Autistic Spectrum Disorders in the genetics clinic

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18.12.2015

Plan

- Genetic basis of ASDs
- Modern dgn techniques
- Clinical markers
- Clinical practice recommendations
- Frequent clinical dilemmas

EXPLANATIONS

ASDs – Autistic Spectrum Disorders (John M. Opitz, 2011)
aCGH – Array Comparative Genomic Hybridization
ID – Intellectual Disability

Genetic basis of ASDs - hypotheses

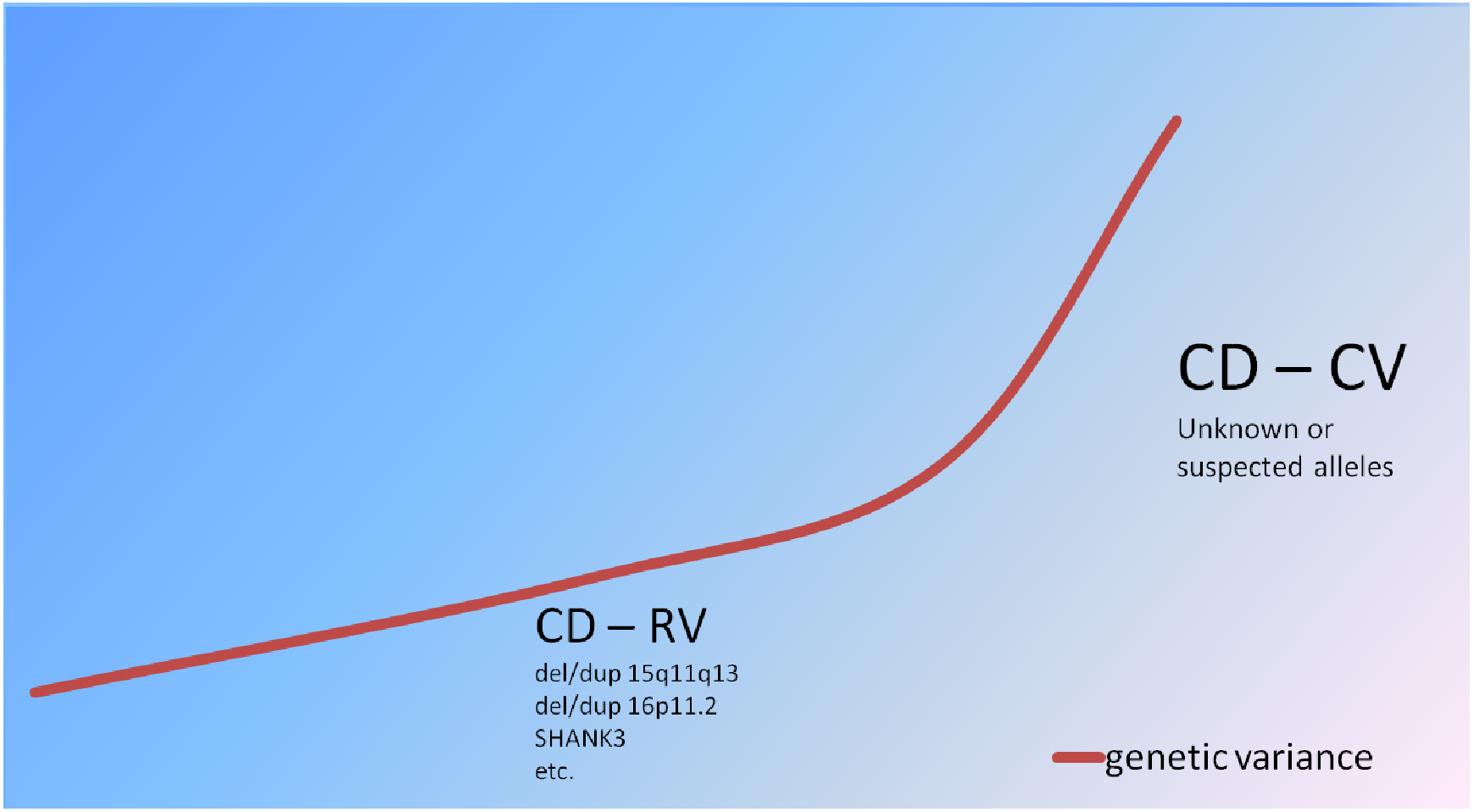
- CD-CV (*common disease-common variant*): the role of common genetic variants (alleles) of individually small effect

