>
<td>ADD/ADHD</td>
<td>0</td>
<td>6 (11%)</td>
<td>2 (5%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>0</td>
<td>2 (4%)</td>
<td>4 (10%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Anxiety and/or depression</td>
<td>12 (11%)</td>
<td>5 (9%)</td>
<td>11 (28%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Any endocrine disorder</td>
<td>4 (4%)</td>
<td>12 (21%)</td>
<td>19 (49%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thyroid dysfunction</td>
<td>4 (4%)</td>
<td>11 (20%)</td>
<td>8 (21%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypocalcemia and/or hypoparathyroidism</td>
<td>0</td>
<td>1 (2%)</td>
<td>17 (44%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Recurrent otitis media</td>
<td>13 (12%)</td>
<td>13 (23%)</td>
<td>15 (38%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Hearing deficit</td>
<td>0</td>
<td>11 (20%)</td>
<td>10 (26%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>In the absence of otitis media</td>
<td>0</td>
<td>3 (5%)</td>
<td>2 (5%)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*ADD/ADHD: Attention Deficit Disorder/Attention Deficit Hyperactivity Disorder
Figure 5. Proportion of adult TOF subjects with one or more congenital extra-cardiac anomaly
Late Outcomes in Adults with Tetralogy of Fallot: Comparison of Syndromic and Nonsyndromic Groups
4.1 Objectives

In STUDY 1, we identified a subgroup of adults with TOF with syndromic features but without 22q11DS or other major genetic anomalies (syndromic group). This group had more complex cardiac disease and a greater prevalence of later onset conditions. Limited studies on pediatric patients with genetic syndromes suggest that syndromic patients are at a high risk for adverse events including mortality (from both cardiac and non-cardiac causes), cardiac reinterventions, infections, and multiorgan failure after repair of TOF (Kyburz, Bauersfeld et al. 2008, Michielon, Marino et al. 2006). We hypothesized that our newly classified syndromic group of adults with TOF would have worse outcomes when compared a group of subjects without these features (nonsyndromic group). Late outcomes in a reference population of subjects with TOF and 22q11DS (22q11DS group) were studied for comparison.

The objectives of this study were to investigate differences in late outcomes between syndromic and nonsyndromic adults with TOF. The primary outcome of interest was all-cause mortality in adulthood (≥18 years of age). Secondary outcomes included: a) adverse nonfatal cardiac events and b) cardiac interventions in adulthood.
4.2 Methods

This study was approved by the institutional ethics boards. This was a retrospective study of adults (≥18 years) with TOF seen at the Toronto Congenital Cardiac Centre for Adults who had undergone prospective clinical screening (Fung, Chow et al. 2008) between 1998 and 2008 [n=447, mean age 39±13 years, 239 males (53%)]. Individuals with major genetic anomalies such as Down syndrome were not included in the initial screening study. Of these 447 patients, 88% (n=395, 12%) had TOF and the remainder (n=52) had TOF-pulmonary atresia.

Subjects were categorized into three groups: syndromic, nonsyndromic, and 22q11DS, as previously described. Using brief clinical genetic screening, subjects were classified as syndromic if they had at least two of the three criteria previously established to identify adults with 22q11DS: an overall impression of dysmorphic facial features (e.g. narrow palpebral fissures), cognitive impairment (e.g. self-reported childhood learning difficulties), and speech problems (predominantly hypernasal speech) (Fung, Chow et al. 2008, Bassett, Chow et al. 2005). Of the initial 447 subjects, 15% (n=66) were classified as syndromic, 9% (n=39) had 22q11DS and the remainder (n=342) formed the entire nonsyndromic group, not meeting syndromic screening criteria on clinical genetic screening (Figure 4). Excluded from the syndromic group were ten subjects with other known genetic syndromes or a history of stroke and acquired speech and/or learning difficulties. Subjects in the remaining syndromic group (n=56) were matched by age (within 5 years) and gender in a 1:2 ratio with subjects who did not meet the syndromic criteria (n=112).

In addition to the prospectively collected screening data, comprehensive chart reviews were performed. Subjects with clinical genetic testing had standard karyotype and fluorescence in-situ hybridization (FISH) testing for 22q11.2 deletions using a TUPLE 1 (Vysis) or N25
Baseline characteristics included: cardiac anatomy, associated extra-cardiac anomalies, and pediatric (prior to age 18 years) surgical and interventional procedures (palliative shunts, intracardiac repairs, pulmonary arterioplasty or pulmonary angioplasty and other cardiac surgeries). Details pertaining to the type of surgeries and the year of surgery were obtained from operative notes. Adverse cardiac events in childhood were recorded including heart failure and arrhythmias.

Follow up details were obtained from medical chart review of clinic notes from the Toronto Congenital Cardiac Centre for Adults and from referring physicians (cardiac and non-cardiac specialists). To determine the cause of death, medical records and post mortem results were reviewed, when available. In addition, results from the last available (or last visit prior to death in deceased subjects) electrocardiography and transthoracic echocardiogram (valvular and ventricular function) were recorded. Functional status at last follow up was based on chart review and graded according to the New York Heart Association (NYHA) functional classification. Electrocardiographic parameters included: rhythm, heart rate, QRS duration, corrected QT interval (QTc), presence or absence of ventricular hypertrophy, and right bundle branch block (RBBB) morphology. Echocardiographic parameters included: a) severity of valvular regurgitation based on Doppler echocardiographic assessment, classified as none, mild, moderate or severe, b) systolic function of the systemic and subpulmonic ventricles, classified as none, mild, moderate, or severe dysfunction, and c) size of the systemic and subpulmonic ventricles, classified as none, mild, moderate, or severe dilation. Medications at last follow up were recorded and categorized as cardiac or non-cardiac.
4.3 Primary outcomes

All-cause mortality was the primary endpoint of interest. Death was further classified as cardiac or non-cardiac. Cardiac deaths included sudden death or death secondary to heart failure, endocarditis, or thromboembolic events (systemic and pulmonary). Sudden cardiac death was defined as unexpected death from a presumed cardiac cause, occurring without any prior condition (Braunwald, 2007). All deaths were adjudicated by two people, SP and CS. Age at death was recorded.

