Novel submicroscopic chromosomal abnormalities: challenges for clinical management and surveillance

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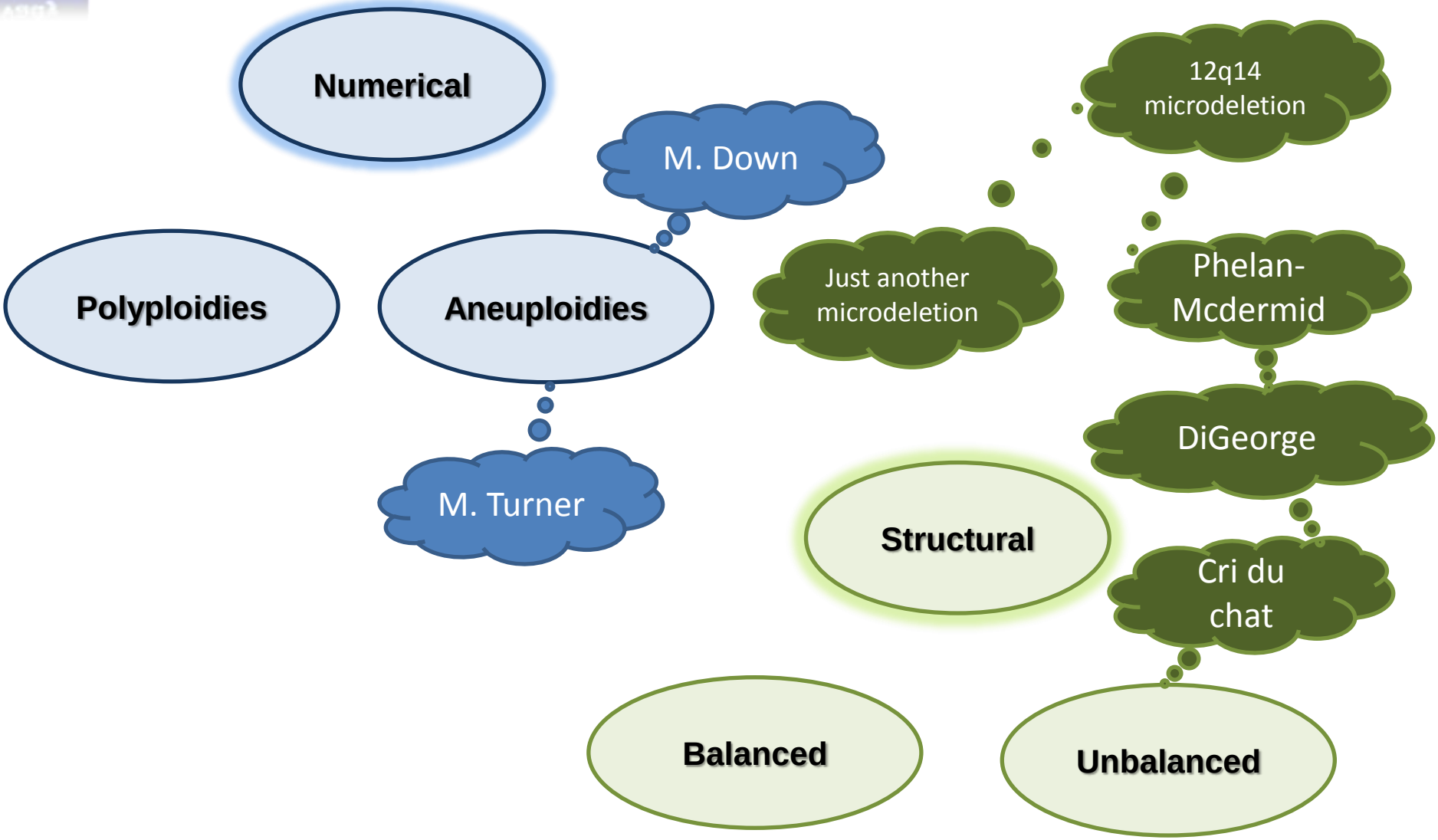
http://www.vrozene-vady.cz/

Vrozené vady

Chromosomal aberrations

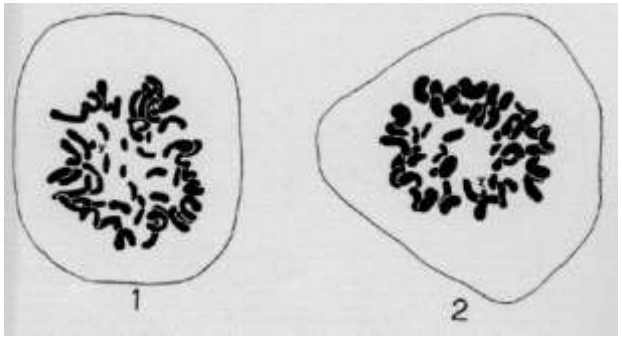


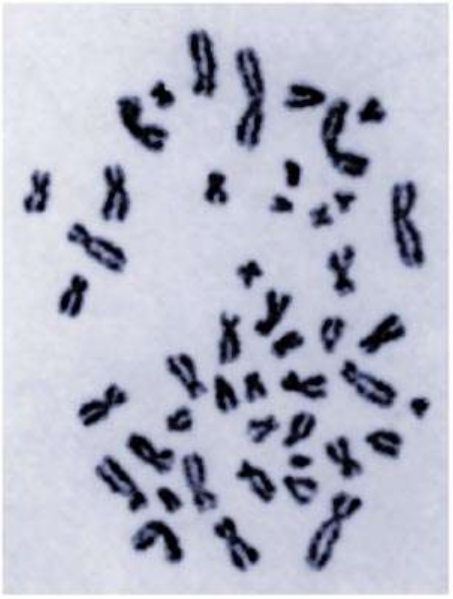
Vrozené vady

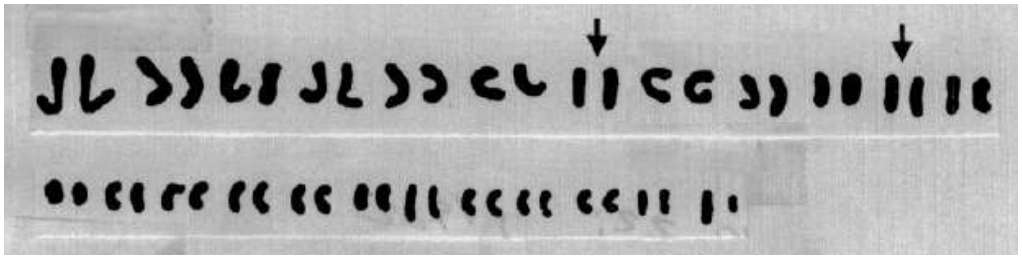
Chromosomes

23 chromosome pairs

19th century







1956

Tjio JH, Levan A. The chromosome number of man. Hereditas vol. 42: pages 1–6, 1956

