

Right Ventricular Overload disorders: Genetic Aspects and Results

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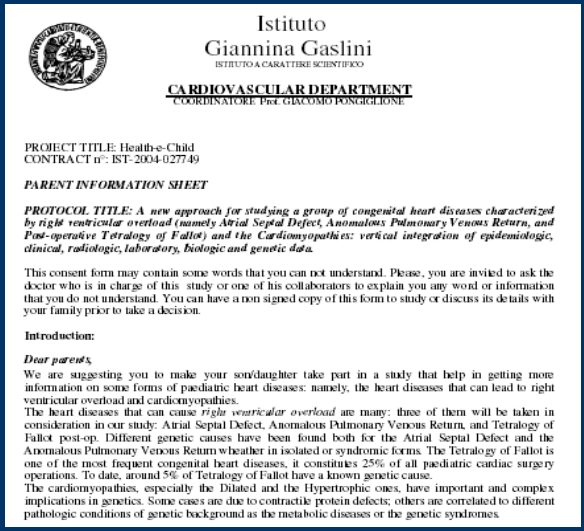
G. Gaslini Institute, Genova, Italy



For each enrolled patient

Informed Consent

Proper Genetic Counselling

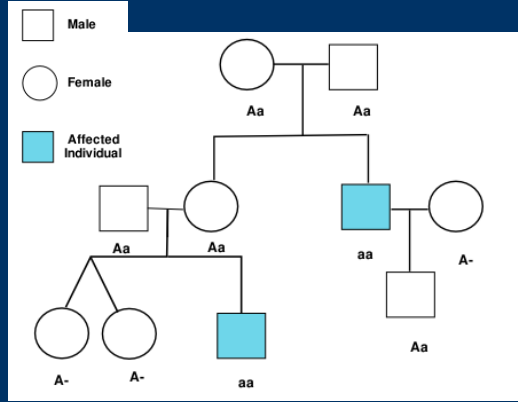


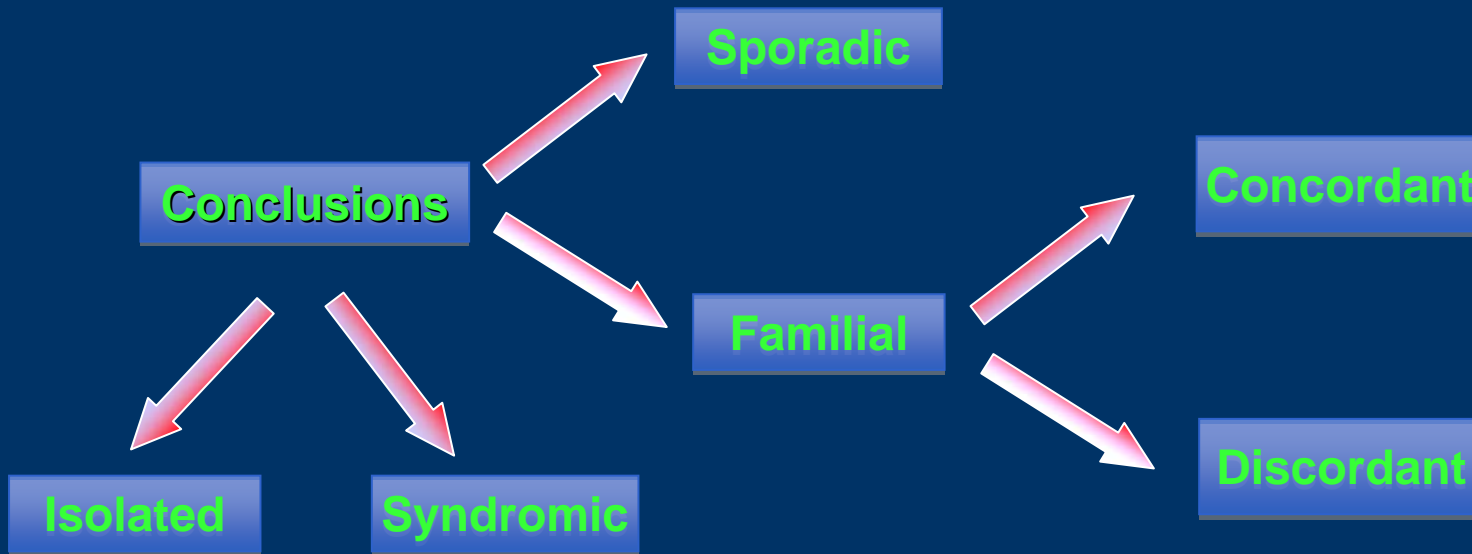
Full History

Family Pedigree

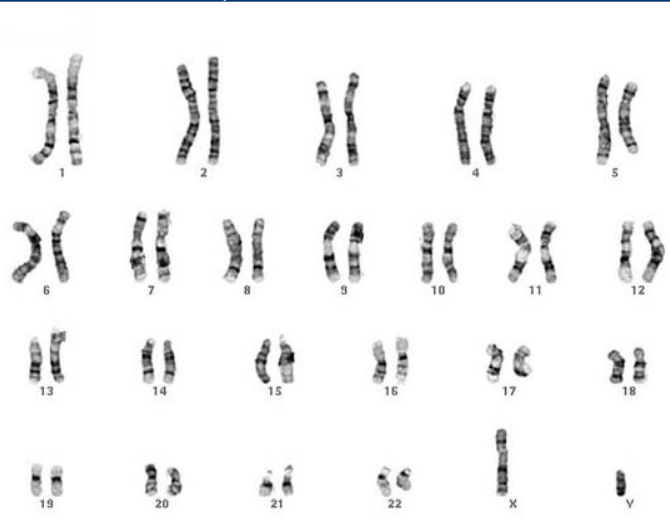
Detailed Physical Examination

- Prenatal History
- Birth History
- Postnatal History
- Developmental milestones