(close relatives = higher ASDs risk, milder ASDs spectrum in relatives)

- CD-RV (*common disease-rare variant*): the role of rare variance = single gene muts / *de novo* chromosome aberrations

(early onset, +ID, high differences in MZ/DZ concordance rates)

Genetics of ASDs – the strategy



Diagnostic techniques in ASDs

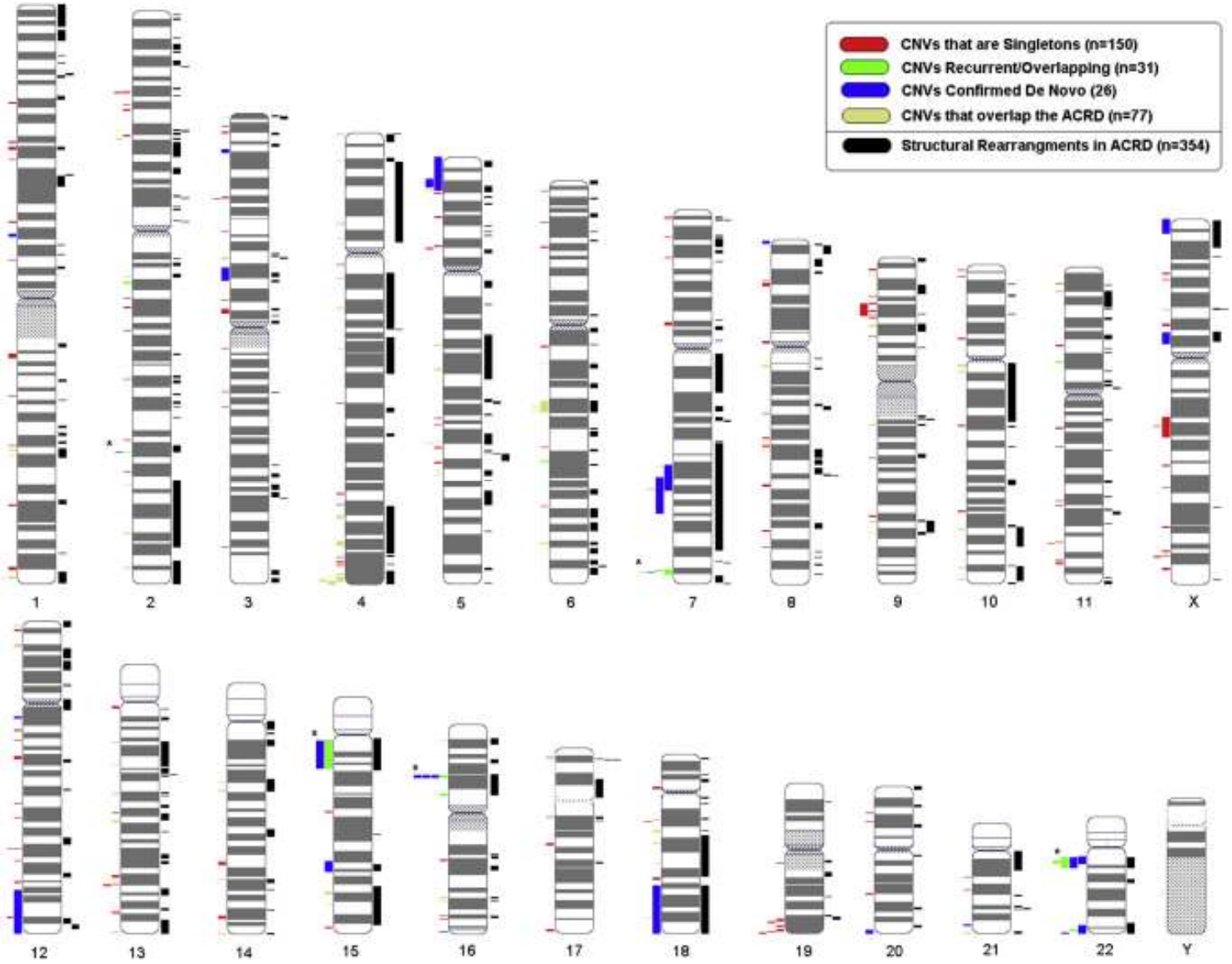
- Molecular analysis ⇒ single gene mutation
- Karyotype analysis ⇒ chromosome aberration
- Molecular cytogenetics (e.g. aCGH) ⇒ chromosome microaberration
- Genomic sequencing ⇒ CD-CV+CD-RV