4.4 Secondary outcomes

The secondary outcomes were: a) adverse nonfatal cardiac events and b) cardiac interventions in adulthood. Adverse nonfatal cardiac events were defined as any of the following: resuscitated cardiac arrest, ventricular tachyarrhythmias, supraventricular tachyarrhythmias, complete heart block, heart failure, stroke or transient ischemic event. We included any sustained (lasting \( \geq 30 \) seconds) or asymptomatic tachyarrhythmias requiring treatment. Heart failure was defined as a) pulmonary edema documented on chest radiograph or b) documentation of heart failure diagnosis and treatment by a physician. Stroke was defined as a thromboembolic or ischemic neurologic event diagnosed by computer tomography or magnetic resonance imaging. Cardiac interventions during adulthood included any surgical, catheter or electrophysiologic procedures. Surgical cardiac interventions included: primary intracardiac repair of TOF, pulmonary valve replacement, aortic valve replacement, tricuspid valve replacement/ or annuloplasty, RV-PA conduit, VSD closure, ASD closure, pulmonary arterioplasty, and unifocalization of MAPCAs. Cardiac catheter procedures included pulmonary angioplasty or percutaneous coronary artery angioplasty and/or stenting. Electrophysiology procedures included
automatic implantable cardioverter/defibrillator (AICD) implantation, pacemaker implantation or ablation procedures (including MAZE procedures and cryoablation).

4.5 Statistical analysis

Statistical analysis was performed using SPSS software (version 16.0, SPSS, Inc., Chicago, Illinois). Continuous variables are reported as mean ± standard deviation. Comparisons of continuous and categorical variables were performed using Student’s t-test, Wilcoxon Rank Sum, Chi-square or Fisher exact tests, as appropriate. Age at last follow up or death was used for the survival analysis. Time to first event (nonfatal cardiovascular event or cardiovascular intervention, as appropriate) after age 18 or time to last follow up was used in the analysis of secondary outcomes. If more than one adverse cardiac event/cardiac procedure occurred, the date of the first event was used in the analysis. Kaplan Meier curves were used to depict: a) all-cause mortality, b) adverse nonfatal cardiac events and c) cardiac interventions in adulthood between the syndromic and nonsyndromic groups. A log-rank test was used to test differences between groups. After confirmation of the proportional hazard assumptions were verified, a Cox regression was used to determine hazards ratios for all cause mortality, adverse nonfatal cardiac events and cardiac interventions in the syndromic group compared to the nonsyndromic group. Hazards ratios could not be computed for all-cause mortality as there were no deaths (n=0) in the nonsyndromic group. Similarly, hazards ratios could not be calculated for RV-PA conduit and unifocalization procedures. P values <0.05 (2-tailed) were considered statistically significant.
4.6 Results

4.7 Screening characteristics

Table V presents the characteristics of the three TOF groups (n=207, 106 males (51%), mean age 36 ±10 years). In the syndromic group (n=56), 62% (35/56) met two and 38% (21/56) met three of the predefined clinical screening criteria (Fung, Chow et al. 2008). All patients with 22q11DS had a positive FISH test. The majority (89%) of subjects from the syndromic group, and a minority (8%) from the nonsyndromic group had karyotype and FISH testing for 22q11.2 deletions, with negative results.

4.7.1 Baseline characteristics in childhood

Tables IX and X and show the baseline anatomic and surgical characteristics (prior to 18 years of age) of the three groups; nonsyndromic, syndromic and 22q11DS. Pulmonary atresia and/or MAPCAs were more common in the syndromic group compared to the nonsyndromic group (25% vs. 13%, p=0.04). The majority (~90 %) of subjects in each of the three groups had had reparative cardiac surgery for TOF. The age at repair was similar between all three groups (p=0.89). The use of transannular patching in childhood was similar in all three groups (p=0.12). The syndromic group did not differ from the nonsyndromic group with respect to history of heart failure (p=0.40), ventricular tachyarrhythmias (p=0.26) or atrial tachyarrhythmias (p=0.26) prior to 18 years of age. All cases of ventricular tachyarrhythmias occurred in the late postoperative period (2-15 years after reparative cardiac surgery). In 4 cases atrial tachyarrhythmias occurred in the late postoperative period (1-3 years after reparative cardiac surgery) and in another case the event occurred in the perioperative period. 22q11DS group had a higher prevalence of perioperative and non-perioperative heart failure in childhood compared to the two main comparison groups (23% vs. 4%, p<0.001).
4.8 Primary outcomes

4.8.1 All-cause mortality

In total, there were five deaths in the syndromic group, six deaths in the 22q11DS group, and none in the nonsyndromic group (Tables XI and XII) (Figure 6). Post mortem was available for only one patient in the 22q11DS group. The median age of death was 44 years; 46 years (range 18-56) in the syndromic group and 31 years (range 22-53) in the 22q11DS group. The median age at last follow up or death was similar between the syndromic and nonsyndromic groups [median 34 (range 19-63) years vs. 32 (18-60) years), p=0.86].

Mortality in the syndromic group. Four of the five deaths in the syndromic group were cardiac-related, although variable in nature (Table XII). None of deaths were sudden. A 44 year old female (case 1) with repaired TOF, replacement of the aortic and pulmonary valves and severe biventricular systolic dysfunction died 14 hours after complicated heart transplant surgery. A 56 year old female (case 2) with repaired TOF and significant residual pulmonary and tricuspid regurgitation, right-sided heart failure and chronic atrial fibrillation/flutter and multiple sclerosis died of an acute respiratory infection and concomitant right-sided heart failure. An 18 year old male (case 3) with repaired TOF and severe pulmonary regurgitation, a VSD and moderate left ventricular dysfunction died after a cardioversion for new onset atrial flutter. Soon after his cardioversion he developed progressive bradycardia, atrioventricular block and then had a cardiac arrest. During the arrest, he developed ventricular fibrillation and could not be successfully resuscitated. A 49 year old female (case 4) with repaired TOF and a pulmonary valve implant died of complications (recurrent heart failure, renal failure, gastrointestinal bleeding and hypothyroidism) after a prolonged hospital admission for endocarditis. She died,
expectedly during sleep, after discontinuation of therapy for endocarditis. A 46 year old female (case 5) with unrepaired TOF and concomitant interstitial lung disease died of pneumonia.

Deceased subjects had more evidence of end stage heart disease compared to the living subjects in the syndromic group. A history of heart failure prior to death was more frequent in deceased subjects compared to living subjects in the syndromic group (n=4, 80% vs. n=5, 10%, p=0.002). All deceased subjects had a history of atrial tachyarrhythmias, which were more common in the deceased subjects compared to the living subjects (n=5, 100% vs. n=7, 14%, p<0.001). Moderate or severe left ventricular dysfunction and tricuspid regurgitation were more common in deceased subjects compared to living subjects in the syndromic group (ventricular dysfunction; n=2, 40% vs. n=1, 2%, p=0.02, and tricuspid regurgitation; n=4, 80% vs. n=7, 14%, p=0.004).