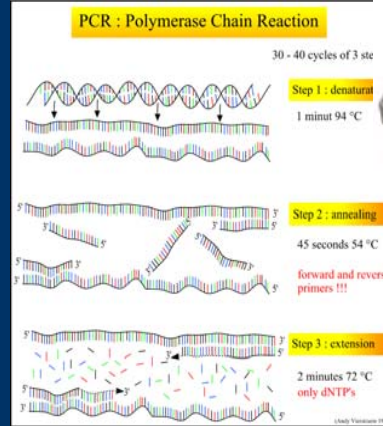


Investigations



Constitutional Karyotyping

Can exclude broad numerical and structural anomalies of chromosomes. Relatively low resolution 5-8Mb (millions basepairs)



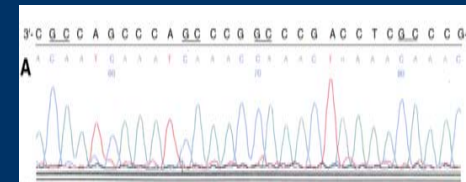
PCR technique allows the amplification of numerous copies of selected segments of human genome

If negative Karyotyping

TBX5
GATA4
NKX2.5

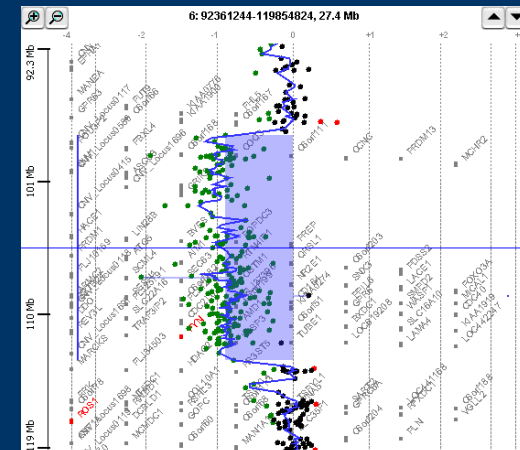
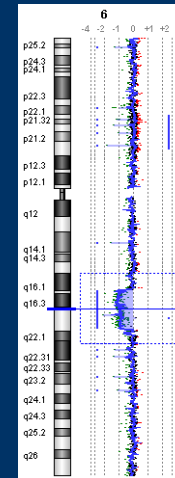
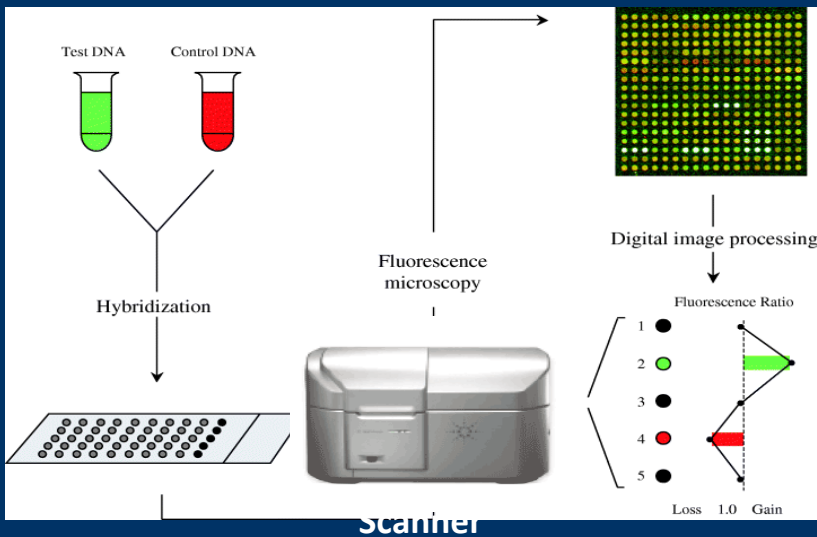


