

Novel submicroscopic chromosomal abnormalities: challenges for clinical management and surveillance

Antonín Šípek Jr

*Institute of Medical Biology and Genetics, 1st Faculty of Medicine, Charles University
and General University Hospital, Prague, Czech Republic*

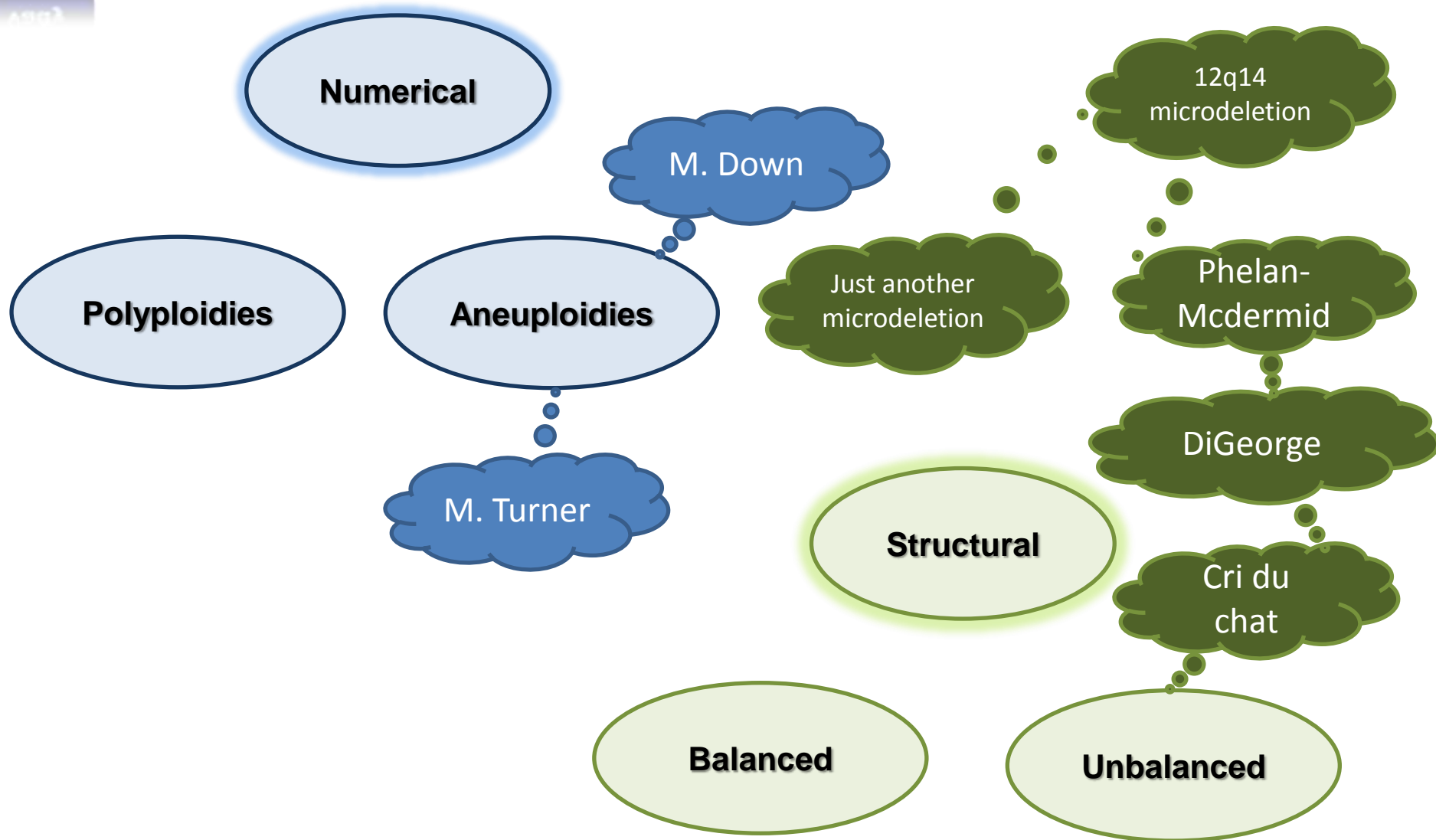
Department of Medical Genetics, Thomayer Hospital, Prague, Czech Republic

National Registry of Congenital Anomalies, Prague, Czech Republic



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

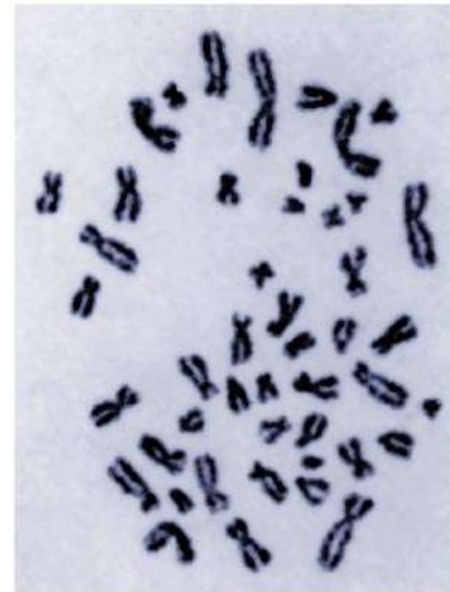
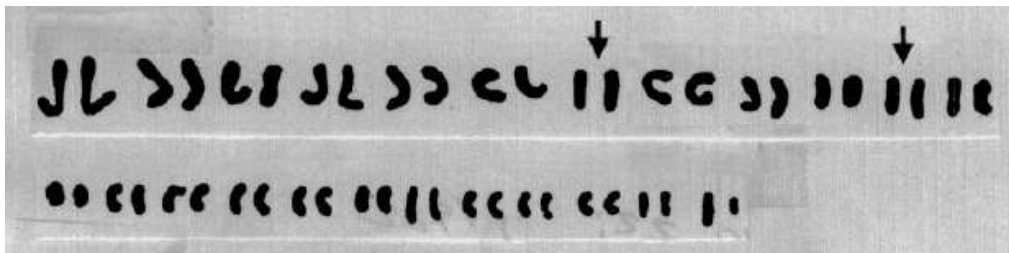
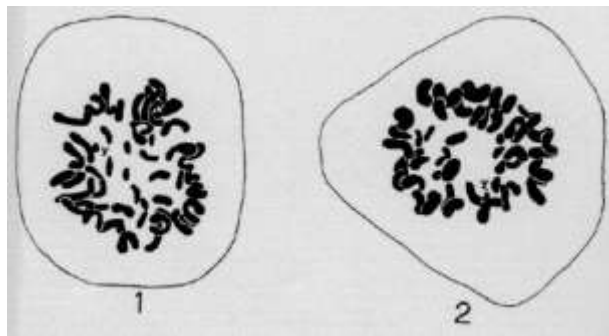
Chromosomal aberrations



