Genetics and Congenital Heart Disease

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The Disease

- 25% of all Congenital anomalies
- Leading cause of death in first year of life
- Prevalence = 6000/1 million American (½ A ; ½ C)
- 35,000 babies are born with CHD in USA (100 NB per 1 million population)
- These numbers are more frequent in our region (Lebanon: 700NB/yr; Prevalence=22,000)
- Little is known about the etiology
Multifactor Inheritance
The interplay between many genetic and environmental factors

J Nora 1968
History-Genetics

From, for example, a drop of blood...

- An individual segment of a DNA molecule is extracted.

- By raising the temperature to about 90°C, the strands are separated.

- Millions of copies of strands are produced in a process called PCR.

- The temperature is lowered about 55°C, and synthetic DNA fragments are added. These bind to the strands at the correct positions.

- The temperature is raised again to separate the strands.

- This whole process works like a copying machine.
Human Genome (2000)
1 – 2% Coding (30,000 genes)

Coding
Non-coding $3 \times 10^9$ bp

Gene A
Gene B

Eukaryotic mRNA gene (DNA)

RNA polymerase II
Transcription
hnRNA
Cap
AAA
AAA
Poly(A) tail
mRNA
5’
AAA
3’

Replication
DNA
mRNA
Translation (protein synthesis)
Transcription (RNA synthesis)
Ribosome
Protein

Start codon
Stop codon
CHD can be related to abnormality of:

- Chromosomes (5 to 6 percent)
- Single gene defects (3 to 5 percent)
- Environmental factors (2 percent)
- In 85 to 90 percent of cases, there is no identifiable cause for the heart defect, and they are generally considered to be caused by multi-factorial inheritance
Chromosomal Abnormalities

- Down Syndrome
- Turner Syndrome
- Trisomy 13 @18
Gene Deletions

- DiGeorge
- Williams
- Cri Du Chat
22q Deletion

- 22q11.2 deletion or CATCH 22 - common genetic disease (1/4000)
- This deletion involves some 30 or so genes (3-MB)
- TBX1 - appears to be important for outflow tract development
- Several syndromes are associated with this deletion
22q11.2 deletion is shared by 4 syndromes:

- DiGeorge syndrome (DGS)
- Velocardiofacial - Shprintzen
- Conotruncal anomaly face syndrome (CTAFS)
- Opitz G syndrome.

- Large proportion of 22q11 patients have CHD (85%).
- 13% of Syndromic TOF has 22q11
- TOF is present in 16% of all patients with CATCH.
Williams
(deletion of the region of 7q11.23)

Positive Williams Syndrome FISH assay
(Chromosome 7)
The elastin gene is found on only one chromosome. The other copy carries an elastin gene deletion.

Negative Williams Syndrome FISH assay
(Chromosome 7)
The elastin gene is found on both chromosomes. This individual does not have Williams Syndrome.
Mutation (PCR + Sequencing)

- Marfan syndrome
- Holt-Oram syndrome
- Alagille syndrome
Mutations - Fibrillin-1 gene

Marfan Syndrome

FBN1

Genetic locus 15q15.2

Imaging findings:
- Dilation of the aorta
- Cardiac abnormalities
- Pectus Excavatum
- Pulmonary changes

Signs of ocular involvement:
- Axenfeld-Rieger anomaly
- Hyperopia

Clinical manifestations:
- Pectus Excavatum
- Aortic aneurysm
- Pulmonary complications
Tbx5 is encoded by the \textit{TBX5} gene (10 spliced exons and is located at 12q24.1).

Multiple mutations in \textit{TBX5} have been identified in patients with \textbf{HOS}.

Genomic DNA will be analyzed for \textit{TBX5} mutations by automatic fluorescent DNA sequencing.
Alagille syndrome (AD)

Bile duct paucity
Cardiac (particularly right sided)
Skeletal, ocular abnormalities;
Characteristic face.
Caused by heterozygous mutations in JAG1(20)
Sporadic / Familial  Non-syndromic CHD
Using linkage analysis, the first disease-related gene for nonsyndromic ASDs has been identified.

**NKX2.5 mutations** were identified in patients with a variety of lesions, ASDs, isolated atrioventricular conduction disturbances, TOF, DORB, Ebstein's anomaly, and muscular VSDs.

**NKX2.5 seems to be a disease-gene for a wide range of CHD (chromosome 5)**.
The Congenital Heart Disease Research
Genetic Program

CHDRGP
Distribution of Cardiac Anomalies

- AA
- ASD
- AVC
- COA
- PA
- PDA
- PS
- SV
- HLHS
- TGA
- TOF
- VSD
- Others

Percentage distribution for AUH and BCH.
Parental Consanguinity and Congenital Heart Malformations in a Developing Country

Mona M. Nabulsi,¹* Hala Tamim,² Maha Sabbagh,¹ Mounir Y. Obeid,³ Khaled A. Yunis,¹ and Fadi F. Bitar¹*

Consanquineous marriage and congenital heart disease: A case control study in the neonatal period.