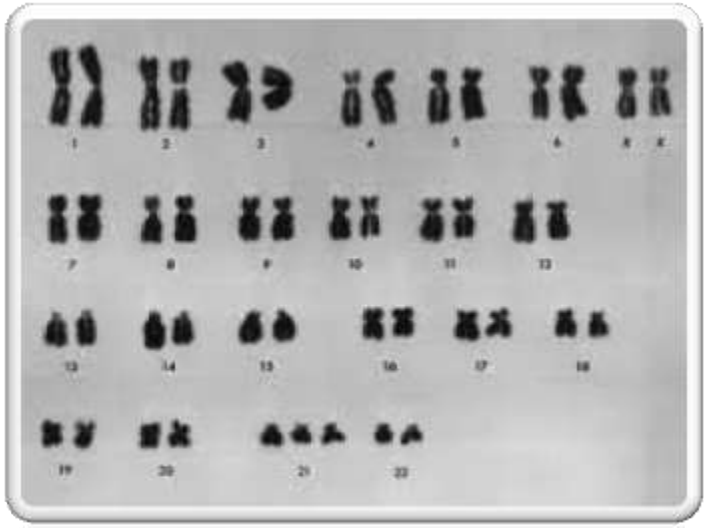
Down syndrome

John Langdon Down – 1866

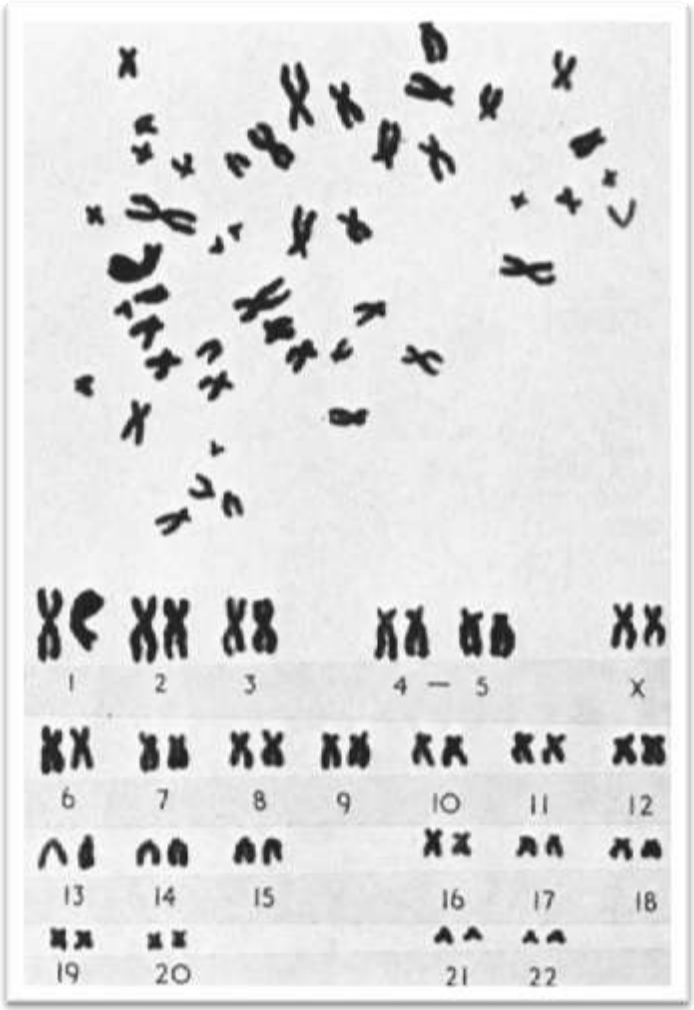
Observations on an Ethnic Classification of Idiots

Jérôme Lejeune – 1959

Etude des chromosomes somatiques de neuf enfants mongoliens



Cri du chat syndrome



Jérôme Lejeune – 1963

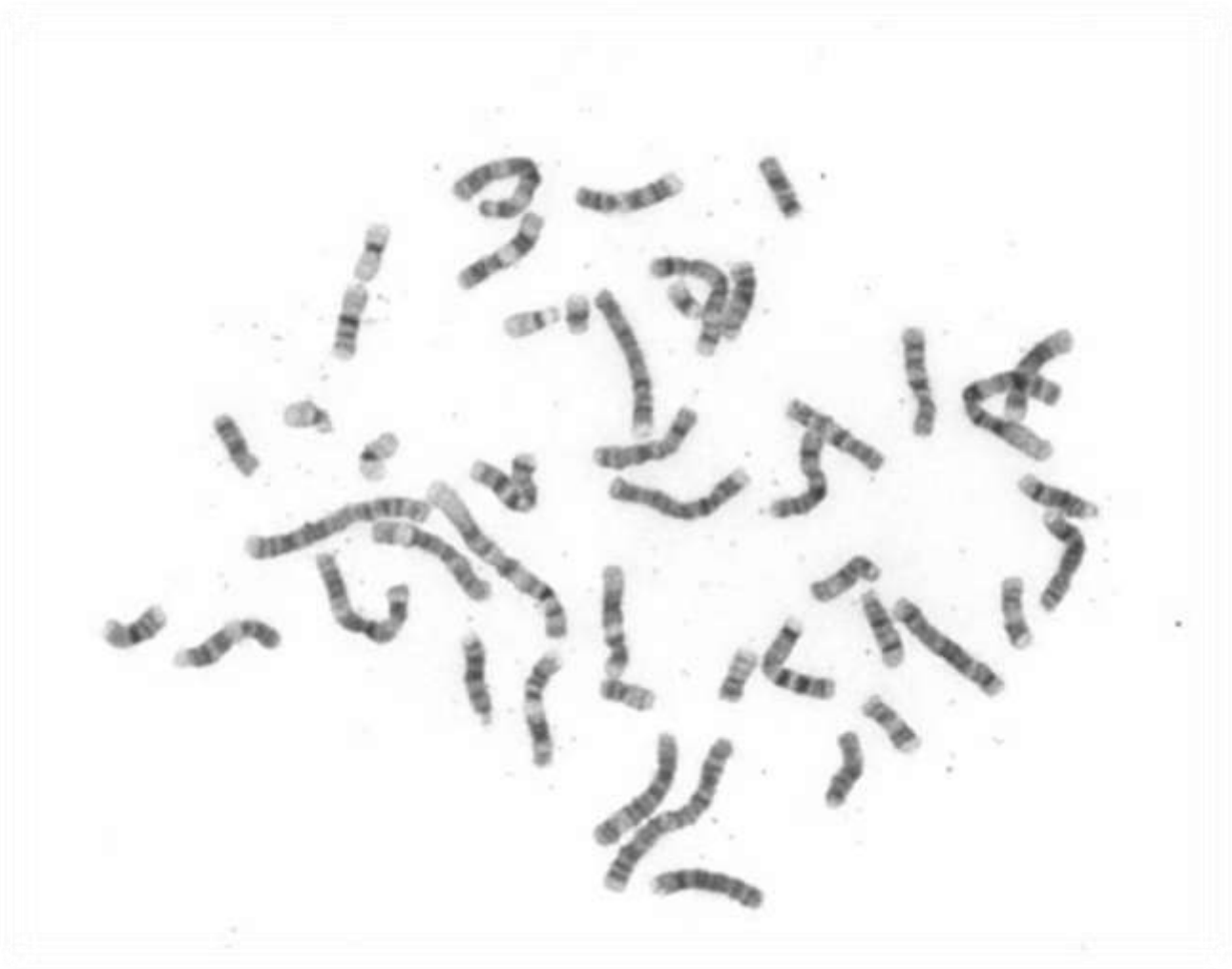
3 Cases of partial deletion of the short arm of chromosome 5

