CJC Pediatric and Congenital Heart Disease 2 (2023) 426–439

Review

Clinically Relevant Genetic Considerations for Patients With Tetralogy of Fallot

Anne S. Bassett, MD, FRCPC,[a,b,c,d,e] Miriam S. Reuter, MD,[a,b] Sarah Malecki, MD,[a,b] Candice Silversides, MD, FRCPC,[a,d,f] and Erwin Oechslin, MD, FRCPC[a,d,f]

[a] *The Dalglish Family 22q Clinic, University Health Network, Toronto, Ontario, Canada*

[b] *Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, Ontario, Canada*

[c] *Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada*

[d] *Toronto Adult Congenital Heart Disease Program, Division of Cardiology, Peter Munk Cardiac Centre, Department of Medicine, University Health Network, Toronto, Ontario, Canada*

[e] *Toronto General Hospital Research Institute, and Campbell Family Mental Health Research Institute, Toronto, Ontario, Canada*

[f] *Department of Medicine, University of Toronto, Toronto, Ontario, Canada*

ABSTRACT

Genetic changes affect embryogenesis, cardiac and extracardiac phenotype, development, later onset conditions, and both short- and long-term outcomes and comorbidities in the increasing population of individuals with tetralogy of Fallot (TOF). In this review, we focus on current knowledge about clinically relevant genetics for patients with TOF across the lifespan. The latest findings for TOF genetics that are pertinent to day-to-day practice and lifelong management are highlighted: morbidity/mortality, cardiac/extracardiac features, including neurodevelopmental expression, and recent changes to prenatal screening and diagnostics. Genome-wide microarray is the first-line clinical genetic test for TOF across the lifespan, detecting relevant structural changes including the most common for TOF, the 22q11.2 microdeletion. Accumulating evidence illustrates opportunities for advances in understanding and care that may arise from genetic diagnosis at any age. We also glimpse into the near future when the multigenic nature of TOF will be more fully revealed, further enhancing possibilities for preventive care. Precision medicine is nigh.

RÉSUMÉ

Dans la population croissante des personnes atteintes de la tétralogie de Fallot (TF), des modifications génétiques influencent l'embryogenèse, le développement, le phénotype cardiaque et extracardiaque, les complications tardives ainsi que les issues de santé et les états comorbides, à court et à long terme. Notre article de synthèse présente l'état des connaissances sur les renseignements génétiques cliniquement utiles pour les patients atteints de la TF tout au long de leur vie. Nous soulignons les découvertes récentes sur les aspects génétiques de la TF qui sont pertinentes pour la pratique clinique quotidienne et la prise en charge lors des différentes étapes de la vie : la morbidité et la mortalité, les caractéristiques cardiaques et extracardiaques (y compris l'expression neurodéveloppementale) et les changements récents touchant le dépistage et les diagnostics prénataux. La technologie de puce à ADN pour le génome entier constitue le test génétique clinique de première intention pour les personnes de tout âge atteintes de la TF, et elle permet la détection de modifications structurelles pertinentes dont celle le plus fréquemment associée à la TF, la microdélétion 22q11.2. L'utilité d'un diagnostic génétique pour améliorer la compréhension de la situation des patients de tous les âges et les soins qui leur sont offerts est de plus en plus mise en évidence. Nous entrevoyons également un avenir pas si lointain dans lequel la nature multigénique de la TF sera entièrement connue, ce qui ouvrira la voie à des soins préventifs bonifiés. La venue de la médecine de précision est imminente.

As the most common of the cyanotic cardiac lesions, comprising 7%-10% of the congenital heart disease (CHD) population, tetralogy of Fallot (TOF) is an important condition that arises early in development, involving a complex spectrum of anatomically defined anomalies (nonrestrictive ventricular septal defect, over-riding aorta of less than 50%, infundibular, valvular, and supravalvular stenosis of the right ventricular outflow tract, and right ventricular hypertrophy),

Received for publication July 21, 2023. Accepted October 5, 2023.

Corresponding author: Anne S. Bassett, MD, FRCPC, the Dalglish Family 22q Clinic, Toronto General Hospital, 8NU-802, 200 Elizabeth St, Toronto, Ontario M5G 2C4, Canada. Tel.: +1-416-340-5145; fax: +1-416-340-5004.

E-mail: anne.bassett@utoronto.ca

https://doi.org/10.1016/j.cjcpc.2023.10.002

with substantial genetic etiology.[1] Advances in CHD surgery and lifelong specialized care have improved outcomes such that TOF is considered a chronic disease, with >90% of individuals with TOF surviving to adulthood and adults vastly outnumbering children.[2] This has led to an increasing focus on improving long-term outcomes, given substantive morbidity risks, especially for heart failure and arrhythmias including sudden cardiac death, and reduced longevity.[3-5] Guidelines recognize the importance of molecular genetic diagnosis for CHD, including adult CHD (ACHD).[6-11] For TOF, parallel to the technological advances in clinical cardiology, congenital cardiac surgery, cardiac congenital anaesthesia, and multimodality imaging, advances in genetics (Fig. 1A) have driven substantial improvements in our understanding of etiology and management. However, the potential impact of genetic diagnosis on management and related clinically relevant issues has had limited attention, and there is relatively little practical guidance available for, and accessible to, busy clinicians.

In this review, we highlight current knowledge around issues of primary clinical relevance to cardiologists who care for patients with TOF. We emphasize the genetic causes of TOF that are currently detectable by broadly available clinical genetic testing. The focus is therefore on major structural variants (deletions and duplications) that account for the largest proportion of known molecular genetic causation. Familiar chromosomal anomalies, such as trisomy 21, are included but not emphasized.

We first provide a brief overview of the genetics of TOF and outline the rationale for and practical guidance on clinical genetic testing of patients with TOF. We summarize issues of active relevance to clinical care of patients with TOF, including testing opportunities across the lifespan (Fig. 1B), with an emphasis on how genetics can help inform risk stratification and outcomes. We outline limitations of the available literature, including genetics-informed outcome data. We nonetheless touch on research that may soon reach clinical relevance as genetic technologies are more broadly implemented and knowledge evolves. As part of a data-driven approach, we rely on the international and domestic literature that is most relevant to current Canadian clinical practice. We also draw on experience with genetic testing and outcomes of the population with TOF followed for decades by Canadian paediatric and ACHD teams, and informed by a geneticist with expertise in TOF genetics.

Overview of the Genetics of TOF

Given the anatomic complexity of TOF and its severity, it is not surprising that genetic factors play a major role in causing TOF and that the genetic architecture is complex and overlaps with, but, to some extent, is distinct from, that of other CHD.[1,12] In general, TOF, like most human conditions, is characterized by genetic heterogeneity (multiple genetic variants that cause the same clinical condition) and less than full penetrance for any individual genetic variant (TOF is not always part of clinical expression).[1,6,13] At the population level, causation can include multiple types of genetic variants, multiple genes, and nongenetic factors. TOF is therefore often described as "multifactorial."