DNA sequencing is obtained for selected genomic segment using the automated sequencer machine. Mutation detection is possible using this instrument



ARRAY - CGH

This technique allows to obtain information on the whole genome (all chromosomes) with single experiment using high resolution (75Kb): it is possible to find Deletions (lost genetic material) or Duplications (gained genetic material)



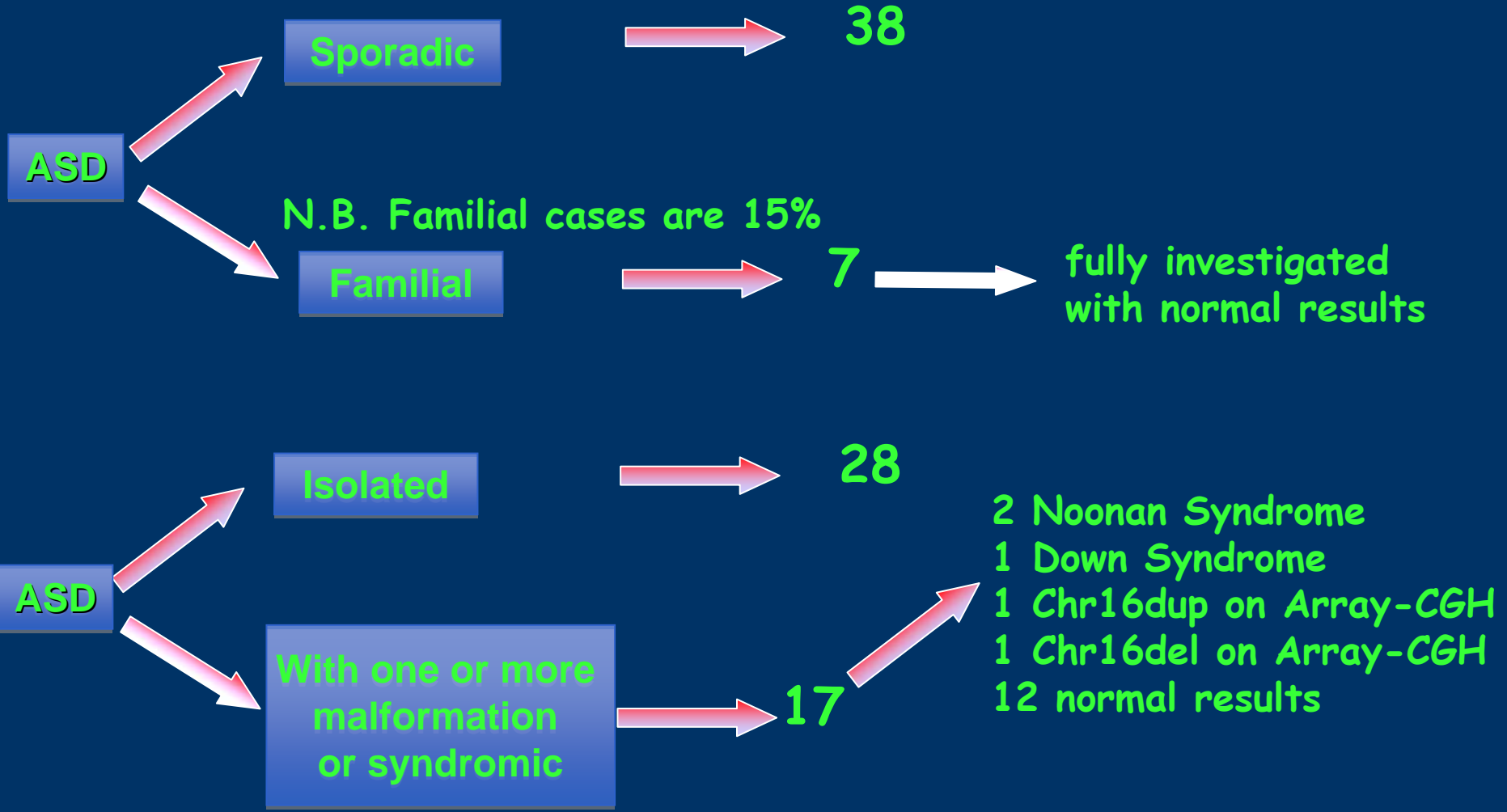
Pediatric Heart Diseases Data Collection Status IGG