Vrozené vady

Classical staining

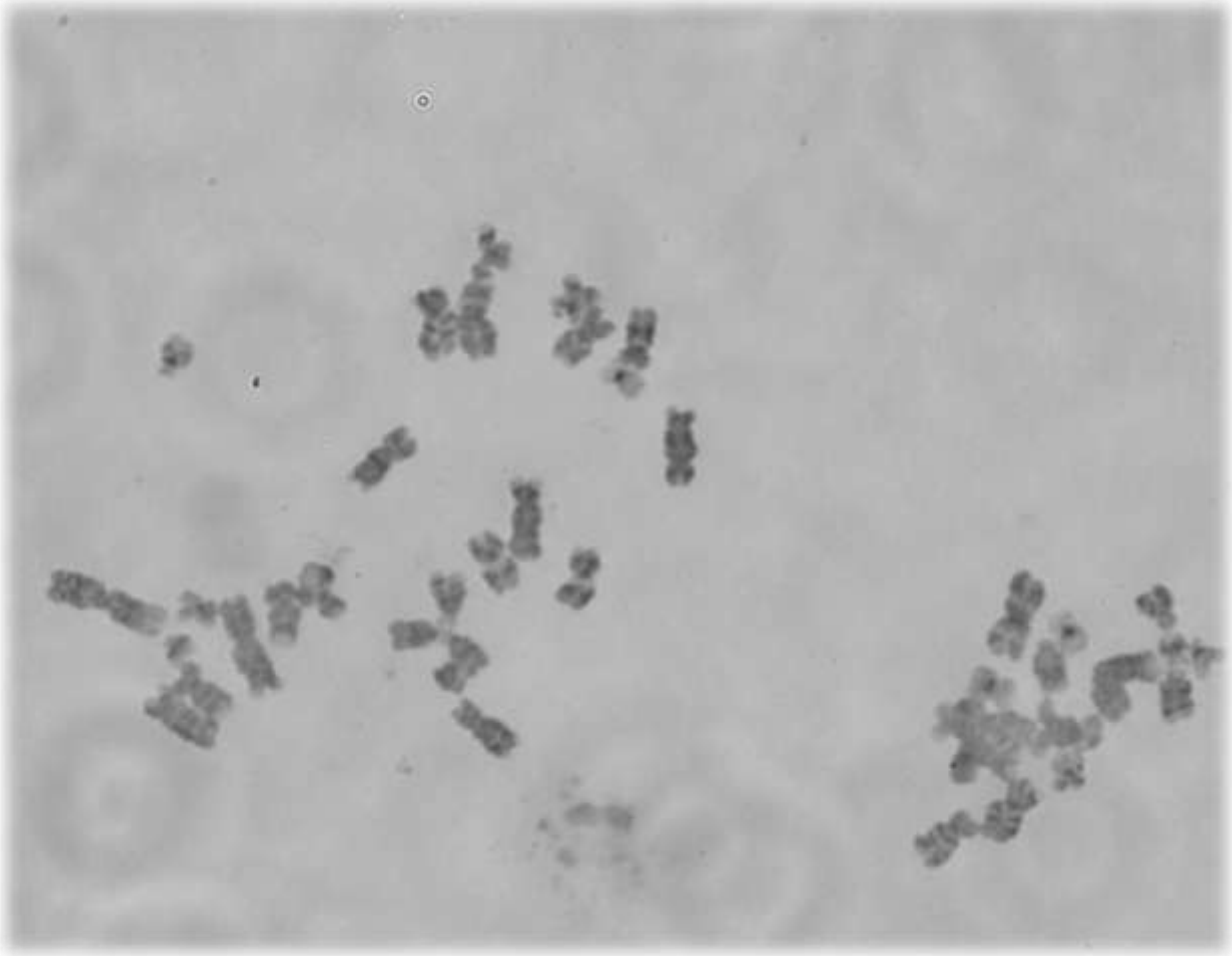
Vrozené vady

G-banding



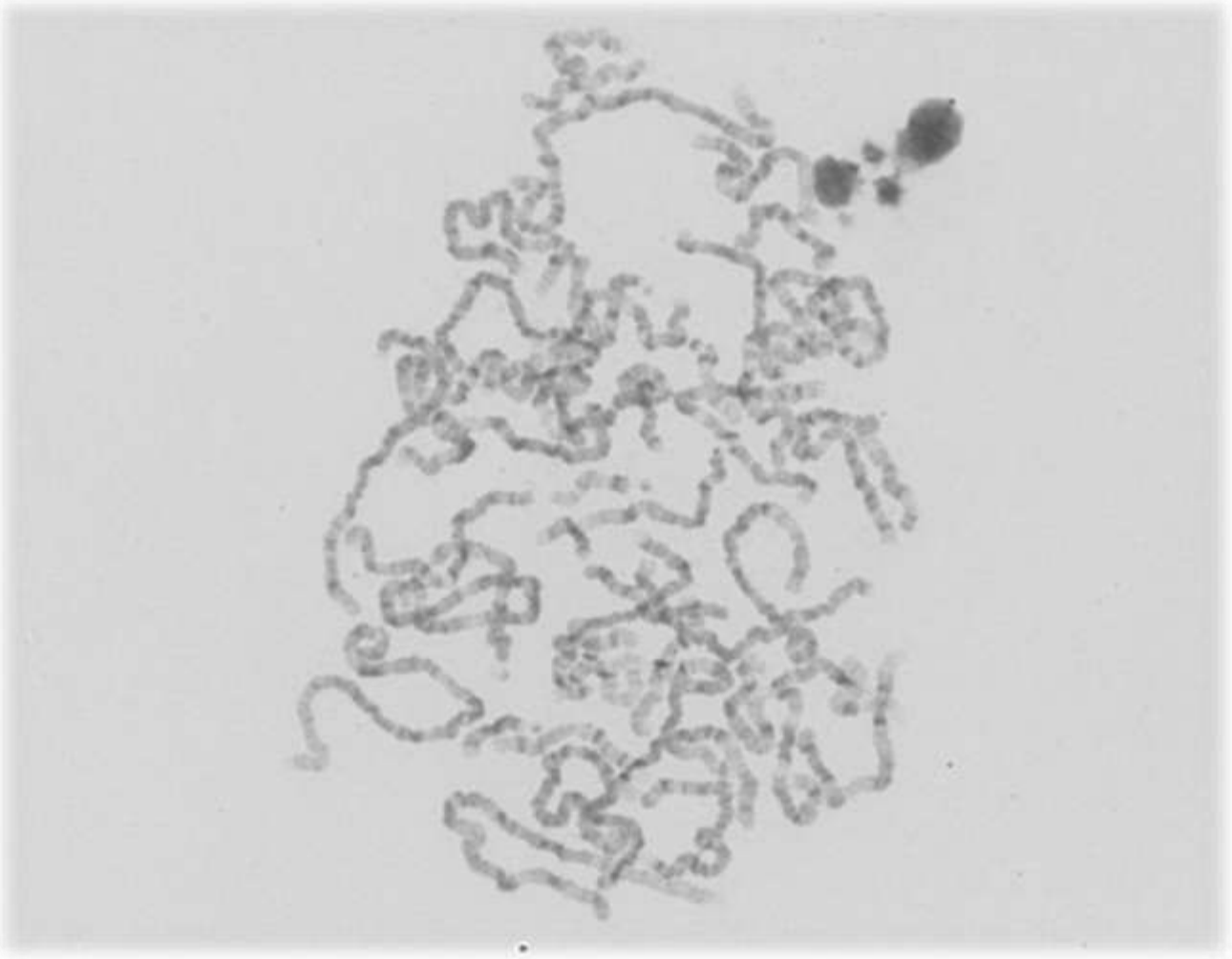
Vrozené vady

G-banding (CVS)



Vrozené vady

HRT



Vrozené vady

FISH

Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 4381–4385, July 1982
Genetics

Immunological method for mapping genes on *Drosophila* polytene chromosomes

(biotin-labeled DNA/anti-biotin/fluorescence microscopy/immunoperoxidase localization)

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Communicated by Alan Garen, April 5, 1982

ABSTRACT A method is described for localizing DNA sequences hybridized *in situ* to *Drosophila* polytene chromosomes. This procedure utilizes a biotin-labeled analog of TTP that can be incorporated enzymatically into DNA probes by nick-translation. After hybridization *in situ*, the biotin molecules in the probe serve as antigens which bind affinity-purified rabbit anti-biotin antibodies. The site of hybridization is then detected either fluorimetrically, by using fluorescein-labeled goat anti-rabbit IgG, or cytochemically, by using an anti-rabbit IgG antibody conjugated to horseradish peroxidase. When combined with Giemsa staining, the immunoperoxidase detection method provides a permanent record that is suitable for detailed cytogenetic analysis. This immunological approach offers four advantages over conventional autoradiographic procedures for detecting *in situ* hybrids: (*i*) the time required to determine the site of hybridization is decreased markedly; (*ii*) biotin-labeled probes are chemically stable and give reproducible results for many months; (*iii*) biotin-labeled probes appear to produce less background noise than do radiolabeled probes; and (*iv*) the resolving power is equal to and often greater than that achieved autoradiographically.

In situ hybridization, initially developed by Gall and Pardue (1) and John *et al.* (2), has proven to be a valuable method for determining the cellular or chromosomal location of hybridized nucleic acids (3–10). Standard *in situ* hybridization protocols use radiolabeled RNA or DNA probes and autoradiographic methods of detection or quantification. By using probes of high specific activity under conditions such that hybridization "saturates" are formed (8–12), it is now possible to localize unique sequences in mammalian chromosome spreads after autoradiographic exposures of 5–22 days (9, 10). However, the inherent drawbacks of radiolabeled probes—notably chemical lability due to radiolytic decomposition, concern for personnel safety, and disposal problems—make it desirable to have sensitive methods for detecting polynucleotide sequences that do not rely on the use of radioisotopes, especially for routine applications in clinical medicine.