Chromosomes

23 chromosome pairs

19th century



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1956

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

Down syndrome

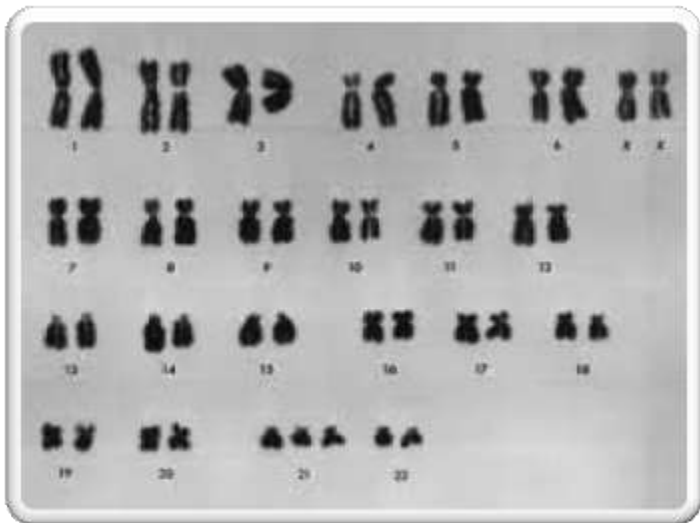


John Langdon Down – 1866

Observations on an Ethnic Classification of Idiots

Jérôme Lejeune – 1959

Etude des chromosomes somatiques de neuf enfants mongoliens



Cri du chat syndrome

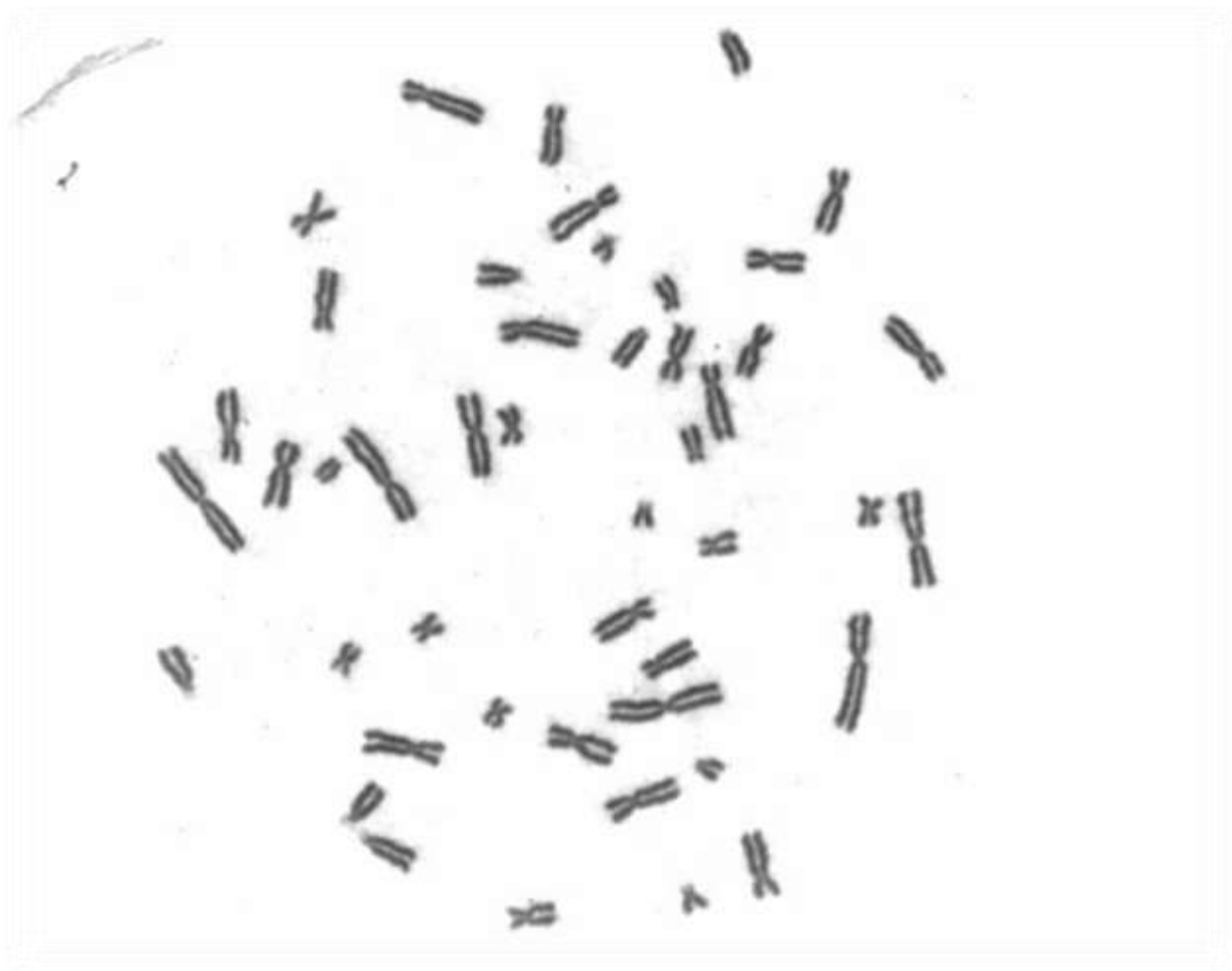


Jérôme Lejeune – 1963

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



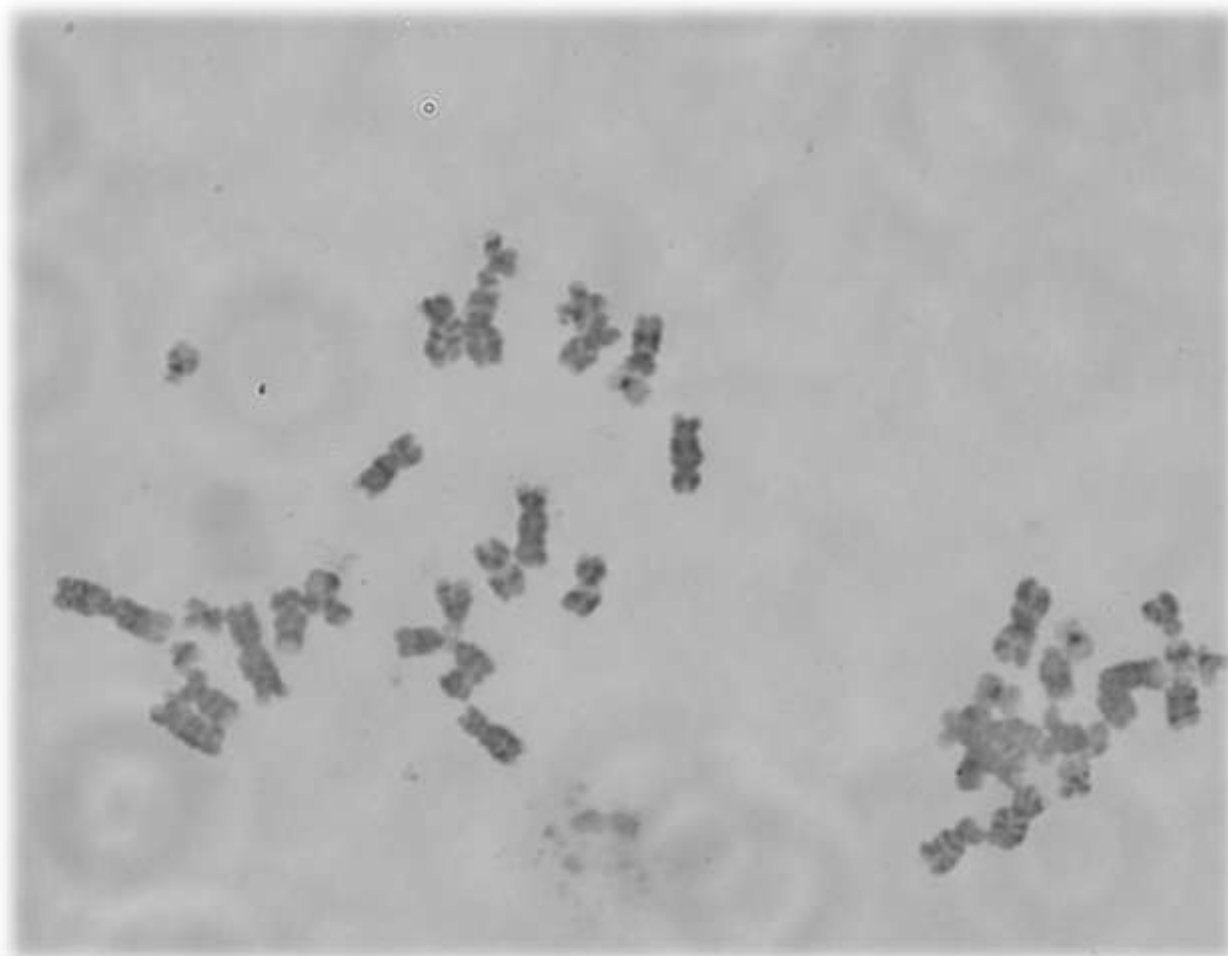
Classical staining



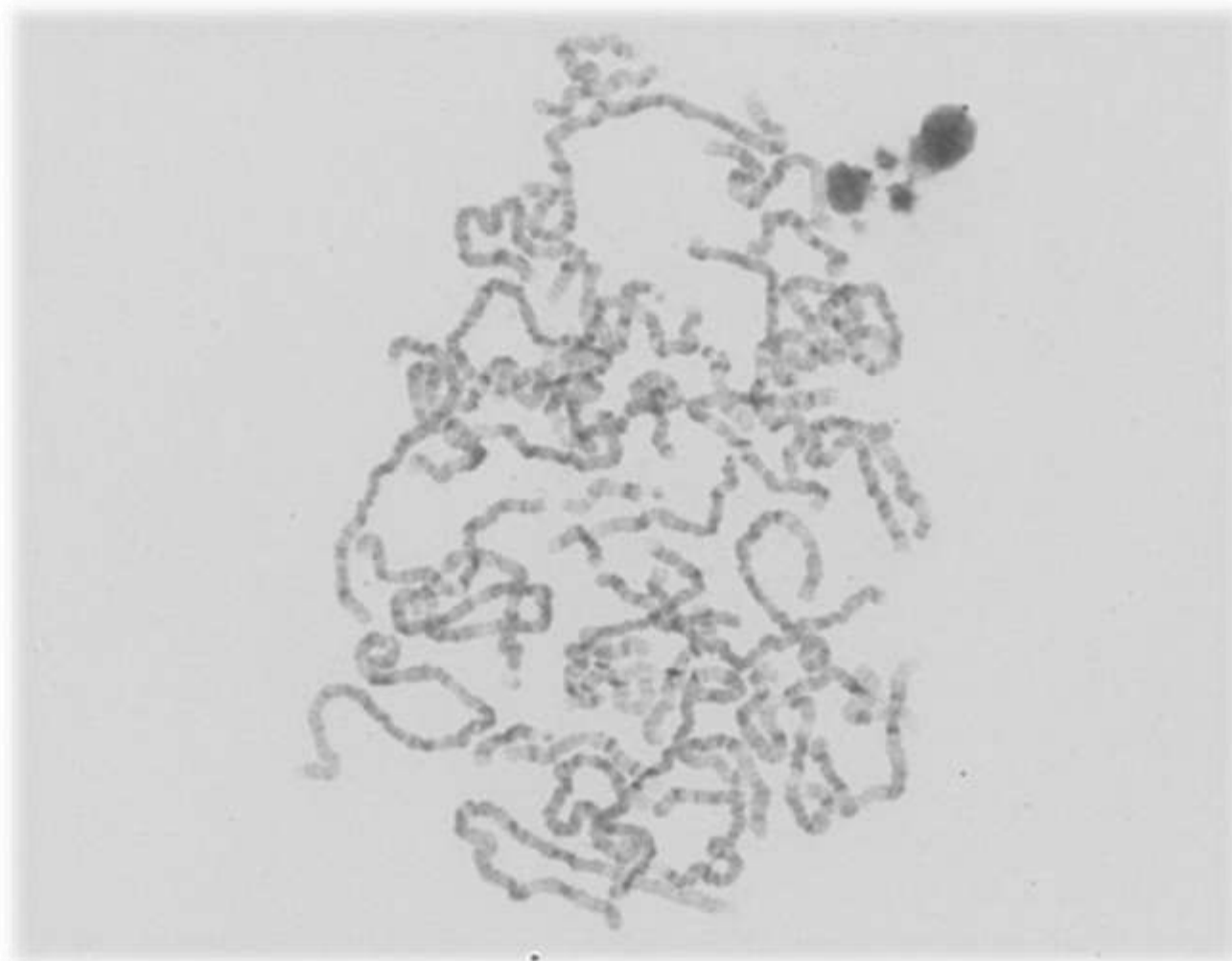
G-banding



G-banding (CVS)



HRT



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.

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)

PENNINA R. LANGER-SAFER¹*, MICHAEL LEVINE¹, and DAVID C. WARD²*

Departments of ¹Human Genetics and ²Molecular Biophysics Biochemistry, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510