Microdeletion syndromes

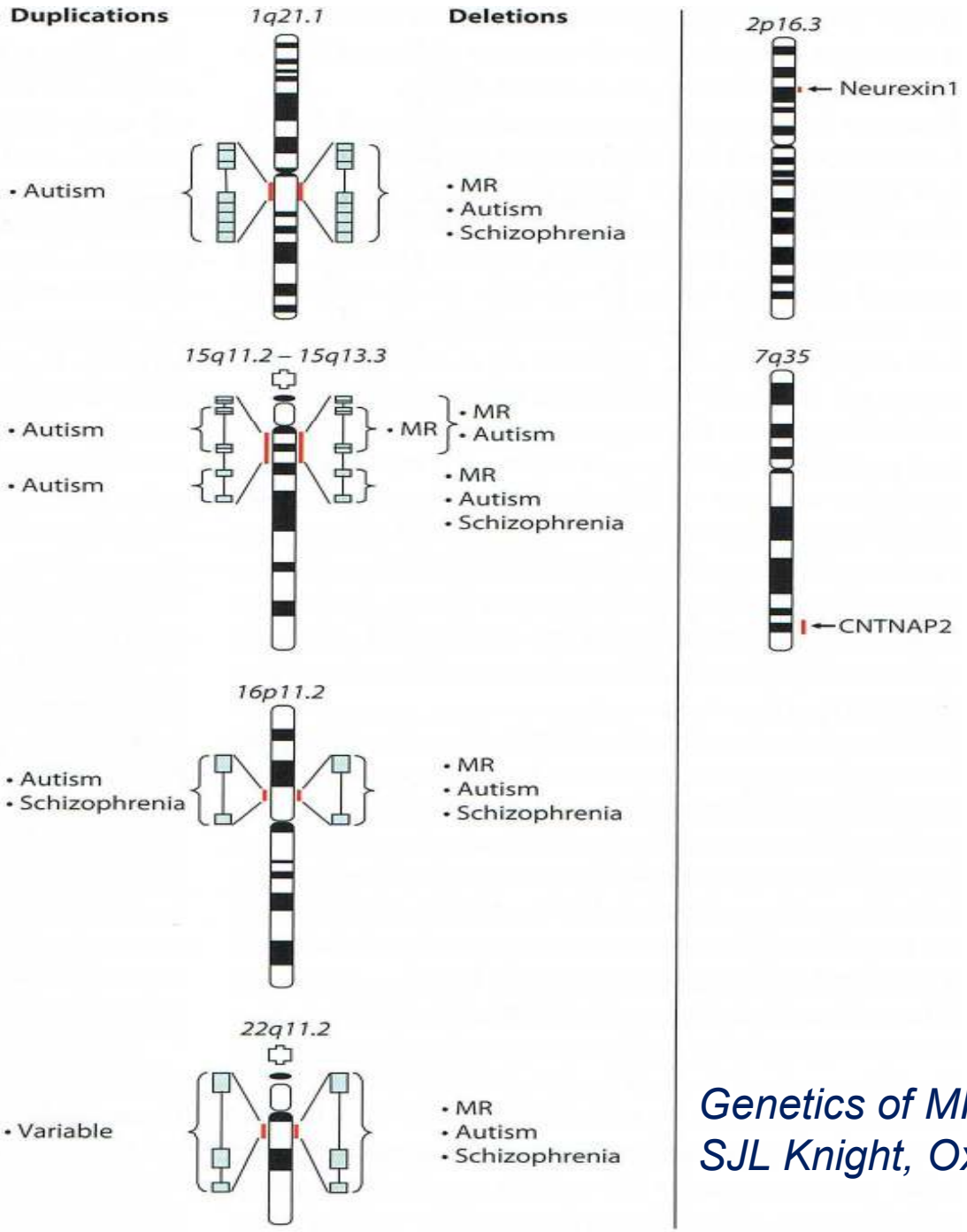
1q21 TAR
1q21.1 ID/Microcephaly
3q29
5q35 Sotos
7q11.23 Williams
8p23.1
15q11.2-q13 PWS/AS (BP1/2-3)
15q13.2-q13.3 ID/Epilepsy(BP4-5)
16p13.11 **Autism**/ID/Schizophrenia
16p11.2 **Autism**
17p12 HNPP
17p11.2 Smith-Magenis
17q12 Diabetes+renal cysts/**Autism**
17q21.31
22q11.2 DiGeorge/VCFS

