VCFSEF 2011 Annual Meeting

When does a 22q11.2 deletion not cause VCFS?

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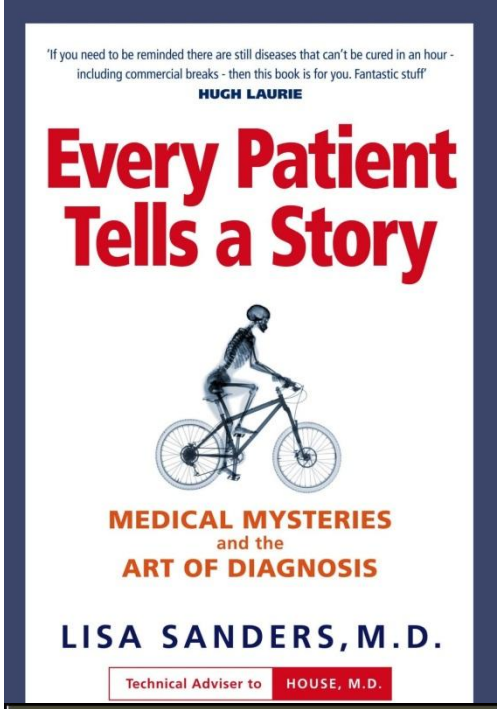
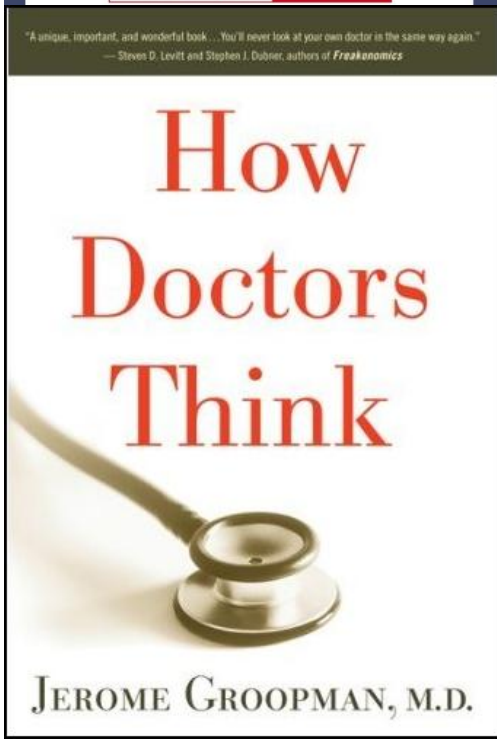
Questions:

1. When does a 22q11.2 deletion not cause VCFS?
2. Why does VCFS have such variability in expression of traits?
3. Phenotype first to genotype?
4. Genotype first to phenotype?
5. Are we there yet?

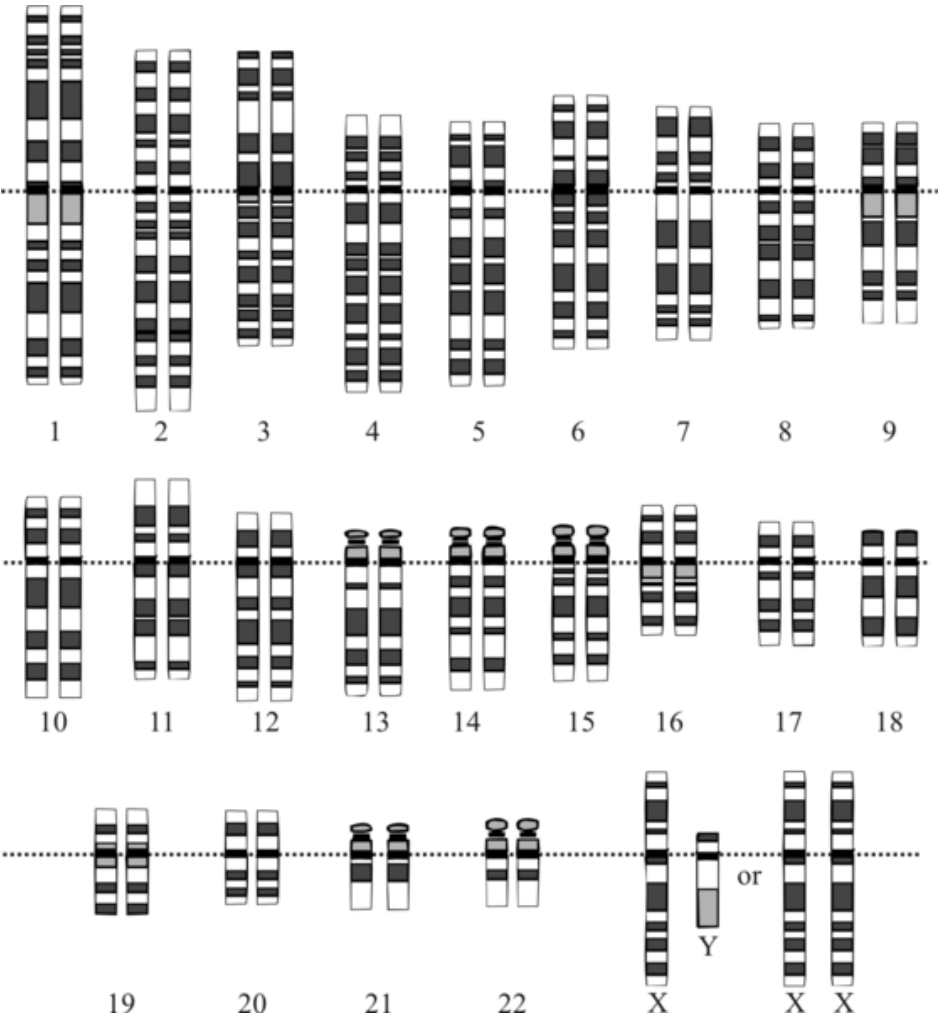


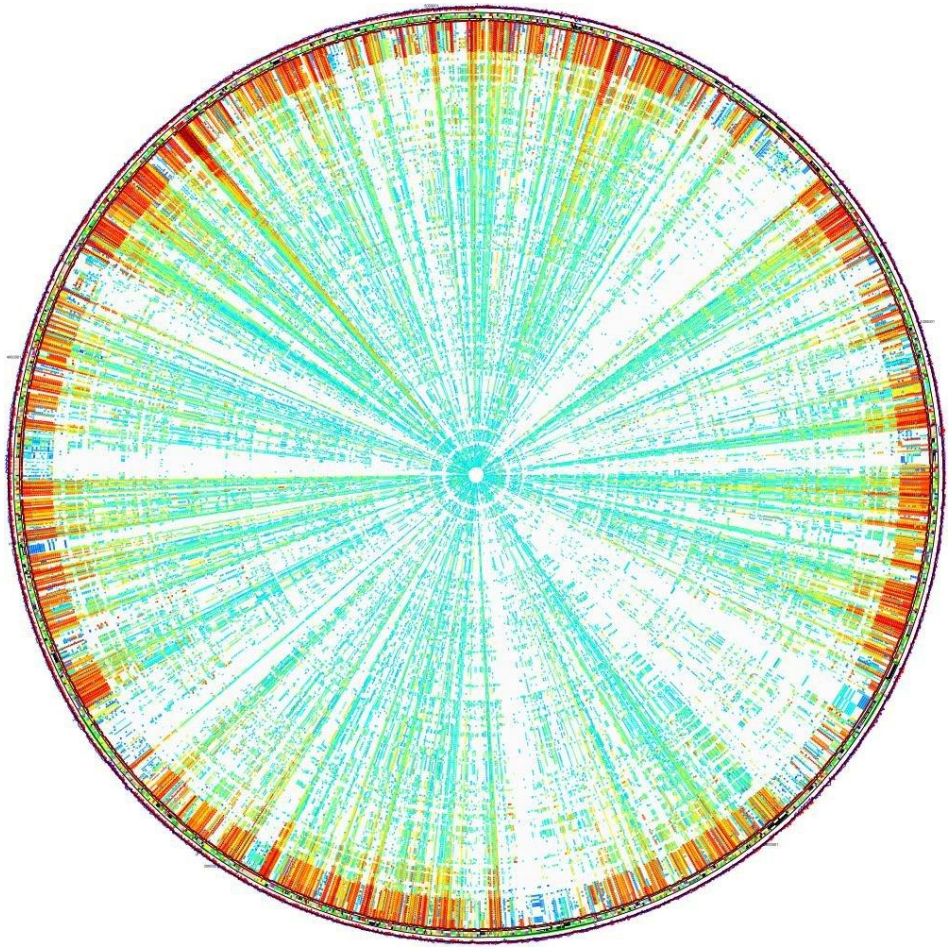


The human genome

-spans about 3 billion DNA base pairs

-contains ca. 23,000 protein-coding genes

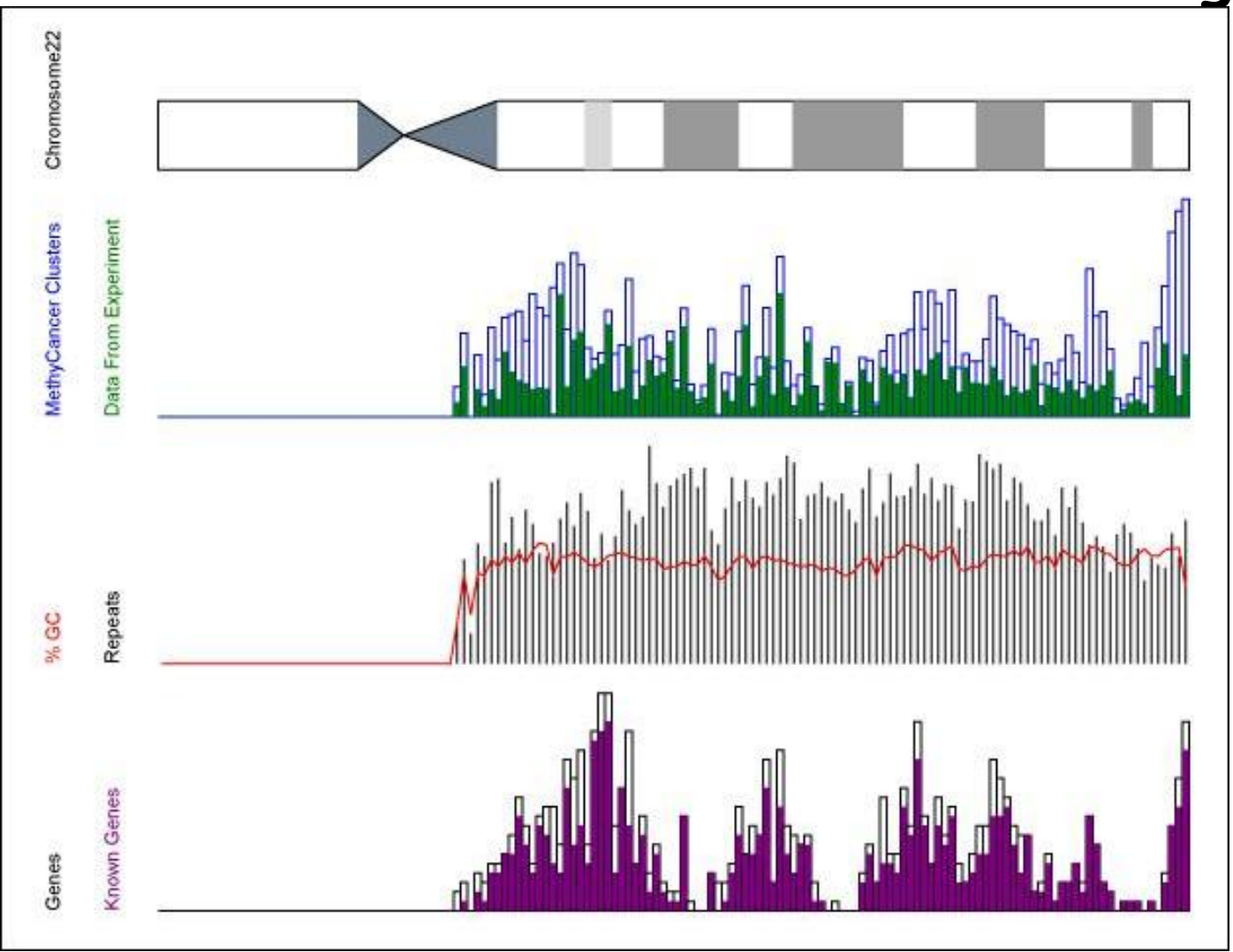


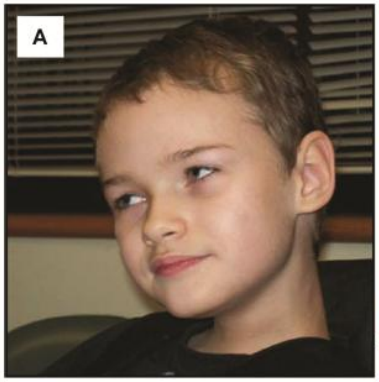


Human chromosome 22

-spans about 50 million base pairs

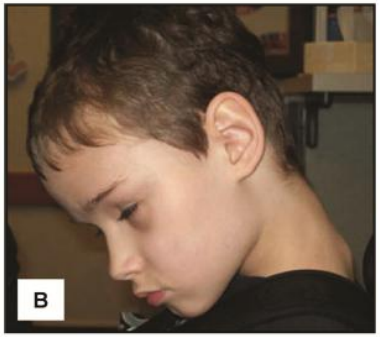
-contains more than 800 annotated genes




A


C


E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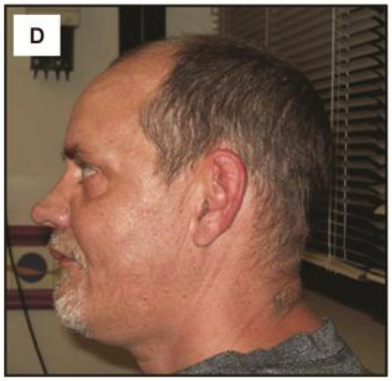
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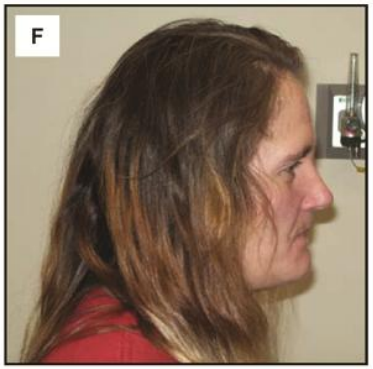
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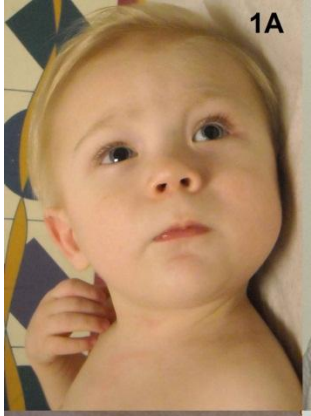
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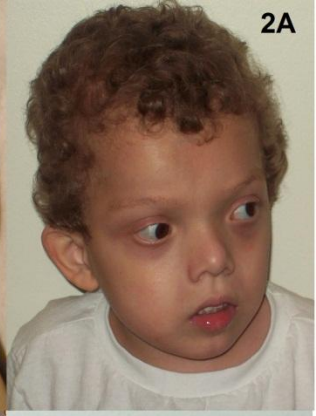
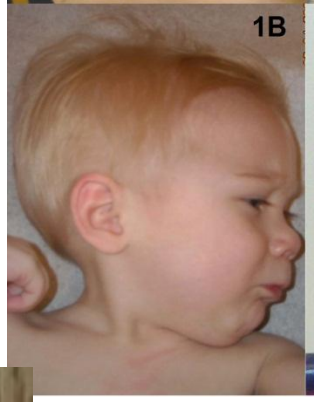