For an individual patient, even where a clinically relevant variant has been identified, background and other factors are likely to be contributory. Such "modifiers" can increase or decrease the risk of the expression of TOF, other cardiac-related risks, and neurodevelopmental and other associated phenotypes (Figs. 2 and 3). Modifying factors include lower impact variants, polygenic background risk, and nongenetic factors.[1]

Whether or not there is a molecularly and/or clinically diagnosable genetic condition present, standard genetic considerations are important. These include a family history of CHD and pregnancy/early loss, ancestral origins, and whether or not there is consanguinity.[1,14,15] Reproductive fitness is somewhat reduced given the severity of the condition, and families segregating TOF in a Mendelian fashion are uncommon,[6] even when taking into account broadly defined CHD.[16] Consanguinity, or originating from a genetic isolate, are features that are discernible from history and/or through advanced genomic methods (Fig. 1A). Both are associated with increased risk of autosomal recessive conditions.

The likelihood of clinically relevant results with any form of genetic testing will vary, depending on associated features. A "spectrum" approach tailored to TOF that considers complexity across the lifespan (Fig. 2) may be more pragmatic than a dichotomy, such as "isolated/nonsyndromic" and "syndromic." Nonetheless, clinically relevant genetic variants are associated across this TOF spectrum, including many in the "baseline" subset. Although the complexity of the genetic architecture means that for the majority of individuals, the main causal factors of their cardiac condition remain to be identified, even on a research basis,[1,17] the most common currently diagnosable causes of TOF are detectable using a genome-wide microarray; thus this is recommended as a first-line clinical genetic test (Fig. 1).

Microarray as First-Line Clinical Genetic Testing for TOF

Since about 2010, genome-wide microarray technology has come into standard practice recommendations, including for TOF, to detect clinically relevant genetic variants.[18] As applied clinically, microarray identifies pathogenic copy number variations (CNVs) now known to be common causes of human diseases, particularly those related to developmental abnormalities such as CHD and neurodevelopmental disorders.[19] CNVs are structural genetic changes involving copy number loss (deletions) and gain (duplications), the vast majority of which are not detectable by karyotype (Fig. 1A). Microarray also supersedes targeted methods, for example, fluorescence *in situ* hybridization, that required ordering a probe for a specific locus (Figs. 1B and 3). A recent Ontario estimate approximated the cost of clinical microarray to that of magnetic resonance imaging.[20]

Although microarray may be offered and performed more during the prenatal/neonatal period than at any time postnatally, overall uptake at the clinic level remains spotty.[19] Opportunities for testing abound across the entire lifespan (Fig. 1B). Recent data reinforce the rationale, clinical relevance, and relative ease of implementation of genetic testing in the clinic for patients with TOF and other major CHD.[21,22] For example, in Indiana, since 2015, most newborns requiring cardiac surgery for CHD had a clinical genome-wide

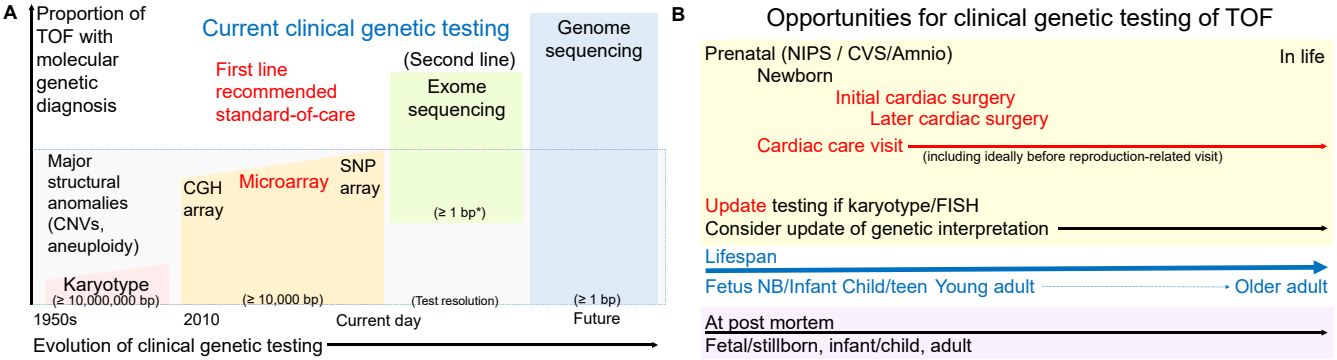

Figure 1. (**A**) Clinical genetic testing for tetralogy of Fallot (TOF)—past, present, and future. The horizontal (*x*) axis represents both the evolution over time (dates nearest the axis) and the increasing resolution of test capability (in base pairs [bp], bottom of each test method), for each of 4 main genome-wide test technologies: karyotype (**pink**), microarray (**orange**; initially comparative genomic hybridization [CGH] methods and more recently using single nucleotide polymorphism [SNP]-based methods), exome sequencing (**green**; *varying methods, usually providing data on single nucleotide variants [SNVs], and small insertions/deletions [indels] for approximately 90% of coding sequence across the genome, and with variable ability to detect copy number variants [CNVs], better for deletions than duplications), and genome sequencing (**blue**) that lies in the future with respect to clinical test capability (providing data on the widest array of variant types: SNVs, indels, tandem repeat expansions (TREs), structural variants, and CNVs, involving both coding and noncoding sequence across the genome). Microarray is the current first-line clinical genetic test; exome sequencing is second-line. The vertical (*y*) axis represents the approximate proportion of individuals with TOF who have molecularly detectable genetic conditions; the top arrow position would be at approximately 40% currently, a figure that would be expected to increase as genetic research advances. The pale grey background with **dotted blue outline** denotes detection of major structural anomalies, predominantly CNVs, but also chromosomal anomalies, that currently comprise the largest group of TOF-relevant genetic variants detectable by microarray. Above the dotted blue line lie other TOF-relevant pathogenic variant types, for example, rare SNVs. SNP data (common variants) used in calculating polygenic risk scores for certain phenotypes would be available from SNP arrays and genome sequencing. This represents a future possibility for TOF and is not yet at the level of broad clinical applicability for any phenotype. Note: targeted clinical genetic testing methods are not shown. For example, fluorescence *in situ* hybridization (FISH), widely available since about 1994, required selection of a probe for a specific region, and gene panels, available since about 2015 for congenital heart disease, assess for certain variants within groups of selected candidate genes, and vary by commercial supplier. Also not included are newer methods of genome-wide sequencing, for example, long-read sequencing that may be superior for detecting some variant types, for example, TREs. (**B**) Opportunities for clinical genetic testing of TOF across the lifespan. Possible time points for clinical genetic testing of individuals with TOF are indicated. In life (**yellow background**), these extend from prenatal screening and diagnostic methods (respectively, NIPS: noninvasive prenatal screening; CVS: chorionic villus sampling, and Amnio: amniocentesis) to newborn (NB), to older adult years. Postmortem time points (**lavender background**) are also shown. In contrast to cell-based methods (karyotype, FISH), and regardless of the tissue origin (eg, venipuncture for blood sample using a lavender-topped ethylenediaminetetraacetic acid, EDTA, tube), DNA extraction at the clinical lab will allow for genetic testing using clinical microarray and all sequencing methods.

microarray, with over 20% found to have a clinically relevant genetic abnormality.[22] Conotruncal defects such as TOF have particularly high yield.[21]

Indeed, a genome-wide microarray exploits the multigenic and innate developmental complexity of TOF (Fig. 2).[19,23] Box 1 outlines the rationale for clinical genetic testing. Multiple studies show that yields of clinically relevant CNVs are high even in "isolated" TOF, here termed "baseline" (Fig. 2).[22] Determining the presence of additional features that further increase the expected yield of pathogenic CNVs, or of other genetic anomalies, will depend on the developmental stage or age of the patient, availability of the information, and the expertise, interest, and time constraints of the clinician.