**Mortality in the 22q11DS group.** Six (15%; 2 F, 4 M) subjects in the 22q11DS group died at a median age of 31 (range 22-53) years. Four of these deaths have been previously reported (Bassett, Chow et al, 2009). Two of the six deceased subjects in the 22q11DS group had pulmonary atresia with MAPCAs; one had absent pulmonary valve syndrome. In three cases death was sudden and unexpected, presumably from arrhythmia. In the other three subjects, causes of death differed: one subject died of heart failure, one died of stroke, and one died of pulmonary hemorrhage (unrepaired TOF with pulmonary atresia and MAPCAs).

**Survival.** The 30-year survival from the date of first clinic visit (survival at age 48 years) was 100% in the nonsyndromic group, 78± 14% in the syndromic group, and 46± 21% in the 22q11DS group (Figure 6). All-cause mortality was significantly higher in the syndromic group compared to the nonsyndromic group (p=0.002).
There was little difference in the clinical, electrocardiographic or echocardiographic parameters between the syndromic and nonsyndromic groups at the last follow up visit (prior to death in deceased subjects) to explain mortality differences between groups. Most adults had good reported functional capacity and few subjects in either group had NYHA functional class III or IV symptoms (syndromic 7% vs. nonsyndromic 3%, p=0.14). The average heart rate in the syndromic group was higher compared to the nonsyndromic group (74 ± 14 vs. 69 ± 12, p=0.04). The QRS duration on the electrocardiogram was similar between both groups (457 ± 59 vs. 468 ± 33, p=0.18); however, QTc interval was greater in the 22q11DS group (489 ± 58, p=0.02). Moderate or severe biventricular dysfunction was similar between the syndromic and nonsyndromic groups (n=11, 20% vs. n=17, 15%, p=0.46). The syndromic group had a higher prevalence of moderate or severe tricuspid regurgitation compared to the nonsyndromic group (n=11, 20% vs. n=6, 5%, p=0.004).

4.9 Secondary outcomes

4.9.1 Adverse Nonfatal Cardiac Events
Overall, 23% (47/207) of subjects had at least one adverse nonfatal cardiac event in adulthood (incidence rate for any cardiac event was 3.0 adverse cardiac events/year). The mean age at the time of the first adverse cardiac event was 34 ± 11 years. The most common adverse cardiac event was atrial tachyarrhythmia. Kaplan-Meier curves showing the freedom from adverse nonfatal cardiac events in adulthood are shown in Figure 7. Overall, freedom from adverse cardiac events was similar in the syndromic and nonsyndromic groups [HR 1.16, 95% CI 0.60-2.25, p=0.65] (Table XIII). Heart failure was more common in the syndromic group compared to the nonsyndromic group [HR 3.91, 95% CI 1.28-11.93, p=0.01]. There were no differences in mean age for heart failure between the syndromic and nonsyndromic groups (31 ± 12 vs. 35 ±
11, p=0.64). Similarly, there was a trend toward higher rates of atrial tachyarrhythmias in the syndromic group compared to the nonsyndromic group [HR 2.12, 95% CI: 0.91-4.96, p=0.07] (Table XIII). Mean age at first atrial tachyarrhythmic event was similar between the main comparison groups (35 ± 12 vs. 36 ± 14, p=0.81). Ventricular arrhythmias were similar between the two main study groups (p=0.79) (Table XIII). There were no differences in mean age for ventricular tachyarrhythmias between the syndromic and nonsyndromic groups (32 ± 11 vs. 33 ± 17, p=0.91).

4.9.2 Cardiac interventions

Overall, 42% (86/207) of subjects had at least one cardiac intervention in adulthood (incidence rate for any cardiac intervention was 5.5 interventions/year) (Table XIV). The mean age at the time of the intervention was 30 ± 9 years. The most common cardiovascular intervention in adulthood was pulmonary valve replacement (n=30-33% for all groups) (Table XIV). Kaplan-Meier curves showing the freedom from cardiac interventions in adulthood are shown in Figure 8. Overall, there were no differences in cardiac interventions between the syndromic and nonsyndromic groups [HR 0.68, 95% CI: 0.40-1.16, p=0.15]. Pulmonary artery angioplasty was significantly more common in the syndromic group compared to the nonsyndromic group (7% vs. 1%, p=0.04).
4.10 Discussion

In this study, we found that adults with TOF with readily recognizable syndromic features, but without 22q11DS or other known genetic syndromes, have a higher mortality rate in adulthood compared to those without syndromic features. This group had similar mortality rates to those observed in patients with 22q11DS (Kyburz, Bauersfeld et al. 2008, Bassett, Chow et al. 2009). Deaths occurred at a relatively young age and were primarily cardiac-related, although variable in nature. While underlying cardiac anatomy and childhood surgical interventions did not explain differences in mortality, clinical heart failure, tricuspid regurgitation, and atrial tachycarrhythmias in adulthood were more common in the syndromic group, particularly in those subjects that died. Mortality differences between clinical subgroup of patients with TOF require further studies in larger populations.

Deaths in both the syndromic and 22q11DS groups occurred much earlier than expected compared to the general population. The median age at death in the syndromic group was 46 years and survival at 30 years (from date of first clinic visit at age 18) was only 78± 14%. Premature death in adults with 22q11DS has been previously reported by Bassett et al, with a median age of 26 years in deceased subjects with TOF (Bassett, Chow et al. 2009). By comparison, the average expected lifespan of Canadians is 80 years; 77 years for males, and 82 years for females (http://www40.statcan.gc.ca/l01/cst01/health26-eng.htm). The increased risk of premature death in both the syndromic and 22q11DS groups is important clinical information for patients and for the treating physician. Additional surveillance may be useful in some patients.

Atrial tachyarrhythmias and significant tricuspid regurgitation were more common in the syndromic group, particularly in those subjects who died (all five subjects who died had atrial tachycarrhythmias and four had significant tricuspid regurgitation). These two findings are likely
interrelated and this association has been previously described (Gatzoulis, 2003). Similarly, heart failure was more common in the syndromic group compared to the nonsyndromic group, especially in those subjects who died. While 2 of the subjects had significant left ventricular systolic dysfunction that would predispose to heart failure, the remaining 2 subjects did not have ventricular dysfunction, but had atrial tachyarrhythmias and tricuspid regurgitation. Previous studies have documented a relationship between heart failure and atrial arrhythmias in TOF patients late after repair (Harrison, Siu et al. 2001). In the study by Harrison et al, almost half of the patients with atrial arrhythmias were reported to have heart failure as a comorbid cardiac event (Harrison, Siu et al. 2001). Despite a frequent focus on pulmonary regurgitation and ventricular arrhythmias and their relationship with adverse outcomes, particularly sudden death, tricuspid regurgitation and atrial arrhythmias, in combination with syndromic features, appear to be important determinants of late outcomes.