2A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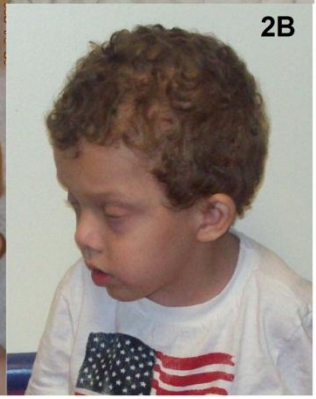
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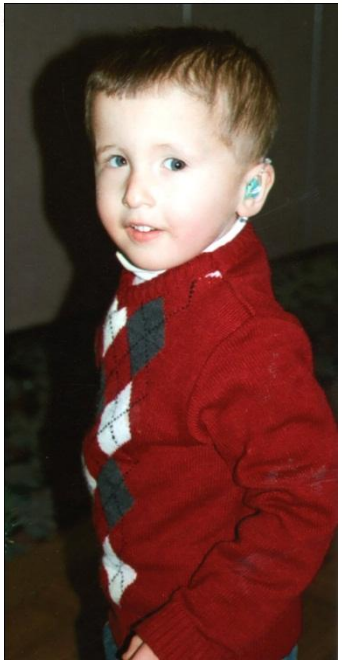
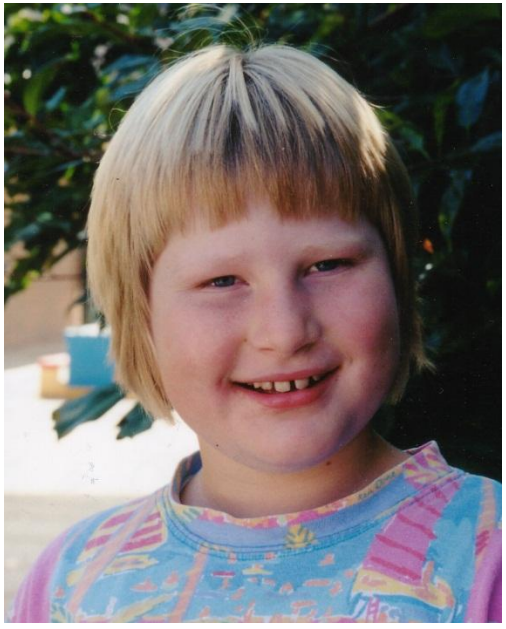
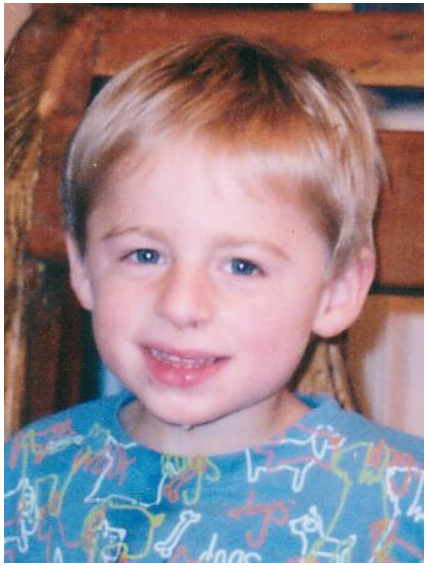






A phenotype is an organism's observable characteristics or traits: such as its morphology, development, biochemical or physiological properties, and behavior

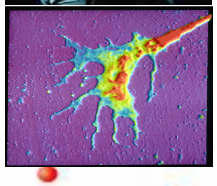
Phenotypes result from the expression genes, the influence of environmental factors and their interactions

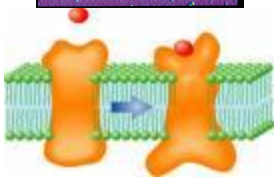
The genotype of an organism is the inherited instructions it carries within its genetic code. Genotype may be modified by environmental conditions

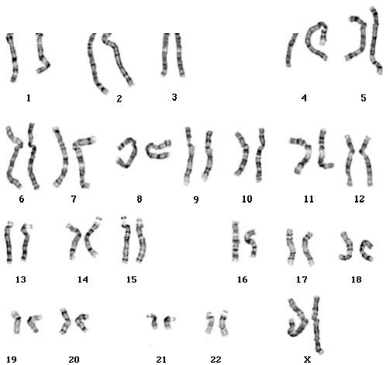
Wilhelm Johannsen. The genotype conception of heredity. American Naturalist 1911;45:129-159

Phenotype by "Level"

Individual Child
Organs
Tissues
Cells
Organelles / Membranes/ Receptors
Proteins
Small molecules
RNA
Chromosomes Genes / DNA







Cognitive phenotypes

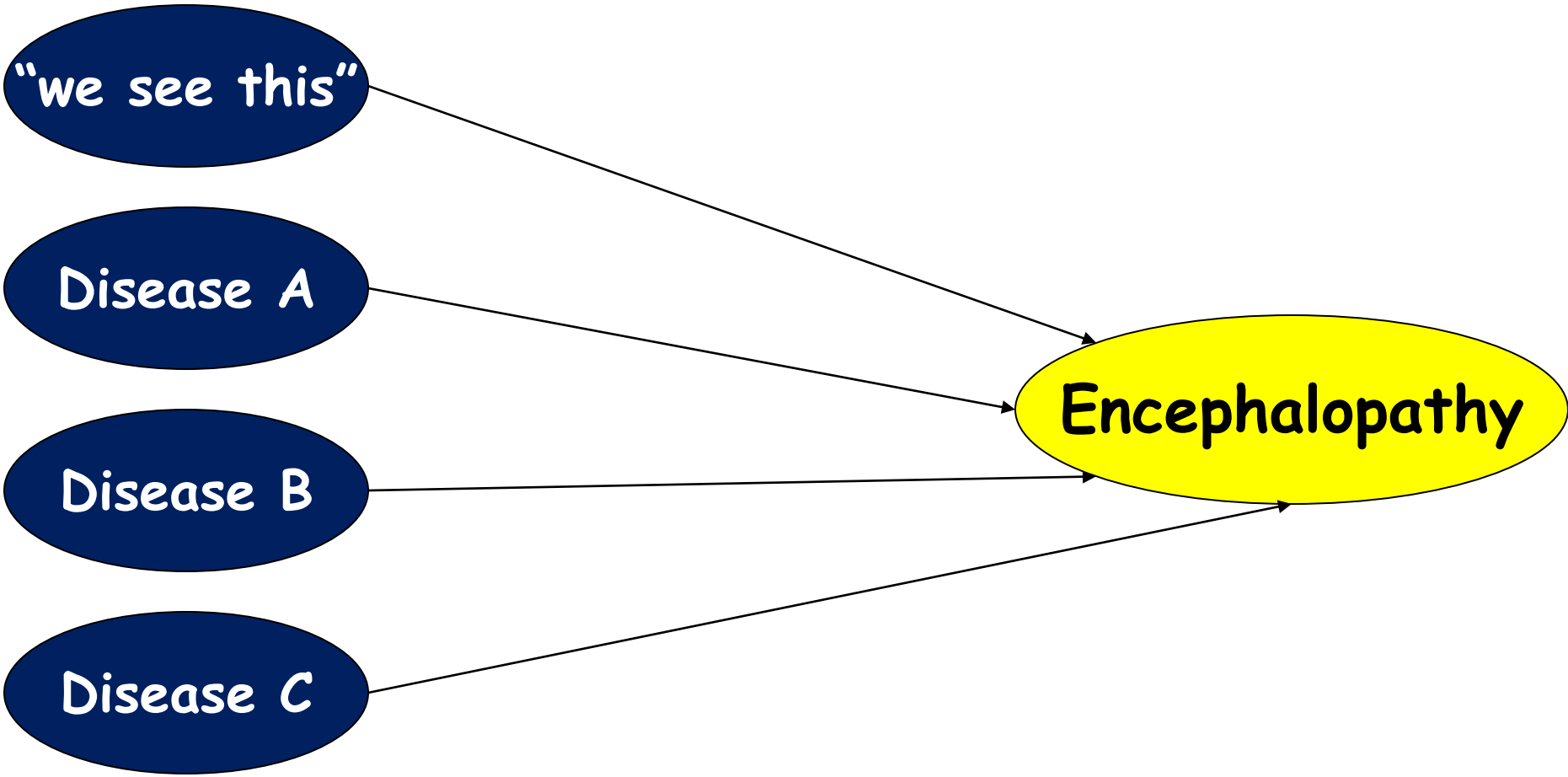
Behavioral phenotypes

Morphological phenotypes

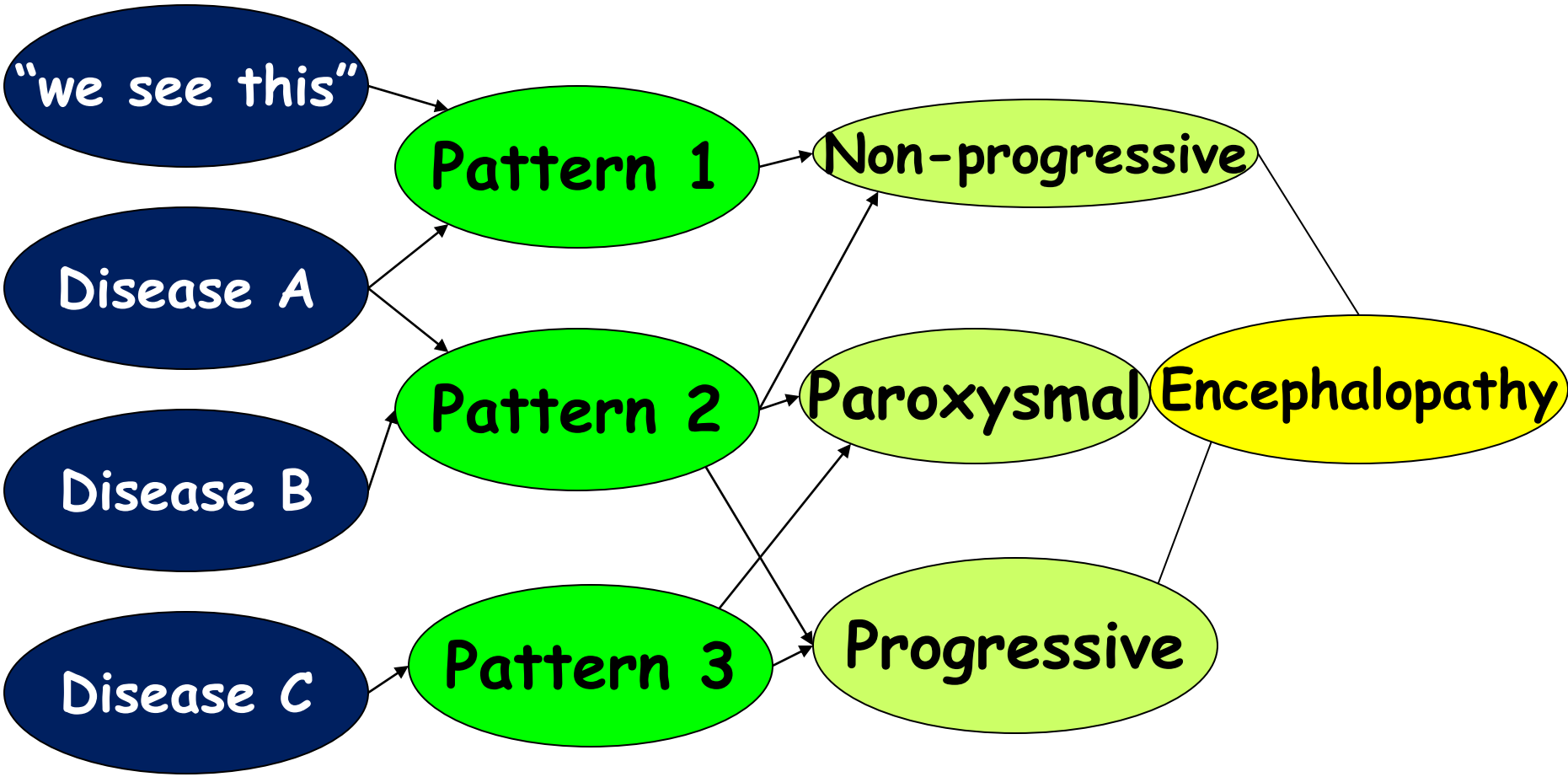
Metabolic phenotypes

"Genomic phenotypes"