Provision of pretest information (genetic counselling) includes basics of the test itself, approximate likelihood of a clinically relevant finding that will help in understanding the etiology of TOF and its management, and the possible implications for other family members (eg, offspring and parent) (Box 1; Figs. 1 and 2). The consent process is comparable to that for any medical test ordered, for example, cardiac magnetic resonance imaging, where the test capacity is circumscribed and there is a possibility of "incidental" findings unrelated to the patient's main condition(s). Though rare with a microarray, a reportable example requiring separate genetic counselling would be a CNV that disrupts a known cancer gene, for example, *BRCA1*.[24]

Individuals with a positive finding on microarray will usually require referral to a genetics expert for genetic counselling and recommendations regarding work-up and follow-up. Information about care implications and prognosis may be available, as may be connection to an international community of others with the same finding (Box 1). Just knowing about a concrete cause, unrelated to pregnancy issues, can come as a relief to a family at any life stage.

Genetics is an ever-advancing area of medicine. For all genome-wide clinical molecular genetic-based tests, including microarray, there are recommendations for periodic reinterpretation of results with updated data, particularly for negative results or variants of uncertain significance (Fig. 1B).[25] Advancing to second-line, higher resolution genetic testing (Fig. 1A) usually involves referral to a clinical geneticist.[19]

What Information Can a Clinical Microarray Provide?

Multiple studies have demonstrated that structural genetic changes, CNVs and chromosomal abnormalities, are the most common of identifiable causes of TOF and related

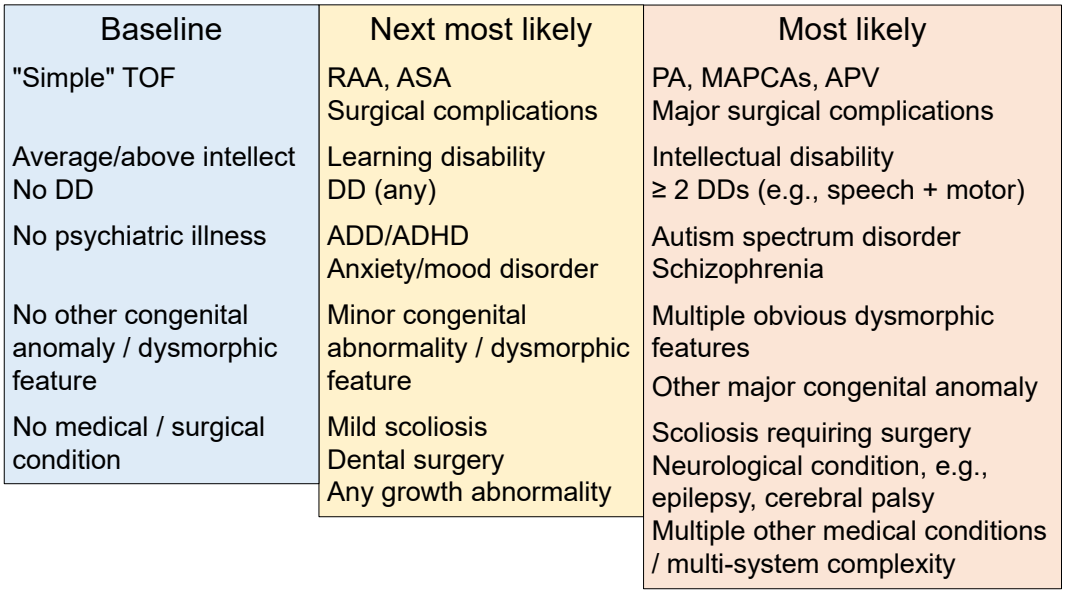

Figure 2. Likelihood of identifying a pathogenic variant with clinical genetic testing. For a patient, of any age, with tetralogy of Fallot (TOF), there is a likelihood of a clinically meaningful result on genetic testing, here termed "Baseline" (**blue box**), even where there are no identifiable additional clinical features, including cardiovascular features (thus, "simple" TOF). Examples of additional cardiac and extracardiac features that increase the prevalence likelihood of a clinically meaningful result on genetic testing are provided in the **yellow box** ("Next most likely"), and those of greater severity that further increase the prevalence likelihood of a clinically meaningful result on genetic testing appear in the **orange box** ("Most likely"). Several of the clinical features would not be apparent for prenatal, or for many paediatric, patients, however. Estimates of relative yield of a genome-wide microarray (ie, report of a clinically relevant CNV) would be 5%-10%, 10%-20%, and 20%-30%, respectively. In general, the more neuro-developmental, severe, and multisystem the condition, the more likely will be a clinical genetic test finding. Notably, the baseline likelihood is sufficiently substantial that prenatal microarray is recommended for patients with TOF and other congenital heart disease. There would often be sufficient rationale to move to second-line clinical genetic testing (Fig. 1A) for individuals with the greatest multisystem severity when there are negative microarray results. ADD/ADHD, attention-deficit disorder/attention-deficit hyperactivity disorder; APV, absent pulmonary valve; ASA, aberrant subclavian artery; DD, developmental delay(s); MAPCAs, major aortopulmonary collateral arteries; PA, pulmonary atresia; RAA, right aortic arch.

conotruncal anomalies.[1,26-29] Most are multigenic,[26] that is, involve a gene dosage decrease (deletions) or increase (duplications) of several to many genes. These changes may occur as a *de novo* (spontaneous) event or may be inherited from a parent who often has a different, usually milder, clinical expression than the affected offspring. All inherited and the vast majority of spontaneous genetic changes are present in the germ cell (sperm or egg) before conception (ie, before pregnancy). Notably, unlike chromosomal anomalies such as trisomies, most CNVs have no relationship to parental age.[30-32]

Several CNVs are recurrent, that is, arise as a new (*de novo*) occurrence in unrelated families. Often this is due to the innate structure of the human genome that in many regions involves low copy repeat (LCR) sequences that predispose to these events.[30-32] Multiple such genomic disorders are now known,[33,34] though why some are more prevalent than others is unknown.

The most common molecularly definable causes of TOF, found on average in approximately 10% of all patients, are recurrent chromosome 22 microdeletions associated with 22q11.2 deletion syndrome (22q11.2DS).[30,35] Targeted testing began in the mid-1990s, detecting the most prevalent 22q11.2 microdeletions, with microarray recently adding far more knowledge (Fig. 3). Thus, substantial outcome information has accrued, providing an example for both the rationale for contemporary clinical genetic testing and the potential advantages afforded by molecular genetic diagnosis (Figs. 1 and 3; Box 1). Much new information can accrue from identifying a homogeneous genetic group (Tables 1 and 2).