Communicated by Alan Coenen, April 5, 1982

ABSTRACT: A method is described for localizing DNA sequences hybridized *in situ* in *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 fluorescently, 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 Pardoll (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 "networks" 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—namely chemical lability due to radiolytic deconjugation, concerns 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. Chong et al. (13) generated a fluorescent signal by coupling latex microspheres containing both poly(U) and denatured fluorochromes to a polyadenylated mRNA probe. Fluorescent signals were also produced by Rodkin and Stollar (14) by using antibodies against DNA-RNA hybrids in conjunction with an immunofluorescent antibody method and by Bauman et al. (15–17) who used RNAs that were labeled at the 5' 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-carbonyl chloride 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 proteins and poly(T)-tailed heteroduplex DNA and used to identify the hybridization sites of poly(T)-tailed *Drosophila* DNA probes. Although each of these approaches was at least partially successful, a simple and more general method for detecting noncovalently 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 aliphatic 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 renaturation characteristics that were compatible 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).

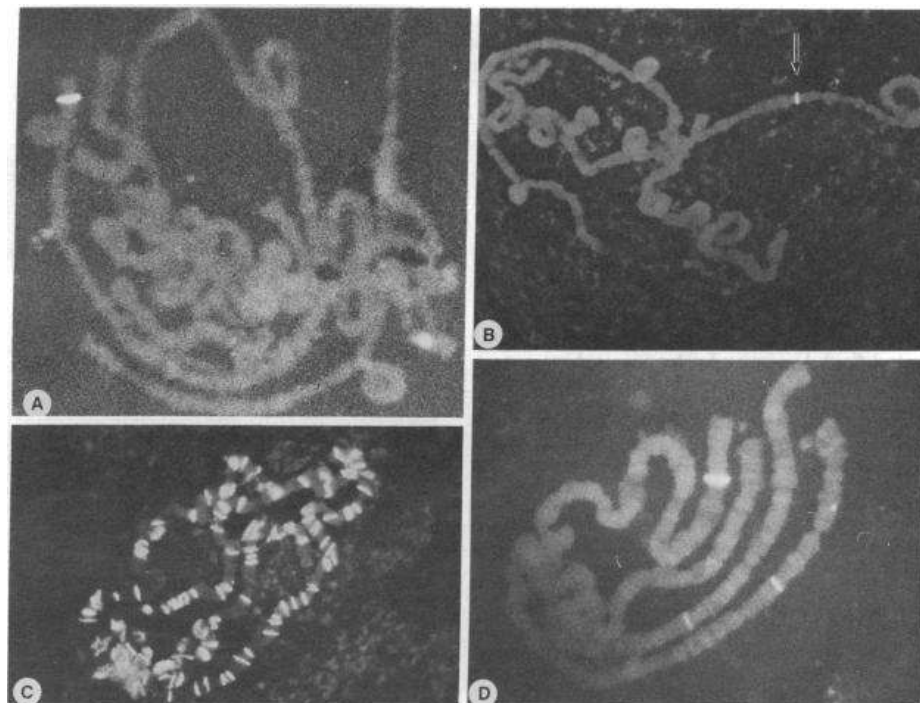
MATERIALS AND METHODS

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, dextrancharcoal, and Baskin-Tanaka magnet were obtained from Sigma. Fluorescein-labeled goat anti-rabbit IgG (FITC-GaIIgG) 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 F. Nakano.