Microduplication syndromes

1q21 TAR
1q21.1 ID/**Autism**
3q29
5q35 Short stature+microcephaly
7q11.23 **Autism**
8p23.1
15q11.2-q13 **Autism** (BP1/2-3)
15q13.2-q13.3 Psychiatric diseases (BP4-5)
16p13.11
16p11.2 **Autism**
17p12 CMT1A
17p11.2 Potocki-Lupski
17q12 Epilepsy
17q21.31 Behavioural problems
22q11.2 ID



AUTISM CHROMOSOME REARRANGEMENT DATABASE
projects.tcag.ca.autism/



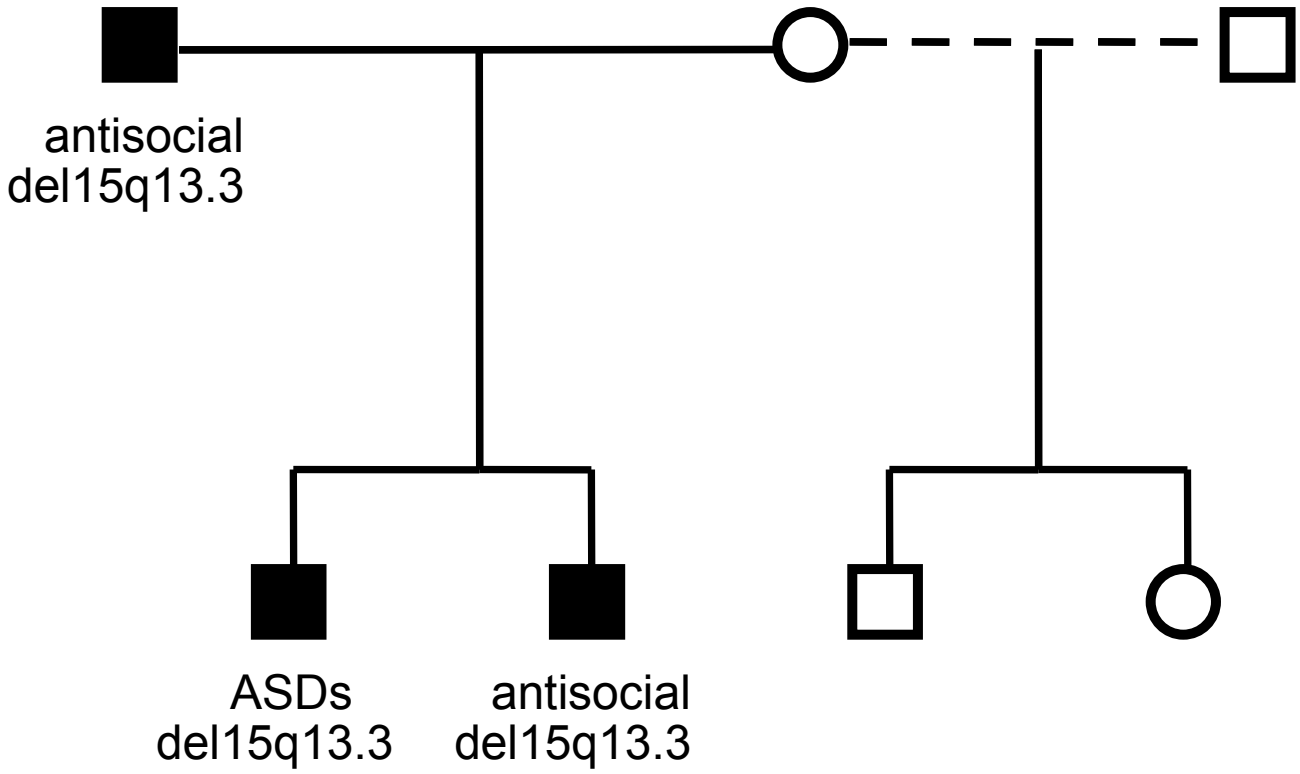
Genetics of MR,
SJL Knight, Oxford 2010

Geneticist – when to refer?

- When parents are asking about recurrence risks!

Affected relative	Schizophrenia	Bipolar Disorder
None (pop risk)	1%	2-3%
Monozygotic twins	40-50%	70%
Both parents	45%	50%
Brother/sister and the parent	15%	20%
Parent	13%	15%
Dizygotic twins	10%	20%
Brother/sister	9%	13%
2nd degree relative	3%	5%
3rd degree relative	1-2%	2-3%

Pedigree!


antisocial
del15q13.3
ASDs
del15q13.3
antisocial
del15q13.3

Diagnostics vs. Research

RESEARCH

A new wave in the genetics of psychiatric disorders: the copy number variant tsunami

Ridha Joober, MD, PhD; Patricia Boksa, PhD

J Psychiatry Neurosci 2009;34(1):55-9

Whole-genome studies (GWAS)

ID, autism, schizophrenia, prostate cancer, breast cancer…