Molecular screening of a contemporary newborn Ontario sample, for example, provided an estimate that 1 in every 2148 live births has a typical 22q11.2 deletion, far higher than previous estimates.[36] Even higher prevalence is documented in prenatal and pregnancy-related data, likely related to disproportionate pregnancy loss associated with the deletion, as for other CNVs.[15,37-40] However, despite its prevalence and its familiarity to many clinicians, the 22q11.2 deletion remains relatively underdetected, even in individuals with CHD. After excluding those with a known diagnosis of 22q11.2DS, research studies using systematic genetic testing continue to identify many previously undetected individuals with the 22q11.2 deletion, even among paediatric cohorts.[41] Although CHD such as TOF may lead to earlier diagnosis, a prolonged diagnostic odyssey remains the norm for most individuals with a 22q11.2 deletion.[42] Reasons include a lack of systematic genome-wide testing (Fig. 1A), limitations of fluorescence *in situ* hybridization (Fig. 3), and variable expression that includes absence of readily recognizable clinical features.

Variability of expression is the norm for CNVs, as for virtually all genetic variants. Notably, most individuals with a 22q11.2 deletion do not have the severe congenital phenotype

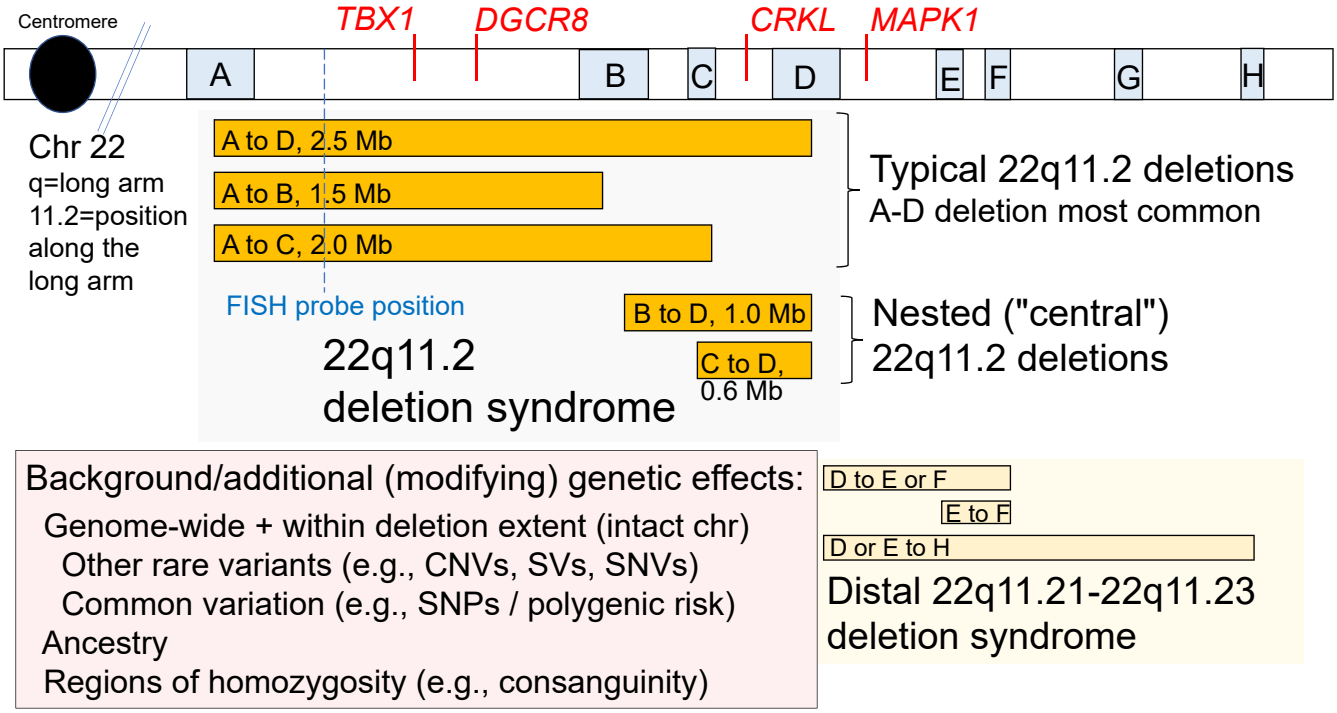

Figure 3. Diagram of the extended chromosome 22 (Chr 22) region with recurrent copy number variants (CNVs). This diagram of the Chr 22 region shows the long (q) arm along with the centromere, which separates the q arm from the short (p) arm of Chr 22. The positions of the low-copy repeats (LCRs, LCR22A to LCR22G) that are present in all human genomes, and predispose to the occurrence of recurrent deletions and duplications usually during gametogenesis, are shown by **blue boxes** enclosing the corresponding letter of the LCR. LCR22A and LCR22D flank the 2.5 megabase (Mb) deletion most commonly associated with 22q11.2 deletion syndrome (22q11.2DS), and this subregion encompasses 2 other LCR22s, LCR22B and LCR22C. Recurrent 22q11.2 deletions are shown (**orange bars**) with their respective sizes in Mb and the flanking set of 2 LCR22s. Rare deletions that are not mediated by LCRs are not shown. The approximate position of the probe used for targeted FISH (fluorescence *in situ* hybridization) testing is indicated by a **dashed vertical blue line** across the CNVs that would be detected by this test; these are termed "typical" 22q11.2 deletions (note that FISH cannot indicate the length of the CNV). Although 22q11.2DS is an umbrella term for all of the deletions across, and nested within, the LCR22A to LCR22D region, the smaller B-D and C-D deletions are rarer, less likely to come to early clinical attention (though still may have associated congenital heart disease, CHD), and more likely to be inherited, than typical 22q11.2 deletions. Recurrent deletions that are distal to the LCR22A to LCR22D region, here shown by **narrow yellow bars**, are associated with a distinct condition termed "Distal 22q11.21-22q11.23 deletion syndrome." Rarer than 22q11.2DS, there may be some overlap of phenotypic features, for example, CHD (Tables 1 and 2). Four protein-coding genes (**red font**) associated with congenital cardiac disease expression are indicated with respect to their relative position (**vertical red lines**) along this Chr 22 region. Wherever the term "deletion" is used, a reciprocal duplication CNV of the same extent is possible, each with its own (variable set of) clinical features. Compared with the deletions, Chr 22 duplications tend to be rarer, less likely to come to early clinical attention (though still may have associated CHD), and more likely to be inherited. Similar mechanisms (eg, flanking LCRs) predispose to other recurrent CNVs across the genome. A genome-wide clinical microarray will detect all of these CNVs. For any genetic abnormality that is clinically detected, the **pink box** highlights some of the modifying factors that may increase or decrease the likelihood of expression, and severity, of a particular associated phenotype, including CHD. *CRKL*, CRK-like proto-oncogene, adaptor protein; *DGCR8*, DGCR8 microprocessor complex subunit; *MAPK1*, mitogen-activated protein kinase 1; SNP, single nucleotide polymorphisms or common variants; SNV, single nucleotide variants; SV, structural variants; *TBX1*, T-box transcription factor 1.

formerly termed "DiGeorge syndrome," nor do they necessarily present with other features classically considered "syndromic."[30-32] Indeed, only about half have a clinically actionable CHD. Though TOF or other conotruncal anomalies are often associated, the range of cardiac anomalies is vast, including (rarely) left-sided lesions; septal defects (including those with early spontaneous closure) are the most common.[30-32] Studies are underway to inform reasons for such variability. These include reduced gene dosage of several genes of known importance in early cardiac development (Fig. 3)[32] and apparent effects of additional genome-wide variants, though there is no evidence that a second 22q11.2 region variant within the intact chromosome 22, for example, in *TBX1*, is needed to affect CHD/TOF expression (Fig. 3).[43,44]

Broader consideration and implementation of clinical genetic testing will enable our ability to observe the relevance of molecular diagnosis to key outcomes, and personalized care, for TOF. Tables 1 and 2 summarize what is currently known about the features and outcomes for specific CNVs and chromosomal anomalies, including those most likely to be clinically reported for patients with TOF. For other, individually rarer reported genetic abnormalities, there will be some clinically relevant information available, with references to these often provided by the reporting clinical genetics lab.