We did not find an association between more complex cardiac disease (i.e. pulmonary atresia and/or MAPCA) and death in the syndromic group, a lesion that has been previously identified as a risk factor for death in patients with TOF (Hickey, Veldtman et al. 2009). However, the study by Hickey et al had a larger sample size (n=1181 with TOF, n=88 with TOF-pulmonary atresia). The small number of adults with TOF-pulmonary atresia and/or MAPCAs (n=28, 14 syndromic and 14 nonsyndromic) may have limited our ability to detect this association. In addition, we included adult patients with TOF in our study, which may have biased the results; some patients with 22q11DS and complex CHD (i.e. pulmonary atresia and/or MAPCAs) will not survive to adulthood (Kyburz, Bauersfeld et al, 2008).

A QRS duration ≥160ms has been associated with an increased risk of atrial arrhythmias, ventricular arrhythmias and sudden cardiac death (Gatzoulis, Balaji et al. 2000, Karamlou, Silber
et al. 2006)(Khairy, Aboulhosn et al. 2010). However, we did not find a relationship between QRS duration and death in the syndromic group. This may reflect the nature of deaths in this group, which were related to advanced heart disease along with other complications and not to sudden death.

In the series by Bassett et al, a five of the twelve deaths occurred unexpectedly during sleep in patients with 22q11DS (including one patient with TOF) (Bassett, Chow et al. 2009). Findings from that study suggest that premature death in some adults with 22q11DS may be arrhythmic in origin, perhaps related to long QT, as is often found in the 22q11DS population. In contrast, there were no sudden unexpected deaths in the syndromic group despite three subjects having significantly prolonged corrected QT intervals (>500 msec).

Besides anatomic differences, underlying genetic factors may contribute to adverse cardiac outcomes. The syndromic group identified in this study may be enriched for genetic causes of TOF such as CNVs (Costain, Silversides et al, 2010, Greenway, Pereira et al, 2009). Previous studies investigating the impact of genetic syndromes on cardiac outcome in pediatric patients with TOF and CHD have demonstrated higher mortality rates in TOF patients with genetic syndromes. In those studies, mortality was related to non-cardiac complications (e.g. multi-organ failure, pneumonia, surgery for extra-cardiac anomalies) (Kyburz, Bauersfeld et al. 2008, Michielon, Marino et al. 2006). In contrast, non-cardiac related causes accounted for only a minority of cases of the deaths in our study sample. This may be due to a survival bias as children with complex extra-cardiac defects may die early of non-cardiac related complications and thus not survive to adulthood.
4.11 Clinical Implications

Although some patients do well after repair of TOF (Norgaard, Lauridsen et al. 1999, Murphy, Gersh et al. 1993), late morbidity remains an important issue in adulthood. This is consistent with our study in which we document high rates of adverse nonfatal cardiac events and cardiac interventions in both the syndromic and nonsyndromic groups, highlighting the need for careful long term follow up. By using a previously established screening protocol to select for patients with 22q11DS, we found a clinically important subgroup of adults with TOF with syndromic features who had a higher mortality rate. This finding is important and suggests that, in addition to the known cardiac risk factors associated with poor survival, there are other non-cardiac variables that can help to identify the high risk adult with TOF.

We would anticipate that the syndromic group is enriched for genetic causes of TOF such as CNVs. Differentiating syndromic from nonsyndromic outcomes, as well as 22q11DS, is of clinical relevance as it may provide some insight into the potential role that genetic variation plays in determining cardiac outcome. Post-mortem molecular and tissue studies could be helpful in identifying potential underlying mechanisms of premature death in the TOF population.

4.12 Study limitations and advantages

The retrospective design of this study, which relied heavily on chart review as the available source of data, limits the quality of information regarding the clinical course of the patient. The retrospective nature of this study is especially relevant in the case where the patient is deceased, when the cause of death is not clear and no post-mortem exam is performed. Of the 11 deaths, only 1 subject had a post-mortem examination. However, in the majority of cases, we were able to obtain reasonable clinical information pertaining to the cause of death. As this study
only focused on adults, a survival bias exists since individuals who died prior to 18 years of age were not included. However, an important advantage to this study is the focus on the adult population, enabling us to study late outcomes and predictors of late outcomes. Most subjects in the syndromic group have not yet had a direct clinical genetics evaluation as an adult to attempt to identify an underlying genetic etiology. Undiagnosed genetic syndromes in the syndromic group may inflate the morbidity and mortality estimates as some known syndromes, such as 22q11DS are known to be associated with worse outcomes. We did not record the age at first clinic visit, but did record all events that had occurred since the age of 18 years.

4.13 Conclusion

In addition to patients with 22q11DS, we have identified a new clinically important subgroup of adults with syndromic features who are at risk for premature mortality. The cause of death in this group is likely multifactorial, but seems, at least in part, to be associated with advanced heart disease. Further, larger studies are required to confirm this observation.
### Table IX. Childhood cardiac and extra-cardiac characteristics in adults with TOF

<table>
<thead>
<tr>
<th>Cardiac anatomy</th>
<th>Nonsyndromic group (n=112)</th>
<th>Syndromic group (n=56)</th>
<th>22q11DS reference group (n=39)</th>
<th>P-value (Nonsyndromic vs. Syndromic groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary atresia and/or MAPCA</td>
<td>14 (13%)</td>
<td>14 (25%)</td>
<td>10 (26%)</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>11 (10%)</td>
<td>11 (20%)</td>
<td>6 (15%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Right aortic arch</td>
<td>28 (25%)</td>
<td>21 (38%)</td>
<td>18 (46%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Left SVC draining into coronary sinus</td>
<td>5 (4%)</td>
<td>4 (7%)</td>
<td>2 (5%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Aberrant subclavian artery</td>
<td>9 (8%)</td>
<td>6 (11%)</td>
<td>14 (36%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Hypoplastic pulmonary artery</td>
<td>20 (18%)</td>
<td>12 (21%)</td>
<td>12 (31%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Other arterial anomalies</td>
<td>17 (15%)</td>
<td>7 (13%)</td>
<td>6 (15%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Absent pulmonary valve</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
<td>2 (5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Other venous anomalies</td>
<td>8 (7%)</td>
<td>4 (7%)</td>
<td>1 (3%)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Associated congenital extra-cardiac anomalies</strong></td>
<td>15 (13%)</td>
<td>14 (25%)</td>
<td>14 (36%)</td>
<td><strong>0.06</strong></td>
</tr>
</tbody>
</table>

MAPCA: Major aorto-pulmonary collateral artery  
SVC: Superior vena cava  
RV-PA: Right ventricle-pulmonary artery  
Pulmonary plasty included pulmonary artery arterioplasty and angioplasty  
Associated extra-cardiac defects were major musculoskeletal (including scoliosis requiring surgery), genitourinary, central nervous system, gastrointestinal, and ear deformities requiring surgery.
Table X. Pediatric cardiac interventions and adverse cardiac events in 207 adults with TOF