	N° Pts enrolled
RVO	98
CMPs	5
Total	103

Pediatric Heart Diseases Data Collection Status IGG

	<i>N° Pts enrolled</i>
ASD	51
TOF post-op.	46
PAPVR	1
Total	98

Total ASD patients 51



No genetic analysis for 1 case

FOR ASD

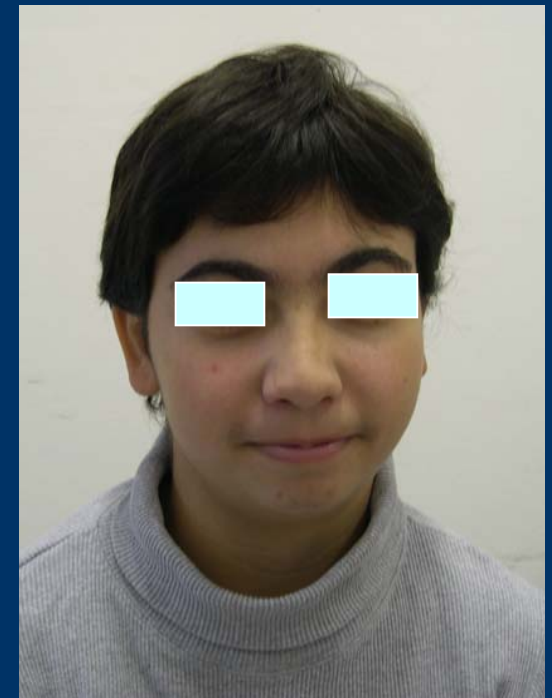
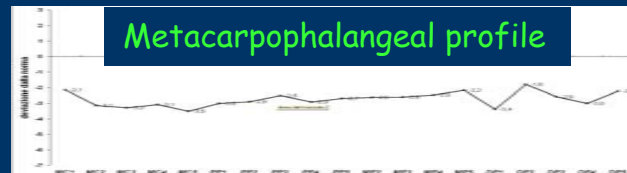
in a total of 50 cases, a molecular or cytogenetic "mark" has been found for 5 (10%)

however

While a trisomy 21 (Down S.) or a mutation in the *PTPN11* gene (Noonan S.) represent "marks" with causative significance, the significance of other "marks" is questionable

ASD-II

- Negative family history of CHD and other congenital abnormalities;
- Normal intelligence;
- Mild dysmorphic features (sloping shoulders, micrognathia, short fingers and toes)



HEC-IT-

del(16)(p11.2) (chr16:29,233,000..30,104,000)

de novo

Could this deletion be causative of the clinical phenotype ?

A region in chromosome 16p11.2 has been found frequently rearranged due to richness of intrachromosomal segmental duplications

A number of articles have reported on association between 16p11.2 microdeletion and mental retardation and/or autism spectrum disorder (Ballif 2007, Ullman 2007, Weiis 2007, Kumar 2008)

Bijlsma et al (2009) have very recently reviewed and discussed theirs and others' results underscoring variable phenotypic presentation, including normal appearance, of individuals who carry the 16p11.2 microdeletion

HEC-IT-

del(16)(p11.2) (chr16:29,233,000..30,104,000)

The region includes the *TBX6* gene, a member of the T-box transcription factor family

Recent work carried out in an animal model of ascidian embryos reports that *Tbx6* is a transcriptional activator of the *Mesp* gene, that encodes a transcription factor defining the heart field