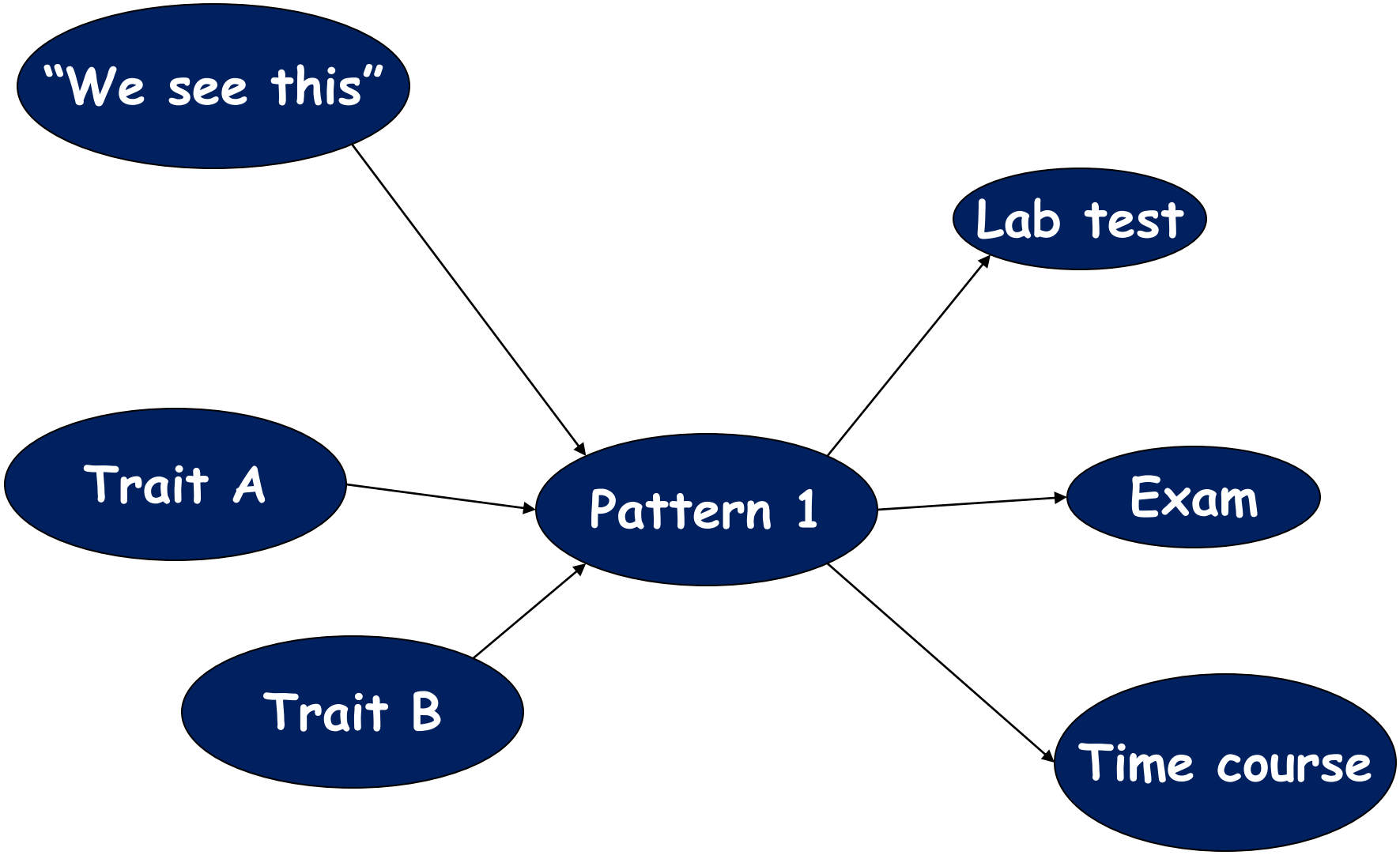
Student-level Pattern Recognition



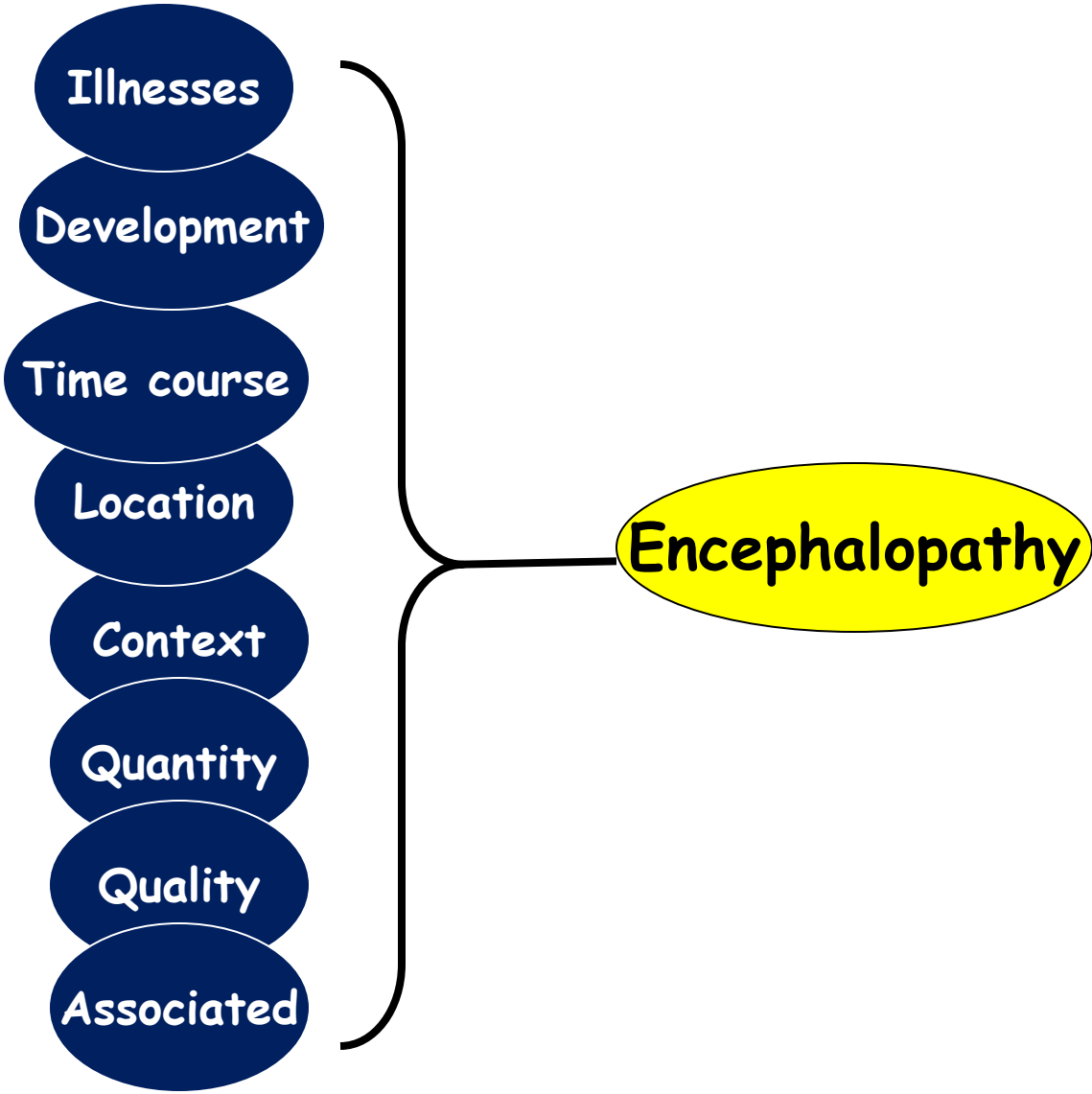
Expert pattern recognition



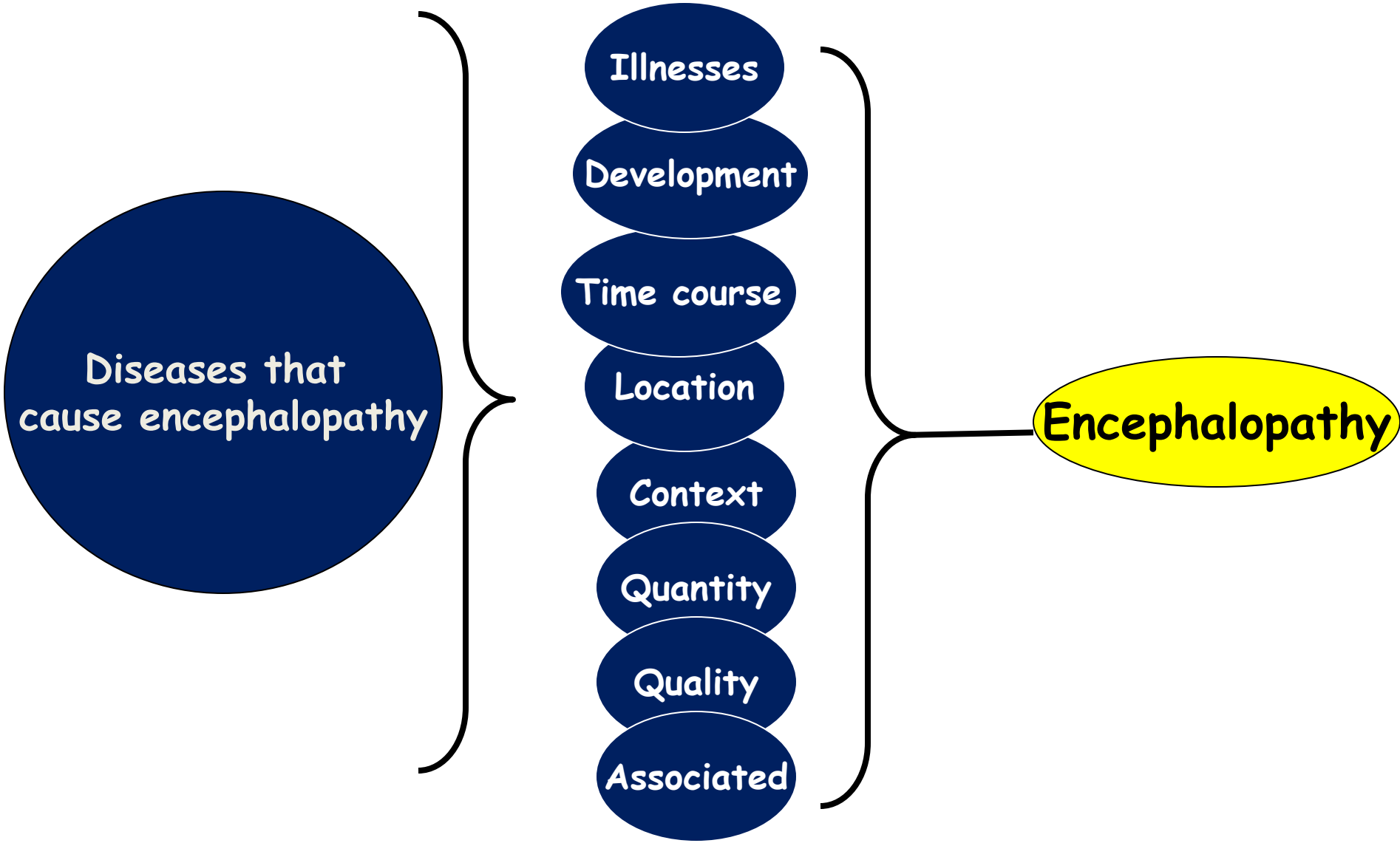
Pattern Recognition / Targeted



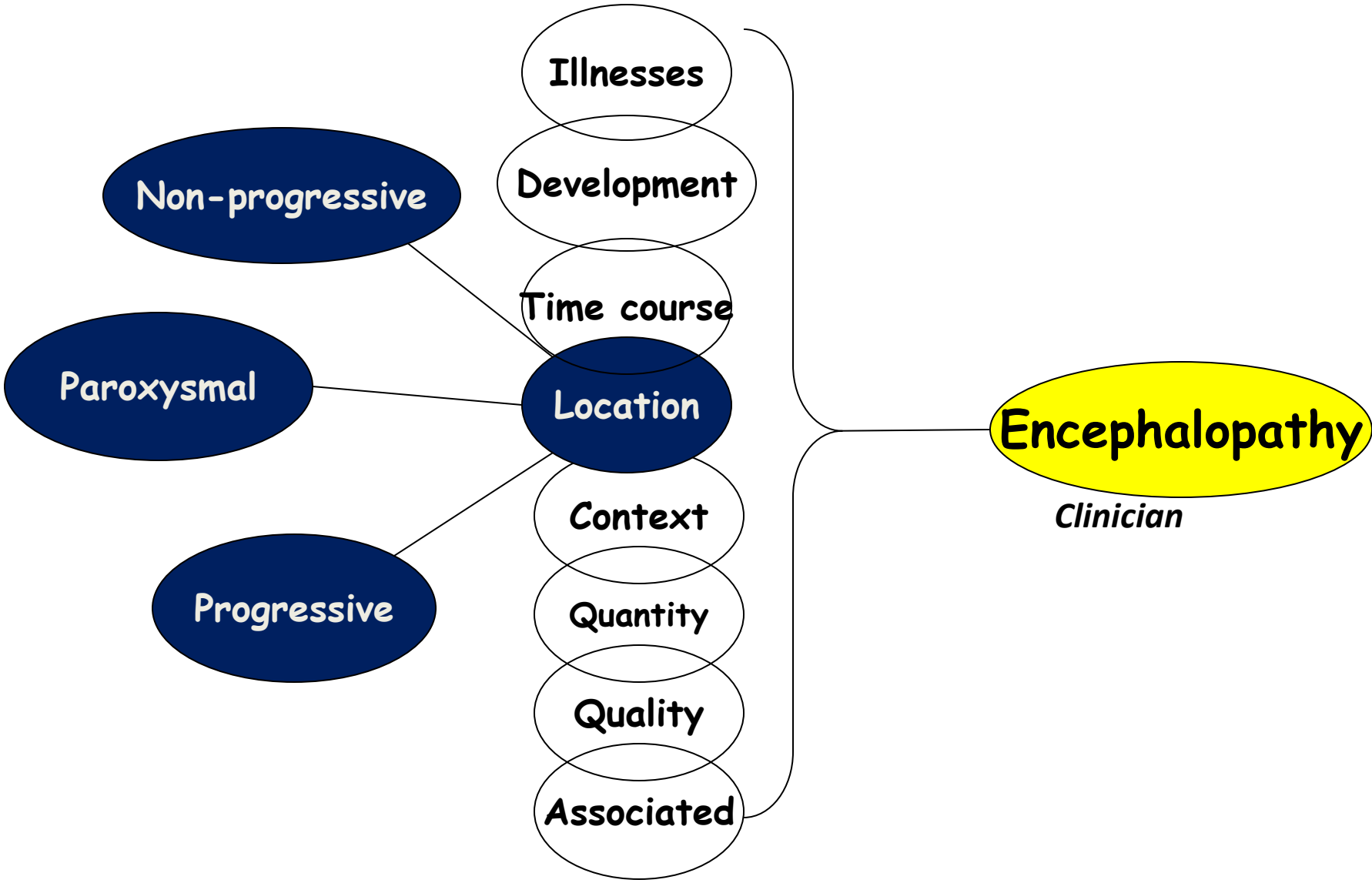
History taking: HPI based on chief complaint



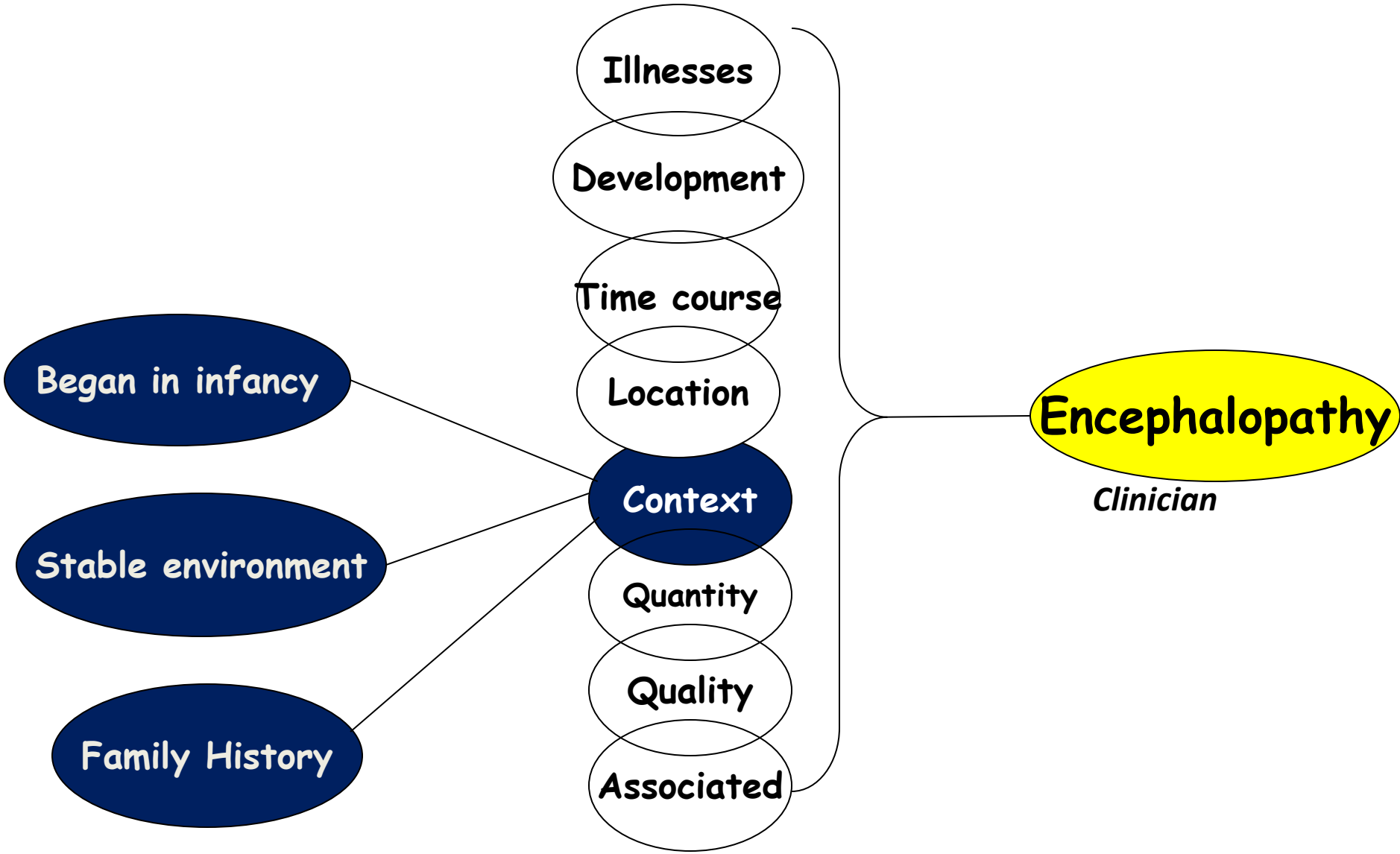
History taking: HPI based on chief complaint