<table>
<thead>
<tr>
<th></th>
<th>Nonsyndromic group (n=112)</th>
<th>Syndromic group (n=56)</th>
<th>22q11DS reference group (n=39)</th>
<th>P-value (Nonsyndromic vs. Syndromic groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pediatric cardiac surgery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shunts</strong></td>
<td>56 (50%)</td>
<td>35 (63%)</td>
<td>15 (38%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Blalock-Taussig</td>
<td>48 (43%)</td>
<td>31 (55%)</td>
<td>15 (38%)</td>
<td>0.23</td>
</tr>
<tr>
<td>Potts and Waterston</td>
<td>10 (9%)</td>
<td>6 (11%)</td>
<td>2 (5%)</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Intracardiac repair</strong></td>
<td>104 (93%)</td>
<td>50 (89%)</td>
<td>35 (90%)</td>
<td>0.43</td>
</tr>
<tr>
<td>Transannular patch</td>
<td>51 (46%)</td>
<td>19 (34%)</td>
<td>21 (54%)</td>
<td>0.15</td>
</tr>
<tr>
<td>RV-PA conduit</td>
<td>10 (9%)</td>
<td>6 (11%)</td>
<td>7 (18%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Age at intracardiac repair</td>
<td>5.9 ± 3.4</td>
<td>5.6 ± 3.2</td>
<td>5.6 ± 3.4</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Pulmonary arterioplasty</strong></td>
<td>37 (33%)</td>
<td>21 (38%)</td>
<td>16 (41%)</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Peri- and nonperioperative events in childhood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart failure</td>
<td>3 (3%)</td>
<td>3 (5%)</td>
<td>9 (23%)</td>
<td>0.40</td>
</tr>
<tr>
<td>Ventricular arrhythmias</td>
<td>1 (1%)</td>
<td>2 (4%)</td>
<td>1 (3%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Atrial tachyarrhythmias</td>
<td>1 (1%)</td>
<td>2 (4%)</td>
<td>2 (5%)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

RV-PA: right ventricle-pulmonary artery
Table XI. Extra-cardiac characteristics in the five deceased subjects in the syndromic group

<table>
<thead>
<tr>
<th>Case</th>
<th>Genetic testing*</th>
<th>Syndromic features</th>
<th>Congenital extra-cardiac anomaly</th>
<th>Late-onset extra-cardiac features</th>
<th>Other late-onset features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Learning difficulties (moderate mental retardation), dysmorphic facial features, voice abnormalities</td>
<td>Ectopic kidney, hydronephrosis, short stature</td>
<td>None</td>
<td>Autoimmune hemolytic anemia</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>Learning difficulties, voice abnormalities</td>
<td>None</td>
<td>None</td>
<td>Multiple sclerosis, neurogenic bladder, hematuria</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Learning difficulties, dysmorphic facial features</td>
<td>None</td>
<td>None</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Learning difficulties, dysmorphic facial features, voice abnormalities</td>
<td>None</td>
<td>Seizure disorder, thyroid disorder</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>Learning difficulties, dysmorphic facial features</td>
<td>Diffuse cerebral and cerebellar atrophy</td>
<td>None</td>
<td>Obstructive airway disease</td>
</tr>
</tbody>
</table>

*Karyotype and FISH testing done with negative results*
<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age at repair (yrs)</th>
<th>Cardiac anatomy</th>
<th>PVR</th>
<th>Heart failure</th>
<th>Arrhythmias</th>
<th>QTc interval (msec)</th>
<th>Echo findings</th>
<th>Age at death (yrs)</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>9</td>
<td>Hypoplastic pulmonary artery, left SVC</td>
<td>Yes</td>
<td>Yes</td>
<td>SVT</td>
<td>500 75</td>
<td>Moderate MR and TR, severe biventricular dysfunction</td>
<td>44</td>
<td>Early post-op death after complicated heart transplant</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>3</td>
<td>Left SVC</td>
<td>No</td>
<td>Yes</td>
<td>Chronic atrial flutter</td>
<td>432 156</td>
<td>Moderate PR and TR</td>
<td>56</td>
<td>Infection and right heart failure</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>4</td>
<td>--</td>
<td>No</td>
<td>Yes</td>
<td>Atrial flutter, VT</td>
<td>500 117</td>
<td>Severe PR, moderate TR, moderate left ventricular dysfunction</td>
<td>18</td>
<td>Post-cardioversion bradycardia and subsequent cardiac arrest</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>10</td>
<td>ASD</td>
<td>Yes</td>
<td>Yes</td>
<td>Recurrent atrial fibrillation</td>
<td>513 138</td>
<td>Moderate TR</td>
<td>49</td>
<td>Endocarditis</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>None</td>
<td>Pulmonary atresia with MAPCA, hypoplastic pulmonary artery, right aortic arch</td>
<td>No</td>
<td>No</td>
<td>Recurrent atrial flutter</td>
<td>416 92</td>
<td>--</td>
<td>46</td>
<td>Pneumonia</td>
</tr>
</tbody>
</table>

SVC: superior vena cava  
ASD: atrial septal defect  
MAPCA: major aorto-pulmonary collateral artery  
PVR: pulmonary valve replacement  
SVT: supraventricular tachycardia  
VT: ventricular tachycardia  
PR: pulmonary regurgitation  
TR: tricuspid regurgitation  
MR: mitral regurgitation
# Table XIII. Adverse nonfatal cardiac events in adulthood

<table>
<thead>
<tr>
<th>Event</th>
<th>Nonsyndromic group (n=112)</th>
<th>Syndromic group (n=56)</th>
<th>22q11DS group (n=39)</th>
<th>Hazards ratio (95% CI) (Syndromic vs. Nonsyndromic groups)</th>
<th>P-value (Syndromic vs. Nonsyndromic groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any cardiac event</td>
<td>25 (21%)</td>
<td>15 (27%)</td>
<td>7 (18%)</td>
<td>1.16 (0.60-2.25)</td>
<td>0.65</td>
</tr>
<tr>
<td>Resuscitated cardiac arrest</td>
<td>2 (2%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>1.00 (0.09-11.07)</td>
<td>1.00</td>
</tr>
<tr>
<td>Ventricular tachyarrhythmias</td>
<td>10 (9%)</td>
<td>5 (9%)</td>
<td>4 (10%)</td>
<td>0.86 (0.27-2.76)</td>
<td>0.79</td>
</tr>
<tr>
<td>Atrial tachyarrhythmias</td>
<td>11 (10%)</td>
<td>12 (21%)</td>
<td>4 (10%)</td>
<td>2.12 (0.91-4.96)</td>
<td>0.07</td>
</tr>
<tr>
<td>Heart failure</td>
<td>5 (4%)</td>
<td>9 (16%)</td>
<td>2 (5%)</td>
<td>3.91 (1.28-11.93)</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>3 (3%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>0.78 (0.08-7.49)</td>
<td>0.83</td>
</tr>
<tr>
<td>Complete heart block</td>
<td>5 (4%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>0.39 (0.05-3.36)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