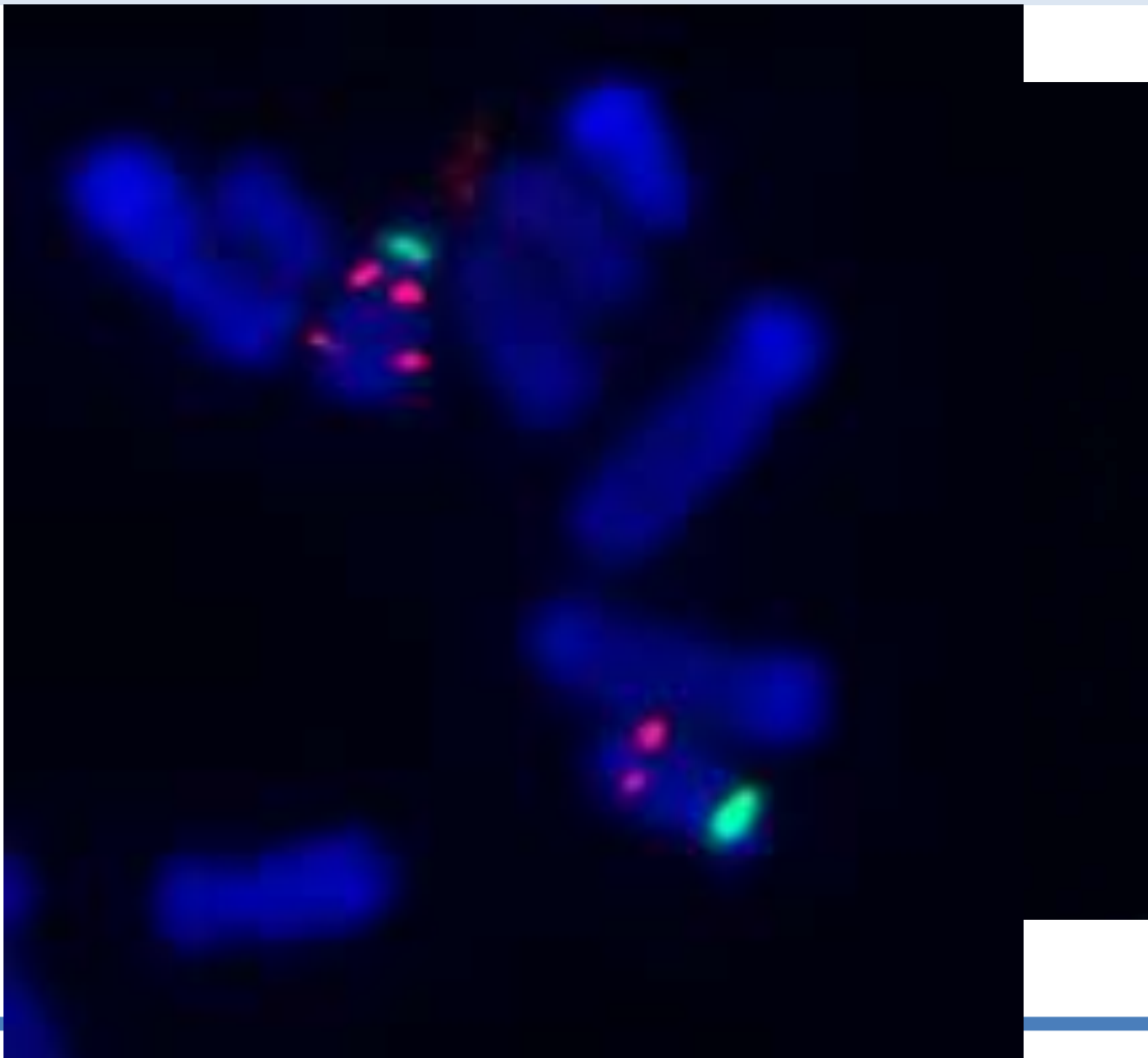
Five of the cloned DNAs used in these studies (ADm 117, ADm 223, ADm 66, ADm 104, and ADm 86) encode genes that are specifically expressed in the fat bodies of third-instar larvae (25). They were obtained from a collection of randomly screened *Drosophila melanogaster* genomic DNA fragments inserted into the Charon 4 phage vector (26). The *Drosophila* DNA is-

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

*Present address: Dept. of Cell Biology, Roche Institute of Molecular Biology, Nutley, NJ 07110.



FISH



Array methods – Main features

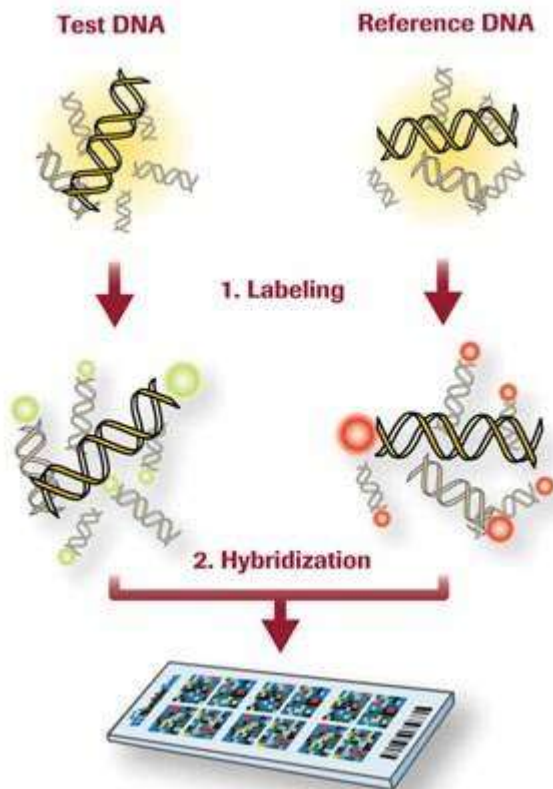
- Whole genome examination for submicroscopic chromosomal copy number variants (deletions/duplications).
- High resolution (up to tens of kBs in gene rich areas).
- SNP-array can detect UPD or LOH.
- Troubles detecting mosaicism.
- Impossible to detect true balanced aberrations (no image of karyotype).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