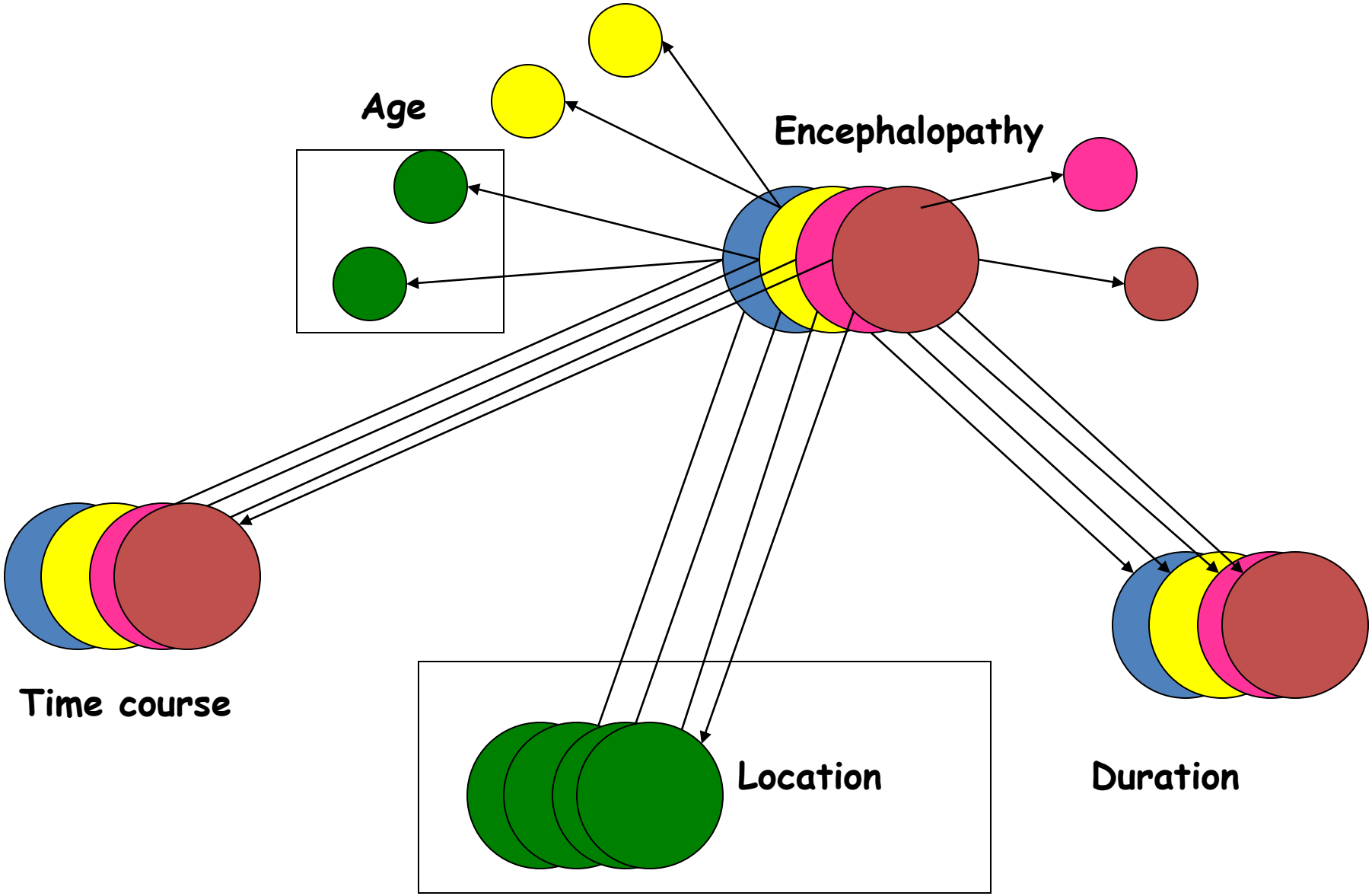


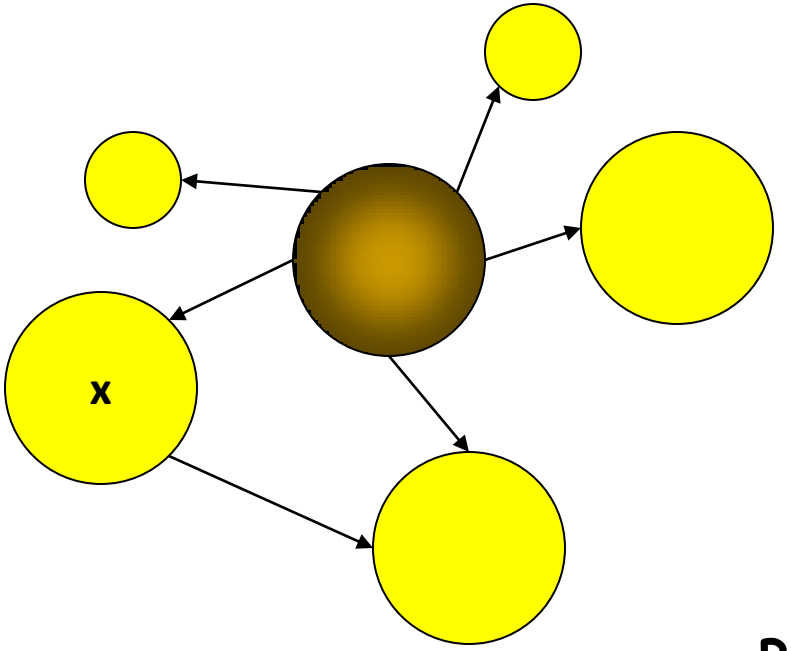
History taking: HPI based on chief complaint

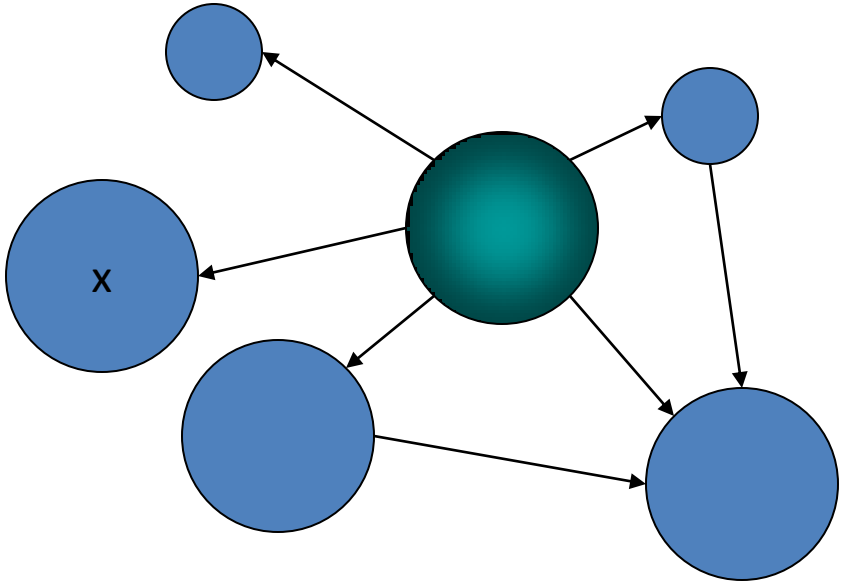


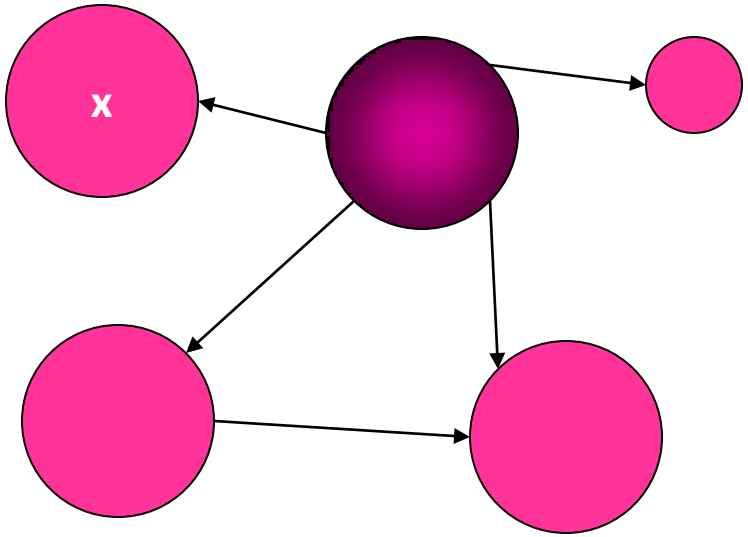
History taking: HPI based on chief complaint




Age
Encephalopathy
Time course
Location
Duration







Biological phenomena are too complex to be represented by ideal cases without destroying their true nature.

If complexity is kept intact, sufficient mathematical techniques will be lacking for their satisfactory handling.

Stanley L. Jaki (1924-2009)
Philosopher of Science

Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities

1. The development of microarray-based comparative genomic hybridization (array CGH) represents the latest advancement in molecular cytogenetics.

2. This new technology highlights the complexity of the human genome.

3. The identification of increasingly more subtle DNA copy gain or loss is also redefining our presumed understanding of some multifactorial conditions.

4. The success of these contemporary molecular cytogenetic techniques has resulted in an exponential increase in genetic information that has far exceeded our ability to understand and use this flood of information in the clinical setting.

5. This technology is an unprecedented reversal of the usual order of the practice and progress of medicine, where clinical suspicion and medical acumen often direct laboratory investigation and suggest specific genetic lesions and mechanisms.

6. Clinicians are now able to scrutinize the genome for guidance in their clinical practice.

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Traditional Path of Discovery

Morphology → Cell → Protein → Gene

The paradigm shift

Gene → Protein → Cell → Morphology

Basic Dogma

One gene → One protein → One phenotype

Basic Dogma

One gene → One protein → One phenotype

Variations of Basic Dogma

Multiple genes → Different proteins → One phenotype

One gene → One protein → Multiple phenotypes

Multiple genes → Different proteins → Multiple diverse phenotypes

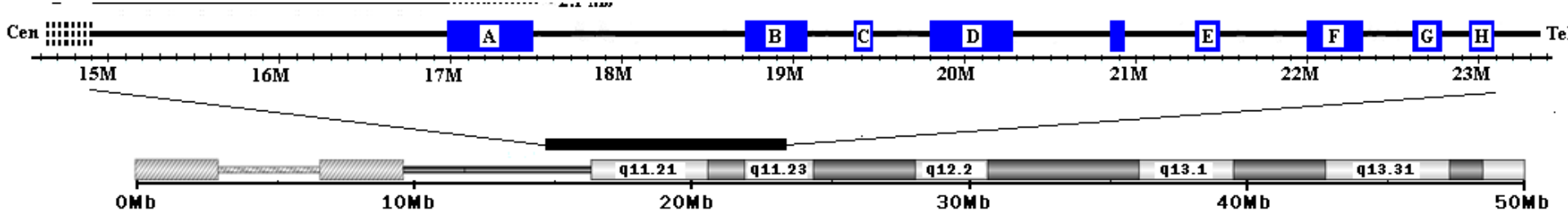
Methods. 1,654 consecutive pediatric patients with a diversity of clinical findings were evaluated by high resolution chromosomal microarray analysis (CMA).

Results. We identified twenty five individuals with abnormal copy number variants (CNVs) on chromosome 22, representing **1.5%** (25/1,654) of the cases analyzed in this cohort.

We detected 1,298 benign CNVs on this chromosome in these individuals.

Twenty one of the 25 abnormal CNVs and majority of the benign CNVs occurred through involvement of the 8 unstable genomic regions enriched with low copy repeats (LCR22A-H).

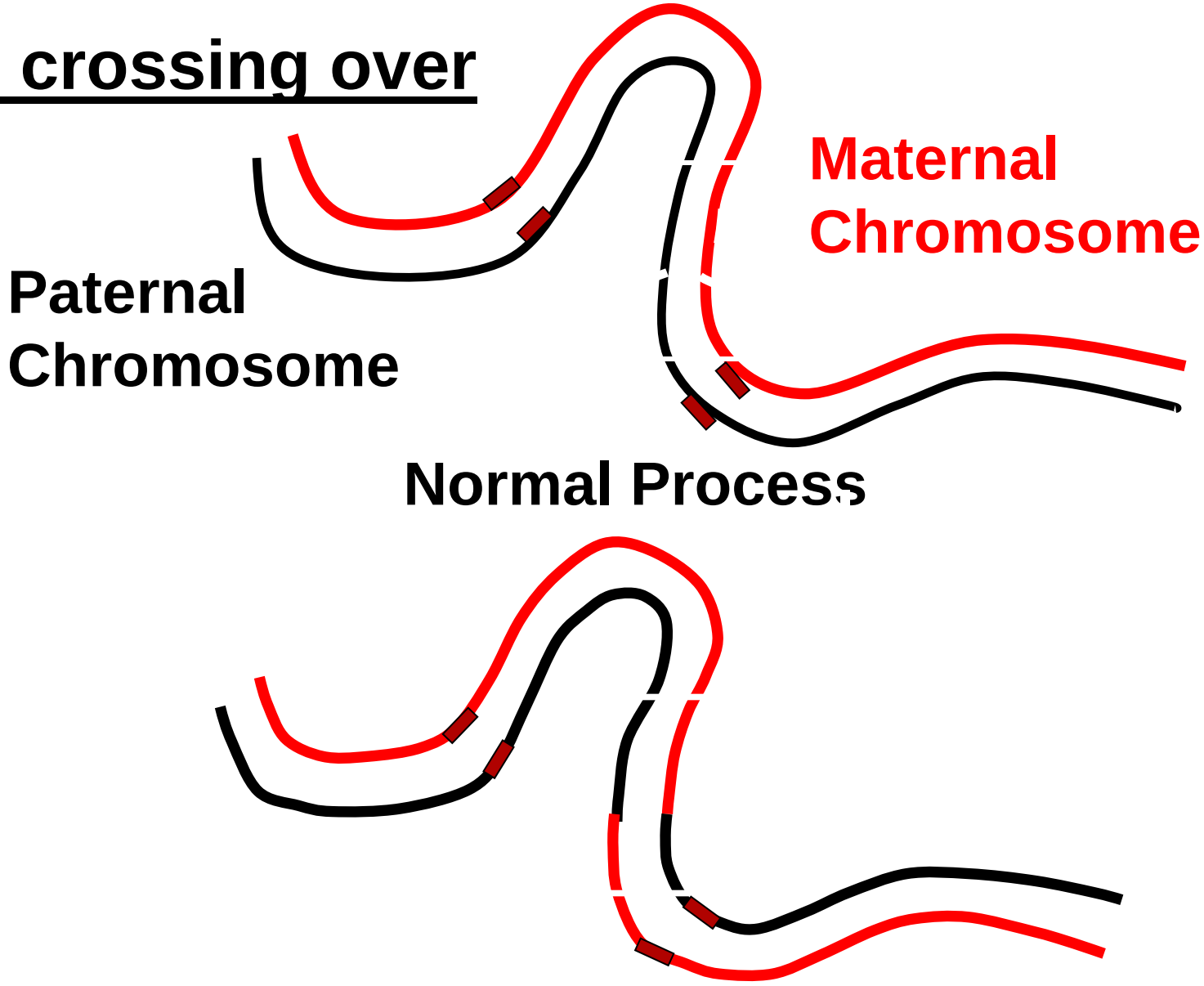
Yu S, Graf WD, et al. Identification of copy number variants (CNV) on human chromosome 22 in patients with a variety of clinical findings. (*in press*)



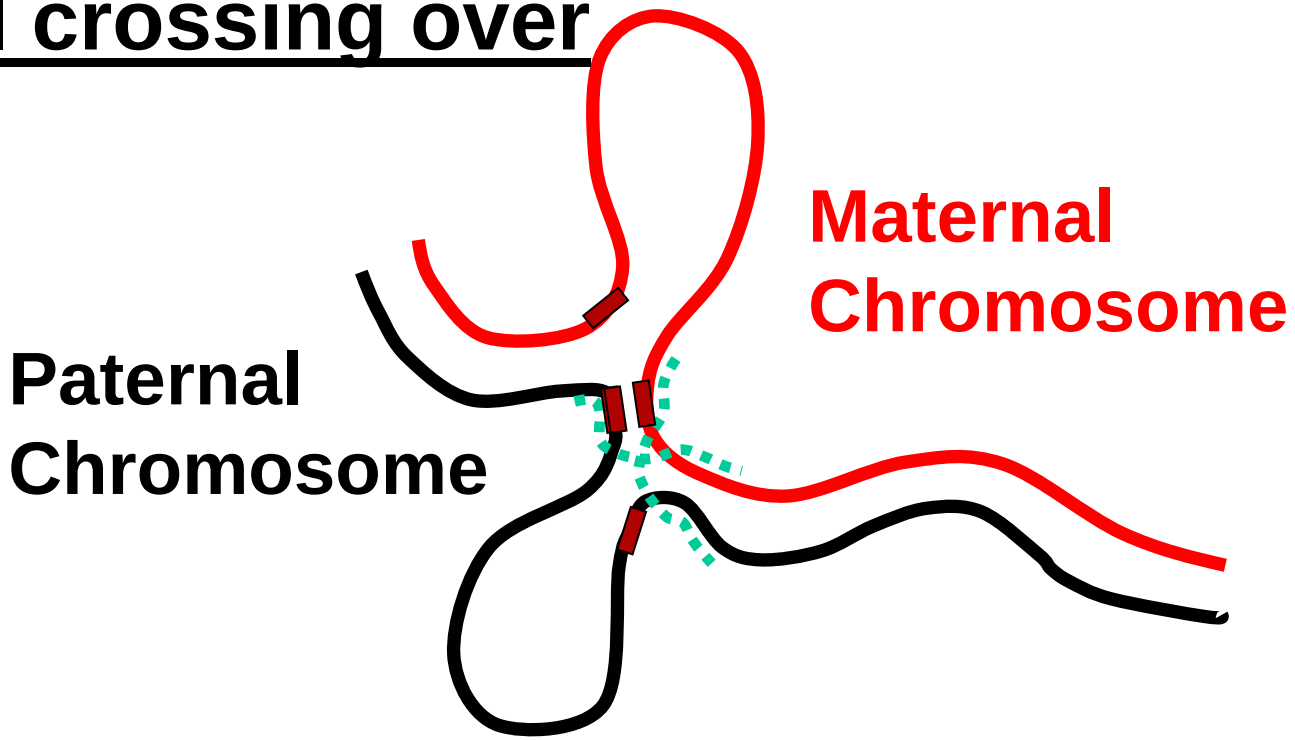
- The proximal region of chromosome 22 is enriched in low copy repeats (LCRs, also called segmental duplications)
- The 22q11.2 region contains at least 8 LCR22s, four of which are localized within the most frequent deletions
- LCR22s are labeled from centromere to telomere:
  - LCR22-2 (or LCR22-A)
  - LCR22-3a (or LCR22-B)
  - LCR22-3b (or LCR22-C)
  - LCR22-4 (or LCR22-D)
  - LCR22-5 (or LCR22-E) to LCR22-8 (or LCR22-H)