TIA: transient ischemic attack
Table XIV. Cardiovascular interventions in adulthood

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Nonsyndromic group (n=112)</th>
<th>Syndromic group (n=56)</th>
<th>22q11DS group (n=39)</th>
<th>Hazards ratio (95% CI) (Syndromic vs. Nonsyndromic groups)</th>
<th>P-value (Syndromic vs. Nonsyndromic groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Any intervention</strong></td>
<td>50 (45%)</td>
<td>19 (34%)</td>
<td>17 (44%)</td>
<td>0.68 (0.40-1.16)</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Surgical intervention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracardiac repair in adulthood</td>
<td>6 (5%)</td>
<td>1 (2%)</td>
<td>1 (3%)</td>
<td>0.23 (0.03-1.94)</td>
<td>0.14</td>
</tr>
<tr>
<td>Pulmonary valve replacement</td>
<td>37 (33%)</td>
<td>17 (30%)</td>
<td>13 (33%)</td>
<td>0.82 (0.46-1.46)</td>
<td>0.49</td>
</tr>
<tr>
<td>Aortic valve replacement/valvotomy</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>1.78 (0.11-30.11)</td>
<td>0.69</td>
</tr>
<tr>
<td>Tricuspid valve replacement/annuloplasty</td>
<td>8 (7%)</td>
<td>4 (7%)</td>
<td>2 (5%)</td>
<td>0.89 (0.27-2.96)</td>
<td>0.84</td>
</tr>
<tr>
<td>RV-PA conduit</td>
<td>4 (4%)</td>
<td>0</td>
<td>1 (3%)</td>
<td>--</td>
<td>0.15</td>
</tr>
<tr>
<td>Pulmonary arterioplasty</td>
<td>22 (20%)</td>
<td>10 (18%)</td>
<td>8 (21%)</td>
<td>0.79 (0.37-1.68)</td>
<td>0.54</td>
</tr>
<tr>
<td>VSD closure</td>
<td>12 (11%)</td>
<td>4 (7%)</td>
<td>2 (5%)</td>
<td>0.58 (0.19-1.81)</td>
<td>0.34</td>
</tr>
<tr>
<td>ASD closure</td>
<td>3 (3%)</td>
<td>4 (7%)</td>
<td>0</td>
<td>2.26 (0.50-10.00)</td>
<td>0.28</td>
</tr>
<tr>
<td>Unifocalization of MAPCAs</td>
<td>0</td>
<td>0</td>
<td>1 (3%)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Catheter laboratory procedure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary artery angioplasty</td>
<td>1 (1%)</td>
<td>4 (7%)</td>
<td>1 (3%)</td>
<td>7.5 (0.83-67.56)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Electrophysiology procedure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AICD</td>
<td>5 (4%)</td>
<td>4 (7%)</td>
<td>0</td>
<td>1.42 (0.38-5.30)</td>
<td>0.60</td>
</tr>
<tr>
<td>Ablation</td>
<td>13 (12%)</td>
<td>6 (11%)</td>
<td>4 (10%)</td>
<td>0.85 (0.32-2.24)</td>
<td>0.74</td>
</tr>
<tr>
<td>Pacemaker implantation</td>
<td>8 (7%)</td>
<td>4 (7%)</td>
<td>2 (5%)</td>
<td>0.87 (0.26-2.92)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

* Interventions are not mutually exclusive

RV-PA: right ventricular-pulmonary artery  
VSD: ventricular septal defect  
ASD: atrial septal defect  
MAPCA: major aorto-pulmonary collateral artery  
AICD: automatic implantable cardioverter defibrillator
Figure 6. Differences in survival in adults with TOF

*log rank test for the syndromic and nonsyndromic groups

<table>
<thead>
<tr>
<th>Number at risk</th>
<th>20.00</th>
<th>30.00</th>
<th>40.00</th>
<th>50.00</th>
<th>60.00</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Syndromic (n=56)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>34</td>
<td>16</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Nonsyndromic (n=112)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>74</td>
<td>31</td>
<td>11</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>22q11DS (n=39)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*p=0.002
Figure 7. Freedom from adverse cardiac events in adults with TOF

*log-rank for the syndromic and nonsyndromic groups

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>53</th>
<th>33</th>
<th>19</th>
<th>1</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syndromic (n=56)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsyndromic (n=112)</td>
<td>111</td>
<td>74</td>
<td>27</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22q11DS (n=39)</td>
<td>33</td>
<td>16</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 8. Freedom from cardiac intervention in adults with TOF

*log-rank for the syndromic and nonsyndromic groups

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>54</th>
<th>31</th>
<th>16</th>
<th>2</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syndromic (n=56)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsyndromic (n=112)</td>
<td>108</td>
<td>63</td>
<td>19</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>22q11DS (n=39)</td>
<td>36</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
5 Summary of Thesis and Future Directions

Using simple clinical genetic screening, we identified a clinically important subgroup of adults with TOF who have a more complex cardiac disease, are at elevated risk for developing neuropsychiatric and endocrine disorders, and have a higher mortality rate compared to an age and gender matched control group. We show that in the absence of major genetic anomalies and syndromes, the prevalence of extra-cardiac features are high in patients with TOF, and in some cases, similar to that found in patients with 22q11DS. The high prevalence of extra-cardiac features in TOF should alert the clinician to the potential multisystem nature of this disease, and prompt a more vigorous approach for early recognition and treatment of extra-cardiac abnormalities. Routine ultrasound (e.g. abdominal ultrasound for gastrointestinal and genitourinary anomalies), magnetic resonance imaging of the brain and spinal cord, and laboratory screening for endocrine abnormalities would be of great diagnostic value for identifying these extra-cardiac features.

While certain extra-cardiac conditions may be present from birth, some develop in late childhood or adulthood, more emphasis on extra-cardiac conditions is required for transition from pediatric to adult care to ensure that care is being followed through from the pediatric to the adult setting. This is important since extra-cardiac anomalies may have important health consequences.