TBX6 mutation screening

Selected patients (27): negative for TBX5 mutation, presenting with cardiac, limb and craniofacial defects

NO MUTATION FOUND IN THIS PATIENTS' GROUP

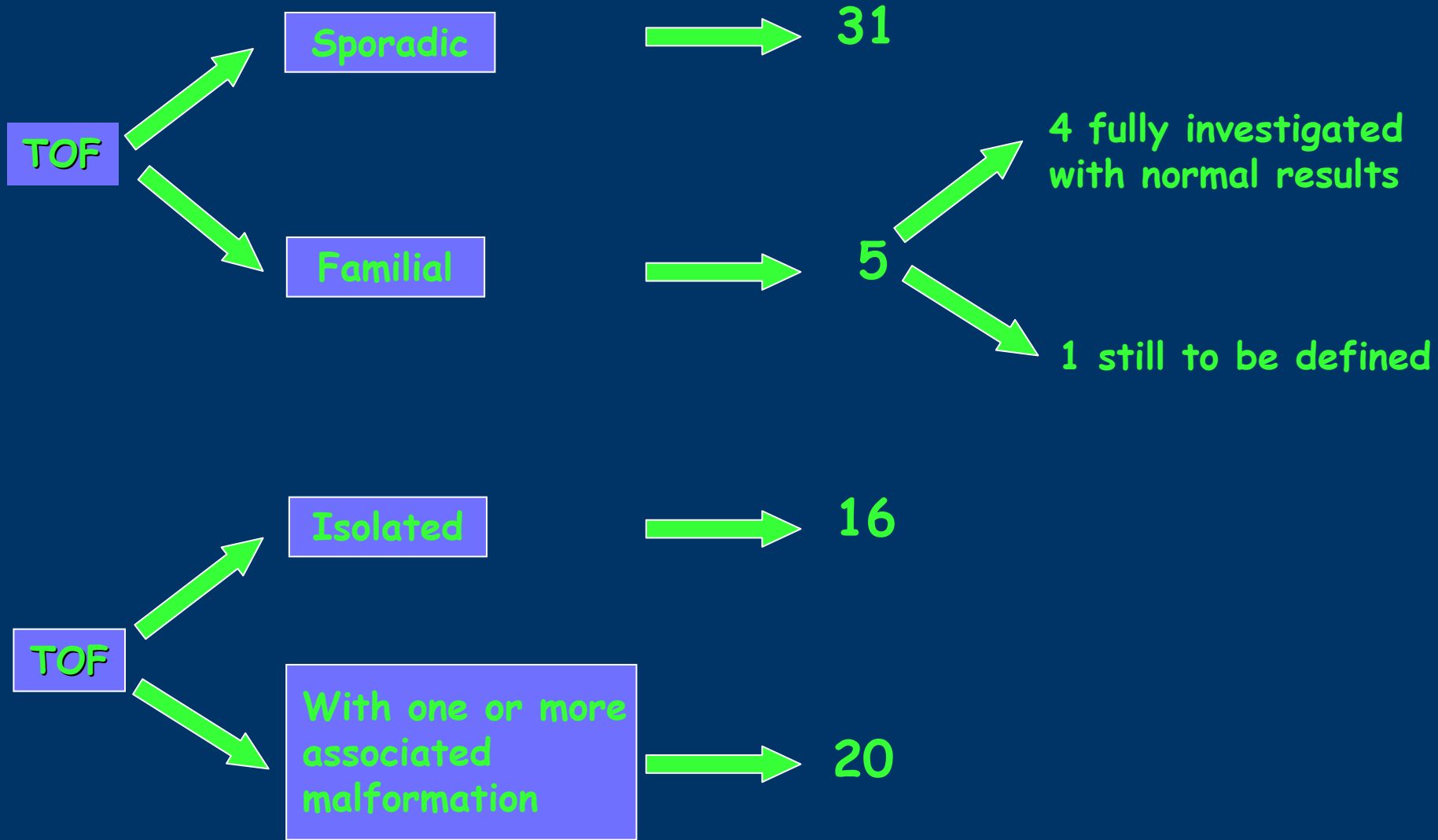
THEN NO CAUSATIVE ROLE ?