Several groups have attempted to develop such procedures. Cheung *et al.* (13) generated a fluorescent signal by coupling latex microspheres containing both poly(U) and dansylated fluorochromes to a polyadenylated mRNA probe. Fluorescent signals were also produced by Rudkin and Stollar (14) by using antibodies against DNA·RNA hybrids in conjunction with an immunofluorescent antibody sandwich and by Bauman *et al.* (15–17) who used RNAs that were labeled at the 3′ end with fluorescein or rhodamine. Davidson and associates (18–20) chemically crosslinked biotin to RNA with cytochrome *c* or polyamine bridges and used these RNA–biotin complexes as hybridization probes. The sites of hybridization were visualized in the electron microscope through the binding of avidin-ferritin or avidin-methacrylate spheres. Wu and Davidson (21) recently described an additional method for gene mapping on *Drosophila* polytene chromosomes by using the electron microscope. Colloidal gold spheres were coated with protein and poly(dT)-tailed heterologous DNA and used to identify the hybridization sites of poly(dA)-tailed *Drosophila* DNA probes. Although each of these approaches was at least partially successful, a simpler and more general method for detecting nonradioactively labeled DNA or RNA probes would be desirable.

The specificity and tenacity of the biotin–avidin interaction (22) makes biotin an attractive candidate as an affinity reagent for tagging nucleic acids. We recently reported the synthesis of dUTP and UTP analogs that contain a biotin molecule covalently attached to the C-5 position of the pyrimidine ring through an allylamine linker arm and demonstrated that these nucleotides can function as efficient substrates for various DNA or RNA polymerases *in vitro* (23). In addition, biotin-substituted polynucleotides were shown to have denaturation and reassociation characteristics that were comparable with their use as hybridization probes (23).

In this report, we describe the first stages in the development of a generalized method for *in situ* hybridization based on biotinized polynucleotides as specifically applied to *Drosophila* polytene chromosomes. A preliminary account of this work was presented elsewhere (24).

MATERIALS AND METHODS

Standard nucleoside 5′-triphosphates were obtained from P-L Biochemicals. Radiolabeled compounds were products of New England Nuclear or Amersham. *Escherichia coli* DNA polymerase I was purchased from Boehringer Mannheim. Egg white avidin, biotin, ovalbumin, diaminobenzidine, and Hunker–Yates reagent were obtained from Sigma. Fluorescein-labeled goat anti-rabbit IgG (FITC-GaRIgG) and goat anti-rabbit IgG conjugated to horseradish peroxidase were purchased from Miles-Yeda. Peroxidase-conjugated rabbit anti-goat IgG was a product of Polysciences (Warrington, PA). Peroxidase-conjugated sheep anti-rabbit IgG was the gift of P. Nakane.

Five of the cloned DNAs used in these studies (ADm 117, ADm 221, ADm 60, ADm 104, and ADm 88) encode genes that are specifically expressed in the fat bodies of third-instar larvae (25). They were obtained from a collection of randomly sheared *Drosophila melanogaster* genomic DNA fragments inserted into the Charon 4 phage vector (26). The *Drosophila* DNA in-

Abbreviations: P_i/NaCl, phosphate-buffered saline; NaCl/Cit, standard saline citrate (0.15 M NaCl/0.015 M sodium citrate); FITC-GaRIgG, fluorescein isothiocyanate-labeled goat anti-rabbit IgG; kb, kilobase(s).

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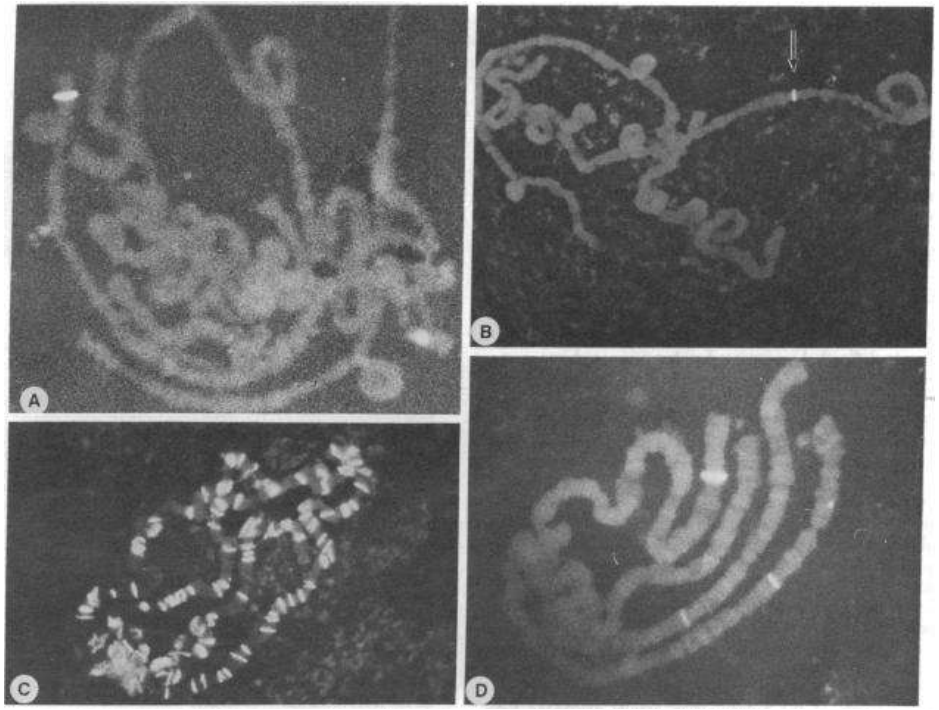
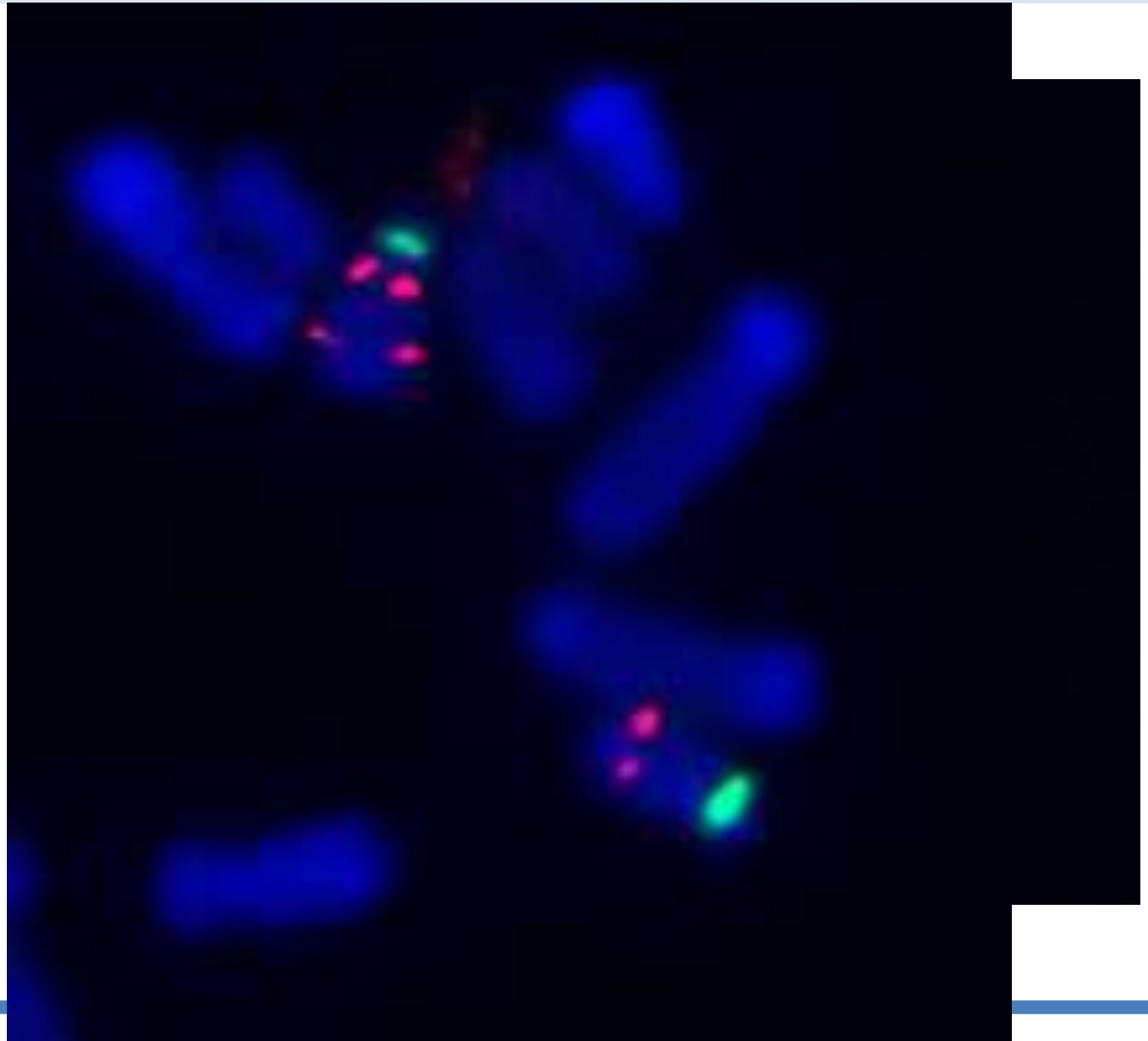
Langer-Safer, P.R., Levine, M. a Ward, D. C. (1982) Immunological method for mapping genes on Drosophila polytene chromosomes. Proc Natl Acad Sci U S A. 79(14):4381–4385.