In agreement with previous studies, we found that adults with 22q11DS have a high mortality rate (Bassett, Chow et al. 2009). We further showed that patients with syndromic features, but without 22q11DS or other major known genetic anomalies, have a significantly
higher mortality rate compared to an age and gender matched control group with TOF. All five deceased subjects in the syndromic group had learning difficulties, and three had extra-cardiac abnormalities. Although not proven, the presence of learning difficulties, CHD, and extra-cardiac anomalies may be due to underlying structural genomic changes altering gene dosage. More recently, copy number variations have been shown to cause developmental disorders such as autism spectrum disorder and CHDs (Greenway, Pereira et al. 2009, Costain, Silversides et al. 2010, Mefford, Sharp et al. 2008b, International Schizophrenia Consortium 2008, Girirajan, Rosenfeld et al. 2010). In particular, CNVs on chromosome 1q21.1 have previously been implicated in cognitive impairment, CHD, and other congenital anomalies (Greenway, Pereira et al. 2009)(International Schizophrenia Consortium 2008, Mefford, Sharp et al. 2008a). This is one mechanism that may explain the connection between cardiac and extra-cardiac anomalies and learning difficulties. CNVs can also increase susceptibility to early mortality, as Bassett et al have shown that adults with 22q11.2 deletions (large CNV) are at a higher risk of death compared to their unaffected siblings (Bassett, Chow et al. 2009). These extra-cardiac features such as learning difficulties, may serve as clinical markers of more complex genetic diseases with an increased risk of premature death in patients with TOF. More studies are needed to examine this relationship.

Studies focusing on phenotype (i.e. clustering patients into meaningful categories), similar to ours (Scutt, Chow et al, 2001), have great clinical relevance and can help in discoveries of genetic pathways to TOF. The majority of patients in our study sample, apart from the 22q11DS group, have not been seen by a clinical geneticist and thus the presence of extra-cardiac features in those patients may be indicative of an unknown or undiagnosed genetic syndrome. In fact, there are current reports that 22q11DS is under-recognized in patients with
CHD (van Engelen, Topf et al. 2010). This signifies the importance of the need for a more vigorous approach to identify patients with 22q11DS and other genetic syndromes in the clinic, as these syndromes have reproductive and clinical implications. Classifying patients with TOF into more homogenous groups, in the absence of major genetic anomalies, may help identify patterns of cardiac and extra-cardiac features that are commonly associated with TOF and which may have relevance to underlying chromosomal or cytogenetic aberrations. Molecular genetic studies in these patients will further our understanding of the genetic causes of TOF.

Recent recommendations suggest that patients with syndromic features should have genome-wide microarray testing to identify pathogenic structural genomic changes (Miller, Adam et al. 2010). These include individuals with syndromic features such as developmental delay, mental retardation, autism and/or multiple congenital anomalies. However, the cost and challenges in interpreting results from genome-wide microarrays suggest that such testing may not be justified for patients who do not have syndromic and/or neurodevelopmental features as they would be expected to have lower yields compared to syndromal patients (Miller, Adam et al. 2010). Genome-wide studies in TOF patients, especially in those with multiple extra-cardiac anomalies, may shed some light on potential pathogenic genes involved in disease formation. Genetic evaluation and hopefully genome-wide testing in families of probands with TOF, especially in cases where there is a positive family history, would also help in our understanding of inheritance and genetic causes of this disease.

5.1 Future directions

This study identified a newly defined clinical subgroup of patients within TOF with more complex CHD, higher prevalence of late-onset conditions who are at greater risk of death compared to a nonsyndromic control group. The syndromic group identified in this study
represents an initial first step towards identifying a more homogenous group of patients who may be enriched for certain genetic causes of TOF. Greenway et al and Costain et al have recently identified CNV(s) in patients with TOF (Greenway, Pereira et al. 2009, Costain, Silversides et al, 2010); genome-wide molecular studies could shed light on the underlying genetic etiology of TOF and help explain the phenotypic variability that exists within this patient population. In addition, further larger studies are needed to determine mechanisms that may contribute to pathologic cause of death in patients late after repair. With the advancement of medical technology, especially genome wide microarrays, genomic medicine will help with surveillance to identify high risk patient groups to optimize clinical management. Further studies will be needed to prospectively follow up with a large number of patients, especially those with syndromic features, to better understand the possible risk factors for death in this patient population. Larger longitudinal studies with appropriate control groups will be required to investigate the impact of genetic aberrations on cardiac and non-cardiac outcomes. Studies using hearts of patients with TOF with extra-cardiac anomalies may shed some light on common genetic factors (e.g. transcription factors) that regulate the development of different organ systems.
6 Appendices

Data abstraction form:

Syndromic Patients with Tetralogy of Fallot-The Clinical Spectrum of Disease

Study ID #: _______________________________ Evaluated by a geneticist? □ Yes □ No

A. DEMOGRAPHICS

Gender: □ Male □ Female Age________

Ethnicity: □ White □ Aboriginal (e.g. North American Indian, Metis, Inuit, Eskimo) □ Chinese □ Black □ Filipino □ Latin American □ Southeast Asian (e.g. Cambodian, Indonesian, Laotian, Vietnames) □ Arab □ West Asian (e.g. Afghan, Iranian) □ Japanese □ Korean □ South Asian (e.g. East Indian, Pakistani, Sri Lankan) □ Other (please specify): ________________ □ Not available

Date and age at last follow-up: ______________________________________________________

Current height (cm)__________weight (kg): _____________________

Demographics at birth:
Gestational age (wks): ________________________________

Weight (kg)/ Height (cm) at birth: _______________________________________

B. CARDIAC ANATOMY

□ Pulmonary stenosis □ Mild □ Moderate □ Severe ________________________________

□ Sub-pulmonary obstruction: _________________________________________________

□ Pulmonary Atresia ________________________________________________

□ Absent pulmonary valve _________________________________________________

□ Ventricular septal defect: ________________________________________________

□ ASD:

□ Patent ductus arteriosis

□ Major aorto-pulmonary collateral artery (MAPCA)

□ Aortic abnormality:
Right aortic arch: __________________________________________________________

Hypoplasia of aorta: _______________________________________________________

Aortic Override: ___________________________________________________________
Aortic Coarctation: _________________________________________________________
Other: _________________________________________________________________

☐ Aberrent Subclavian Artery: _____________________________________________________
☐ Other Vascular abnormalities: _________________________________________________________

☐ Other cardiac anomalies: _________________________________________________________

C. OTHER CONGENITAL ANOMALIES
☐ Facial feature: Ocular hypertelorism
☐ narrow palpebral fissures/
☐ bloated eyelids
☐ low nasal bridge
☐ small mouth
☐ deformed earlobe

Other: _________________________________________________________

☐ Otolaryngeal abnormality: cleft palate (hard/soft/submucous)
☐ velopharyngeal insufficiency

Other: _________________________________________________________

☐ Developmental delay (Intelligence quotient) :