..... NO ASSOCIATION WITH THE CLINICAL PHENOTYPE ?

several cryptic rearrangements, even de novo occurring in patients with particular phenotypes, are also found in unaffected individuals

BUT it is conceivable that part of these cryptic rearrangements have a role as susceptibility factors that can affect the carrying individual when a combination of susceptibility alleles in genes concurring to the determination of phenotype, are also present

Total TOF patients 46



No genetic analysis for 7 cases

FOR TOF

in a total of 39 cases, a molecular or cytogenetic "mark" has been found for 14 (36%)

ISOLATED TOF

Positive findings (3/17 cases)

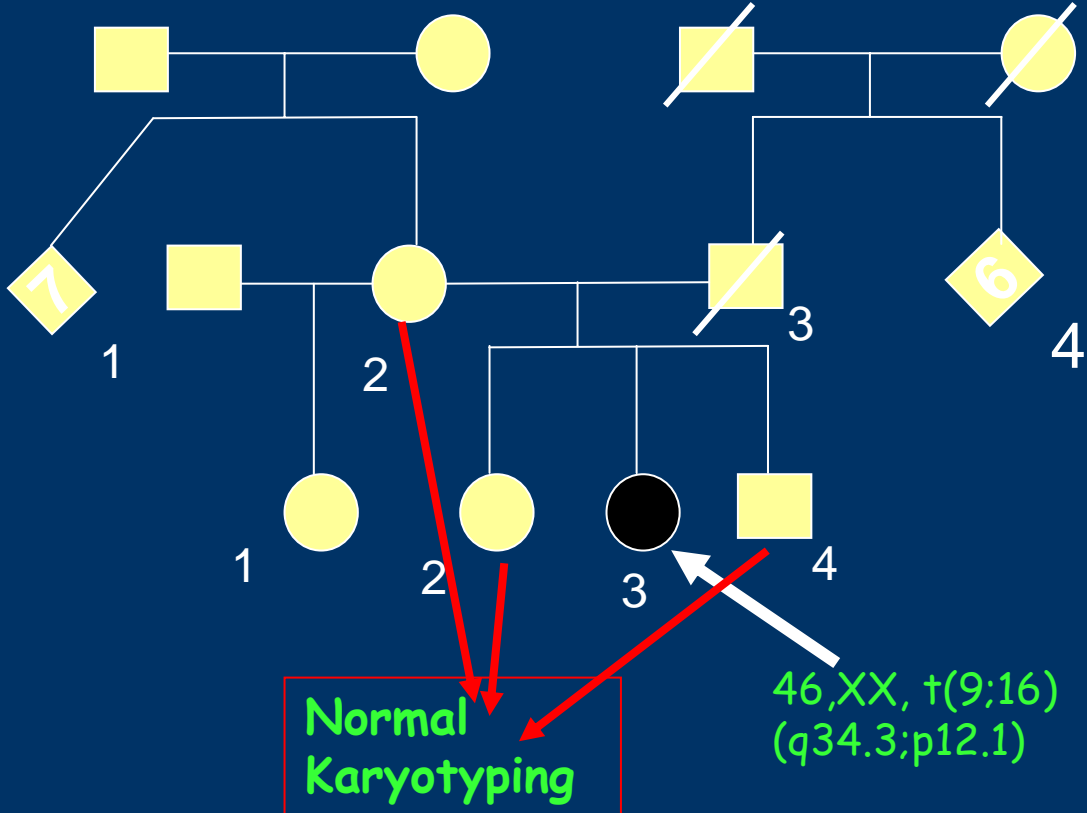
1 balanced translocation
1 TBX5 mutation
1 chr10 deletion

TOF WITH ASSOCIATED ANOMALIES

HECIT-██████████	Scoliosis, speech difficulties and undescended testes	Microdeletion 22q11
HECIT-██████████	Scoliosis, speech delay, MR, and dental problems	Microdeletion 22q11
HECIT-██████████	Mild MR and nasal speech	Microdeletion 22q11
HECIT-██████████	Moderate MR, dysmorphic features, speech delay, nasal speech, scoliosis	Microdeletion 22q11
HEC-IT-██████████	Down syndrome	47,XX+21
HEC-IT-██████████	Dysmorphic features and global psychomotor delay	46,XX,del(6)(q16.1-q21)
HEC-IT-██████████	Scoliosis, high arched palate	46,XY, del(2)(q11.2)mat
HEC-IT-██████████	Osteogenesis imperfecta	Mutation at alpha chain of COL1 gene
HEC-IT-██████████	Dysmorphic features and mild MR	46,XY,dup4q32pat
HEC-IT-██████████	Kabuki Syndrome	46,XX,dup(16)(q23.3)pat
HEC-IT-██████████	Dysmorphic features and global psychomotor delay	GATA4 mutations de novo 46,XX, del(14)(q31.3)mat