Vrozené vady

FISH



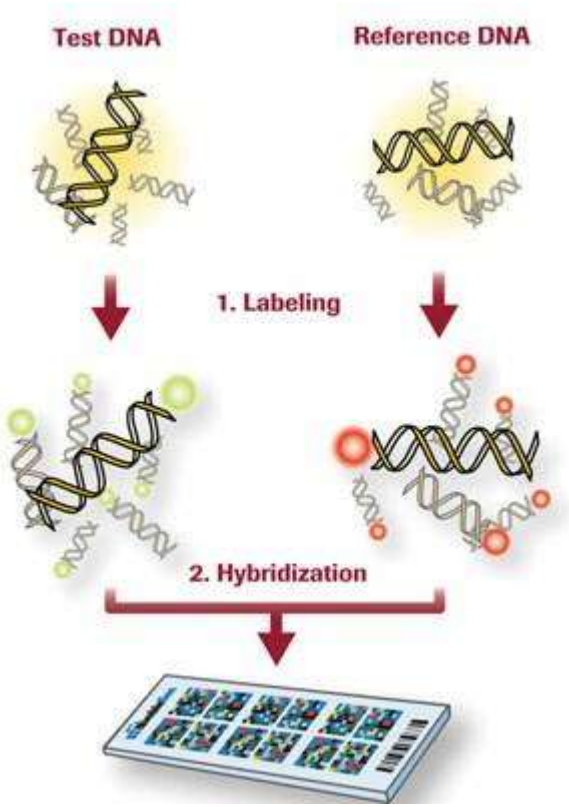
Array methods – Main features

- Whole genome examination for submicroscopic chromosomal copy number variants (deletions/duplications).
- High resolution (up to tens of kBs in gene rich areas).
- SNP-array can detect UPD or LOH.
- Troubles detecting mosaicism.
- Impossible to detect true balanced aberrations (no image of karyotype).
- Challenging for interpretation.

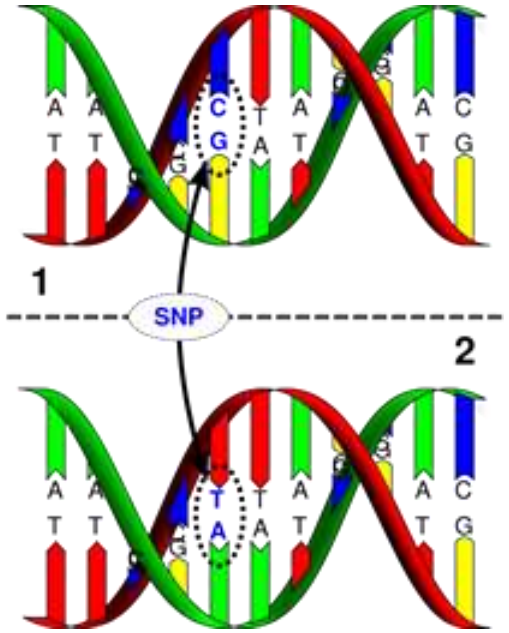
FISH vs. Array

Array Methods

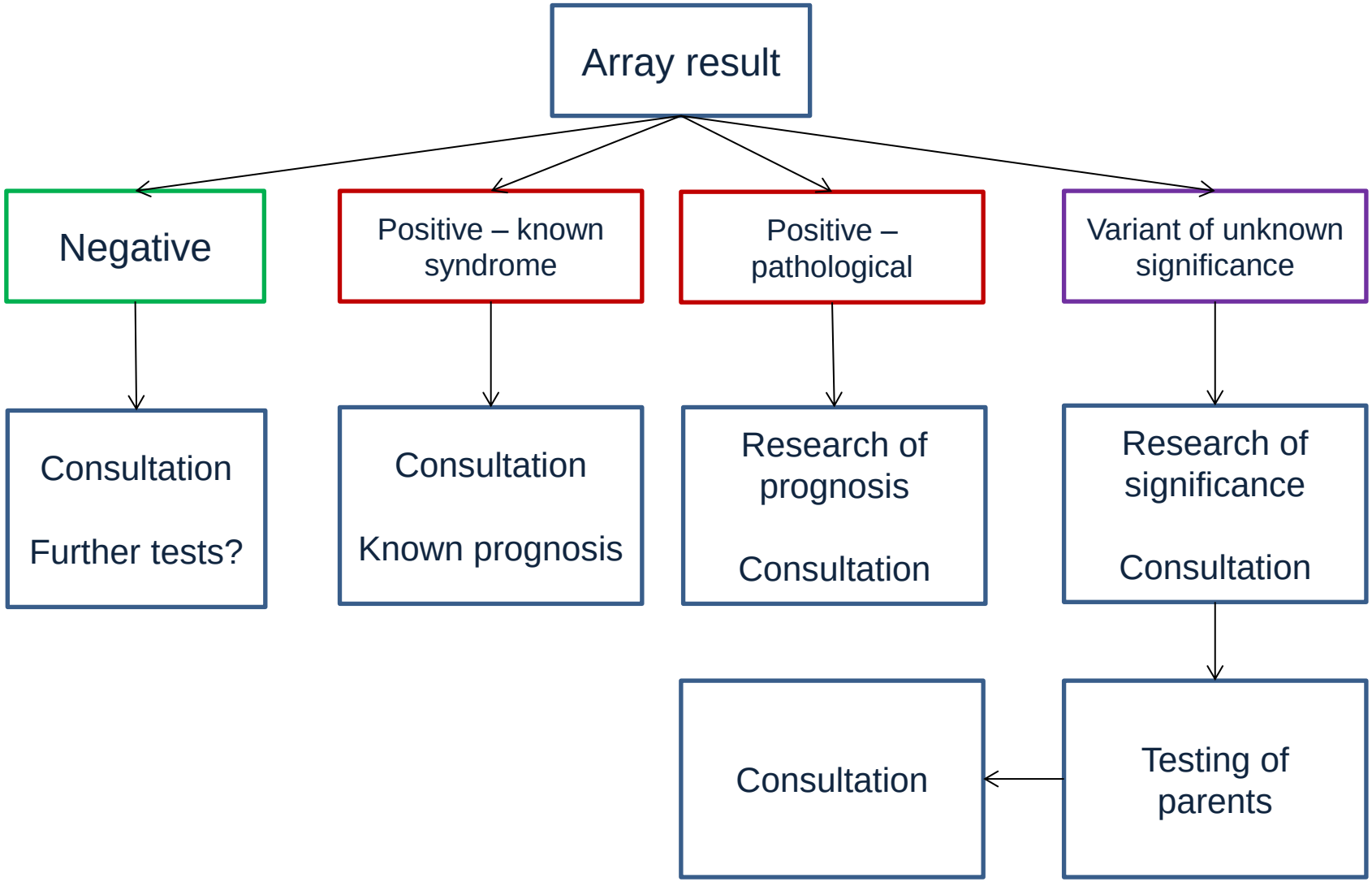
Array-CGH



SNP-Array



Strategy


Array result
Negative
Positive – known syndrome
Positive – pathological
Variant of unknown significance
Consultation
Further tests?
Consultation
Known prognosis
Research of prognosis
Consultation
Research of significance
Consultation
Consultation
Testing of parents

VUS – Variants of unknown significance

- Submicroscopic chromosomal copy number variants.
- Not listed in lists of benign variants.
- Not listed in lists of proved pathological changes.
- Poor or none correlations in databases.
- Extremely hard for interpretation.
- „unexpected result“
- Testing of relatives (parents) „can“ be useful.