Box 1. Rationale for clinical genetic testing using standard genome-wide microarray.

Potential benefits

- Can improve understanding about the root cause of the tetralogy of Fallot, and other developmental/health issues that are present
- Can help plan for short- and long-term management
- Can help inform what other investigations and care may be helpful now and in the future
- May provide new support opportunities for patients and families
- Could provide important new information about recurrence risk (likelihood that a child/pregnancy may be affected)

Likelihood of a clinically relevant copy number variation (CNV) to be reported

- For about 5%-25% of individuals (Fig. 2), a clinically relevant CNV will be reported
- Thus, the most likely result is that no structural imbalance variation is detected that is currently known to be clinically relevant
  - Note: This does not mean there is no genetic abnormality/explanation present (eg, there may be a clinically relevant single nucleotide variation ("point mutation") that this test cannot detect)

The microarray test is limited to chromosomal imbalances (deletions or duplications), usually those over a certain size (e.g., >10,000 base pairs, Fig. 1A), and those for which sufficient information is available to warrant their clinical reporting.

Further information about clinically relevant implications of microarray testing for TOF appears below, including the special circumstances of reproduction/recurrence risk and prenatal/perinatal considerations, and key paediatric and adult morbidity- and mortality-related outcomes. We begin however by summarizing limitations of the current literature.

Prevalence Estimates and Limitations of the Available Literature

Knowledge about the genetics of TOF, including estimates of overall prevalence and mortality/morbidity of specific aberrations, is affected by the research design, including cohort studied, birth/surgical era, and methods used. Data are almost always more complete for cardiac anatomy than for genetic or other parameters. To our knowledge, there are no studies that have applied uniform genetic testing of all individuals with TOF, without a priori excluding individuals with "syndromic" features or known syndromes such as 22q11.2 deletions and/or more recognizable and severe autosomal anomalies.

Parallel to considering the CHD "surgical era" will be consideration of the "genetic testing era" (Fig. 1A). One can reasonably assume that most individuals with trisomy 21, 13, or 18 would be clinically recognizable, and thus the vast majority would be molecularly diagnosed at birth (by karyotype for the past 60+ years). On the other hand, many with pathogenic CNVs or sex chromosome aneuploidies would be expected to have no or long delayed (eg, into late adulthood) molecular diagnosis.[30,42] Although this is especially likely for the majority of individuals with TOF who are now adults, it may include several in the paediatric age range (Fig. 1A). Molecular diagnosis postmortem is also rare (Fig. 1B).

Other limitations of available research relate to whether genetic diagnoses were recorded at all, or recorded accurately, in medical records, let alone in health administrative data where even Down syndrome documentation and/or International Classification of Disease codes are known to be subpar.[45] Consistent with this, for individuals with a confirmed 22q11.2 deletion, we have unpublished data showing that only a minority were ever domiciled with an International Classification of Disease code for a 22q11.2 deletion related syndrome in health administrative data. This, in addition to expected inadequate clinical genetic testing and other factors, may help explain reported low prevalence of those with a recorded diagnosis of 22q11.2DS in some recent population-based studies of TOF.[46,47]

Clinical Genetic Results and Relevance to Clinical Care of Individuals with TOF

Reproduction and recurrence risk

Over time, more and more individuals with TOF are in the reproductive age range and are having children.[48,49] This is in the context of overall reduced reproductive fitness of TOF recently reported in a Canadian study (70%) that excluded individuals with 22q11.2 deletions and major chromosomal anomalies,[48] and in a Danish study of conotruncal anomalies (71%-77%).[50] Within individuals with a 22q11.2 deletion, while there is little evidence of infertility, reproductive fitness is somewhat reduced for those with major CHD such as TOF, and for men, and there are more substantial reductions in those with severe intellectual disability or psychotic illness.[30,40]

For individuals without a 22q11.2 deletion or other major chromosomal anomaly, family history data on recurrence risk indicate a general increased risk of CHD, encompassing a broad spectrum of associated conditions, in offspring of individuals with TOF.[48] In general, the reported risk to offspring is low (<5%) and may be somewhat lower for affected fathers than for affected mothers with TOF.[48,50] Background heritable susceptibility to CHD related to family history has also been observed in families of individuals with 22q11.2DS as a likely modifier of the baseline risk imparted by the 22q11.2 deletion.[51]

Importantly, for individuals with a pathogenic variant, who are at a 50% *a priori* risk of transmitting the variant at every pregnancy, an affected parent's clinical expression does not predict the breadth of expression (cardiac, neurodevelopmental, or otherwise), or its severity, in the offspring.[30] Rather, expression in an offspring who inherits a major genetic variant will be influenced by the (variable) effects of that variant itself and by other genetic and nongenetic factors. This includes transmitted alleles from the affected parent's partner who may have a different genetic condition and/or neurodevelopmental disorder (assortative mating).[40]

General guidelines are available for genetic counselling and management, preconception, and during pregnancy, delivery, and postpartum for individuals with CHD.[6,8] There are new prenatal guidelines available specifically for 22q11.2DS that provide further information about both

Table 1. Clinically relevant copy number variation (CNV) and aneuploidies in TOF that are detectable by microarray—Genetic-, prenatal-, and reproduction-related features

Genetic variant	Estimated prevalence within TOF (%)*	Inherited	Prenatal genetic screening†	Clinically recognizable at/ before birth	Advanced parental age associated	Parental testing	Perinatal findings	Cardiac features	Reproduction affected	Offspring affected (%)‡
Typical 22q11.2 deletion	10	5%-10%	Yes†	Some	No	Recommended	SGA/low birth weight Prematurity	PA MAPCAs RAA ASA	Rarely	50
1q21.1 duplication or deletion	1	Often	No	—	No	Recommended			No	50
8p23.1 deletion	<1	Rarely	No	—	No	Recommended	IUGR		No	50
22q11.2 duplication	<1	Often	No	—	No	Recommended			No	50
Distal 22q11 deletion	<1	Rarely	No	No	No	Recommended	Prematurity, IUGR			50
45, X0 (Turner syndrome)	<1	—	Yes	—	No	Not usually	IUGR		Usually	—
47, XXY (Klinefelter syndrome)	<1	—	Yes	—	Maternal	Not usually	—		Usually	—
Trisomy 21 (Down syndrome)	6	—	Yes	Yes	Maternal	Not usually	Prematurity, IUGR		Usually	—

For several of the emerging genetic conditions in the table, there is insufficient information as yet; thus boxes are left blank. Many other recurrent genetic variants detectable on clinical microarray that may be relevant to TOF are not included here, for example, 15p11.2 deletion, a CNV that may be considered a variant of uncertain significance or "risk" variant. Also not included are the many very rare variants that are pathogenic and clinically relevant to the individual patient but so rare that little is yet known about them, whether CNVs or single nucleotide variants (SNVs) of individual genes.