☐ Visual/Hearing Problems (please specify what):

☐ Central nervous system/ Psychiatric illness (please specify what):

☐ Endocrine (please specify what):
☐ Hyperthyroid ☐ Hypothyroid
☐ Hypoparathyroidism ☐ Hypocalcemia

☐ GI (please specify what):

☐ Renal (please specify what):

☐ Pulmonary disease (please specify what):

☐ Muskoskeletal (please specify what):

☐ Hematologic / Immunologic (please specify what):

☐ Reproductive (please specify what):

☐ Other (please specify): _________________________________________________________

D. CARDIAC SURGERY
☐ BT Shunt: Year
<table>
<thead>
<tr>
<th>Procedure</th>
<th>Year</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterston shunt: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potts shunt: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSD patch: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annular patch/pulmonary artery patch: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVOT Infundibulectomy: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVR: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryoablation: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maze procedure: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP ablation: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD implantation: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pacemaker (type): Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV to PA conduit (type): Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left pulmonary artery angioplasty (type): Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left pulmonary artery stent (type): Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other: Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**E. NONCARDIAC SURGERY**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Year</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palatal flap year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scoliosis year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choleyslectomy year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hernias year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other year</td>
<td></td>
<td>TYPE</td>
</tr>
<tr>
<td>year</td>
<td></td>
<td>TYPE</td>
</tr>
<tr>
<td>year</td>
<td></td>
<td>TYPE</td>
</tr>
<tr>
<td>year</td>
<td></td>
<td>TYPE</td>
</tr>
</tbody>
</table>
F. MEDICATIONS AT LAST FOLLOW UP

<table>
<thead>
<tr>
<th>Drug Category</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitors</td>
<td></td>
</tr>
<tr>
<td>Antiarrhythmics (specify)</td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td></td>
</tr>
<tr>
<td>Beta blocker</td>
<td></td>
</tr>
<tr>
<td>Coumadin</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td></td>
</tr>
<tr>
<td>Heparin sc</td>
<td></td>
</tr>
<tr>
<td>Nitrates</td>
<td></td>
</tr>
<tr>
<td>Synthroid</td>
<td></td>
</tr>
<tr>
<td>Calcium supplement</td>
<td></td>
</tr>
<tr>
<td>Magnesium supplement</td>
<td></td>
</tr>
<tr>
<td>Vitamin D analog</td>
<td></td>
</tr>
<tr>
<td>Antidepressant</td>
<td></td>
</tr>
<tr>
<td>Anxiolytic</td>
<td></td>
</tr>
<tr>
<td>Antipsychotic</td>
<td></td>
</tr>
</tbody>
</table>

- Other cardiac (specify):

- Other non-cardiac (specify):

G. FUNCTIONAL STATUS AT LAST FOLLOW UP

<table>
<thead>
<tr>
<th>NYHA</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
</table>

Percent predicted: ...............  

H. ECG AT LAST FOLLOW UP

1. ECG available:
- No  
- Yes

Normal sinus rhythm:
- No  
- Yes  
If no, specify: _____________________________

QRS duration:
QT interval: □ No  □ Yes  If yes, specify: ________________________________________________

Ventricular hypertrophy: □ No  □ Yes  If yes, specify: ________________________________________________

Other: __________________________________________________________________________

I. OXYGEN SATS AT LAST VISIT
Oxygen saturation: _____________%

J. ECHOCARDIOGRAM AT LAST VISIT

1. Ventricular size and function.
Systemic ventricle size: □ Normal □ Mildly dilated □ Moderately dilated □ Severely dilated
Systemic ventricular function: □ Normal □ Mildly reduced □ Moderately reduced □ Severely reduced
Sub-pulmonary ventricle size: □ Normal □ Mildly dilated □ Moderately dilated □ Severely dilated
Sub-pulmonary ventricular function: □ Normal □ Mildly reduced □ Moderately reduced □ Severely reduced

2. Systemic AV regurgitation: □ None □ Mild □ Moderate □ Mod-severe □ Severe
   Venous AV regurgitation: □ None □ Mild □ Moderate □ Mod-severe □ Severe
   Aortic regurgitation: □ None □ Mild □ Moderate □ Mod-severe □ Severe
   Pulmonic regurgitation: □ None □ Mild □ Moderate □ Mod-severe □ Severe

3. Systemic AV stenosis: □ None □ Mild □ Moderate □ Severe
   Venous AV stenosis: □ None □ Mild □ Moderate □ Severe
   PV stenosis: □ None □ Mild □ Moderate □ Severe
   Subvalvular PV stenosis: □ None □ Mild □ Moderate □ Severe
   Supra PV stenosis: □ None □ Mild □ Moderate □ Severe
   Aortic stenosis: □ None □ Mild □ Moderate □ Severe

5. Hemodynamics / Shunts:
□ No  □ Yes  If yes, then level and direction: □ atrial □ ventricle □ great artery
   □ L-R □ R-L □ Bidir.
   Peak VSD gradients: .................. mmHg
   RV systolic pressure: ............ mmHg
   Assuming RA pressure of ......... mmHg

K. LABORATORY RESULTS AT LAST FOLLOW UP

Details

TSH: .................. 
Creatinine: ..................
Calcium (serum vs ionized)
Hemoglobin
Platelet
### L. CARDiac EVENTS AT LAST FOLLOW UP

<table>
<thead>
<tr>
<th>Event</th>
<th>Date/Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Coronary Syndrome</td>
<td></td>
</tr>
<tr>
<td>Left heart failure</td>
<td></td>
</tr>
<tr>
<td>Right heart failure</td>
<td></td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td></td>
</tr>
<tr>
<td>Aortic dissection</td>
<td></td>
</tr>
<tr>
<td>Syncope</td>
<td></td>
</tr>
<tr>
<td>Endocarditis</td>
<td></td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td></td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td></td>
</tr>
<tr>
<td>Ventricular arrhythmias</td>
<td></td>
</tr>
<tr>
<td>Supraventricular arrhythmias</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
</tbody>
</table>

### M. FAMILY HISTORY

- **TOF**

- Other Congenital heart disease:

- Other health problems (please specify what):
7 References


Circulation, 92(2), 158-159.

Clinical genetics, 50(2), 89-92.

Human pathology, 18(4), 355-360.

Lancet, 351(9099), 311-316.

European journal of epidemiology, 18(8), 773-780.

129, 1218-1228.

British heart journal, 67(6), 470-473.

British heart journal, 33(6), 899-904.


22q11.2 syndrome and congenital heart defect: clinical course and cardiac outcome. *Pediatric cardiology*, **29**(1), 76-83.


Adults with genetic syndromes and cardiovascular abnormalities: clinical history and management. *Genetic medicine*, 10(7), 469-494.