HEC-IT-

1 patient 46,XX, t(9;16)(q34.3;p12.1) in apparently isolated TOF



Chromosome 16p12.1: rich of segmental duplications

Chromosome 9q34.3: one candidate gene interrupted by the translocation breakpoint

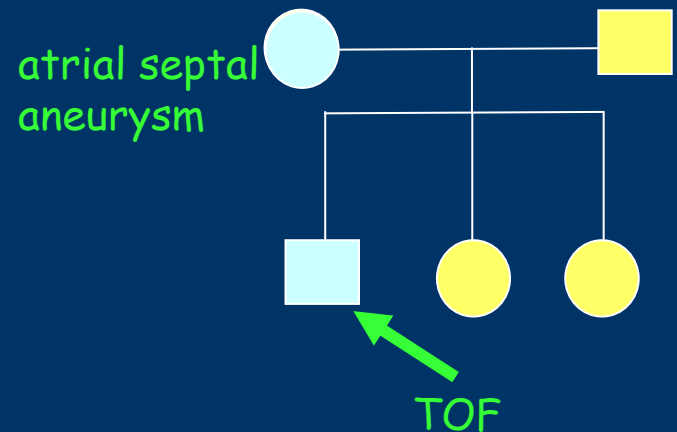
HEC-IT-

Phenotype: isolated TOF

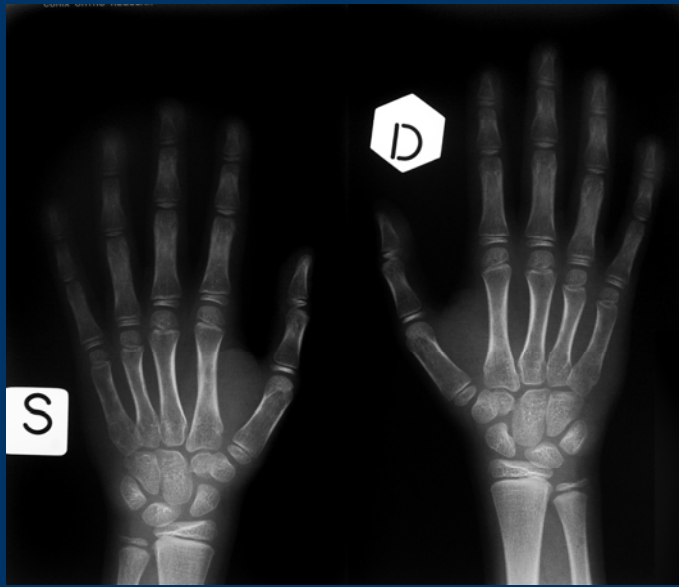
Normal screening of *GATA4* and *NKX2.5*

Screening of *TBX5* gene showed a maternally inherited variation in exon 9 causing the S372L substitution

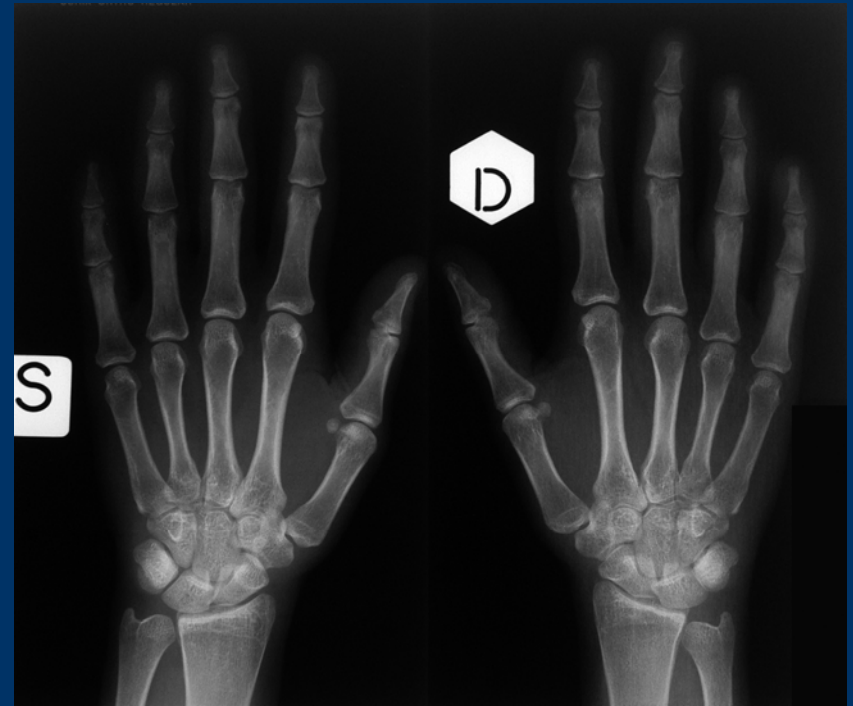
Detailed physical examination of the mother, ECG, Echocardiography: atrial septal aneurysm