ASA, aberrant subclavian artery; IUGR, intrauterine growth retardation; LCR, low copy repeat; MAPCAs, major aortopulmonary collateral arteries; PA, pulmonary atresia; RAA, right aortic arch; SGA, small for gestational age; TOF, tetralogy of Fallot.

* Rough estimates that will vary by age (eg, due to mortality) and availability of high-quality prevalence data.

† Prenatal diagnostic genetic testing is available for all CNVs and anueploidy (by chorionic villus sampling or amniocentesis, and microarray), in contrast to current noninvasive prenatal screening (NIPS, see text); beyond large chromosomal anomalies, NIPS is currently reliable only for LCR22A-LCR22D 22q11.2 deletions (Fig. 3).

‡ Likelihood of genetic affected status, with variable expression in the offspring that is expected to be unrelated to the clinical expression of the affected parent. (Occurence of offspring in individuals with sex chromosome anomalies and trisomies is very rare due to reduced fertility in these conditions; otherwise recurrence risk would be up to 50%.)

Table 2. Clinically relevant detectable copy number variants and aneuploidies in TOF—Extracardiac features* likely to affect the management of TOF

Genetic variant	Intellectual/learning disability (ID/LD)†	Psychiatric†	Neurologic†	Respiratory	Endocrine Metabolic	Skeletal, renal, other
Typical 22q11.2 deletion	LD, mild ID (moderate-severe-rare) (average-some)	Anxiety Schizophrenia ADD Autism spectrum	Seizures, any age/ type‡ Epilepsy (5%) Early onset Parkinson disease Other movement disorders Neural tube defects	Asthma OSA Small airways Laryngomalacia	Hypocalcaemia (70%) Hypothyroidism (25%) Obesity Type 2 diabetes mellitus Hyperlipidaemia	Velopharyngeal insufficiency Hearing loss Scoliosis (40%) Single kidney (rare) Other urogenital Thrombocytopenia (ITP rare)
1q21.1 duplication	Mild-moderate ID, LD	Anxiety Schizophrenia Autism spectrum ADHD	Seizures (rare) Carpal tunnel syndrome Hypoplasia of corpus callosum	–	Obesity Hypercholesterolaemia Type 2 diabetes mellitus	Macrocephaly Hypospadias Scoliosis Benign cysts
8p23.1 deletion	Mild-moderate ID, LD	ADHD	Seizures (rare) Hypoplasia of corpus callosum Hypotonia	Asthma	Low birth weight Feeding difficulties in infancy	Microcephaly Congenital diaphragmatic hernia High palate Hypospadias, cryptorchidism
22q11.2 duplication	Mild or no LD	Behavioural difficulties	Hypotonia	–	–	Velopharyngeal insufficiency Hearing loss Growth delay Gastro-oesophageal reflux
Distal 22q11.21 deletion	Variable	Behavioural difficulties	Hypotonia	–	–	Growth delay
45,X0 (Turner syndrome)	Normal IQ	Anxiety Schizophrenia	–	–	Gonadal dysgenesis (delayed puberty, infertility) Obesity Hyperlipidaemia Type 2 diabetes mellitus Hypertension Autoimmune disorders Thyroid disease	Short stature Hearing loss Vision problems Urinary tract malformations
47,XXY (Klinefelter syndrome)	Normal IQ or mild LD	Anxiety Schizophrenia ADD	Hypotonia Intention tremors	–	Gonadal dysfunction (delayed puberty, infertility)	Tall stature
Trisomy 21 (Down syndrome)	Mild-severe ID	Autism spectrum	Seizures Hypotonia Alzheimer disease	Narrow trachea Recurrent infections Pulmonary hypertension	Obesity Hypothyroidism Type 1 diabetes mellitus	Short stature Umbilical hernia Hearing loss Vision problems Leukaemia

ADD, attention deficit disorder; ADHD, attention deficit hyperactivity disorder; ITP, immune thrombocytopenia; OSA, obstructive sleep apnea; TOF, tetralogy of Fallot.

* Detection of extracardiac features will depend on the age/developmental stage of the affected individual and to a lesser extent the rigour of examination. For example, prenatal detection would be limited to anatomic features detectable on fetal ultrasound, neonatal features to obvious congenital anatomic anomalies; important neurodevelopmental features may not be evident until school age and neuropsychiatric and metabolic/endocrinological associations not until adolescence or adulthood. In all cases, specific extracardiac features may or may not be present given variable expression.

† On brain magnetic resonance imaging, findings associated with typical 22q11.2 deletions include increased prevalence of white matter hyperintensity signals, regardless of congenital heart disease presence or severity, and developmental findings such as cavum septum pellucidum/cavum vergae, enlarged ventricles and/or sulci, and disordered neuronal migration, for example, neuronal heterotopias, polymicrogyria.

‡ Including neonatal seizures/cyanosis often related to hypocalcaemia; generalized, focal, absence, myoclonic, febrile, etc.

screening and diagnostic methods and related findings, as well as details about genetic counselling and reproductive options for individuals with this condition.[52] These complement practical recommendations with respect to genetic counselling, reproductive, and sexual health-related issues.[30,40] Such guidelines would be helpful for all TOF-related genetic conditions.

Prenatal and perinatal considerations

Diagnosis of complex CHD such as TOF in a fetus requires special considerations for pregnancy management, including consideration of a potential genetic diagnosis.[7,9-11] Genetic testing options include diagnostic testing of fetal cells or tissue (eg, chorionic villous sampling or amniocentesis), and prenatal cell-free DNA screening (noninvasive prenatal screening). Prenatal cell-free DNA screening is now available from the first trimester for all pregnancies (at a cost). Recently published clinical guidelines by the American College of Medical Genetics and Genomics[53] recommend that prenatal cell-free DNA screening be offered to all pregnant patients to screen for the main autosomal trisomies (21, 13, and 18), sex chromosome anomalies, and conditionally for 22q11.2 deletions (meaning that most patients would request this and most clinicians would offer noninvasive prenatal screening for this purpose, after discussing the benefits and limitations). It is important to recognize that screening does not take the place of prenatal genetic diagnosis. For example, in cases of indications such as fetal ultrasound anomalies (including CHD), diagnostic testing with genome-wide microarray should be offered.[54]

If a fetus is diagnosed with a particular genetic disease, pregnancy, delivery, and postpartum monitoring for associated conditions can help elucidate and prevent potential complications (Table 1).[30,52,55] For fetuses with a 22q11.2 deletion, for example, there is an elevated risk for prenatal growth abnormalities (small for gestational age at birth) and other conditions (eg, polyhydramnios), regardless of the presence/absence of CHD or the affected status of parents.[36,55,56] Reproductive outcomes may also include increased risk of pregnancy loss at any stage, including stillbirths, related to a fetus with a pathogenic variant,[15,37-39] emphasizing the importance of genetic diagnosis at these developmental stages (Fig. 1B). Specialist care and delivery at a tertiary care facility are often recommended, regardless of the presence or absence of CHD or of the parental affected status.[30,31,52]

In contrast to prenatal genetic screening, there is no comparable newborn screening as yet for TOF-relevant genetic conditions (many not clinically recognizable at birth), including the 22q11.2 deletion.[36] Newborn pulse oximetry screening is however expected to increase early diagnosis of TOF in liveborn infants,[57] which could possibly trigger clinical genetic evaluation.

