# Congenital Heart Disease
## – Genetic Aspects –

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# Congenital Heart Disease (CHD)

- A complex mixture of environmental and genetic factors
- Due to the multifactoral nature of the disease, the etiologies of CHD are largely unknown.
- A heterogenous group, caused in some cases by single-gene or chromosomal mechanism and in others by exposure to teratogens

# Congenital Heart Disease (CHD)

- A frequency of about four to eight cases/1,000 births (one in every 100 infants each year)
- Often occur in the setting of multiple congenital anomalies :
  - ✓ abnormal facial features
  - ✓ limb anomalies
  - ✓ other organ malformations
  - ✓ developmental abnormalities
  - ✓ growth abnormalities

# Genetic Basis of CHD

- Advanced cytogenetic techniques => detect subtle rearrangements in chromosomes
- Molecular instruments such as linkage analysis and positional cloning => identify genes causing Mendelian monogenic syndromes with CHDs

# Genetic Basis of CHD

- CHD are going to live to adulthood and may have the opportunity to reproduce.
- For the clinician caring for a child with CHD, it is very important to determine whether there is an underlying genetic pattern (eg, deletions, duplications, or mutations)

# Current genetic techniques for evaluation of CHDs

- A number of genetic tests that can assist the clinician in diagnosing genetic alterations in the child with CHD
  - ✓ Cytogenetic techniques
  - ✓ Fluorescence in situ hybridization (FISH)
  - ✓ DNA mutation analysis

# Chromosome analysis

- Standard chromosome analysis : revealed chromosomal aberration in 8% to 13% of neonates with CHD
- CHD : At least 30% of all children with chromosomal abnormalities (nearly 100%, as in trisomy 18)

✓ An important part in children with various types of CHD of medical evaluation

# Chromosome analysis

- Standard metaphase karyotype (450 to 550 bands), especially those of chromosome number (7 to 14 days)
- High-resolution banding, in prometaphase (550 to 850 bans) : more sensitive test (up to 3 weeks)

  => better defines chromosomal structural abnormalities (duplications, translocations, and interstitial or terminal deletions)

# Chromosome analysis

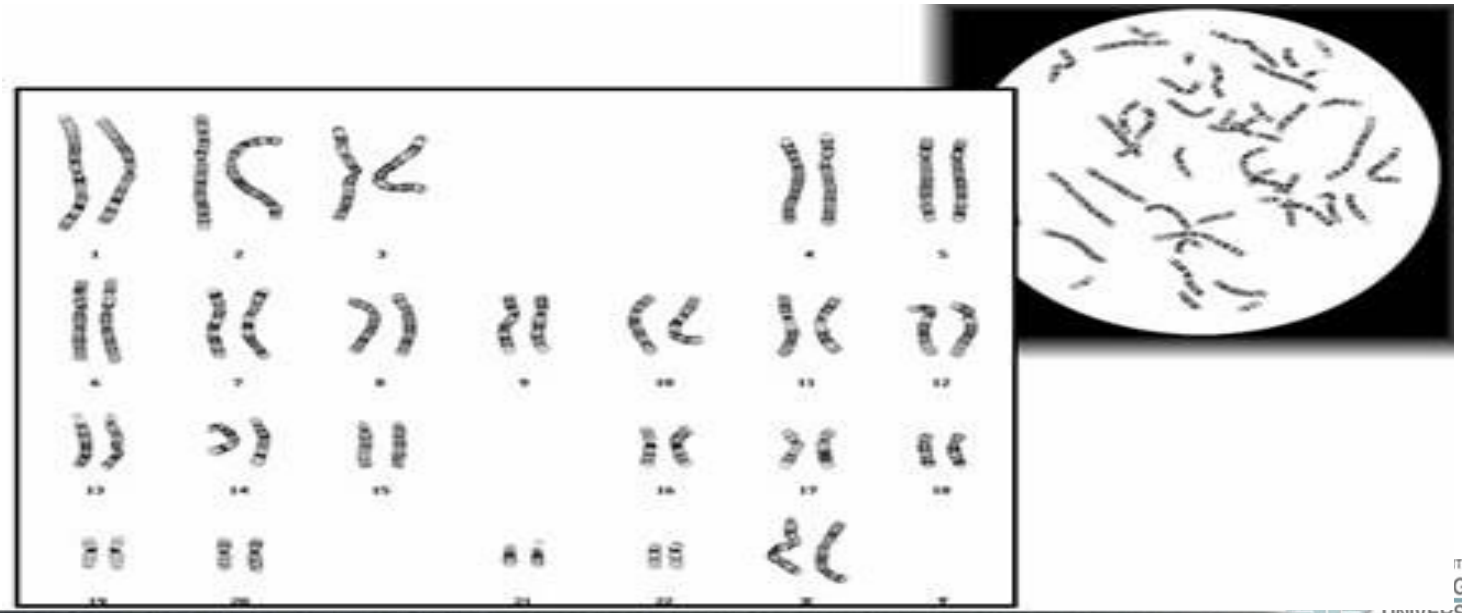
- The standard karyotype, G-banding : the staining technique most commonly used in the clinical cytogenetics laboratory, metaphases

  => GTG-banding (G-banding with trypsin and Giemsa)

- Specimens : P.P. blood lymphocytes, cord blood, skin fibroblasts, amniotic fluid, chorionic villi, and bone marrow

# Chromosome analysis

- A light microscope and the images acquired by a digital camera for computer analysis
- Using specialized software, investigators pair the chromosomes according to size, centromere position, and banding pattern, to produce a karyotype.