VUS – Variants of unknown significance

Questions:

Does that cause the impariment?

What shall we tell the parents?

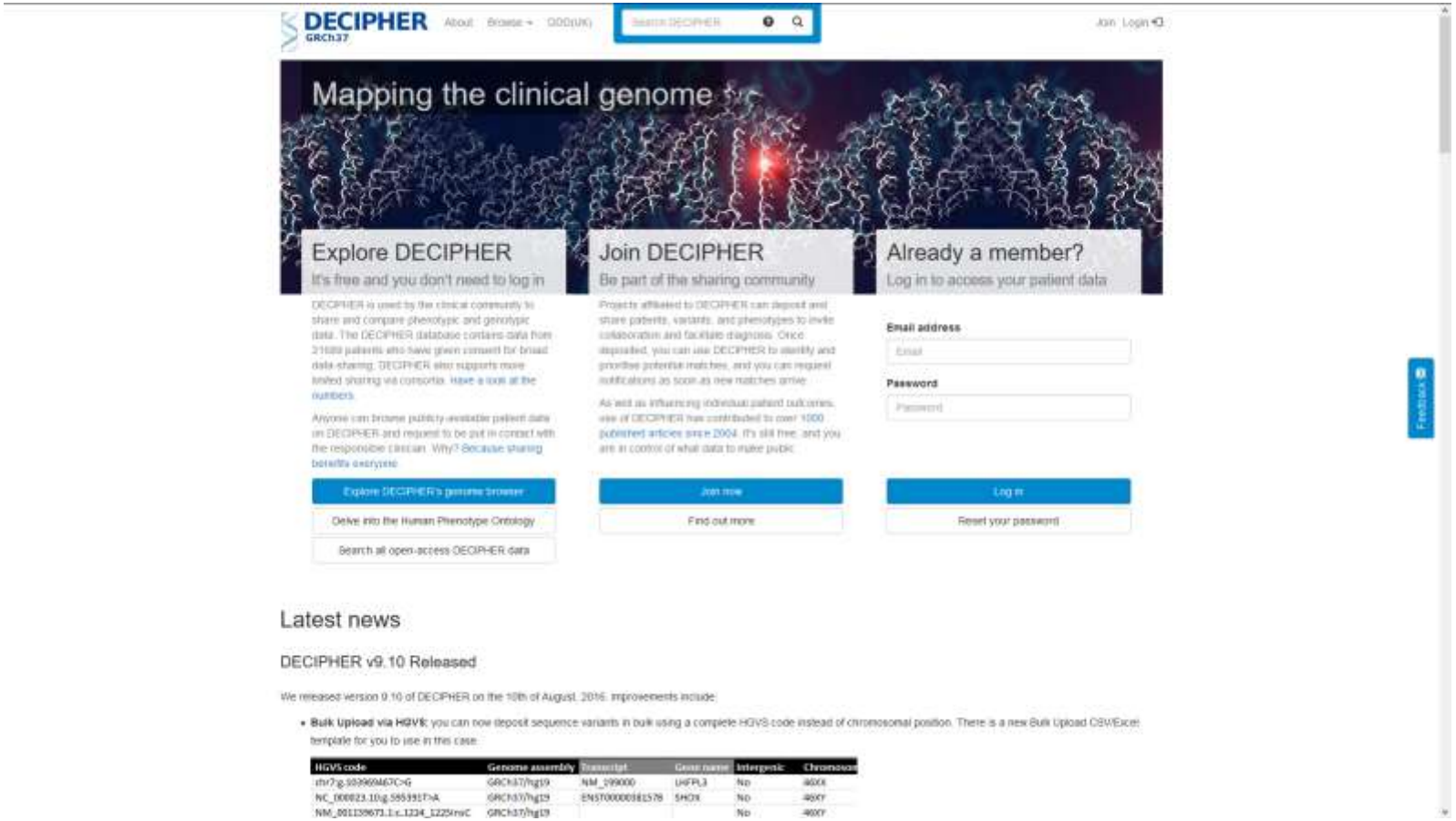
They want another child! So what shall we tell them!?

How to code that case?