Surgical and other cardiac-related outcomes

Multiple studies have shown that perioperative complications, though usually not immediate postoperative survival of congenital cardiac surgery, are adversely affected in individuals with CHD who have 22q11.2 deletions.[58] These complications encompass infections, bleeding, seizures, atelectasis, airway issues, vocal cord paralysis, need for dialysis, and so on. In TOF, individuals (median age 17 years) with pulmonary atresia (PA) and recorded as having a 22q11.2DS diagnosis had significantly more cardiovascular interventions.[46] 22q11.2 deletions also affect patient-reported outcomes and exercise tolerance.[59,60] In a US study, CNVs of *de novo* origin at 22q11.2, but also at 15q25.2 and 3p25.2, were associated with prolonged time to final extubation.[41] For individuals with 22q11.2DS, for example, attention to increased risk of hypocalcaemia (and thus seizures), especially at surgery, childbirth, or serious infection, may be clinically relevant.[61] Tables 1 and 2 provide multiple examples of associated features pertinent to clinical care for this and other genetically defined conditions. Collectively, these data suggest that some tailoring of management according to genetic etiology could improve cardiac outcomes.

Neurodevelopmental and related outcomes

Neurodevelopmental disabilities that are relatively common in individuals with TOF are highly related to the underlying genetic etiology.[62] These include developmental delays and intellectual disability/learning disabilities. For example, the 22q11.2 deletion lowers IQ on average by 30 points, regardless of CHD, though the outcome may be modified somewhat by additional factors, including the intellectual level of unaffected parents.[30-32,63] Many other CNVs also affect the cognitive outcome and the likelihood of developing treatable illnesses such as attention-deficit disorder, anxiety, and depression.[62,64-66] Anxiety and depression are common in TOF.[59,67] Severe neurodevelopmental disorders such as psychotic illness (eg, schizophrenia) are far rarer, but, for example, the 22q11.2 deletion increases the risk by 20- to 25-fold.[30-32,63] Genetic diagnosis can thus help with appreciating risk and accessing early treatment (Table 2).[30,68]

Collectively, neurodevelopmental/neuropsychiatric conditions may complicate care in the cardiac clinic, as patients may be less able to independently make and keep appointments, communicate, and follow recommendations.[30] This is especially the case for patients who have treatable but untreated neuropsychiatric conditions and/or those without family or other tangible support systems. Families, too, are often overburdened, further complicating the situation.[69] Likewise, dropout at transition and transfer from paediatric to adult care may be more likely.[70] Genetic diagnosis may help identify those needing additional supports.

Other extracardiac features / multimorbidity

TOF is among the most likely CHD to be associated with extracardiac features that are increasingly evident as patients age and are pertinent to potential personalizing of care (Table 2).[71]

One example relates to lung function, of known importance to outcomes in adults with CHD.[72] Recently, the 22q11.2 microdeletion was found to be enriched in adults with TOF and abnormal spirometry, even when accounting for complex CHD, scoliosis, and asthma.[73] The findings suggested both restrictive and obstructive defects, supporting the potential value of early pulmonary function testing.[73] Possible broader relevance is suggested by a recent report of increased risk for chronic obstructive pulmonary disease in otherwise healthy individuals with mild forms of CHD.[74]

Also, scoliosis, like early neurodevelopmental outcomes, was originally believed to be secondary to the effects of congenital cardiac surgery. Genetically informed studies have revealed that the prevalence of scoliosis is predominantly related to the presence of a 22q11.2 deletion.[75]

Multimorbidity is prominent in 22q11.2DS, including conditions highly relevant to cardiac care and functioning, with many only evident later in life, for example, intellectual/learning disability, hypothyroidism, hypoparathyroidism, and metabolic conditions (obesity, type 2 diabetes, and dyslipidaemia).[76-78] Evidence outside of the TOF context suggests that each clinically relevant CNV will have its own multimorbidity profile.[34]

Mortality

There are replicated findings that genetic causation is relevant to early and late mortality and longevity expectations for TOF, though the limitations of current clinical genetic testing data are also evident.

Mortality in the first year of life for liveborn infants with TOF in North Carolina identified neonatally to have a 22q11.2 deletion (n = 34) was reported to be 27% (vs 15% mortality overall for the total 496 infants).[79] There may be some parallels with infant mortality findings in a Québec TOF birth cohort study. Nearly half of all-cause mortality (at median age 17 years) occurred in infancy before surgical repair or in those for whom such repair was unavailable; in those without PA, this infant mortality was associated with genetic conditions, including a recorded diagnosis of 22q11.2DS.[46] In a recent US study of patients with TOF, in a subset without a documented extracardiac anomaly, a genetic abnormality (most discovered on microarray) was independently associated with increased infant operative mortality.[22] Another US study reported that CNVs at 15q25.2 and 15q11.2 that arose as *de novo* events were associated with worse transplant-free survival compared with the rest of a large general, mostly paediatric, CHD sample.[41] Also potentially pertinent to early mortality is a report showing high yield of CNVs in infants with sudden unexplained death (none with CHD).[23]

With respect to late outcomes, in a Canadian study of adults (average age approximately 30 years) with TOF, the 22q11.2 microdeletion was found to be a significant predictor of adult all-cause mortality, even after accounting for the higher prevalence of PA, compared with other (mostly unknown; Down syndrome was excluded) etiologies; the probability of survival to age 45 years in those without PA was 72% and 98%, respectively.[80] Excess mortality in those with a 22q11.2 deletion was evident only after age 30 years and was primarily related to cardiovascular causes.[80] Findings were comparable in a European study of similarly-aged individuals with TOF or PA/ventricular septal defect.[81] Adolescent/young adult mortality was also reported to be elevated in a multicentre US study[82] and in a Swedish population-based study,[47] even though the prevalence of those with a recorded diagnosis of 22q11.2DS was low (2.2% and 4.1%, respectively). An independent Québec TOF birth cohort study (average age 17 years) found excess lifetime mortality in the 71 individuals with a recorded diagnosis of 22q11.2DS but only for the subset of 27 with PA.[46] No adult mortality data are yet available outside of major chromosomal anomalies and 22q11.2DS.

