# Y&H CCN Approach to Genetic Testing in Congenital Cardiac Disease

Congenital heart disease may occur as part of a genetically-identifiable syndrome. In many cases the first signs of the syndrome may be the discovery of congenital heart disease. Thus, there is an opportunity to diagnose genetic conditions at this time. Timely diagnosis ensures that the appropriate medical management is instigated, but also helps prepare families for future clinical manifestations of the conditions in question. Providing a genetic diagnosis may also allow families to make better-informed choices regarding future pregnancies.

It must be borne in mind; however, that most congenital cardiac disease is **not** associated with an underlying identifiable genetic abnormality. Please use the following advice when considering genetic investigations for children with congenital cardiac disease.

**It is the responsibility of the Consultant that suspects a child with a congenital heart defect and associated clinical features may have an underlying genetic diagnosis, to send appropriate investigations. CONSENT FROM PARENTS FOR GENETIC INVESTIGATIONS SHOULD BE OBTAINED IN ALL CASES. It is the referring clinician's responsibility to follow-up the results once they are reported, and feed these back to the family.**

Prior to any child with a congenital cardiac lesion and suspected genetic condition undergoing surgery, or having a blood transfusion, the responsible clinical team at that time should check that appropriate genetic investigations (or a sample of DNA for storage) have been received in the genetics lab. (Contact details for Leeds Genetics Laboratory and Sheffield Diagnostic Genetic Services).

Please also note that genetic conditions associated with cardiac disease can be due to a chromosomal or single gene causes. This means that a normal chromosome test (such as array CGH), does not exclude single gene disorders that require other molecular tests for confirmation. **If you are informed by a colleague "the genetics are normal" – specify which test(s) is/are negative, and consider whether further genetic opinion is required.**

Referral forms for Leeds Genetics Laboratory or Sheffield Diagnostic Genetic Service.

In complex cases where a diagnosis is unclear or there is relevant family history, the Clinical genetic services based in Leeds and Sheffield (depending on which hospital you are in) can provide advice on the most suitable testing strategy. This may include a clinical review by their services.

Below is the quick reference guide for suspecting genetic diagnosis in a child with congenital cardiac disease. There is more detailed, supporting information in Appendix A.

Version 1 - Y Gargani, E Brown, R Jewell, M Parker
Approved 28/06/2017 – Review July 2020

# Quick Reference Guide

1. **It is the responsibility of the Consultant that suspects a child with a congenital heart defect and associated clinical features may have an underlying genetic diagnosis, to send appropriate investigations**
2. **<u>CONSENT FROM PARENTS FOR GENETIC INVESTIGATIONS SHOULD BE OBTAINED IN ALL CASES</u>**
3. **It is the referring clinician's responsibility to follow-up the results once they are reported, and feed these back to the family.**

**For any child with congenital cardiac disease and a suspected underlying genetic condition, send array CGH in the first instance. If this is normal, refer to clinical genetics for further tests. If a Trisomy is suspected, such as Trisomy 21 (Down syndrome), then a much quicker result will be obtained from QF-PCR testing; with confirmatory karyotype to identify translocations.**

Referral forms for Leeds Genetics Laboratory or Sheffield Diagnostic Genetic Service.

There are certain cardiac defects and phenotypic presentations that may point towards specific underlying syndromes. The list below is by no means exhaustive, and a variety of cardiac anomalies can appear in each of the syndromes detailed below. However, certain lesions are more strongly associated with those syndromes.

| ***Cardiac Anomalies*** | ***Other clinical Features*** | ***Possible Diagnosis*** |
|---|---|---|
| • Conotruncal*/ arch abnormalities | • Palatal anomalies<br>• Dysmorphism | Suspect 22q11DS** |
| • AVSD | • Hypotonia<br>• Dysmorphism | Trisomy 21 |
| • Arch /Aortic valve anomalies<br>• **In a Female Child** | • Short stature<br>• Lymphoedema | Turner Syndrome |
| • Supravalvular Aortic Stenosis<br>• Peripheral Pulm Artery Stenosis | • Dysmorphism | Williams Syndrome*** |