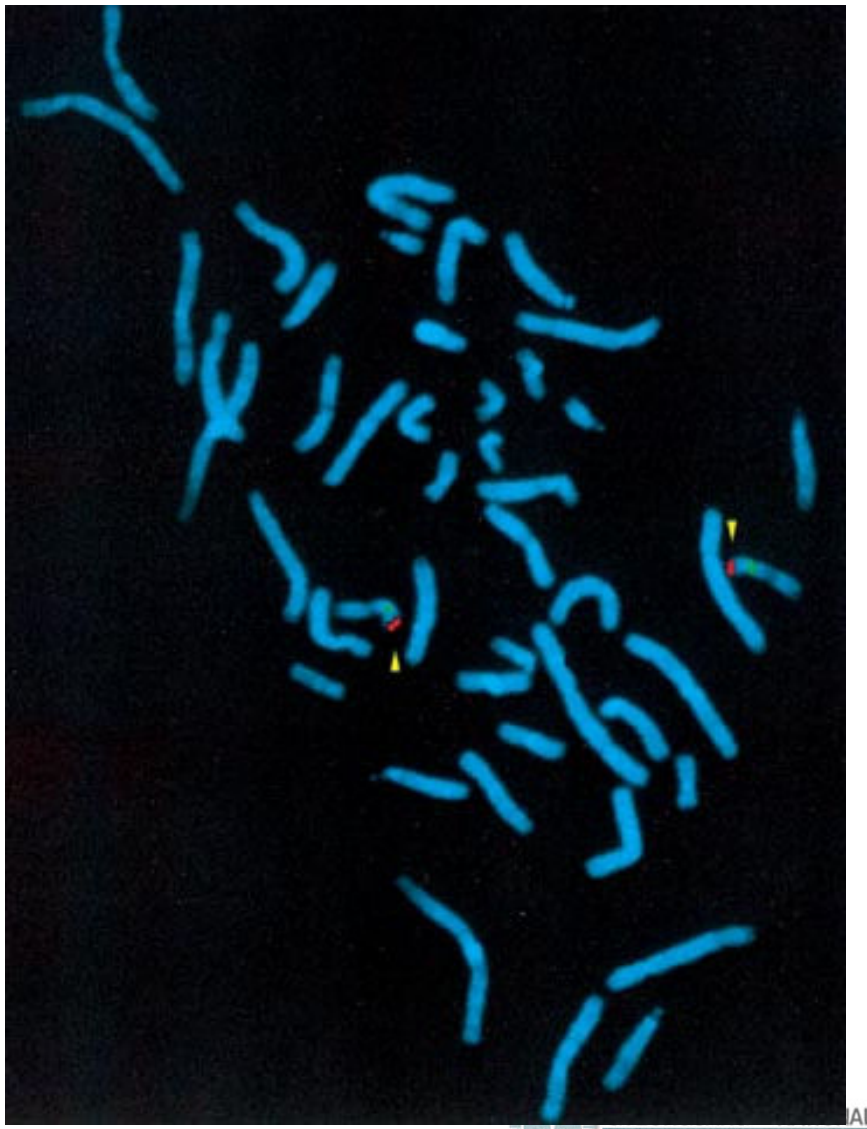


# FISH technology

- Molecular cytogenetic technique : uses fluorescently labeled DNA sequences (gene, locus, loci) in metaphase chromosomes
- A specific location in the genome ~ determine the presence or absence of these sequences
- Detect microdeletions : too small (*ie*, < 3 to 4 Mb) to be seen by GTG-banding.(esp, normal karyotypes by GTG-banding.)

# FISH Analysis

- Chromosomes are stained blue with a dye such as DAPI (4′,6-diamidino-2-phenylindole dihydrochloride hydrate) for visualization.
- Fluorescent label : fluoroscein isothiocyanate (green) or rhodamine (red)



# Telomere analysis by subtelomere FISH

- Tiny deletions, duplications, or subtle translocations involving the most distal ends of each chromosome (telomeres) : quite difficult to detect by standard or high-resolution karyotype techniques.
- Newly developed fluorescent DNA probes for many interstitial chromosomal regions now provide the ability to detect abnormalities that involve the subtelomere-telomere regions (subtelomere FISH).