These LCRs facilitate the formation of majority of the chromosome 22 genomic abnormalities through LCRs-mediated non-allelic homologous misalignment and unequal recombination (NAHR) inter- or intra-chromosomally during meiosis

Equal crossing over



Unequal crossing over

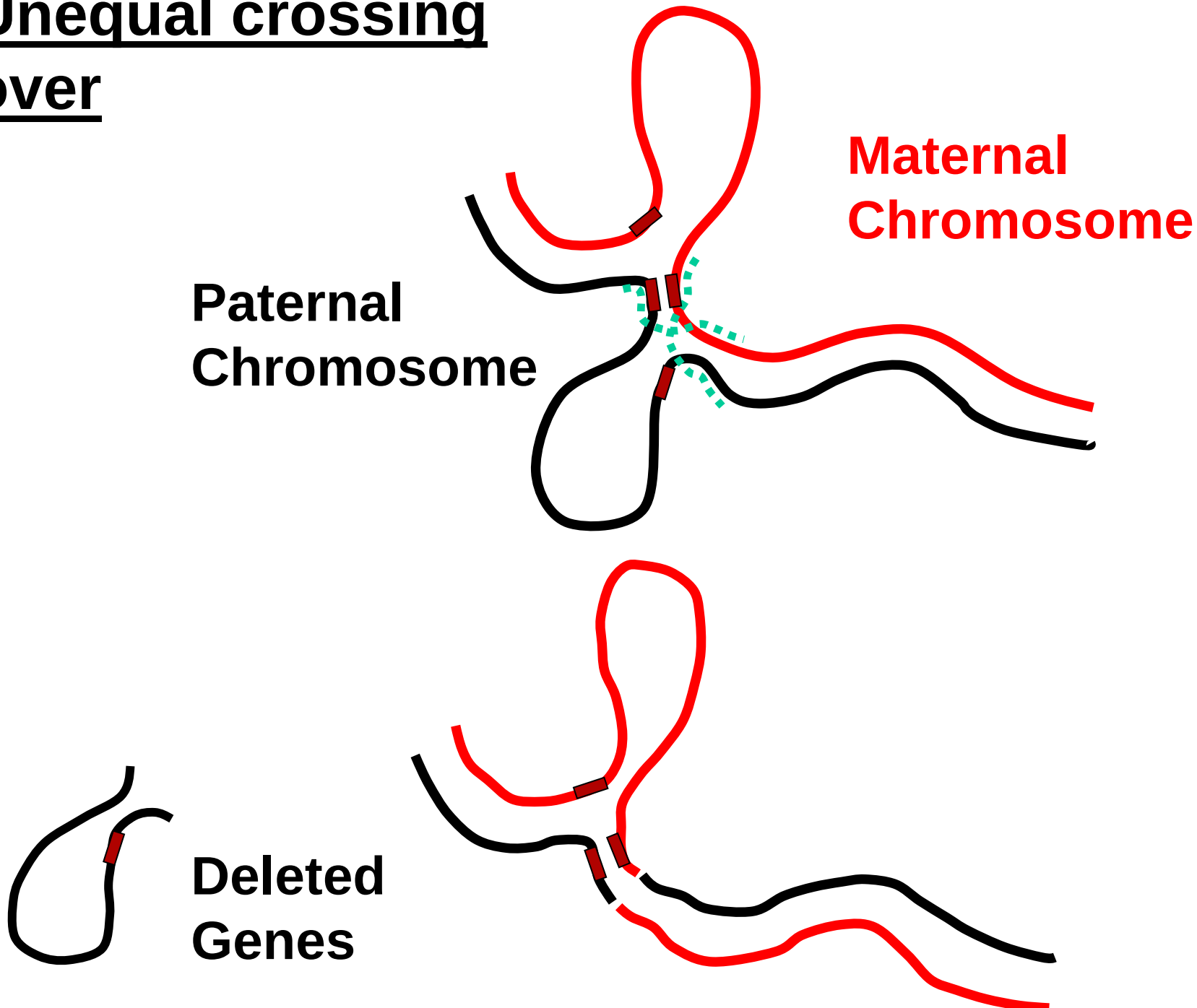


Susceptible breakpoints:

Low-copy-repeat gene clusters flank the region of microdeletion.

Breakpoints in 22q11.2 del syndrome are almost always identical.

Unequal crossing over



Known genomic disorders on chromosome 22 include:

1)cat eye syndrome (CES) caused by extra copies of the proximal region of chromosome 22

2)22q11.2 deletion syndrome (Velocardiofacial / DiGeorge syndrome)

3)22q11.2 duplication syndrome

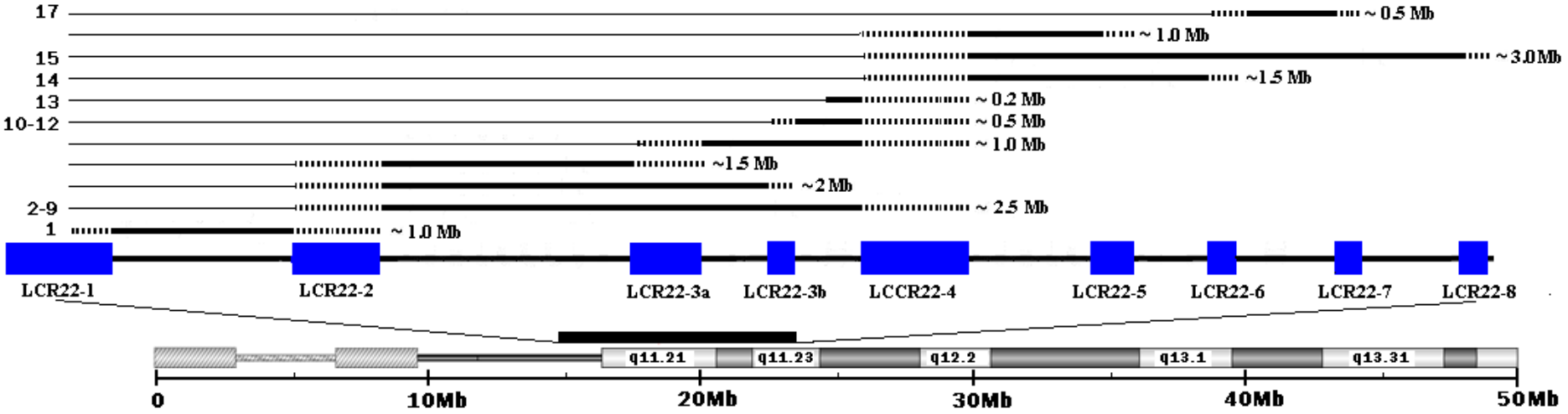
4)22q11.2 distal deletion syndrome

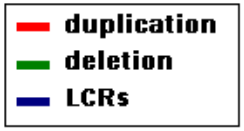
5)supernumerary der(22)t(11;22) syndrome derived from t(11;22) malsegregation

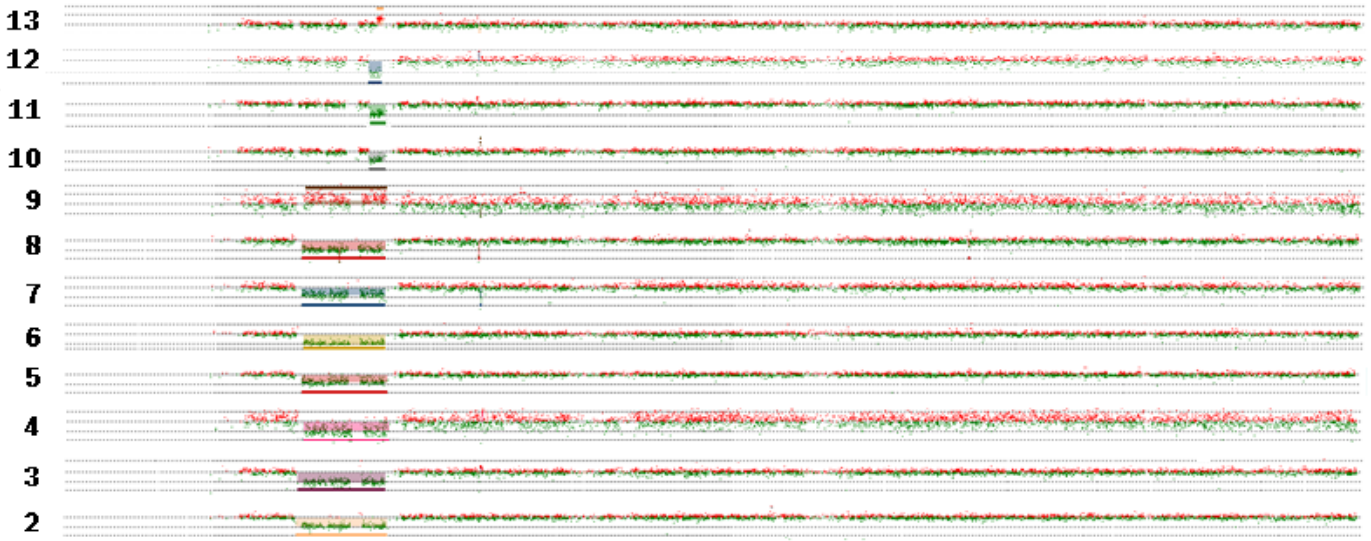
6)22q13.3 deletion syndrome

In addition to these defined genomic disorders, other pathogenic genomic abnormalities on human chromosome 22 have been identified while numerous genomic imbalances are considered as copy number variants (CNV) of unknown clinical significance (CNVUS) or copy number variants without apparent clinical significance (benign CNV) (http://projects.tcag.ca/variation)

Eight 22q11.2 characterized LCRs are labeled LCR22-1 thru LCF22–8 from the centromeric to telomeric ends







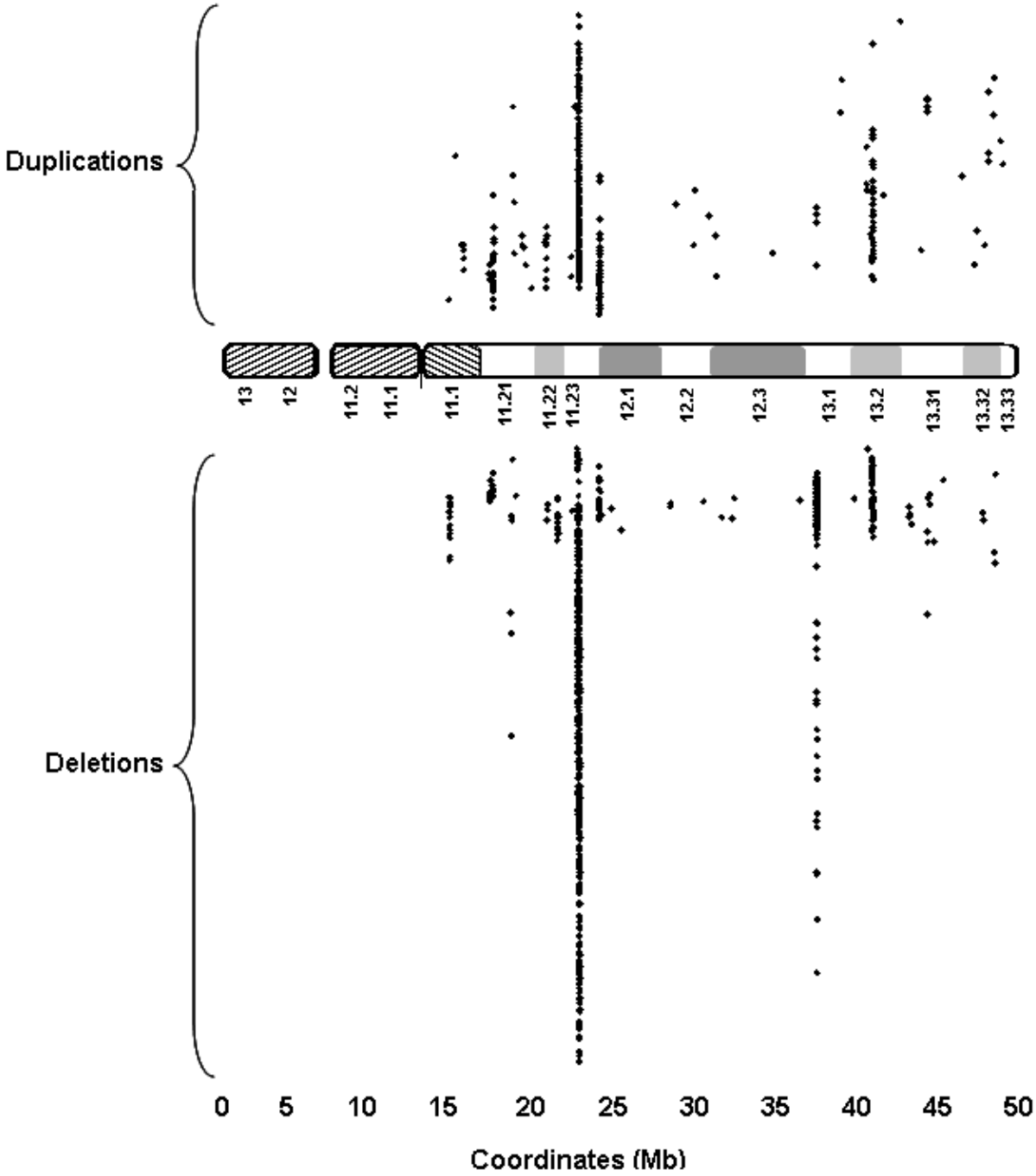
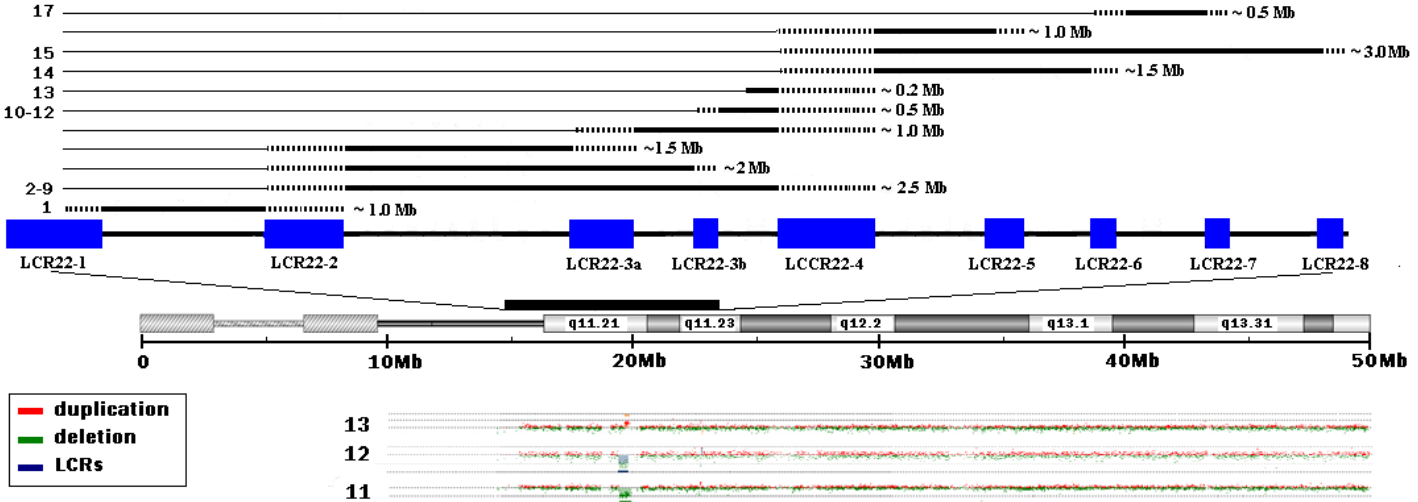


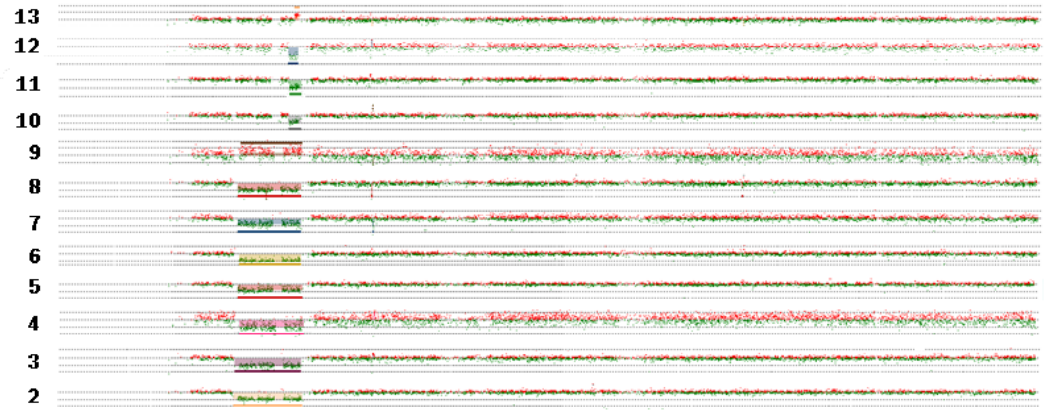
Figure 3. **Distribution of benign CNVs across chromosome 22**