Yunis KA, Mumtaz G, Bitar FF, Chamseddine F, Kassar M, Rashkidi J, Makhoul G, Tamim H.
Cardiac Crescent
Heart Tube
Looping Heart
Chamber Formation
Septation/Maturation

GATA-4
Nkx2.5
Mesp1,2

GATA-4
Nkx2.5
Hand1,2
MEF2c
Pitx2

Irx4
Tbx5
RAR/RXR

NFATc
Pax3
FOG2
Hey2
HF1b
TEF1
CITED2
Tbx1
Zic3

d15
d21
d25
d28
d50

Ao
PA
LA
RV
LV
CT
AS
A
RV
LV
CT
AS
A
MUTATION IN BRIEF

A Novel Mutation in the GATA4 Gene in Patients With Tetralogy of Fallot

Georges Nemer¹*, Fatimah Fadlalah¹, Julnar Usta¹, Mona Nemer², Ghassan Dbaibo³, Mounir Obeid³, and Fadi Bitar³

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Exon 2. Heterozygous mutation in 2 patients with TOF resulting in a E216D substitution. **A:** Three chromatograms showing the results of sequencing with the reverse primer of a normal individual, and the two patients with TOF in which a C is substituted by a G (arrow). The resulting codon GAC will encode an aspartic acid instead of glutamic acid (GAG).
Characterization Of The E216D Mutation

A) NPPA $\text{700bp}$

B) 

C)
Inject ES cells with (-) gene $X$ into early mouse embryo

Transfer embryos to surrogate mothers

Resulting chimaras have some cells with (+) gene $X$ and (-) gene $X$.

Mate them with normal mice

Lucky you, if germline contain (-) gene $X$

Screen pups to find -/+ and mate them

Next generation will split as 3:1 (Mendelian)
Differential duplication of an intronic region in the \textit{NFATC1} gene in patients with congenital heart disease

Amin Yehya, Ramzi Souki, Fadi Bitar, and Georges Nemer

Genome 49(9): 1092–1098 (2006)
Letter to the Editor

Exclusive cardiac dysfunction in familial primary carnitine deficiency cases: a genotype–phenotype correlation

Abir A. Yamak, Fadi Bitar, Pascale Karam, and Georges Nemer
Arginine stop-code

PCD is caused by mutations of the SLC22A45 gene that encodes the sodium-dependent organic cation transporter OCTN2 (5q31.1) R254X.
Families with more than 4 children with CHD

Note: * refers to the individual carrying the polymorphism
Docking of dHAND with Tbx 5. (A) Interaction of the two proteins with emphasis on the interface. (B) Docking of Tbx5 with wt dHAND with Tbx bundled with c-terminal of dHAND. (C) Docking of Tbx5 with Mt dHAND, the interface of interaction is abrogated as a result of the new modified conformation.
Cardiac Tissue Bank (AUBMC)

- Bank of tissues from patients with CHD.
- Analyze the DNA from genomic DNA or mRNA.
- Look at somatic mutations (occurring only in tissues where the damage is) as opposed to germ mutations.
- Extract proteins.
- Utilize Protein microarrays to determine whether the rate of synthesis of a given protein is the disease-causing factor.
Competitive Genomic Hybridization (CGH)
CHD

Syndromic
- Karyotype
  - Williams; Marfan; Alagille
  - Asplenia; Char; HOS; CM

Conotruncal
- 22q11.2 deletion

Non-Syndromic
- Research
  - Tbx5; Tbx1;
  - NKx2.5; Gata-4; ZIC3;
  - Affymetrix others
DHFMCR Collaborative Center for the Genetic Analysis of Congenital Anomalies

Site Work Flow / Personnel

KFSRH
Clinical + Molecular
• Ascertainment + Banking
• Molecular Karyotype
• Genomic Mapping
• Sequencing

HMS
Clinical + Molecular
• Ascertainment + Banking
• Cell Lines (Selective)
• Molecular Karyotype
• aCGH
• Genome-wide SNPs + CNVs
• High Throughput Sequencing
• Bioinformatic Pathways
• Functional + Expression Studies

Research study coordinator

Kuwait
Clinical
Ascertainment Banking Karyotype

Qatar
Clinical
Ascertainment Banking Karyotype

UAE
Clinical
Ascertainment Banking Karyotype

AUB
Clinical + Molecular
Ascertainment + Banking
• Molecular Karyotype
• Genomic Mapping
• Sequencing

CENTRAL DATABASE: Web-based Password Protected

Fellow

Fellow

Fellow
What is all of this Good for?

- Genetic Screening and Counsel for the mutated gene
- Premarital counseling in the contest of the affected families.
- Prevention and therapy
- Establishing a genetic abnormality, not only defines a cause but implicate on genetic counseling prognosis and surveillance for potential complications.
طبيبان يكتشفان الخلل في العامل الوراثي GATA4 المسبب لتشوهات قلوب الأطفال

"العلوم في الإسلام" تقنية القلب عند ابن سينا تستلزم الطب الحديث

"المستشفى الملك فهد

عملية جراحية القلب في سبع سنوات

مستشفى

رفيق الحريري الجامعي

544 سريراً

و14 غرفة عمليات

د. فادي بيطار د. جورج نمر

يضحكان لغز تشوهات القلب لدى الأطفال

أطباء قلب سعوديون يبرؤوا في المستشفيات العالمية

تقنيات الرنين المغناطيسي والجهازي والنووي

قسطرة الشرايين في مستشفى دبي الأول على مستوى الدولة والعالم على مستوى العالم