# DNA Mutation Analysis

- Mutation analysis : identifies changes in the coding sequence of the gene, including small deletions, insertions, or substitutions of nucleotides (that alter the encoded amino acid and consequently protein structure)
- Most methods : PCR (polymerase chain reaction) – based assays

# DNA Mutation Analysis

- Indirect screening methods :
  - ✓ HPLC (high-performance liquid chromatography)
  - ✓ Single-strand conformation polymorphism
- Exon-by-exon sequencing of genomic DNA : More expensive
- Direct sequence analysis methods : newer, more cost-effective methods
- Specimens : P.P. blood lymphocytes, other tissues (skin, liver, muscle, buccal cells, or saliva)

# Loci and genes associated with CHDs identified to date

*Deletion syndromes identified by FISH technology*

- ✓ *DiGeorge syndrome*
- ✓ *Williams-Beuren syndrome*

# DiGeorge syndrome

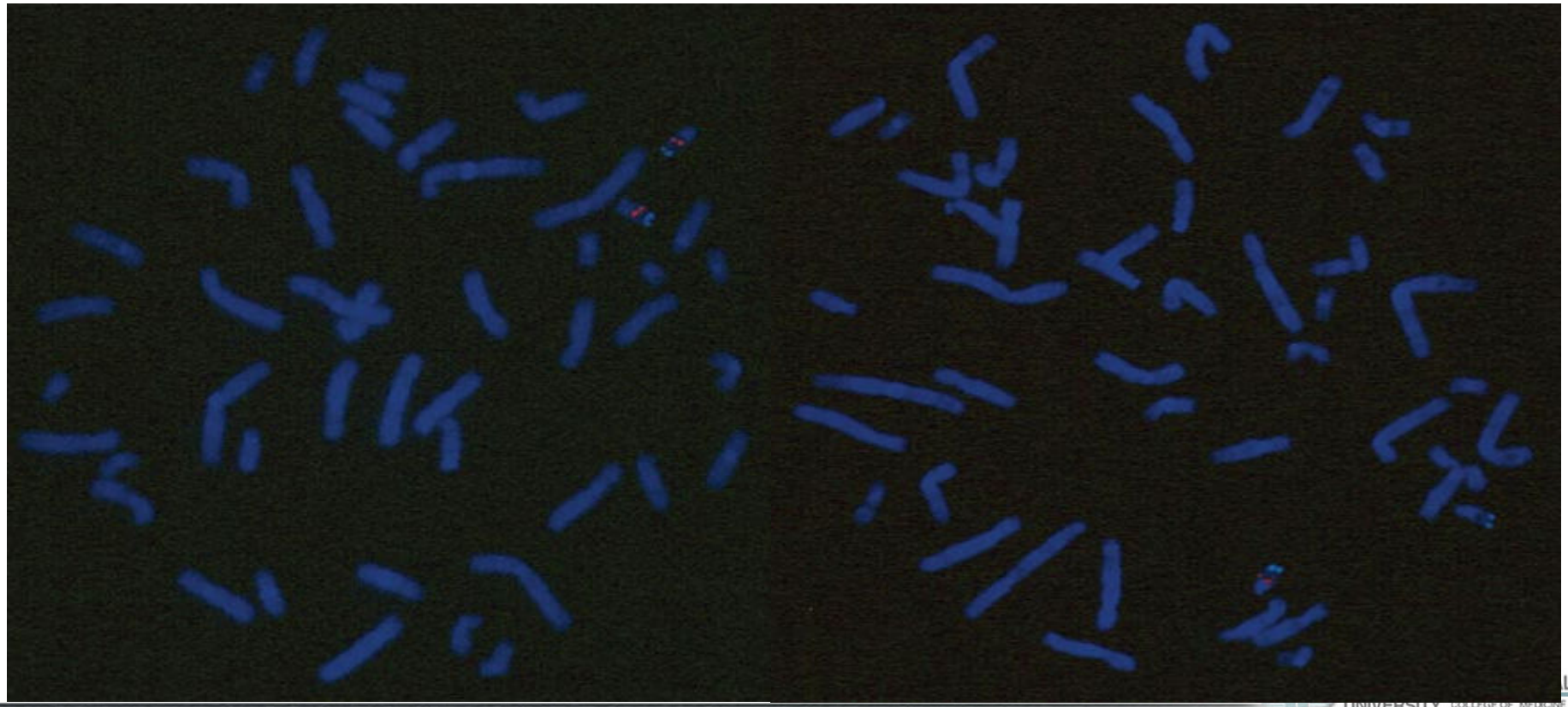
- There are many different organs affected by DiGeorge.
- Known as Velocardiofacial syndrome
- The CHD involve derivatives of the neural crest or second heart field (SHF), including interruption of the aortic arch (IAA), persistent truncus arteriosus (PTA), TOF, DORV and TGA.

# DiGeorge syndrome

- On FISH, ≈90% of patients with the DiGeorge phenotype have a microdeletion of part of 1 copy of chromosome 22.
- Prevalence : 1 per 5,950 live births
- A chromosomal deletion of 22q11 : the most common human chromosomal deletion syndrome
- Metaphase with 22q11 microdeletion

# DiGeorge syndrome

- Chromosomes 22 : the *green signals* (from an internal control probe for the ends of chromosome 22)
- The *red signals* : for sequences for gene *TUPLE1* and adjacent loci within the 22q11 region.