Databases - ECARUCA

http://umcecaruca01.extern.umcn.nl:8080/ecaruca/ecaruca.jsp

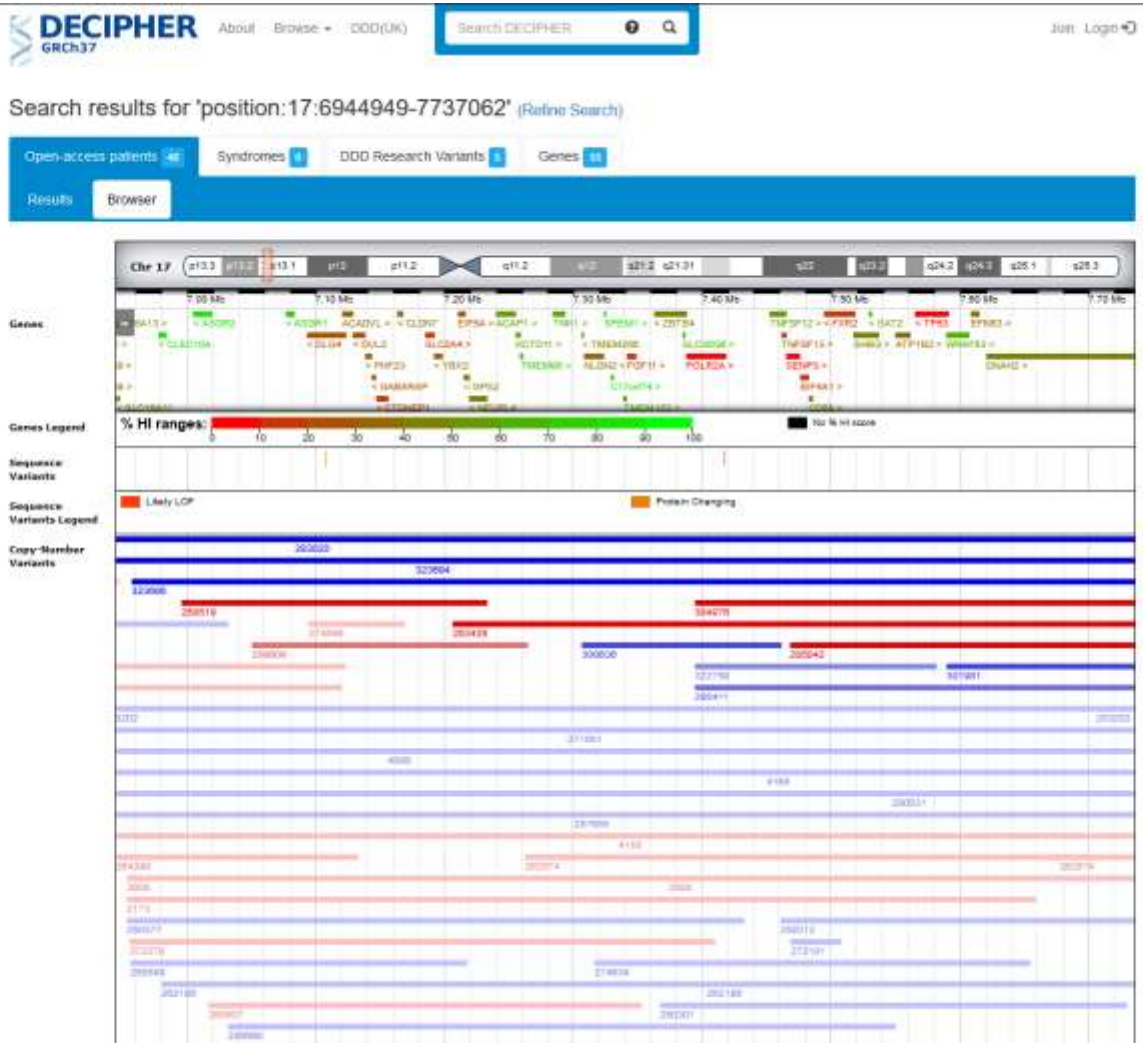
Databases - DECIPHER



https://decipher.sanger.ac.uk/

Vrozené vady

Databases - DECIPHER



Databases - DECIPHER

Search results: 1 to 48 of 48

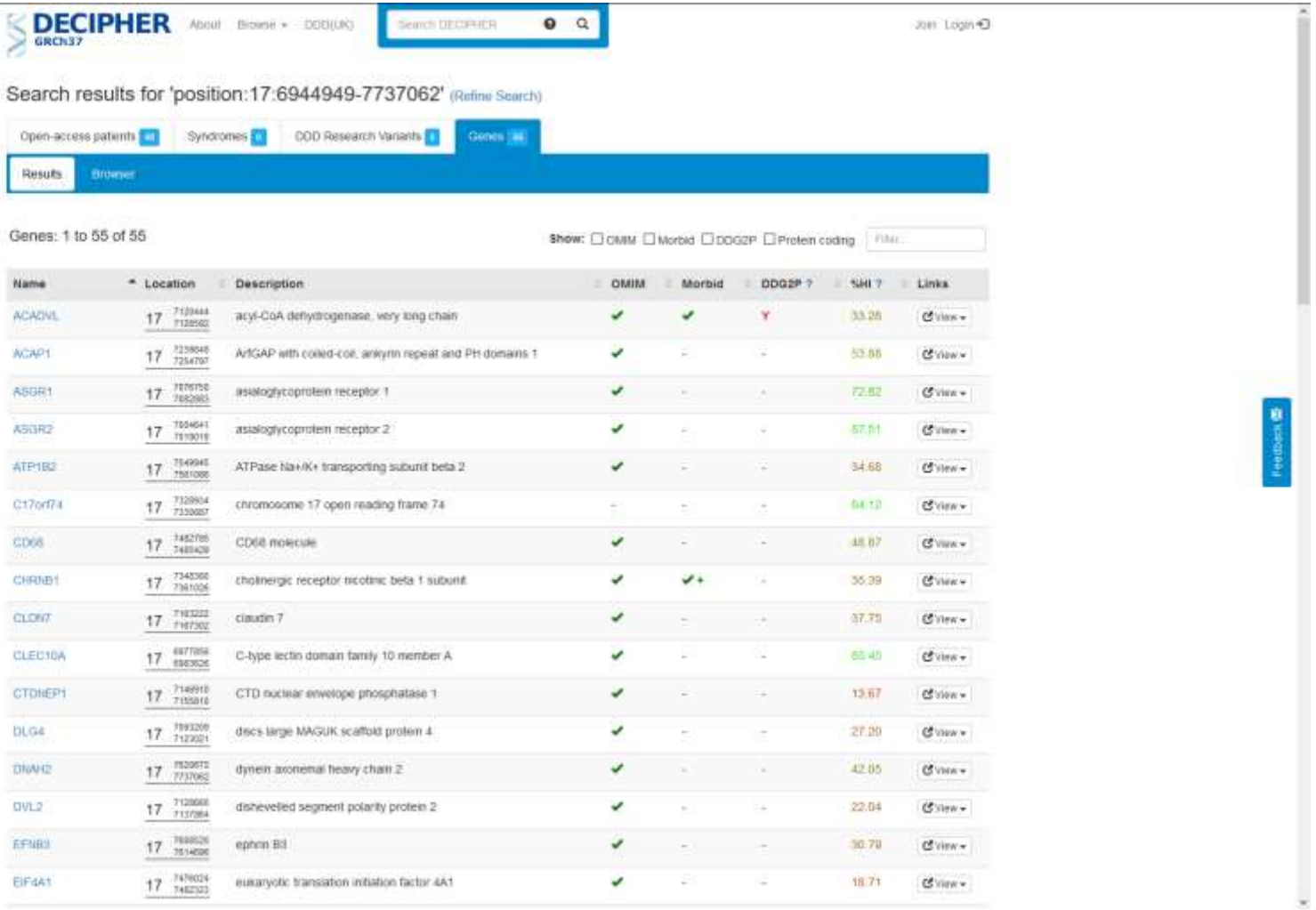
Show: All

Filter...

DECIPHER ID	Variant	Sex	Size	Pathogenicity Contribution ?	Inheritance	Phenotype(s)	Patient Open-Access Variants	Contact
2009	17 6953276 7793076 loss	46XY	839.80 kb	Unknown	De novo constitutive	Cerebral atrophy, Delayed speech and language development, Hydrocele testis, Intellectual disability, Micropenis, Plagiocephaly, Spina bifida occulta	2	
2055	17 7081062 7282862 gain	46XY	201.80 kb	Unknown	Inherited from normal parent	Intellectual disability	91	
2173	17 6953276 7658354 loss	46XY	705.08 kb	Unknown	De novo constitutive	Intellectual disability	1	
2203	17 3294806 7122528 loss	46XY	3.83 Mb	Unknown	De novo constitutive	Delayed speech and language development, Flexion contracture, Intellectual disability, Muscle weakness, Myopia, Narrow forehead, Strabismus	2	
2346	17 7113664 7400253 deletion	46XX	286.59 kb	Unknown	De novo constitutive	Intellectual disability, Leukodystrophy, Muscular hypotonia, Nystagmus, Scoliosis, Seizures	1	
2857	17 46285 7031638 gain	other	6.99 Mb	Unknown	De novo constitutive		1	
3474	17 7050325 7268888 deletion	46XX	218.56 kb	Unknown	De novo constitutive	Arachnodactyly, Delayed speech and language development, Intellectual disability, Joint laxity, Microcephaly, Micrognathia, Prominent nasal bridge, Triangular face	1	
4155	17 6840241 7828868 deletion	46XX	988.63 kb	Unknown	De novo constitutive	Broad forehead, Cerebral atrophy, Convex nasal ridge, Delayed speech and language development, Feeding difficulties in infancy, Hypertelorism, Intellectual disability, Joint laxity, Low-set ears, Microcephaly, Multiple joint dislocation, Myopia, Short hard palate, Wide nasal bridge	1	

Vrozené vady

Databases - DECIPHER



Challenges

International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10)-WHO Version for ;2016

Chapter XVII
Congenital malformations, deformations and chromosomal abnormalities
(Q00-Q99)

Chromosomal abnormalities, not elsewhere classified
(Q90-Q99)

Q90	**Down syndrome**
Q90.0	**Trisomy 21, meiotic nondisjunction**
Q90.1	**Trisomy 21, mosaicism (mitotic nondisjunction)**
Q90.2	**Trisomy 21, translocation**
Q90.9	**Down syndrome, unspecified** Trisomy 21 NOS

Q91	**Edwards syndrome and Patau syndrome**
Q91.0	**Trisomy 18, meiotic nondisjunction**
Q91.1	**Trisomy 18, mosaicism (mitotic nondisjunction)**
Q91.2	**Trisomy 18, translocation**
Q91.3	**Edwards syndrome, unspecified**
Q91.4	**Trisomy 13, meiotic nondisjunction**
Q91.5	**Trisomy 13, mosaicism (mitotic nondisjunction)**
Q91.6	**Trisomy 13, translocation**
Q91.7	**Patau syndrome, unspecified**

Challenges

Q92 **Other trisomies and partial trisomies of the autosomes, not elsewhere classified**

Incl.: unbalanced translocations and insertions

Excl.: trisomies of chromosomes 13, 18, 21 (Q90-Q91)

Q92.0 **Whole chromosome trisomy, meiotic nondisjunction**

Q92.1 **Whole chromosome trisomy, mosaicism (mitotic nondisjunction)**

Q92.2 **Major partial trisomy**
Whole arm or more duplicated.

Q92.3 **Minor partial trisomy**
Less than whole arm duplicated.