Results: Each patient had genomic abnormalities within the 22q11.2 region, and none had copy number variants of biological significance in other regions. We found:

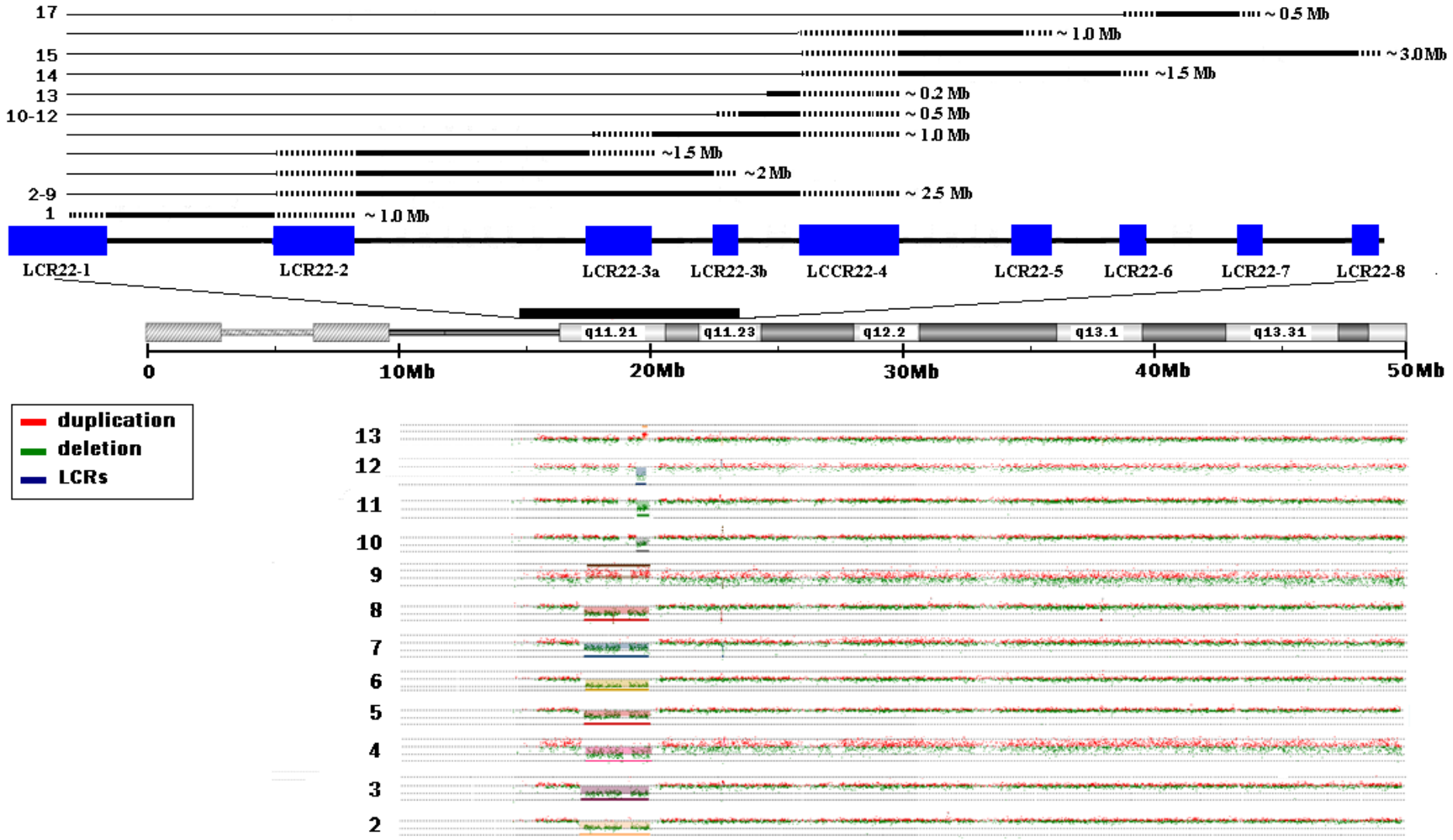
- **Seven "typical" 22q11.2 deletions and one duplication between LCR22-2 and LCR22-4**
- **The seven typical patients have an average 22q11.2 deletion of 2.66 (range 2.56—2.79) Mb and common features of VCFS**

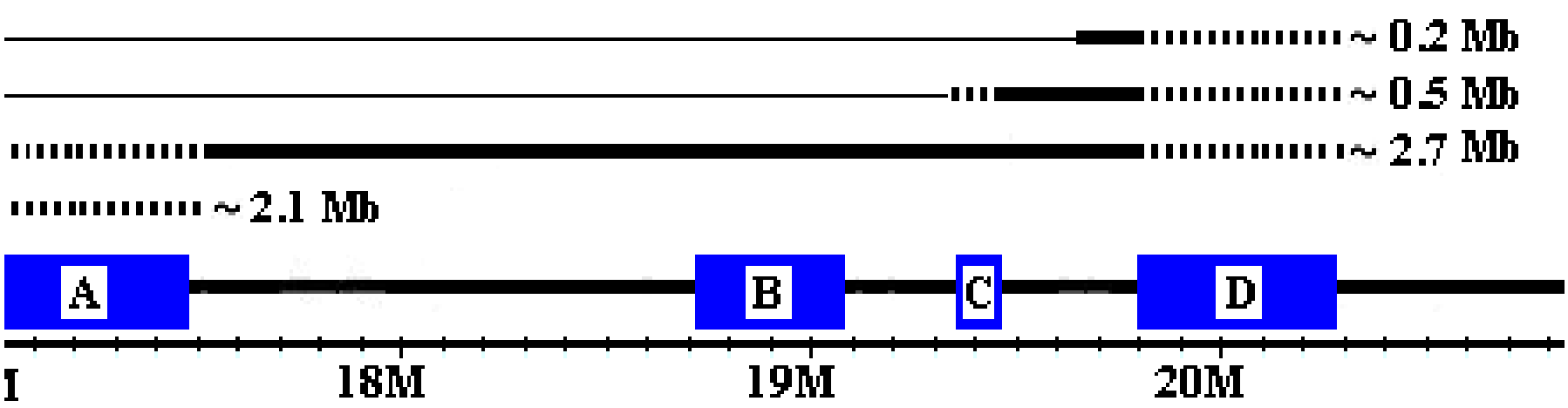
Two atypical deletions between LCR22-3b & LCR22-4





Atypical patients were found serendipitously by aCGH


17
~0.5 Mb
~1.0 Mb
15
~3.0Mb
14
~1.5 Mb
13
~0.2 Mb
10-12
~0.5 Mb
~1.0 Mb
~1.5 Mb
~2 Mb
2-9
~2.5 Mb
1
~1.0 Mb
LCR22-1
LCR22-2
LCR22-3a
LCR22-3b
LCCR22-4
LCR22-5
LCR22-6
LCR22-7
LCR22-8
q11.21
q11.23
q12.2
q13.1
q13.31
0
10Mb
20Mb
30Mb
40Mb
50Mb
duplication
deletion
LCRs
13
12
11
10
9
8
7
6
5
4
3
2



Only two patients were previously reported to have deletion between LCR22-3b LCR22-4*

We report 3 additional cases with this microdeletion.

The clinical features in these four patients are variable, but neurodevelopmental problems occurred in all cases

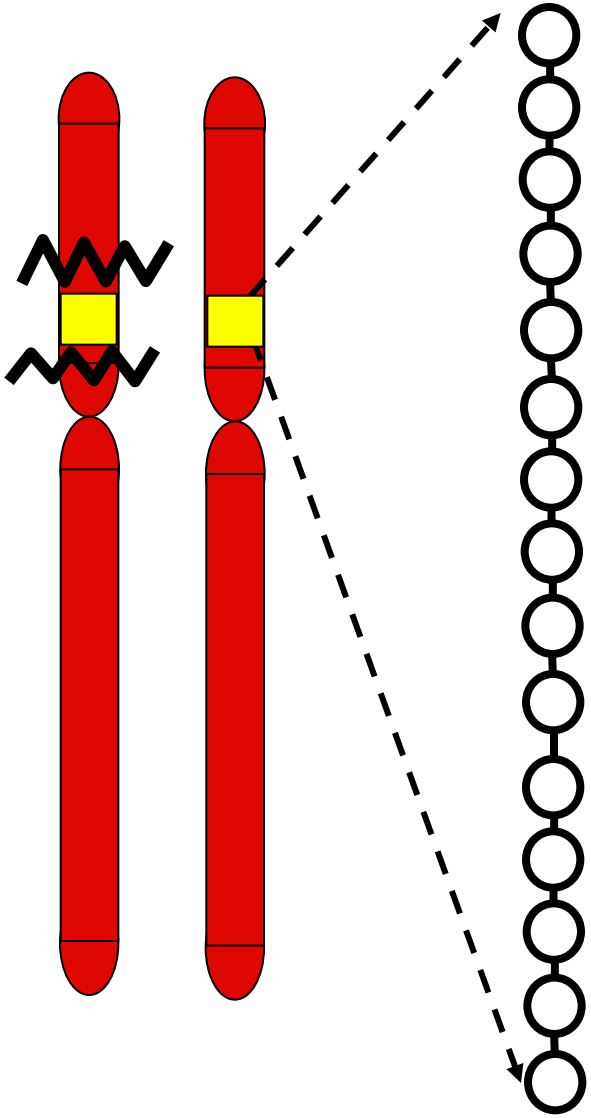
HOWEVER, THE PHYSICAL PHENOTYPES IN THESE CASES ARE NOT CONSISTENT WITH VCFS.

(D'Angelo et al., 2007; Kurahashi et al., 1997)

Findings in 4 persons w/ chr 22q11.21 LCR22C-D del

Abnormal Finding / Patient	1	2	3	4	Kurahashi et al.	D'Angelo et al.
Conotruncal CHD	-	-	-	-	+	-
Palate, VPI, or speech disorder	-	-	-	-	-	+
Endocrine or Ca++ disorder	-	-	-	-	-	-
Immune system	-	-	-	-	-	-
VCFS facial features	-	-	-	-	-/+	?
Learning Disability	+	++	+	-	?	+
Behavioral or Ψ Disorder, or ADHD	-	++	+	+	?	++
Tics [T], Seizures [S], Hearing [HL]	HL	T, S	HL	-	?	?

Some genes within the chromosome 22q11.2 typically deleted region

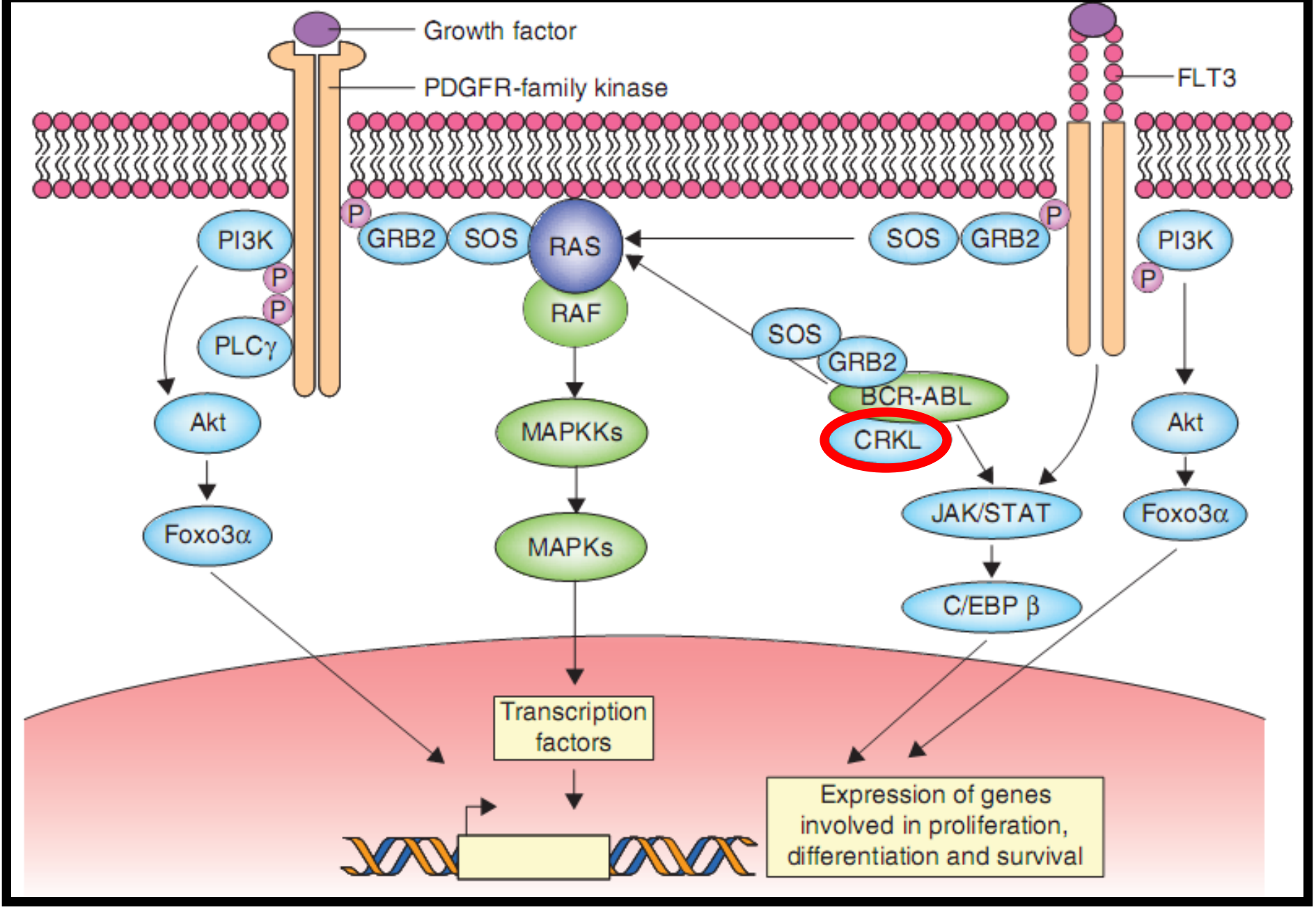


Gene	Function
CLTD	clathrin heavy-chain homologue
COMT	**catechol-O-methyltransferase**
CTP	mitochondrial citrate transport
DGCR2 (LAN)	adhesion receptor protein
DGCR6	laminin gamma-1 chain homologue
DVL-22	disheveled segment homologue
GNB1L	G-protein beta-subunit polypeptide
GSCL	goosecoid-like homeobox gene
HIRA	transcription repressor homologue
TBX1	***T-box transcription factor***
TMVCF	encodes a transmembrane protein
TUPLE1	a putative transcription factor
UFD1L	ubiquitinated protein degradation
ZNF74	a putative transcription factor