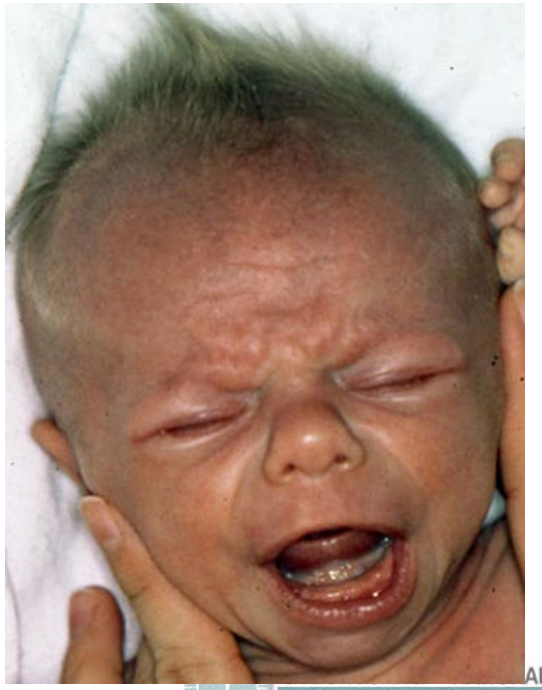


# DiGeorge syndrome and TBX1

- Evidence in mice suggests that disruption of one of the two copies of the T-box gene, *TBX1*, causes the cardiac and pharyngeal arch anomalies observed in 22q11 deletion syndrome.
- This provided strong evidence that *TBX1* was the gene responsible for CHD in DiGeorge syndrome.
- *TBX1* mutations were subsequently found in DiGeorge patients that lacked the 22q11 chromosomal deletion.