Another means of examining the outcome is to look within a molecularly defined genetic subgroup. Within 22q11.2DS, for example, longevity of adults is significantly reduced when a major CHD such as TOF is present (survival to age 45 years: 72%, vs those with no such CHD 95%).[83] Late adult mortality is reported to be elevated in the presence of several other recurrent CNVs, compared with their absence, in a relatively healthy, that is, without major CHD, elderly sample (UK Biobank).[34] At the other end of life, within 22q11.2DS, paediatric mortality is estimated at 5%-15%, with most deaths occurring during the first year of life, the majority related to complex CHD.[31]

Collectively, these studies suggest that patients with premature mortality are likely to be enriched for potentially identifiable genetic causation, whether or not there is detectable cardiac or extracardiac complexity (Fig. 2). Further research, including postmortem genetic analysis at all life stages (Fig. 1B), is needed in this important area.

Variants at the Individual Gene Level—Potential Clinical Implications

The genetic architecture of TOF, where pathogenic CNVs discoverable by clinical microarray dominate, clearly differs from that of cardiomyopathy where deleterious variants in defined, functionally related genes are at the forefront of causation.[84] Although the advent of genome-wide sequencing with systematic adjudication of variants with respect to pathogenicity has substantially increased our knowledge about individual genes for TOF, there remains no standard recommendation for clinical genetic testing in this regard.[85] Clinical geneticists will use American College of Medical Genetics and Genomics criteria to adjudicate second-line (usually exome sequence-based) testing for patients with TOF, relying on the literature, beyond genes for known TOF/CHD syndromes, and updated guidance for secondary findings.[24]

Numbers remain relatively small for individual genes or variants, with limited data on clinical features, key outcomes, or segregation within families. For example, multicentre exome sequencing studies of, mostly paediatric, TOF samples have reported a notable prevalence of deleterious variants in the *FLT4* gene, encoding vascular endothelial growth factor receptor 3 (VEGFR3).[86,87] In the US study, 6 of the 10 probands reported had inherited the *FLT4* variant from an "unaffected" parent (ie, with no serious CHD).[86]

The first ever whole genome sequencing study of TOF was Canadian, using a subset of an adult cohort with TOF previously studied using microarrays, and excluding those with a 22q11.2 deletion.[16] Highly damaging variants in *FLT4* were found in 5.1% of individuals, with discovery of comparable variants in 7 other genes (including *KDR*), thus totalling 11.4% with variants in a VEGF signalling pathway.[16] Clinical relevance was suggested by enrichment for absent pulmonary valve and right aortic arch, but not for PA, and with only rare extracardiac anomalies and intellectual disability.[16]

Genome-wide sequencing studies place previously identified genes and variants, some for clinically recognizable rare syndromes where referral to clinical genetics would be expected, in a broader context. Gene networks implicated for TOF highlight VEGF/Notch dysregulation, including

deleterious variants in *FLT4*, *KDR*, and *NOTCH1*, but also rarer variants in other genes, for example, *TBX1* (22q11.2 deletion region), *RAF1, RASA1* (RASopathy/Noonan syndrome), *JAG1* (Alagille syndrome), *CHD7* (CHARGE syndrome), and genes encoding other transcription factors (eg, *NKX2-5* and *GATA4*).[12,16,85,87-93] When TOF-associated noncardiac phenotypes, for example, neurodevelopmental disorders and multiple congenital anomalies, or consanguineous cohorts, are considered, the spectrum of genetic findings becomes even wider.[94] Collectively, these results further illustrate the genetic heterogeneity of TOF and support future gene discovery efforts. The individual rarity of variants, each potentially conveying its own risk profile, promotes the value of international collaborative efforts to better understand their impact and variability of expression.[94]

As for pathogenic CNVs, the identification of lower impact (modifying) factors (Fig. 3) will be important. Assessing a global common variant (single nucleotide polymorphism—based) background effect awaits the availability of summary statistic data to calculate polygenic risk scores for TOF.[95] Some initial studies are demonstrating the potential clinical utility of genome sequencing that can assess multiple variant types simultaneously, including sequence-based variants and CNVs.[96,97] Genetically designated homogeneous groups also provide the opportunity to perform clinical trials of prenatal neuroprotective strategies, which may be able to be informed by animal or cellular models.[98]

These genetic advances have led some to predict a shift from a phenotype-first to a genotype-first approach in clinical medicine for conditions of a large societal impact, given the expected yields and benefits.[99] Although futuristic at this point in clinical practice, implementing the recommendation to consider clinical microarray for all patients with TOF (Fig. 1) could be considered a fundamental step forward in helping prepare for the sea change that genome sequencing will bring to medicine as a whole.

Conclusion—Opportunities for Clinical Care

TOF genetics remain a relatively young area with much promise for cardiologists to be at the forefront of molecular and personalized medicine that can improve patient care. The highest impact genetic variants with the most severe cardiac and extracardiac expression have been the first to be identified, and their clinical relevance clearly established. Because most are CNVs or other chromosomal imbalances, the vast majority will be detectable using genome-wide microarray.

This standard, yet underutilized, test is available to frontline clinicians with the recommendation that clinical microarray be ordered and basic information (genetic counselling/expected outcome) about this provided (Box 1, Figs. 1 and 2, Tables 1 and 2) ahead of referral to medical genetics.[19] For the rarest CNVs and for other genetic changes, for example, those affecting individual genes, there is accumulating knowledge about their role in causing TOF but less information available yet about their associated, especially long-term, outcomes and variability of expression. Clinical elucidation of such variants will contribute to our understanding.

Looking ahead, in the relatively near future, genome sequencing will come into the clinical realm, likely first in paediatrics and later for adults, with further downstream enhancement of understanding etiology, pathogenesis, and clinical trajectory of TOF. Knowledge about baseline cardiac anatomy, surgical remodelling, and cardiac functioning will remain foundational, with genetics providing an important "plus" for patient care, for families, and for clinicians. Genetic advances promote consideration of a multidisciplinary team approach (eg, CHD cardiologist, psychiatrist, social worker, endocrinologist, and other specialists as necessary) for the increasing numbers of patients with multisystem conditions at a "hub" centre of excellence. The Dalglish Family 22q Clinic (https://22q.ca) provides a model that is integrated with the ACHD programme at Toronto General Hospital.

Old ideas about restricting genetic consideration and testing to particular phenotypes (eg, based on facial features) or time periods (eg, perinatal) should be discarded. For all patients with this complex CHD, regardless of age and co-occurring conditions, identification of genetic causation provides the opportunity for preventive care, other clinical options, and increasing knowledge that will be meaningful for understanding outcomes and lifelong management.

Acknowledgements

The authors thank all of the patients with TOF and their families for inspiring our work, and wish to acknowledge the contributions of the many physicians and other clinicians involved over the years, particularly the late and assuredly great Dr Gary Webb, who provided encouragement and vision to so many.

Ethics Statement

This review paper adhered to the journal guidelines for review papers.

Patient Consent

The authors confirm that patient consent is not applicable to this article as this is a review article.

Funding Sources

This work was supported by the Dalglish Chair in 22q11.2 Deletion Syndrome (ASB), with contributions to cited work supported by Canadian Institutes of Health Research grants (MOP-313331 and MOP-111238 to ASB), a McLaughlin Centre Accelerator Grant (to ASB and CS), and the W. Garfield Weston Foundation (to ASB and CS).

Disclosures

ASB holds the Dalglish Chair in 22q11.2 Deletion Syndrome at the University Health Network and the University of Toronto. The other authors have no conflicts of interest to disclose.

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