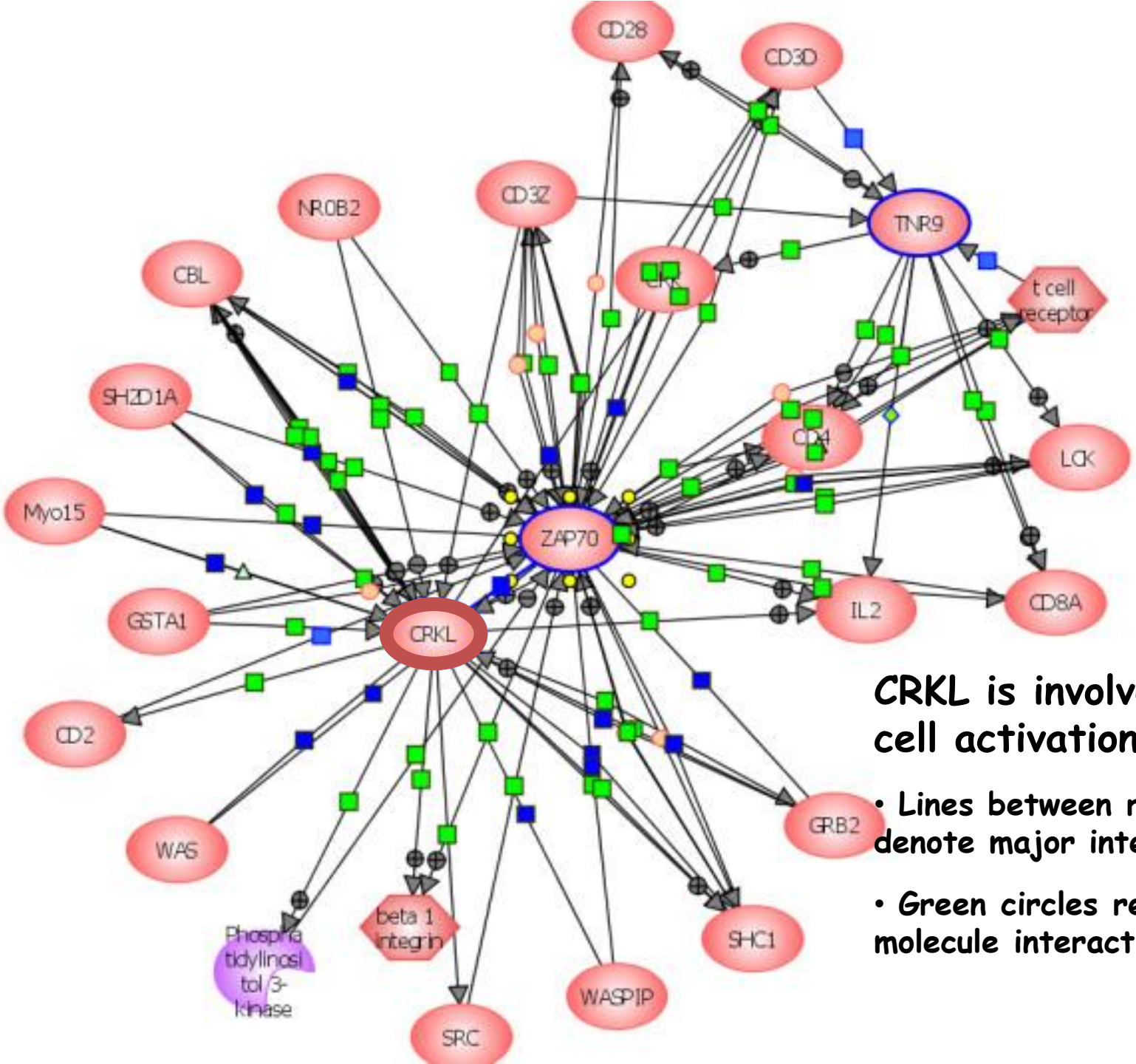
Symbol	Description of genes in the 508-kb (LCR22-3b & LCR22-4) region	Associated genetic diseases in OMIM
TMEM191A	transmembrane protein 191A.	NA
PI4KA	phosphatidylinositol 4-kinase, catalyzing the first committed step in the biosynthesis of phosphatidylinositol 4,5-bisphosphate.	NA
SERPIND1	serpin peptidase inhibitor, clade D (heparin cofactor), rapidly inhibits thrombin in the presence of dermatan sulfate or heparin.	heparin cofactor II deficiency
SNAP29	synaptosomal-associated protein, binding a syntaxin protein and mediating synaptic vesicle membrane docking and fusion to the plasma membrane with involvement in multiple membrane trafficking steps.	cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratodermia
CRKL	v-crk sarcoma virus oncogene homolog – a protein kinase shown to activate the RAS and JUN kinase signaling pathways and transform fibroblasts in a RAS-dependent fashion. **Possible interactions between CRKL and TBX1 influencing VCFS phenotype**	NA
AIFM3	apoptosis-inducing factor, mitochondrion-associated, 3.	NA
LZTR1	leucine-zipper-like transcription regulator 1:localizing exclusively to the Golgi network where it may help stabilize the Gogli complex.	NA
THAP7	THAP domain containing 7.	NA
P2RX6	purinergic receptor P2X, ligand-gated ion channel, 6:The protein belongs to the family of P2X receptors, which are ATP-gated ion channels and mediate rapid and selective permeability to cations.	NA
SLC7A4	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4	NA

CRK-Like Adapter Protein (CRKL):

- Essential for activation of MAPK3 – it sustains phosphorylation of many proteins required for mito-genesis, cell proliferation, differentiation and migration
- A member of an adapter protein family that binds to various tyrosine-phosphorylated proteins
- Has several Src-homology domains (SH2 and SH3) which recruit cytoplasmic proteins in the vicinity of tyrosine kinase through SH2-phosphotyrosine interaction. Thus, CRKL can bind to multiple sites of various signaling proteins and activate enzymatic cascades through their links to PI3K and other proteins
- Forms multimeric complexes with several growth promoting proteins involved in enhanced cell growth
- Coordinates expression of multiple tyrosine kinases and other enzymes (ERBB2, GRB2, MAPK3, PKC, PI3K and FAK2)



CRKL is a protein kinase that activates the RAS and JUN kinase signaling pathways and transforms fibroblasts in a RAS-dependent fashion.



CRKL is involved in T-cell activation pathways:

- **Lines between red ovals denote major interactions;**
- **Green circles represent small molecule interactions**

So what?

- The *CRKL* gene within the deleted region could be responsible for the onset of some clinical findings
- *CRKL* gene encodes a protein kinase which activates the *RAS* and *JUN* kinase signaling pathways and transforms fibroblasts in a *RAS*-dependent fashion.

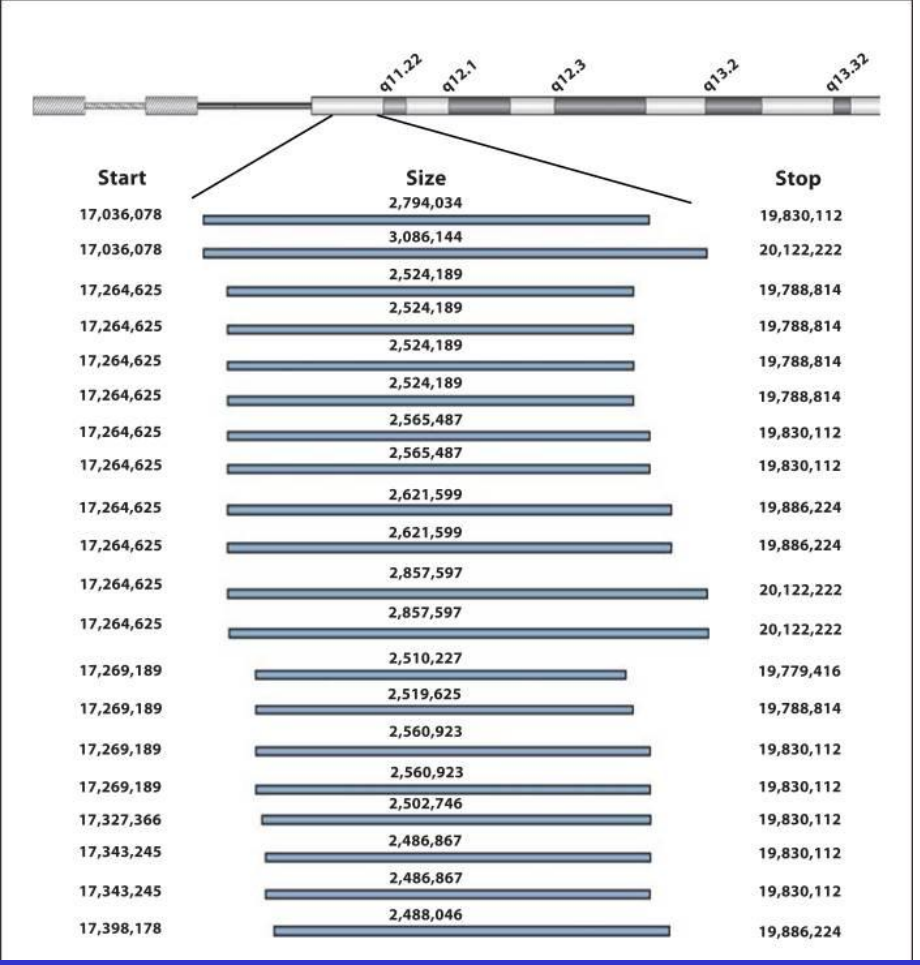
- crkl-/- mice die in utero with cranial nerve and aortic defects and 100% have VSD with overriding aortic arch, and may also have defective thymus, thyroid and parathyroid
- The heterozygote mouse (crkl+/-) only has craniofacial and/or thymic defects, but when combined with heterozygosity for tbx1, VCFS-like heart defects are seen
- Haploinsufficient effects are strain- and species-dependent; the CRKL+/- state in humans could give some of the defects seen in the crkl+/-;tbx1+/- mouse, which could be quite variable between individuals. The deletion interval we describe thus provides a tighter focus on this gene about its involvement in the underlying molecular pathogenesis leading to the clinical findings in patients who harbor the genetic defect.

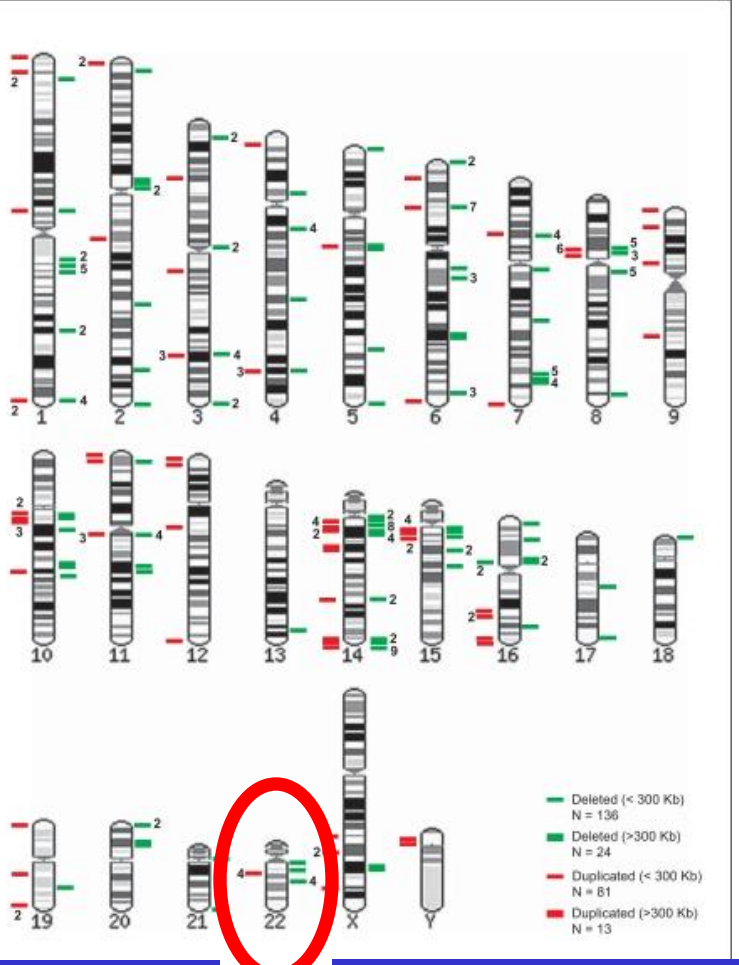
What does this type of study demonstrate?

- Nosology of these disorders: "VCFS" and "22q11.2 deletion syndrome" should not be synonymous. Patients with distal deletions that do not include the proximal 1.5 Mb do not resemble VCFS.
- Understanding genetic causes of variable VCFS phenotypes will be aided through the study of atypical deletions (such as our patients with the 508-kb (LCR22-3b & LCR22-4) deletion) – the clinical features in these patients are variable but all had neurodevelopmental problems

Variability in expression of traits: Why?

- The role of gene dosage within the 22q11.2 region
- Variants (SNPs or polymorphisms) the genes in the 22q11.2 region on the non-deleted chromosome
- Downstream and upstream interaction between genes in the deleted region as well as other genes (e.g. TBX1, with VEGF, PITX, and FGF10)





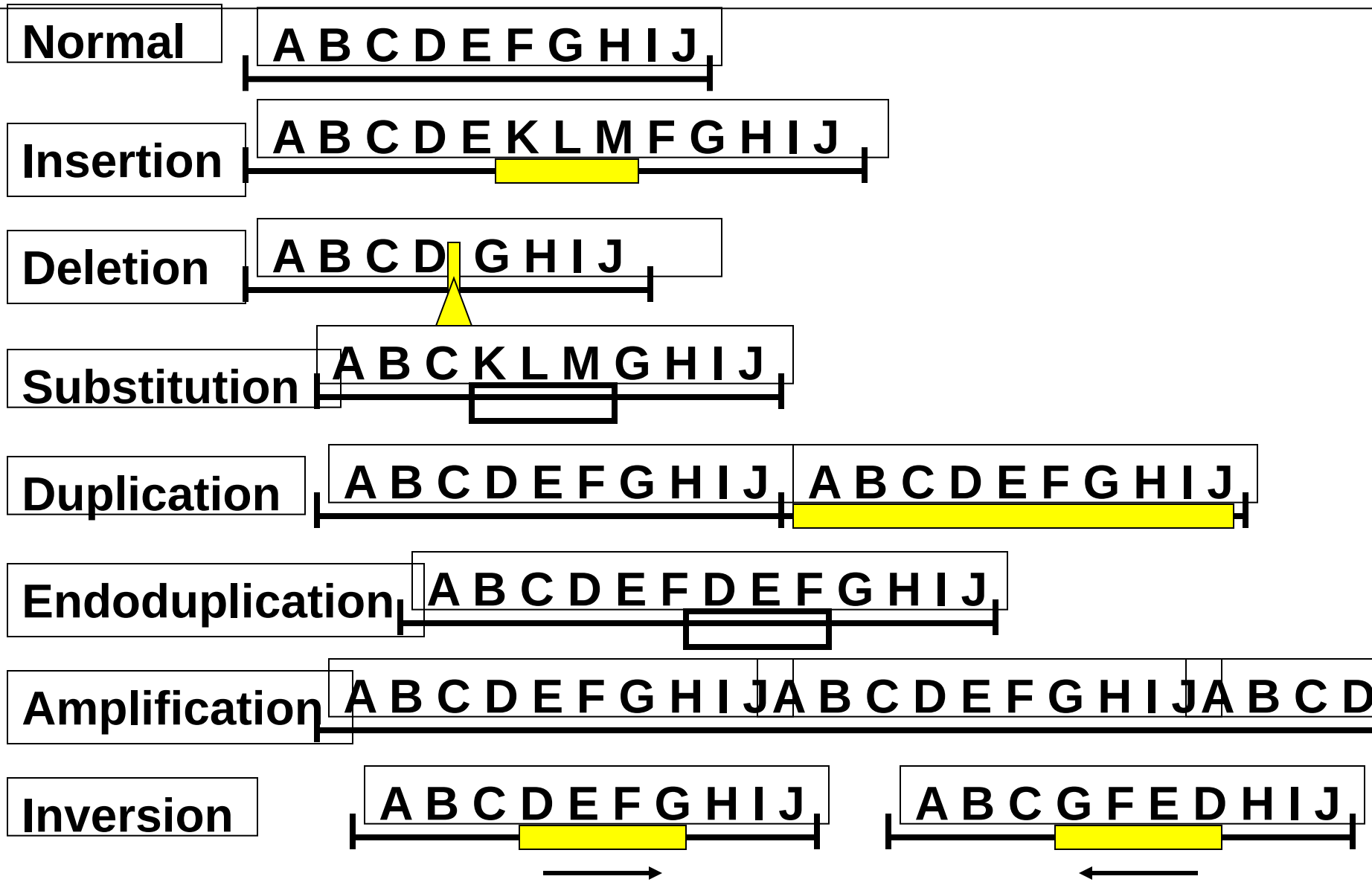
Deletions range from 2.49 to 3.09 Mb

Other CNVs?