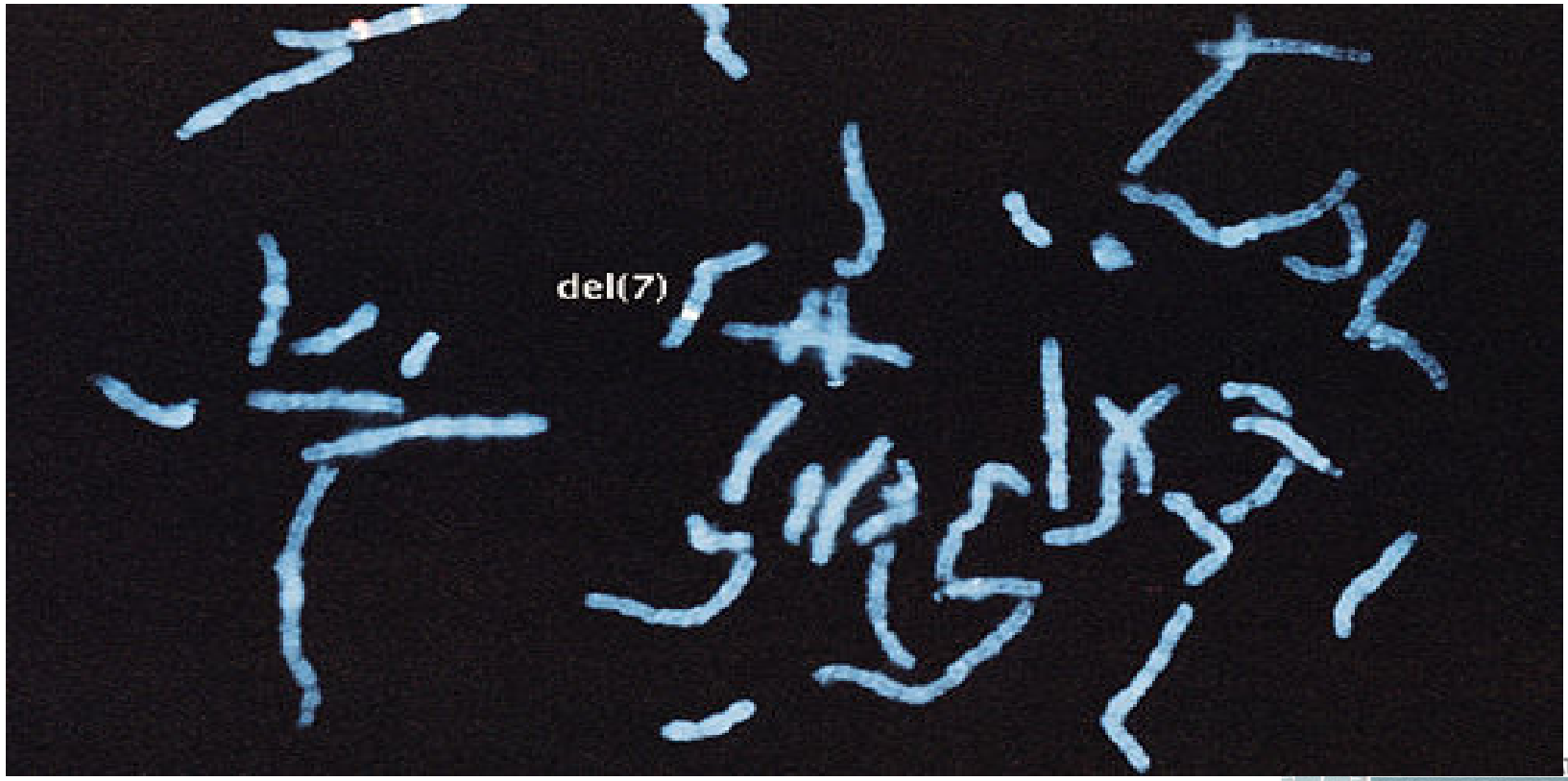
# Williams-Beuren syndrome

- Williams syndrome : an autosomal dominant disorder
- specific cardiovascular defects (supravalvular aortic stenosis, p.p. pulmonary stenosis..), infantile hypercalcemia, skeletal and renal anomalies, cognitive deficits, "social personality," and elfin facies.
- Most cases arise de novo due to a chromosomal microdeletion.



# Williams-Beuren syndrome

- Approximately 90% of individuals with a clinical diagnosis of Williams syndrome : by FISH to have a microdeletion at chromosome 7q11.23



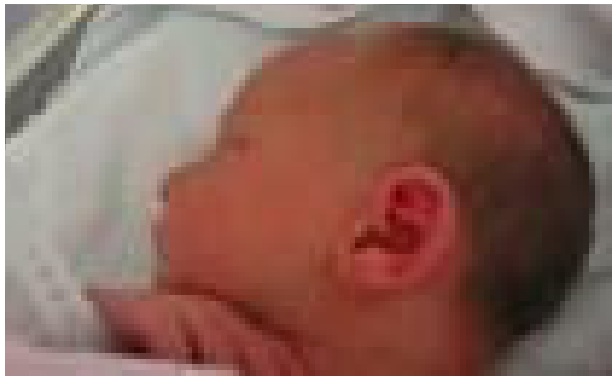
# Loci and genes associated with CHDs identified to date

*Single-gene disorders*

- ✓ *Alagille syndrome*
- ✓ *Noonan syndrome (NS)*
- ✓ *Holt-Oram syndrome (HOS)*
- ✓ *Nonsyndromic single-gene disorders*

# Alagille syndrome

- A complex disease : characterized by liver disease (chronic cholestasis; reduced bile flow), pulmonic artery stenosis (PS), and occasionally TOF & CoA
- A subset of Alagille patients (3% to 7%) : deletions of chromosome 20p12 detectable by karyotype or FISH analysis



# Alagille syndrome

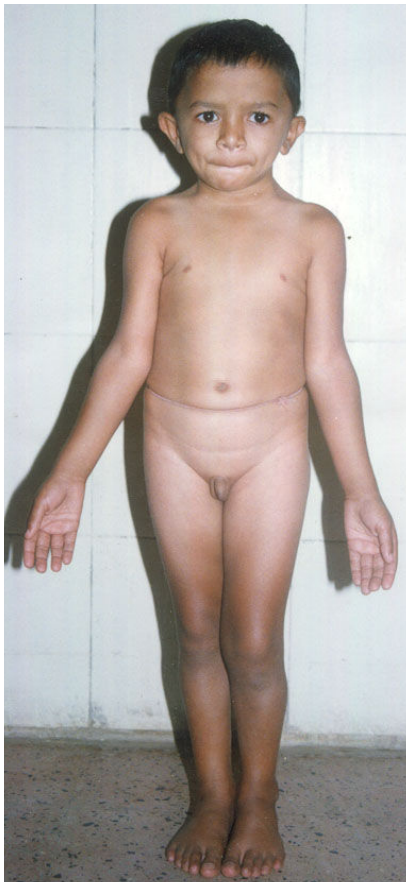
- The gene *JAG1*, which encodes a Notch ligand protein product, has been mapped into the commonly deleted region of 20p12.
- Mutations of *JAG1* : identified in patients with a broad spectrum of clinical phenotypes (including patients with a predominant cardiac phenotype)

# Alagille syndrome and the Notch pathway

- Mutations in JAGGED1 (JAG1) : a transmembrane ligand for the Notch family of receptors, are found in most cases of Alagille syndrome.
- The most recent gene linked to isolated CHD also belongs to the Notch signal transduction pathway.

# Noonan syndrome (NS)

- A genetic multiple malformation disorder : short stature, typical facial dysmorphism, webbed neck, chest deformity, and cardiovascular abnormalities
- Cardiac involvement : in 80% to 90% of affected individuals
- The most common of CHD : valvar pulmonic stenosis and hypertrophic cardiomyopathy



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# Noonan syndrome (NS)

- Genetically heterogeneous : there are at least 3 NS disease genes, *PTPN11*, *SOS1*, and *KRAS.92*.
- With genetic linkage analysis and then positional candidacy, an NS disease gene on chromosome 12 was identified.
- It is *PTPN11*, which encodes a protein tyrosine phosphatase called SHP-2.