**Conotruncal abnormalities that should prompt suspicion of 22q11DS are: Fallot's Tetralogy, PS with VSD, double-outlet right ventricle, truncus arteriosus, and interrupted aortic arch.*
***Please check **calcium levels** in all children with suspected 22q11DS. Give only **Irradiated blood** products, and avoid live vaccines until condition ruled out, or seen by immunologist.*

***Please check calcium levels in all children with suspected Williams Syndrome.

- Pulmonary stenosis
- Hypertrophic cardiomyopathy

⇨

- Webbed neck
- Sternal defect

⇨ Noonan Syndrome

- Peripheral Pulm artery stenosis
- Tetralogy of Fallot

⇨

- Cholestasis
- Butterfly vertebrae
- Dysmorphism

⇨ Alagille Syndrome****

****Please ensure that the paediatric hepatology team are involved in in all suspected Alagille syndrome cases.

- Congenital cardiac lesion (often ASD)
- Heart block

⇨

- Upper limb abnormalities

⇨ Holt-Oram Syndrome*****

*****Please perform and ECG, paying close attention to PR interval.

- Congenital cardiac lesion

⇨

- Coloboma
- Choanal atresia
- Genital
- Ear anomalies

⇨ CHARGE Syndrome

Dysmorphic child with cardiac lesion and possible, unidentified genetic disease ⇨ Send CGH array and Consult local genetic colleagues regarding review and appropriate testing. Consider 100,000 genomes project.

## Sample type and turnaround times

| Genetic Test | Sample type | Volume | Turnaround time |
|---|---|---|---|
| **Karyotype** | Lithium Heparin | 1-2mL neonates<br>2-5mL children | Up to one month |
| **QF-PCR** | Lithium Heparin | 1-2mL | 24 hours |
| **Array CGH** | EDTA | 1-2mL neonates<br>2-5mL children | Up to one month |
| **Molecular DNA testing** | EDTA | 1-2mL neonates<br>2-5mL children | Up to six months |

# Appendix A – Supporting Information

## 22q11.2 Deletion syndrome (DiGeorge syndrome, velocardiofacial syndrome)

22q11.2 Deletion syndrome (22q11DS), previously known as DiGeorge syndrome, results in a range of clinical features. Most patients with 22q11DS will have a form of congenital heart disease, with conotruncal abnormalities (truncus arteriosus, interrupted aortic arch, Fallot's tetralogy, pulmonary atresia with VSD and double-outlet right ventricle) and aortic arch abnormalities being the most commonly associated defects. Other features of the syndrome are palatal defects, characteristic facial features, developmental delay and immune deficiency.

The characteristic facial features of 22q11DS are often subtle in infancy. They include a high and broad nasal bridge, prominent nose with bulbous tip and hypoplastic nares, narrow and upslanting palpebral fissures and micrognathia.

Diagnosis of 22q11DS is now made using a targeted CGH array in the first instance, with a full array being undertaken if this is negative. Rarely, whole exome sequencing is needed for point mutations.

**Please note** Calcium level should be measured in all children with suspected 22q11DS. Children with suspected or confirmed 22q11DS should only receive irradiated blood. Avoid live vaccines until immunological testing has been untaken and immunologist consulted. Please consult the Max Appeal consensus document for further information.

**Children with conotruncal abnormalities/aortic arch abnormalities OR any cardiac defect with other signs suggestive of 22q11DS (palatal abnormalities or dysmorphic facial features) should have blood sent for CGH array specifying 22q11DS. Monitor their calcium levels, avoid live vaccines and use only irradiated blood.**

## Trisomy 21 (Down syndrome)

Trisomy 21 (T21) is a common, lifelong condition that presents in infancy with characteristic clinical features. The characteristic clinical features include: hypotonia, brachycephaly, loose skin at the nape of the neck, epicanthic folds with almond-shaped eyes and up slanting palpebral fissures, relative macroglossia, clinodactyly, single-palmar crease and a "sandal gap" between the first and second toe.