Table 2 | Global CNV burden analysis: event type and frequency

			CNV burden (number)			CNV burden (gene count)		
CNV type	Frequency	CNV (*n*)	*P*	Case/control ratio	Baseline rate (controls)	*P*	Case/control ratio	Baseline rate (controls)
Deletions and duplications	All	6,753	3×10^{-5}	1.15	0.99	2×10^{-6}	1.41	2.01
	Single occurrence	890	5×10^{-6}	1.45	0.11	0.005	1.67	0.32
	2–6 occurrences	2,465	0.003	1.16	0.35	5×10^{-4}	1.36	0.80
Deletions only	All	2,652	0.11	1.08	0.40	3×10^{-5}	1.55	0.72
	Single occurrence	470	0.01	1.29	0.06	0.001	1.77	0.12
	2–6 occurrences	994	0.048	1.15	0.15	0.13	1.38	0.21
Duplications only	All	4,101	2×10^{-5}	1.20	0.59	10^{-4}	1.28	1.94
	Single occurrence	734	8×10^{-7}	1.58	0.09	0.015	1.60	0.30
	2–6 occurrences	1,532	0.011	1.16	0.22	0.012	1.30	0.69

Diagnostics vs. Research

DIAGNOSTICS

Autistic Spectrum Disorder in a 9-Year-Old Girl With Macrocephaly

Martin T. Stein, MD,* Ellen Roy Elias, MD, FACMG,† Margarita Saenz, MD,† Laura Pickler, MD,† Ann Reynolds, MD‡

J Dev Behav Pediatr 31:632–634, 2010

- Clinical suspicion/exclusion of KNOWN genetic condition
- Molecular analysis of the defect in KNOWN gene and/or KNOWN chromosomal defect