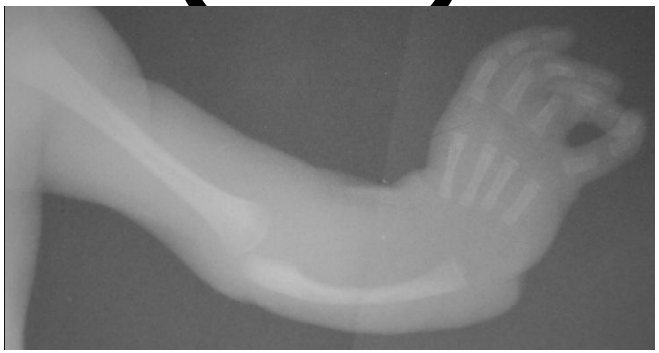
# Noonan syndrome (NS)

- SHP-2 : an important role in signal transduction for a wide variety of biological processes, including the formation of the semilunar valves.
- Mutations in the *PTPN11* gene : in 40% to 50% of NS patients (more prevalent among familial cases and among NS patients with pulmonary valve stenosis)

# The Noonan syndrome family (Cardio-Facio-Cutaneous, Costello, and Noonan)

- Three syndromes with many overlapping clinical symptoms have revealed a signal transduction pathway controlling formation of the pulmonary valve.
- Two other syndromes, Cardio-Facio-Cutaneous syndrome (CFCS) and Costello syndrome, have similar clinical features but no mutations in *PTPN11* could be linked to them.

# Holt-Oram syndrome (HOS)



- An autosomal dominant

  "heart-hand" syndrome :

  characterized by CHDs in patients with upper-limb deformities
- Occurs in approximately 1 per 100,000 individuals
- Arm and hand abnormalities : about 100% (Defects in the bones of the hand and/or arm)
- Heart abnormalities : About 75% (a heart defect such as an atrial septal defect or ventricular septal defect, abnormal heart rhythms may also be present.)

# Holt-Oram syndrome (HOS)

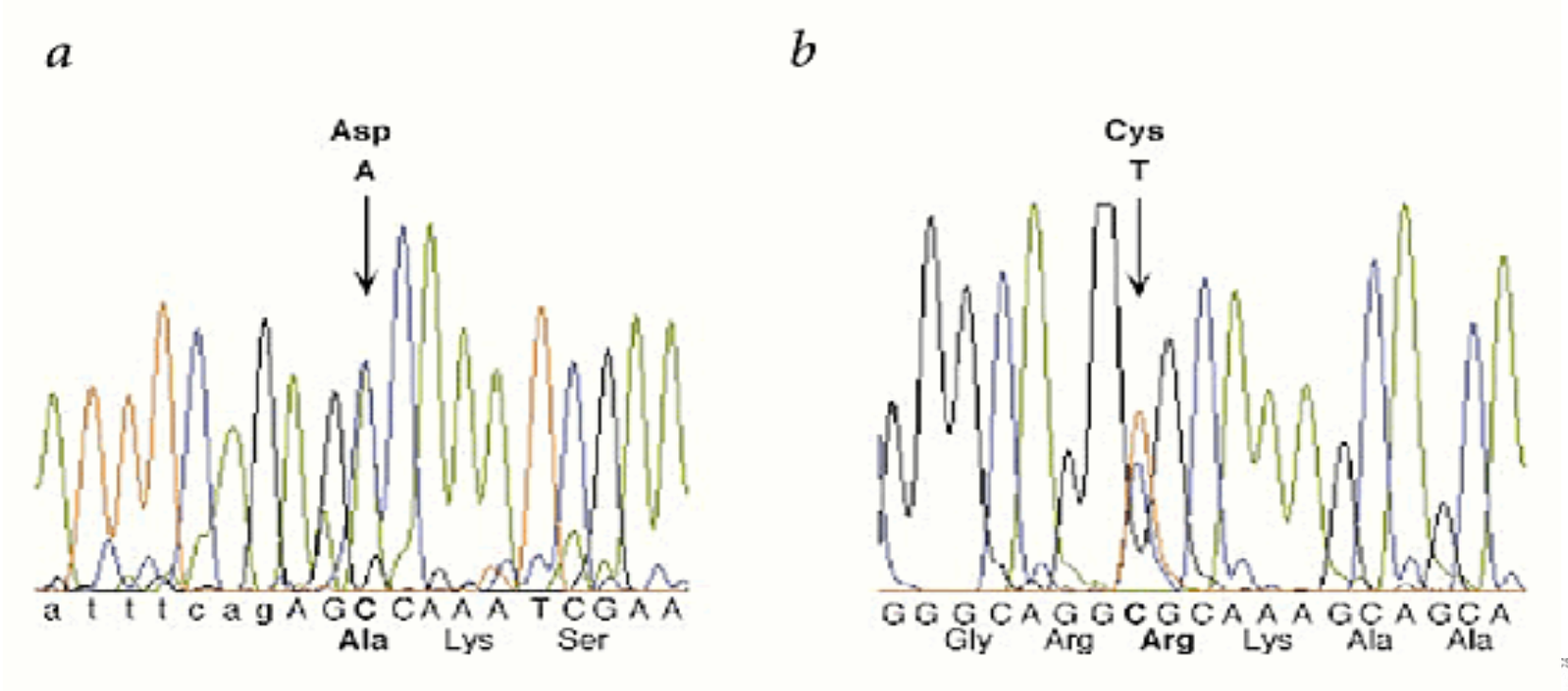
- Mutations in the *TBX5* transcription factor gene (chromosome 12q24.1)
- *TBX5* transcription factor : a key regulator, particularly in combination with other transcription factors such as *NKX2.5* and *GATA-4*, of gene expression during embryogenesis
- Loss of its activity markedly impairs development of the heart and limb.