FISH vs. Array

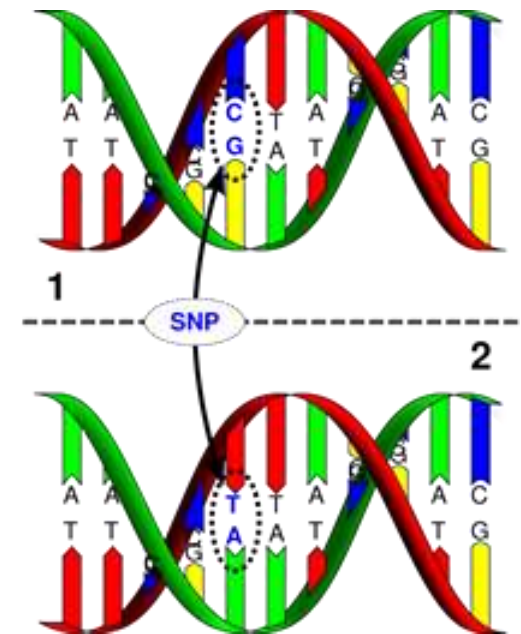


Array Methods

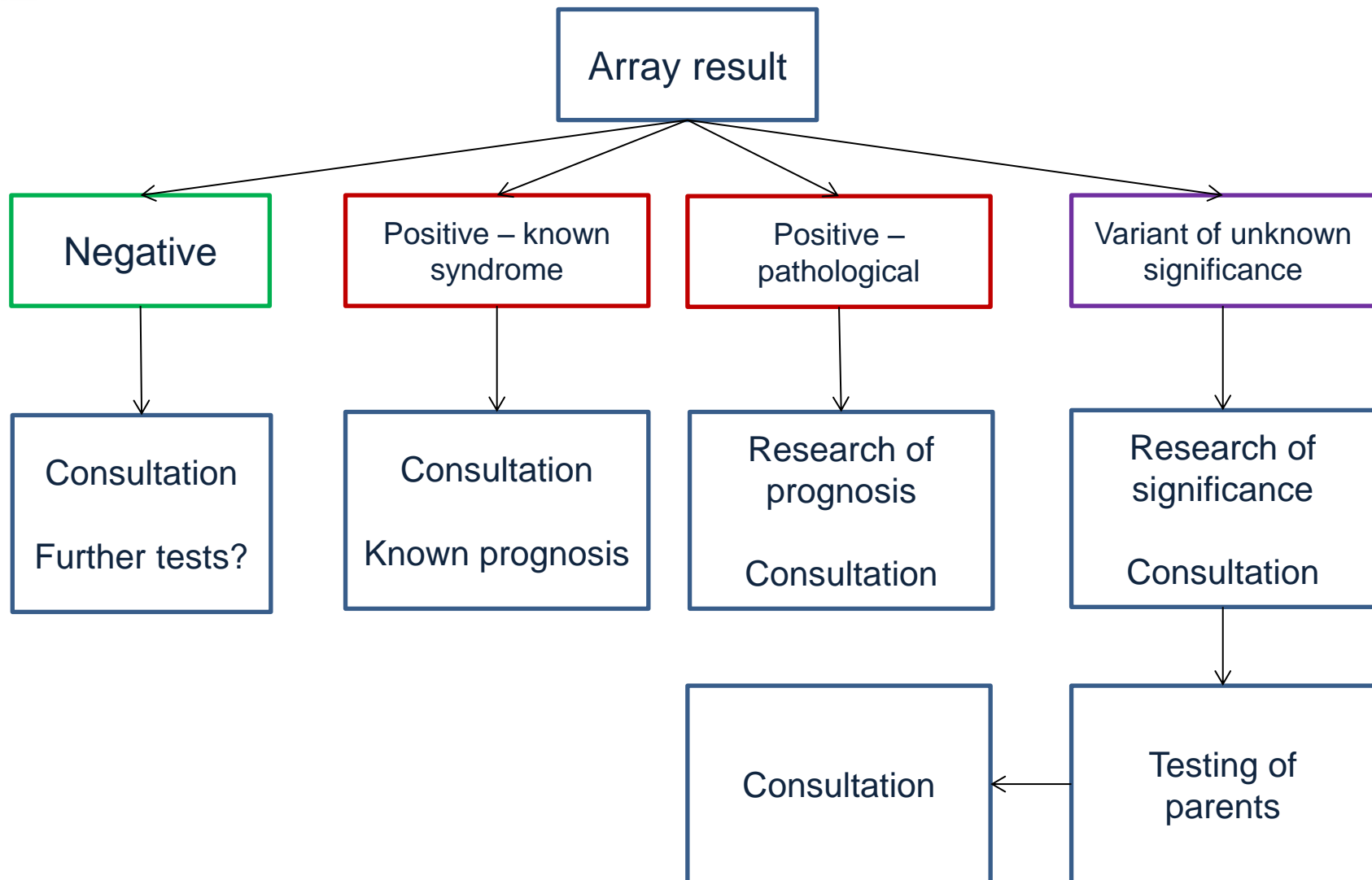
Array-CGH



SNP-Array



Strategy



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 impairment?

What shall we tell the parents?

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

How to code that case?

Databases - ECARUCA



ECARUCA



European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations

[Submit Cases](#)
[Query Database](#)
[FAQ](#)

ECARUCA

- What is ECARUCA?
- Main Participants
- Collaborators
- National Coordinators
- Regulations
- Funding
- Research Platforms

Member

- Log in

Parent

- Parent account

Register

- Submit cases
- Query Database
- Manuals
- Overview Data

News

- Progress
- Introduce Yourself
- Publications
- Presentations
- Interesting case report

Various

- Forms
- Contact
- Links
- Archive
- Message Board
- FAQ

Welcome to ECARUCA, a database which collects and provides cytogenetic and clinical information on rare chromosomal disorders, including microdeletions and microduplications.