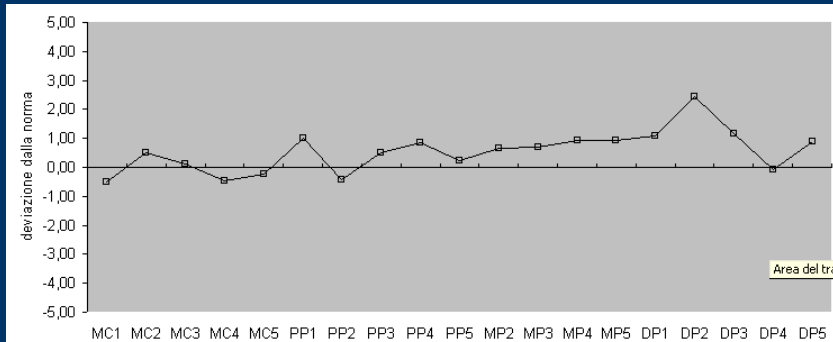
HEC-IT-



proband



mother



Metacarpophalangeal profile of the proband

Hands X-ray of the patient and mother: normal structure and metacarpophalangeal profile .

HEC-IT-

Screening of 100 normal controls for this variant: absence of S372L

Functional assay by co-transfection of a Luciferase vector containing the ANF promoter and an expression plasmid with the mutated TBX5 cDNA

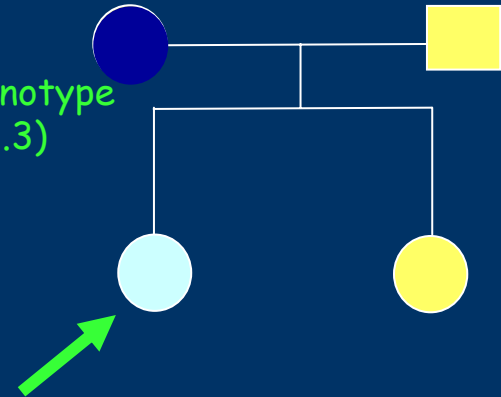
The S372L showed an infrequent functional feature, i.e. a gain of function

Could a gain of function mutant produce a different phenotype than more frequent loss of function TBX5 mutations in Holt-Oram ?

HEC-IT-

- TOF
- Mild mental retardation
- Increased liver enzymes

-normal phenotype
-del(14)(q31.3)



-*de novo* *GATA4* mutation N352S
-maternally inherited
del(14)(q31.3), very small,
containing only part of the
NRXN3 gene

Is the deletion, present in the unaffected mother,
totally neutral with respect to the clinical phenotype
or contributes to it ?

CONCLUSIVE REMARK

The integrated system developed by the Health-e-child program is becoming a tool available to the scientific community

If and when it will be possible to collect large number of cases, the integration of genetic data with functional or morphological data will provide the basis for correlation not only with the most known and obvious findings

Also those "marks" that now are considered of dubious or no significance might acquire significance if found related to particular groups or subgroups of disorders

CONTRIBUTORS

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All the Cardiology team at Gaslini

The Genetic Counseling team at Gaslini





The VPH Network of Excellence (VPH NoE) is designed to foster, harmonise and integrate pan-European research in the field of

i) patient-specific computer models for personalised and predictive healthcare

ii) ICT-based tools for modelling and simulation of human physiology and disease-related processes.

Individualized // Individual

Genome // Genes // Coding // Non-coding

Response to physiological changes

Response to environmental changes (including drugs)

Types of genetic data and their use

Modelling & simulation of organs/systems targeting specific biomedical issues