# TFAP2β and Char syndrome

- Char syndrome, another heart-hand syndrome like Holt-Oram syndrome, is characterized by PDA.
- TFAP2 β was mapped to the previously described Char syndrome locus, and dominant negative proteins that impaired DNA binding of TFAP2 β appear to cause many of the defects observed in Char syndrome. (chromosome 6p12-p21)

# TFAP2β and Char syndrome

- Direct sequence analysis of amplified DNA containing the TFAP2 β exon 5 and flanking intronic boundaries revealed heterozygous changes in the ARK (C-> A transversion) and SCOT families(C-> T transition).



# Genetic etiologies of syndromic CCVM

| Gene | Syndrome | Cardiac malformation |
|---|---|---|
| Fibrillin1/2 | Marfan | Aortic aneurysm |
| TGFBR2 | Marfan | Aortic aneurysm |
| NF1 | Neurofibromatosis | PS |
| NF1 | Neurofibromatosis-Noonan syndrome | PS |
| Elastin | William | SVAS |
| TBX5 | Holt-Oram | ASD, VSD, conduction |
| JAG1 | Alagille | PS, TOF |
| NOTCH2 | Alagille | PS, TOF |
| ZIC3 | Heterotaxy syndrome | d-TGA with heterotaxy |
| CFC1 | Heterotaxy syndrome | d-TGA with heterotaxy |
| EVC/EVC2 | Ellis-van Creveld | ASD |
| TFAP2β | Char | PDA |
| TBX1 | DiGeorge/22q11.2 deletion syndrome | VSD, PTA, IAA, TOF |
| VEGF promoter (−2578A/−1154A /−634G) | DiGeorge modifier | VSD, PTA, IAA, TOF |
| PTPN11/Shp2 | Noonan | PS |
| MEK1 and MEK2 | Cardio-Facio-Cutaneous | PS |
| B-Raf | Cardio-Facio-Cutaneous | PS |
| H-Ras | Costello | PDA, PS |
| K-Ras | Cardio-Facio-Cutaneous | PS |
| K-Ras | Noonan | PS |
| CHD7 | CHARGE association | ASD, VSD, mitral valve defects |
| Sema3E | CHARGE association | ASD, VSD, mitral valve defects |

# Nonsyndromic single-gene disorders

- Studies have recently shown that nonsyndromic CHD can result from single-gene defects.
- The mutations were found only in affected individuals, were not present in control samples, and were demonstrated to change protein structure or function.

# Nonsyndromic single-gene disorders

- These studies identify critical molecular pathways involved in cardiovascular development and disease, given that the proteins encoded by *NKX2.5*, *GATA4*, and *TBX5* are known to interact with one another in experimental systems.

# Nonsyndromic single-gene disorders

- Many cases of nonsyndromic CHD : unlikely to result from simple single-gene disorders
- Instead, many cases of CHD are likely the result of multiple genetic alterations that increase susceptibility to CHD and interact with environmental factors.

# Genetic etiologies of non-syndromic CHD

| GENE | Inheritance | Cardiac malformation |
|---|---|---|
| NKX2.5 | Autosomal dominant | ASD, conduction |
| JAG1 | Partial penetrance | TOF |
| GATA4 | Autosomal dominant | VSD, ASD |
| PROSIT240 | Chromosomal translocation | d-TGA |
| MYH6 | Autosomal dominant, partial penetrance | ASD |
| VEGF promoter (−2578A/−1154A/ −634G) | Modifier gene | TOF |
| NOTCH1 | Autosomal dominant | BAV, calcification |

# Genetic etiologies of isolated CHD (CCVM)

- Outflow tract defects and NOTCH1
- Septal defects
  - ✓NKX2.5
  - ✓ GATA4
  - ✓ Myosin heavy chain 6

# Outflow tract defects and NOTCH1

- A family with a history of OFT defects : predominately BAV and /or early-onset aortic valve calcification
- Linked to chromosome 9q34-35
- Premature stop codons in the NOTCH1 gene : found to segregate with the CCVM in this family & a second unrelated family with a similar phenotype

# Septal defects : NKX2.5

- Linkage of four families with histories of ASD & atrioventricular conduction block to mutation in NKX2.5 => the first example of single gene mutations causing non-syndromic CCVM

  => The conduction defect may be due to progressive loss of specialized conduction cells at the atrioventricular node.