Variable phenotype: presence or absence of genes at the proximal (e.g.DGCR6 and PRODH) or distal (e.g. GGT2 and HIC2) breakpoints depend on the size of the deletion, plus variation in the rest of the genome due to other CNVs

- 90% of persons with VCFS have a deletion that spans ~ 3 Mb (with much variability)
- At each end of this typical deletion are identical sets of low copy repeats (LCRs). The presence of these two sets of LCRs coinciding with the breakpoints for the deletion directed researchers to discover the mechanism of the deletion to be either an inter-chromosomal recombination error during stage 1 meiosis during gametogenesis or an intra-chromosomal event resulting in material being spliced out of one copy of the chromosome.
- The inter-chromosomal event is more common
- < 10% of persons with VCFS inherited the deletion from an affected parent

Genetic Rearrangement in Contiguous Gene Syndromes



All persons with VCFS have a chromosome 22q11.2 del,

But all persons with a chr 22q11.2 del do not have VCFS

- **Approximately 90% of affected individuals have a 3 Mb deletion with identical breakpoints at each end.**
- **<10% of individuals with VCFS have smaller nested deletions with the same proximal breakpoint, but a distal breakpoint that encompasses only 1.5 Mb**
- **It has been reported that there is no difference in the clinical expression between the 3 Mb and 1.5 Mb deletion, but it may be that subtle differences do exist that have not been studied sufficiently because of the expansiveness of the phenotype and the number of genes in the deletion**

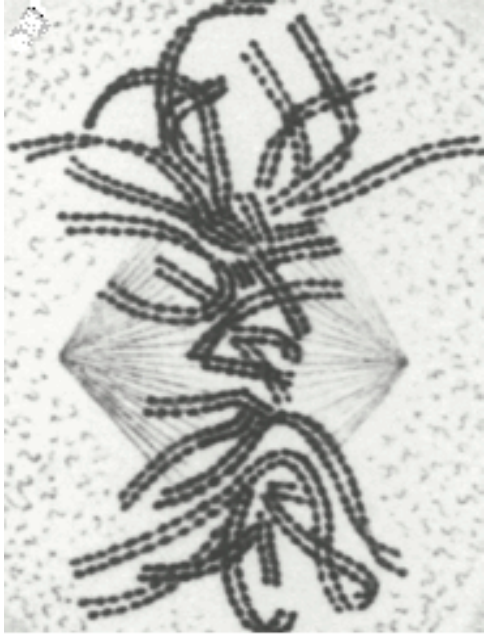
Changes in Cytogenetic Technology over Decades

Category	1960s	1970s	1980s	1990s	2000s
Technology	Prebanding	Banding	High-resolution banding	FISH *	aCGH
Resolution	10-20 Mb	5-10 Mb	3-5 Mb	100 kb	50-100 kb
Known **phenotype** to have a specific **genotypic** basis	Down syndrome Turner syndrome		Prader-Willi Miller-Dieker **VCFS**	Williams syndrome	
Known **genotype** to have a specific **phenotypic** basis	Trisomy 18 Trisomy 13 5p- 4p-	WAGR Jacobsen	Smith-Magenis	1p36 22q.13	17q21.3

* FISH has a resolution of ~100 kb but is limited to a targeted approach involving individual genomic clones, as compared with the genomewide assessment achievable through aCGH (equivalent to thousands of FISH probes)

Modified from Ledbetter DH. N Engl J Med 2008;359:1728-30

Evolution of Human Cytogenetics

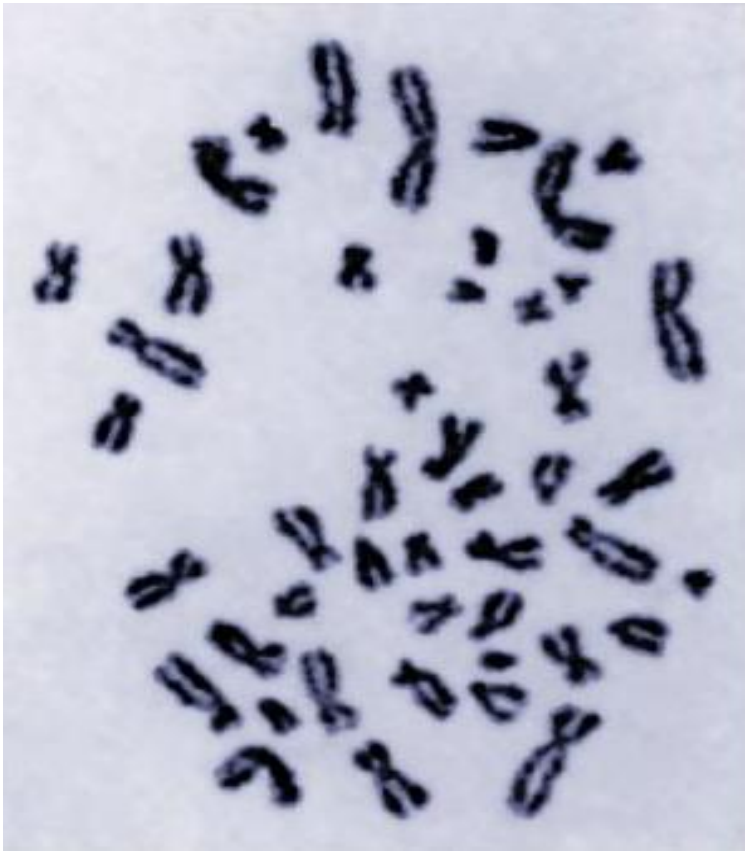


Walther Flemming 1882

Theophilus Painter 1921

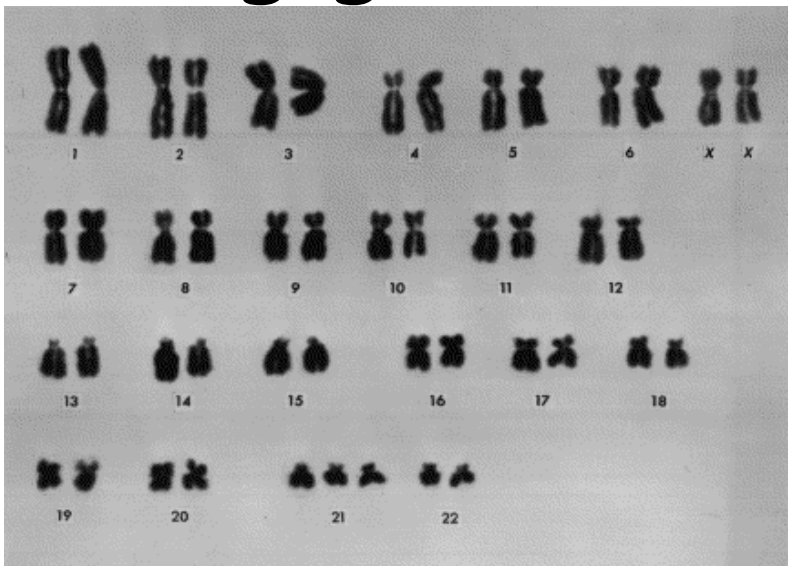
Birth of Human Cytogenetics

Joe Hin Tjio (1919–2001)

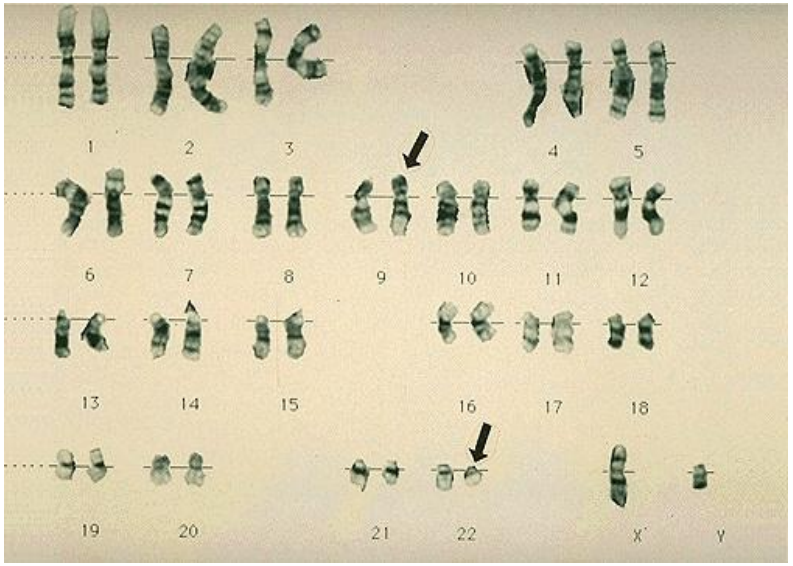


1956

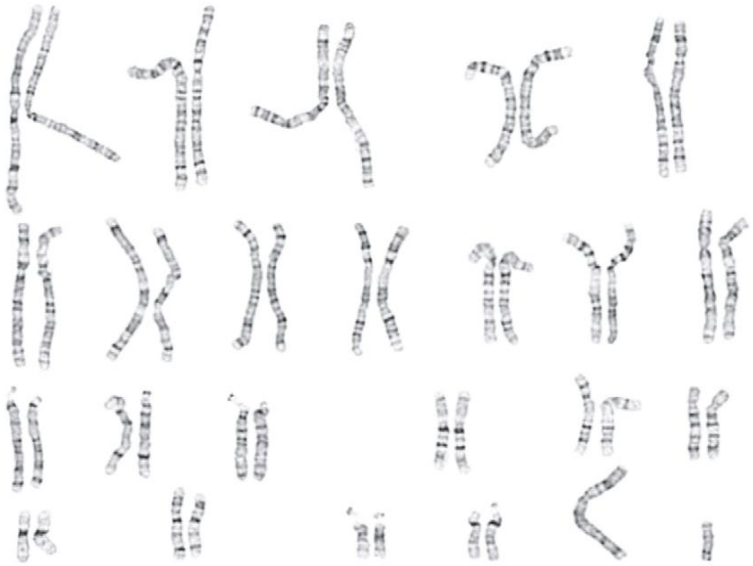
Scanning genome based on chromosomes



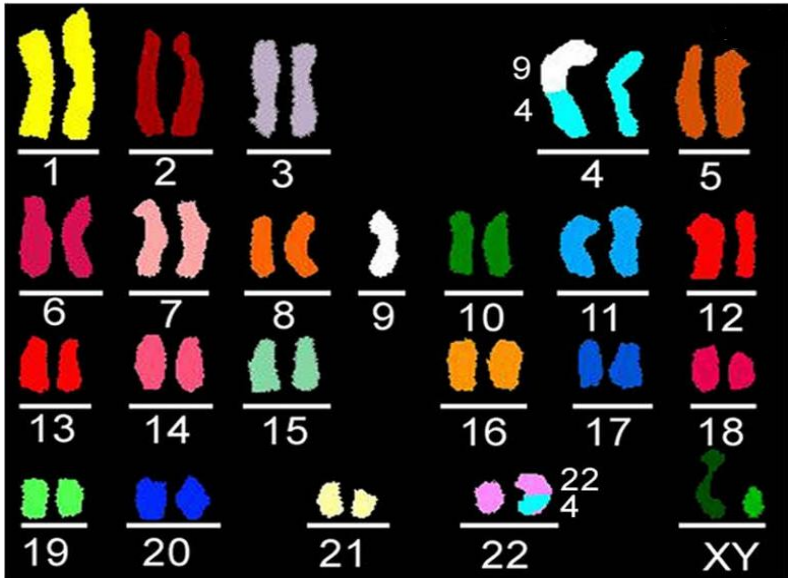
1959



1972

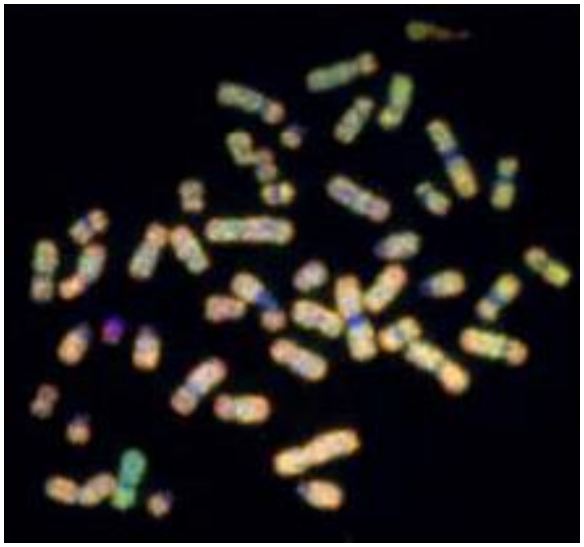


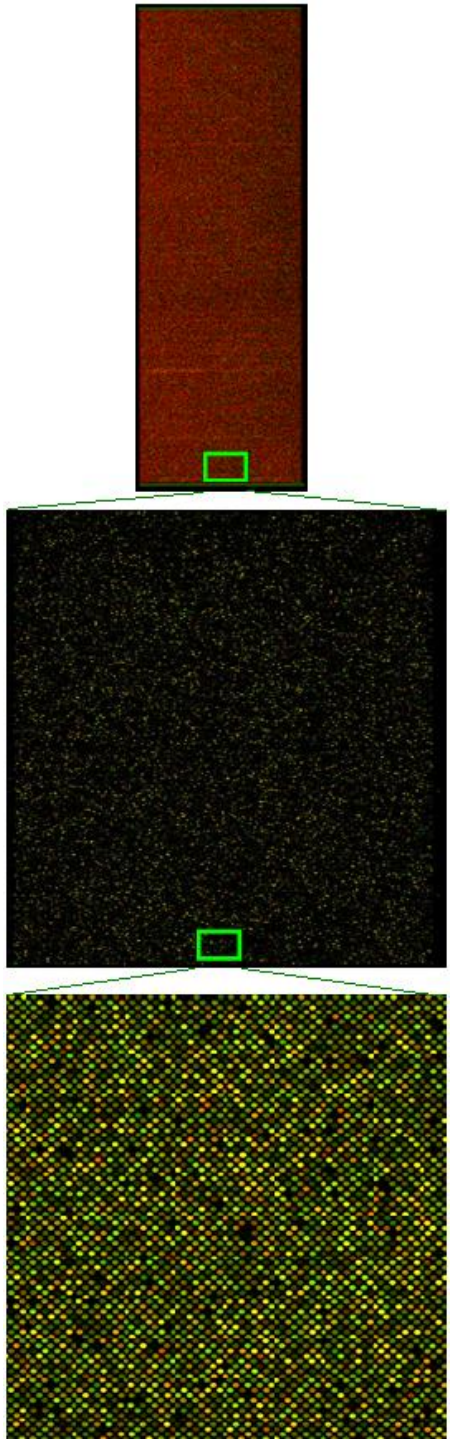
1978

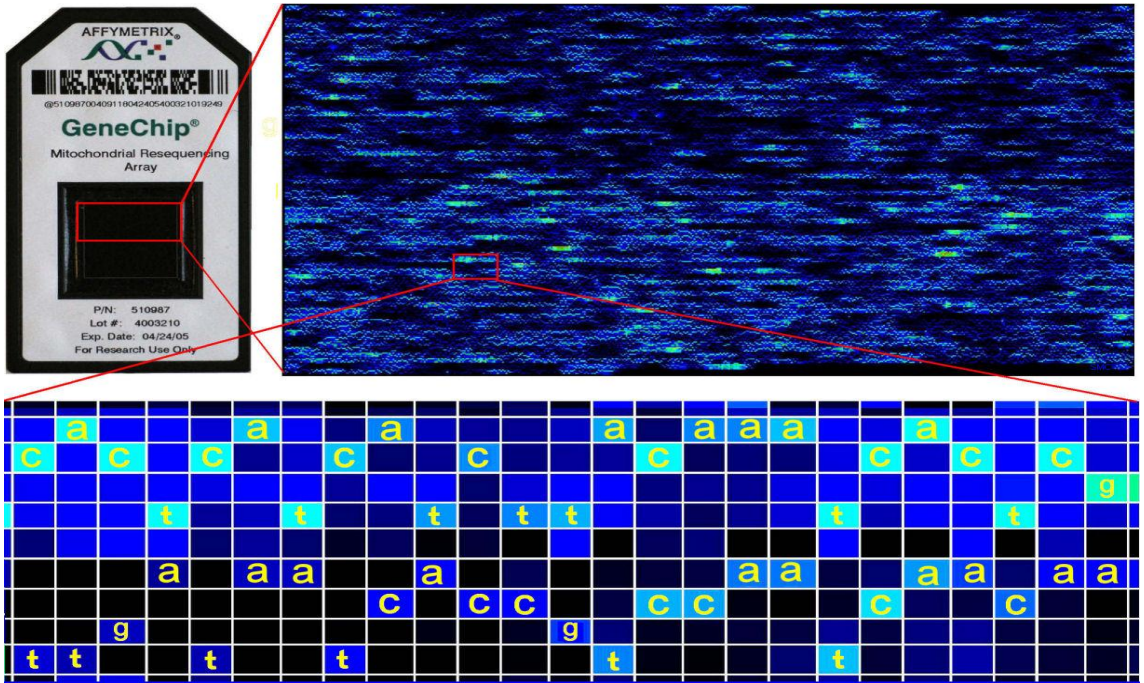


1996

CGH and aCGH: from low to high resolution to array-sequencing







Fruit

Apples

Grannies

Schizophrenia

Genes associated with schizophrenia

COMT deficiency in VCFS

Answering the "what" and "why" questions through advanced technology