- ★ ECARUCA aims to be a database that is easily accessible for all participants and encourages information exchange as well as exchange of technical knowledge.
- ★ ECARUCA wants to improve patient care and collaboration between genetic centres in the field of clinical cytogenetics.
- ★ ECARUCA collects the results of cytogenetic research & the accompanying clinical features, but NOT the patient material used for the analysis; this stays in the centre where the research was carried out.

Content of the ECARUCA database



Currently the microarray data in ECARUCA is stored based on probe IDs which map to genomic locations either in hg17, hg18 or hg19 depending on the microarray platform used. Please take this into account when searching the database via base position.

View all ECARUCA cases smaller than 30Mb in one of the following Genome Browsers:




Acknowledgements:

- We would specifically like to thank Professor Albert Schinzel and his team in Zurich, Switzerland, for their outstanding contribution to the ECARUCA database. Their data is the foundation on which the database is being built and Professor Schinzel and his team continue to contribute a large number of cases to the ECARUCA database.
- We would also like to record our appreciation of Professor Robin Winter (1950-2004) and Dr Michael Baraitser for allowing us to use their search strategy, based on the Winter-Baraitser Dysmorphology Database (WBDD) of the London Medical Databases, to query the ECARUCA database.

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



Databases - DECIPHER

DECIPHER About Browse GDD(UK) Search DECIPHER Join Login

Mapping the clinical genome

Explore DECIPHER

It's free and you don't need to log in

DECIPHER is used by the clinical community to share and compare phenotypic and genotypic data. The DECIPHER database contains data from 21000 patients who have given consent for broad data sharing. DECIPHER also supports more limited sharing via consortia. Have a look at the numbers.

Anyone can browse publicly available patient data on DECIPHER and request to be put in contact with the responsible clinician. Why? Because sharing benefits everyone.

[Explore DECIPHER's genome browser](#)

[Dive into the Human Phenotype Ontology](#)

[Search all open-access DECIPHER data](#)

Join DECIPHER

Be part of the sharing community

Projects affiliated to DECIPHER can deposit and share patients, variants, and phenotypes to invite collaboration and facilitate diagnosis. Once deposited, you can use DECIPHER to identify and prioritise potential matches, and you can request notifications as soon as new matches arrive.

As well as influencing individual patient outcomes, use of DECIPHER has contributed to over 1000 published articles since 2004. It's still free, and you are in control of what data to make public.

[Join now](#)

[Find out more](#)

Already a member?

Log in to access your patient data

Email address

Email

Password

Password

[Log in](#)

[Reset your password](#)

Latest news

DECIPHER v9.10 Released

We released version 9.10 of DECIPHER on the 10th of August, 2016. Improvements include:

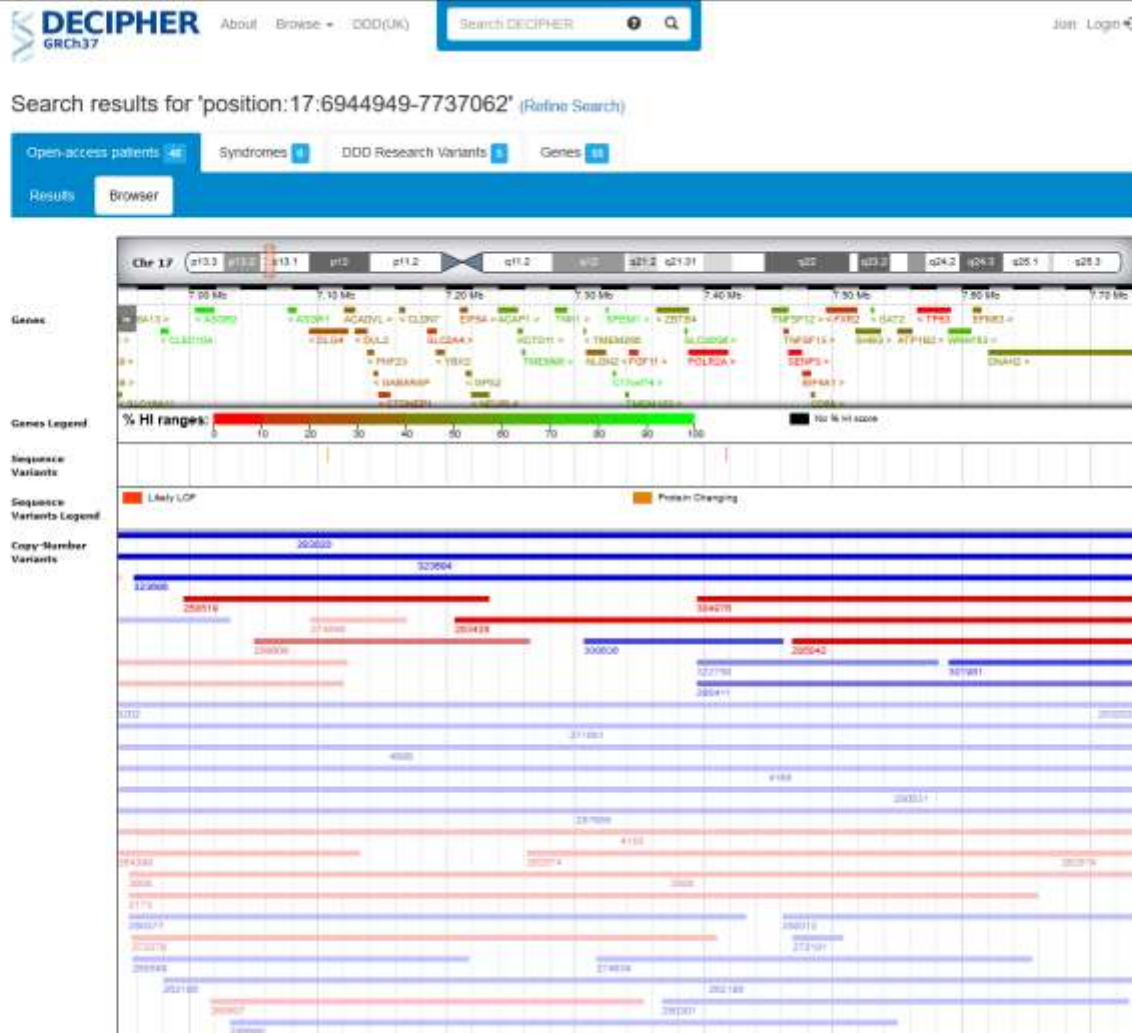
- Bulk Upload via HGVS:** you can now deposit sequence variants in bulk using a complete HGVS code instead of chromosomal position. There is a new Bulk Upload CSV/Excel template for you to use in this case:

HGVS code	Genome assembly	Transcript	Gene name	Intergenic	Chromosome
chr7:g.55960467C>T	GRCh37/hg19	NM_099000	USP13	No	46XX
hvc_000023.10:g.555351T>A	GRCh37/hg19	ENST00000181578	SHOX	No	46XY
NM_001139673.L1:c.1234_1225insC	GRCh37/hg19			No	46XX

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



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	

Databases - DECIPHER

DECIPHER GRCh37 About Browse > DDD(UK) Search DECIPHER 2011 Login

Search results for 'position:17:6944949-7737062' (Refine Search)

Open-access patients Syndromes DDD Research Variants Genes

Results Browser

Genes: 1 to 55 of 55 Show: ☐ OMIM ☐ Morbid ☐ DDG2P ☐ Protein coding Filter

Name	Location	Description	OMIM	Morbid	DDG2P ?	SHI ?	Links
ACADVL	17 718444 718582	acyl-CoA dehydrogenase, very long chain	✓	✓	Y	33.26	View
ACAP1	17 7258048 7254707	ArfGAP with coiled-coil, ankyrin repeat and PH domains 1	✓	-	-	53.86	View
ASGR1	17 7878758 7882863	asialoglycoprotein receptor 1	✓	-	-	72.82	View
ASGR2	17 784641 7819018	asialoglycoprotein receptor 2	✓	-	-	87.81	View
ATP1B2	17 7849945 7841086	ATPase Na+/K+ transporting subunit beta 2	✓	-	-	34.68	View
C17orf74	17 7328604 7329287	chromosome 17 open reading frame 74	-	-	-	64.12	View
CD68	17 7482788 7488429	CD68 molecule	✓	-	-	48.87	View
CHRNA1	17 7342368 7361026	cholinergic receptor nicotinic beta 1 subunit	✓	✓+	-	55.39	View
CLDN7	17 7182222 7187382	claudin 7	✓	-	-	37.75	View
CLEC10A	17 887788 8863826	C-type lectin domain family 10 member A	✓	-	-	85.46	View
CTDNEP1	17 7149918 7155818	CTD nuclear envelope phosphatase 1	✓	-	-	13.67	View
DLG4	17 7883208 7122621	discs large MAGUK scaffold protein 4	✓	-	-	27.29	View
DRH42	17 8529672 7737062	dynein axonemal heavy chain 2	✓	-	-	42.05	View
DVL2	17 7126688 7137864	dishevelled segment polarity protein 2	✓	-	-	22.04	View
EFNB3	17 7886326 7814086	ephrin B3	✓	-	-	30.79	View
EIF4A1	17 7476024 7482323	eukaryotic translation initiation factor 4A1	✓	-	-	18.71	View

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

0 nepracujících lidí 0 nových zpráv Antonín Špek jr VFTI Ústav biologie a lékařské genetiky - Lékař. genetik (C, Lékař. genetik) / Zapisovatel - PZS 17. 9. 2016 22:46

BRN: BRN_VV; verze: 1.2.11

Národní registr vrozených vad - Vrozená vada plodu nebo dítěte

I. Identifikace zařízení

Číslo a PČZ zdravot. zařízení: 00064165000 - Všeobecná fakultní nemocnice v Praze Zdravotnické oddělení: 0006416500004137000 - Lékař. genetik (C, Lékař. genetik)

II. Hlášená diagnóza - VV, GPO

☒ Vrozená vada ☐ GPO

III. Vrozené vady (VV) a Geneticky podmíněná onemocnění (GPO) u plodu

Zjištění vrozené vady/GPO: Těhotenství: Dokončený týden těhotenství při zjištění VV: Ukončení těhotenství:

Spontánní potrat:

IV. Vrozené vady (VV) a Geneticky podmíněná onemocnění (GPO) u dítěte nebo dospělého

Zjištění vrozené vady/GPO: Rodné číslo dítěte: Státní občanství: Porodní hmotnost v gramech:

Porodní délka v cm: Datum úmrtí: Výsledek těhotenství: Pohlaví:

ICD-10 ORPHANET OMIM SIEM

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

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

ICD-10

No OMIM nr.

ORPHANET

:: Turner syndrome

ORPHA881		ICD-10:	Q96.0 Q96.1 Q96.2 Q96.3 Q96.4 Q96.8 Q96.9
Synonym(s):	45,X syndrome 45,X/46,XX syndrome	OMIM:	-
Prevalence:	1-5 / 10 000	UMLS:	C0041408
Inheritance:	Not applicable or Unknown	MeSH:	D014424
Age of onset:	Infancy Neonatal Antenatal Childhood	MedDRA:	10045181

:: Turner syndrome due to structural X chromosome anomalies

ORPHA99413		ICD-10:	Q96.1 Q96.2
Synonym(s):	-	OMIM:	-
Prevalence:	-	UMLS:	-
Inheritance:	-	MeSH:	-
Age of onset:	-	MedDRA:	-

Challenges – Rare chromosomal CNV

Potocki-Lupski syndrome

:: 17p11.2 microduplication syndrome

ORPHA1713

Synonym(s): Potocki-Lupski syndrome
Trisomy 17p11.2

Prevalence: -

Inheritance: -

Age of onset: -

ICD-10: Q92.3

OMIM: [610883](#) [↗]

UMLS: C2931246

MeSH: C536578

MedDRA: -

#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!



antonin.sipek@lf1.cuni.cz

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