  => Familial ASD populations may benefit from periodic electrophysiologic monitoring

# Septal defects : GATA4

- Another cause of septal defects (ASD, VSD, and AVSD) without conduction abnormalities : identified in two families that had mutations in the GATA4 transcription factor
  a. A frameshift mutation with a premature stop codon : in non-sense mediated decay of the mRNA
  b. A missense point mutation, Gly295Ser
- Potential protein-protein interactions based on the common phenotype observed in human with GATA4, TBX5 or NKX2.5 mutations

# Septal defects : MyHC6

- A mutation in myosin heavy chain 6 (MyHC6) can also cause ASDs.
- MyHC6 : a known target of all three-transcription factors
- Only a single kindred has been identified with MyHC6 distruption (binding to its myosin light chain counterpart)

# Genetic risk factors for sporadic CHD

- Most cases of CCVM : sporadic, no immadiate family history => multifactorial with no single gene being totally responsible
- In part to mutation in NOTCH1, JAGGED1, GATA4, MHC6, or TBX5 (less than 5% of CCVM)
  - ✓ VEGF promoter
  - ✓ A cautionary note : methylenetetrahydrofolate reductase (MTHFR)

# VEGF promoter

- Deletion of the 164 amino acid VEGF isoform from mice : revealed defects that mimicked most of the DiGeorge syndrome phenotype, including TOF.
- In the same study, a haplotype of three SNPs in the VEGF promoter and 5'-UTR was tentatively linked as a modifier of DiGeorge syndrome CCVM in a small cohort of patients.

# VEGF promoter

- More recent work, using transmission disequilibrium testing of the VEGF promoter haplotype found a 1.8-fold increased risk for sporadic TOF in a single cohort of approximately 250 Caucasian patients, but this has yet to be confirmed by other groups.

# A cautionary note: MTHFR

- 5,10-Methylenetetrahydrofolate reductase (MTHFR) is one of the enzymes necessary for metabolizing homocysteine into methionine.
- A common variant of MTHFR, 677C > T : present in 10-20% of Caucasians in a homozygous state and is a risk factor for defects of the neural tube.
- At the prevalence of homozygous 677C>7 in sporadic cases of CCVM

# Evaluation for genetic basis in children with CHD

- Chromosome analysis and FISH testing for specific deletions : now accepted tools for the clinician
- If the clinician finds a specific chromosome abnormality

  => provide the family with a clear explanation of the cause

  => allow the clinician to provide appropriate counseling about recurrence or lack of recurrence

  => prompt the physician to investigate other potential medical problems known to be associated with the particular chromosomal anomaly

# Evaluation for genetic basis in children with CHD

- Specific assessment for physical features : should focus on
  - ✓dysmorphic facies
  - ✓eye and ear abnormalities
  - ✓limb reduction defects
  - ✓polydactyly
  - ✓other skeletal defects
  - ✓gastrointestinal and urologic defects
  - ✓neurological status

# Evaluation for genetic basis in children with CHD

Genetic testing :

- ✓ determine a genetic mechanism of disease
- ✓ provides an important opportunity for genetic counseling (benefits the entire family)

Cytogenetic testing should be considered in the following situations:

1. Any infant or child with the phenotype of a recognizable chromosomal syndrome (eg, trisomy 21 or 18)

# Evaluation for genetic basis in children with CHD

2. Any infant or child with a congenital heart defect combined with

   (a) dysmorphic features

   (b) growth retardation that cannot be explained by the heart defect

   (c) developmental delay or mental retardation

   (d) multiple congenital anomalies

# Evaluation for genetic basis in children with CHD

3. Infants or children with a family history of multiple miscarriages and/or siblings with birth defects

4. If major cardiac and/or other visceral organ malformations are documented by prenatal ultrasound and/or fetal echocardiogram

# Future directions

- Become increasingly accepted that genetic variations and mutations play a major role in the development of CHD
- Implicated in syndromic and familial non-syndromic CHD (mutated in cases of sporadic CHD)
  - ✓ Candidate gene screen
  - ✓ Haplotype mapping of CCVM

# Candidate gene screen

- Over 150 genes : implicated in the cardiogenesis of model organisms (so far, and the number continues to grow)
- A clear bias for genes that act as nodal points : Transcription factors (NKX2.5 and GATA4), Signal transduction pathways (VEGF and NOTCH1), Translational regulators (such as the newly discovered microRNAs)

# Haplotype mapping of CCVM

- The full list of heart development genes is unknown.
- The HapMap project has identified 10,000,000 single nucleotide polymorphisms (SNPs)

  => occur in at least 5% of the general population
- The HapMap project identified a core group of 500,000 common SNPs.
- Using this core list, one could investigate cohorts of CCVM patients and controls to determine which haplotypes occur more often in the disease state.

# Haplotype mapping of CCVM

- The incorporation of gene copy number (a potential genetic risk factor) : important

  => It is becoming clear that relatively small genetic duplications or deletions may segregate with disease.

- A combination of copy number evaluation with re-sequencing and genome-wide SNP associations : ultimately begin to reveal the genetic basis for common forms of CHD.

# Future directions

- Patients with CHD require multidisciplinary care.
- Their families deserve up-to-date genetic information
  - ✓ as it relates to their child's prognosis
  - ✓ to the kindred's risk for future inheritance of genetic abnormalities associated with cardiac defects.