Around half of children with T21 will have a congenital cardiac lesion, and so **all children with T21 should have an ECHO performed.** The majority of associated congenital cardiac lesions are (complete) AVSDs; with VSDs, ASDs and Fallot's Tetralogy also being associated with T21.

In addition to cardiac defects, children with T21 often have hearing loss, visual, thyroid, gastrointestinal and orthopaedic issues.

Diagnosis can be made quickly using QF-PCR analysis, which can bring about a result within 24 hours. A karyotype is required to exclude a translocation causing trisomy 21, as this may be inherited from a parent carrying a balanced translocation. This will increase risks of the parents having another child with trisomy 21.

Positive diagnosis should be communicated sensitively to the parents, followed by signposting to support groups available and referral to the child's local Child Development Centre.

**Consider T21 as a diagnosis in all children with a complete AVSD or children that have congenital heart lesions and hypotonia/features of T21. Diagnosis is made using QF-PCR and karyotype.**

## Turner syndrome

Turner syndrome is a relatively common chromosome abnormality, affecting female children, and is caused by monosomy or structural rearrangement of X chromosome. Clinical features of Turner syndrome include: short stature, webbed-neck, shield-shaped chest with widely-spaced nipples and cubitus valgus. Neonates with Turner syndrome often have lymphoedema of the hands and feet, nail dysplasia and a high-arched palate.

Turner syndrome is associated with cardiac disease, ovarian failure, skeletal abnormalities, hearing difficulties and renal problems. The congenital cardiac lesions most-commonly associated with Turner syndrome are aortic valve defects and aortic arch abnormalities (principally coarctation). Later in life, affected individuals are at increased risk of hypertension and aortic dissection.

Diagnosis of Turner syndrome is usually made through performing a karyotype.

**In a female child with aortic valve/arch anomalies OR unexplained short stature OR neonatal hand/foot lymphoedema consider Turner syndrome. Diagnosis is made using karyotype, but it will also be picked up using CGH array.**

## Williams Syndrome

Williams syndrome is a multi-system condition that can result in characteristic facial features (small, upturned nose, periorbital puffiness, long philtrum, and full lips), connective tissue abnormalities, developmental delay with characteristic personality, growth failure and cardiovascular abnormalities (elastin arteriopathy).

The most commonly-found cardiovascular abnormality in children with Williams syndrome is supravalvular aortic stenosis. In infancy, peripheral pulmonary artery stenosis can also occur.

Williams syndrome is caused by a deletion of 7q11.23 including the elastin gene. This is detected using array CGH.

**Supravalvular aortic stenosis OR peripheral pulmonary artery stenosis and presence of characteristic facial features should prompt suspicion of Williams syndrome. Diagnosis is made using CGH array testing.**

## Noonan Syndrome

Noonan syndrome is a common genetic condition characterised by cardiac defects, short stature, webbed neck, sternal deformity (pectus carinatum superiorly and pectus excavatum inferiorly), cryptorchidism and coagulopathy.

Most children with Noonan syndrome have a cardiac defect. The most frequent lesion seen is a stenotic/dysplastic pulmonary valve. A third may also have hypertrophic cardiomyopathy. Other cardiac lesions include ASDs, VSDs, Coarctation and Tetralogy of Fallot.

Noonan syndrome is diagnosed clinically, but can be confirmed using molecular genetic testing, as their chromosomal analysis is usually normal.

Noonan syndrome is inherited in an Autosomal dominant manner.

**Pulmonary stenosis AND/OR hypertrophic cardiomyopathy in infancy, in addition to webbed neck, sternal abnormalities and cryptorchidism should raise suspicion of Noonan Syndrome. Send a CGGCH array and DNA for storage. If the array is negative, liaise with clinical genetics for further molecular diagnostic testing.**

## Alagille Syndrome

Alagille syndrome is a multisystem disorder affecting principally the liver, in addition to the heart, eyes, skeletal development and the kidneys.