Q92.4 **Duplications seen only at prometaphase**

Q92.5 **Duplications with other complex rearrangements**

Q92.6 **Extra marker chromosomes**

Q92.7 **Triploidy and polyploidy**

Q92.8 **Other specified trisomies and partial trisomies of autosomes**

Q92.9 **Trisomy and partial trisomy of autosomes, unspecified**

Q93 **Monosomies and deletions from the autosomes, not elsewhere classified**

Q93.0 **Whole chromosome monosomy, meiotic nondisjunction**

Q93.1 **Whole chromosome monosomy, mosaicism (mitotic nondisjunction)**

Q93.2 **Chromosome replaced with ring or dicentric**

Q93.3 **Deletion of short arm of chromosome 4**
Wolff-Hirschorn syndrome

Q93.4 **Deletion of short arm of chromosome 5**
Cri-du-chat syndrome

Q93.5 **Other deletions of part of a chromosome**
Angelman syndrome

Q93.6 **Deletions seen only at prometaphase**

Q93.7 **Deletions with other complex rearrangements**

Q93.8 **Other deletions from the autosomes**

Q93.9 **Deletion from autosomes, unspecified**

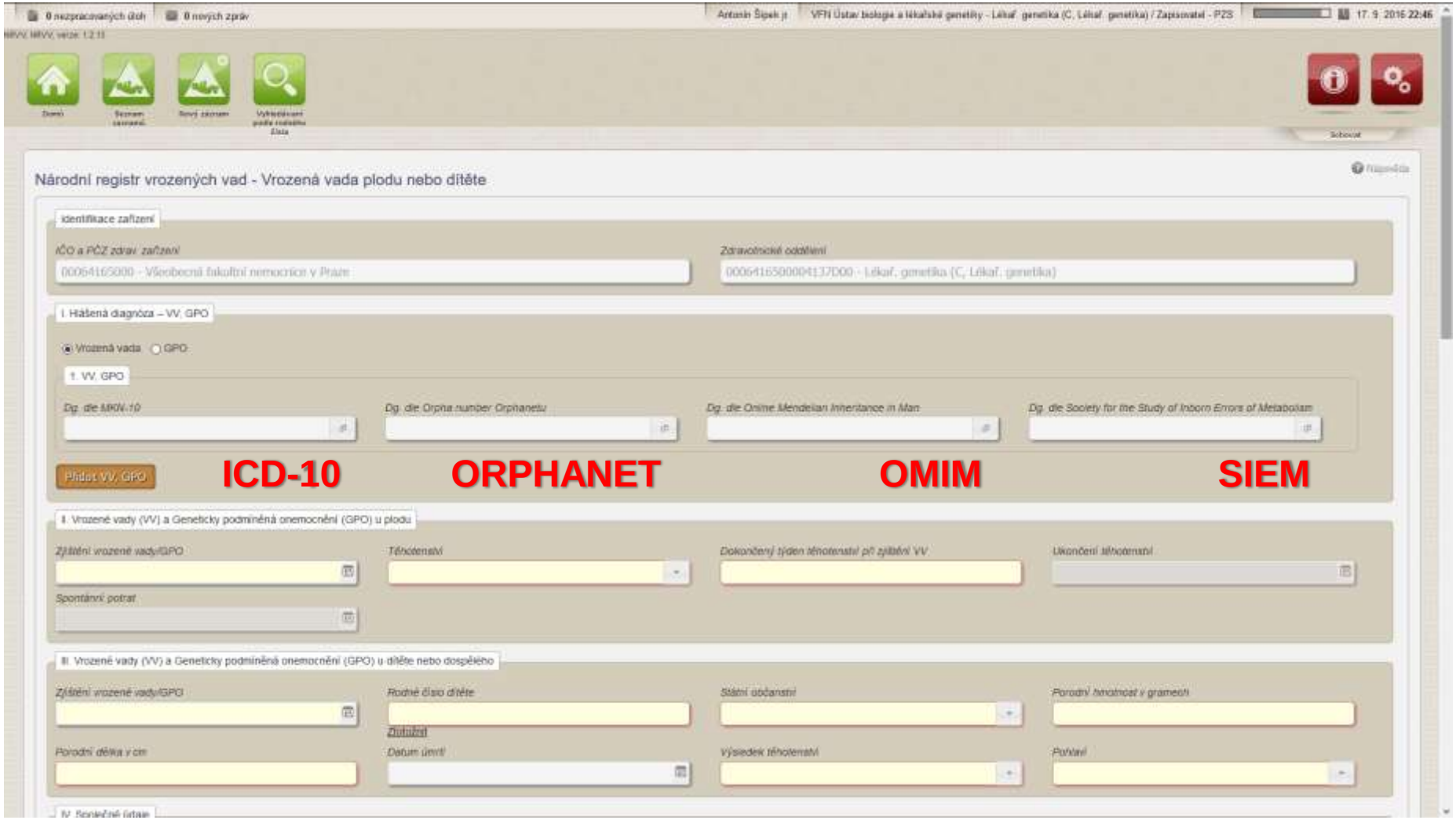
D82 **Immunodeficiency associated with other major defects**

Excl.: ataxia telangiectasia [Louis-Bar] (G11.3)

D82.0 **Wiskott-Aldrich syndrome**
Immunodeficiency with thrombocytopenia and eczema

D82.1 **Di George syndrome**
Pharyngeal pouch syndrome
Thymic:
- alymphoplasia
- aplasia or hypoplasia with immunodeficiency

New reporting tool - CZE



Challenges – Down syndrome

Down syndrome – 47,XY+21

ICD-10: **Q900**
ORPHA: 870
OMIM: 190685
SIEM: NA

Down syndrome – 46,XY,der(14;21),+21

ICD-10: **Q902**
ORPHA: 870
OMIM: 190685
SIEM: NA

Challenges – Turner syndrome

ICD-10

Q96	Turner syndrome
	Excl.: Noonan syndrome (Q87.1)
Q96.0	Karyotype 45,X
Q96.1	Karyotype 46,X iso (Xq)
Q96.2	Karyotype 46,X with abnormal sex chromosome, except iso (Xq)
Q96.3	Mosaicism, 45,X/46,XX or XY
Q96.4	Mosaicism, 45,X/other cell line(s) with abnormal sex chromosome
Q96.8	Other variants of Turner syndrome
Q96.9	Turner syndrome, unspecified

No OMIM nr.

ORPHANET

Turner syndrome

ORPHA881

Synonym(s):	45,X syndrome 45,X/46,XX syndrome	ICD-10:	Q96.0 Q96.1 Q96.2 Q96.3 Q96.4 Q96.8 Q96.9
Prevalence:	1-5 / 10 000	OMIM:	-
Inheritance:	Not applicable or Unknown	UMLS:	C0041408
Age of onset:	Infancy Neonatal Antenatal Childhood	MeSH:	D014424
		MedDRA:	10045181

Turner syndrome due to structural X chromosome anomalies

ORPHA99413

Synonym(s):	-	ICD-10:	Q96.1 Q96.2
Prevalence:	-	OMIM:	-
Inheritance:	-	UMLS:	-
Age of onset:	-	MeSH:	-
		MedDRA:	-

Challenges – Rare chromosomal CNV

Potocki-Lupski syndrome

17p11.2 microduplication syndrome

ORPHA1713

Synonym(s):	Potocki-Lupski syndrome Trisomy 17p11.2	ICD-10:	Q92.3
		OMIM:	610883 [↗]
		UMLS:	C2931246
Prevalence:	-	MeSH:	C536578
Inheritance:	-	MedDRA:	-
Age of onset:	-		

#610883

POTOCKI-LUPSKI SYNDROME; PTLS

Alternative titles; symbols

CHROMOSOME 17p11.2 DUPLICATION SYNDROME

Gene-Phenotype Relationships

Location	Phenotype	Phenotype MIM number	Inheritance (in progress)	Phenotype mapping key
17p11.2	Potocki-Lupski syndrome	610883	IC	4

Clinical Synopsis

ICD-10: **Q923**
ORPHA: 1713
OMIM: 610889
SIEM: NA

Conclusions

Detailed research on VUS.

Testing of the parents.

Follow up (checking new information).

Detailed counseling.

Information for GP.

Conclusions

Classification:

Existing (most appropriate) code for particular diagnose (somewhere).

ICD-10 – NS codes.

Karyotype formulas in registires?


Thank you for your attention!
Vrozené vady
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http://www.vrozene-vady.cz/