Alagille syndrome can be diagnosed using its clinical and histological findings. The vast majority of affected individuals will have a paucity of bile ducts on histological analysis, this results in cholestasis clinically. This is in addition to heart defects (principally peripheral pulmonary artery stenosis), butterfly vertebrae, posterior embryotoxon and characteristic facial features. These facial features include deep set eyes, a wide forehead and a small, pointed chin.

Alagille syndrome is known to be caused by mutations in two genes: *JAG1* and *NOTCH2.* With most affected individuals having mutations in one of these two genes. These can be detected on molecular genetic testing.

Alagille syndrome is an Autosomal dominant condition.

**Suspect Alagille syndrome in children with peripheral pulmonary artery stenosis/tetralogy of Fallot AND cholestasis OR dysmorphism OR butterfly vertebrae. Send a CGH array and DNA for storage. If the array is negative, liaise with clinical genetics for further molecular diagnostic testing.**

**Please note that both Williams syndrome and Alagille syndrome are associated with peripheral pulmonary artery stenosis, but have very different phenotypic presentations.**

**If Alagille syndrome is suspected, contact your local paediatric hepatology team for advice.**

## Holt-Oram Syndrome

Holt-Oram syndrome (HOS) is characterised by upper limb malformations (radial, thenar or carpal bone anomalies) associated with structural cardiac lesions and/or conduction disease.

The structural cardiac lesions associated with HOS are ostium secundum ASDs and VSDs.

Diagnosis can be made using single-gene testing, in the form of sequence analysis of the *TBX5* gene.

HOS is inherited in an autosomal dominant fashion and so pregnancies created by affected individuals have a 50% risk of being affected.

**Any child with upper limb anomalies AND structural heart lesions OR heart block should be suspected of having Holt-Oram syndrome. Send a CGH array and DNA for storage. If the array is negative, liaise with clinical genetics for further molecular diagnostic testing.**

**If Holt-Oram is suspected, please perform an ECG, paying close attention to the PR interval.**

## CHARGE syndrome

CHARGE syndrome causes a recognised collection of congenital defects. It is a mnemonic for the classical clinical features seen in the syndrome: coloboma, heart defects (including conotruncal abnormalities, aortic arch and AV canal defects), atresia of the nasal choanae (palatal defects in some individuals), retarded growth, genital anomalies and ear abnormalities.

Diagnosis is made clinically, but most cases will be due to mutations in the *CDH7* gene, which can be detected using molecular genetic techniques. However, there is some cross-over with other genetic syndromes and chromosomal conditions and so array CGH is indicated in the first instance.

**Suspect CHARGE syndrome in any child with cardiac defects in addition to choanal atresia, coloboma, genital or ear anomalies. Send a CGH array and DNA for storage. If the array is negative, liaise with clinical genetics for further molecular diagnostic testing.**

## VACTERL association

VACTERL association is a recognised association of congenital defects that can occur together. The name is a mnemonic for the classical clinical features found: vertebral anomalies, anal atresia, cardiac defects (a range of defects are seen), trachea-(o)esophageal fistula, renal anomalies and limb defects. Children with this association will have at least 3 of the classical features, and may have other defects in addition. VACTERL is a diagnosis of exclusion; therefore, array CGH and review by a clinical geneticist is indicated to investigate other genetic causes for the child's features.

## Marfan syndrome

Marfan syndrome is an Autosomal dominant, multi-system condition. The main cardiac abnormalities associated with Marfan syndrome are dilatation of the aortic root and mitral valve prolapse. If Marfan syndrome is suspected please refer to a clinical geneticist.

## Dysmorphic children with possible genetic conditions

Some children will have dysmorphic features and cardiac lesions or associated conditions, that don't appear to fit the syndromes listed above. If there is diagnostic uncertainty, an array CGH in the first instance is a sensible test. Following that, local genetic colleagues should be consulted, and advice sought regarding clinical review and appropriate genetic testing.

# Children with family history of structural cardiac disease

For children with both a personal and family history of congenital cardiac disease, consider referral to clinical genetics for recruitment to the 100,000-genome project.

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Approved 28/06/2017 – Review July 2020