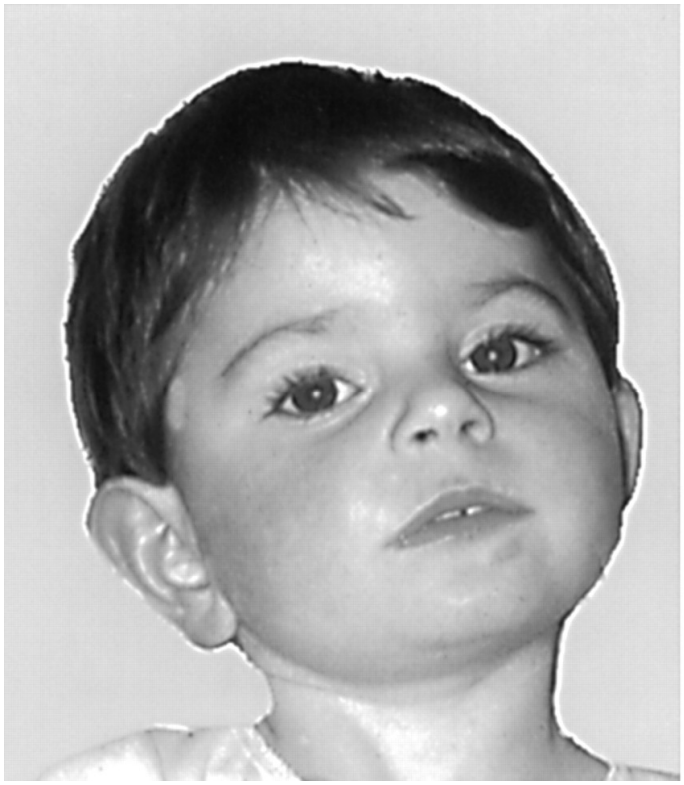
Autism and else…

Some referrals for aCGH chromosome analysis in Baylor TCM, Texas, Sep 2009

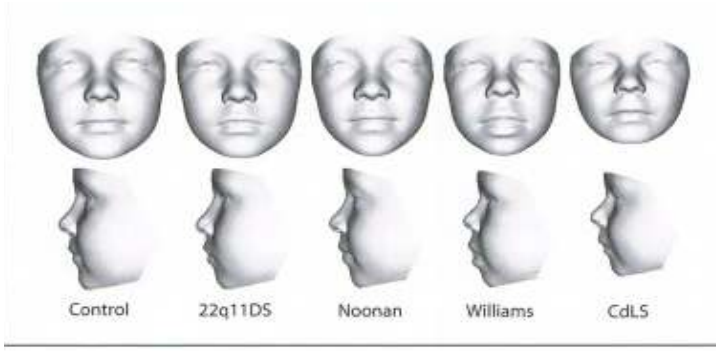
Dev Delay / ID	n=1711	16,65%
Multiple congenital anomalies	1503	14,62%
Autism / ASD	643	6,26%
Seizures / Epilepsy	454	4,41%
ADHD	29	0,28%

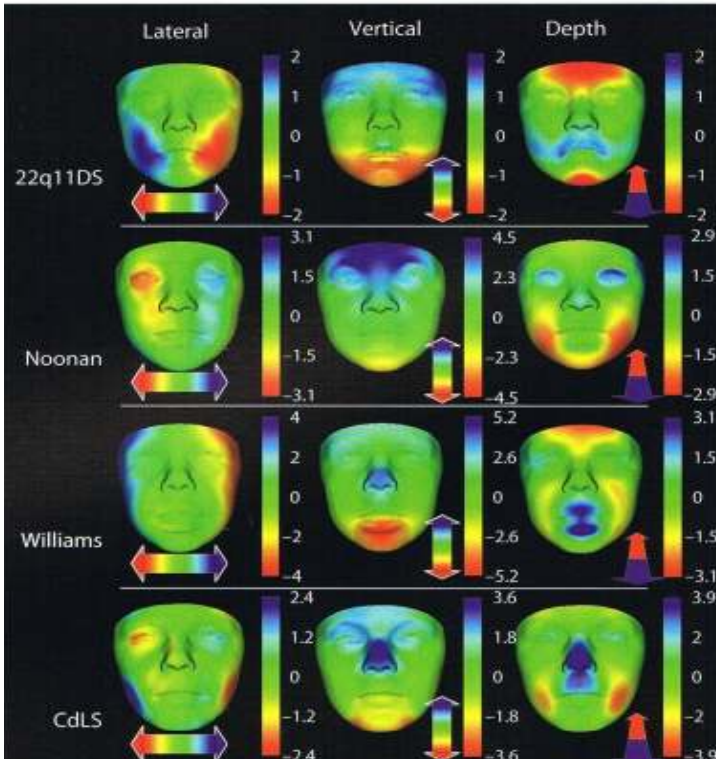
Dysmorphism?

Dysmorphism in a clinical geneticist's view

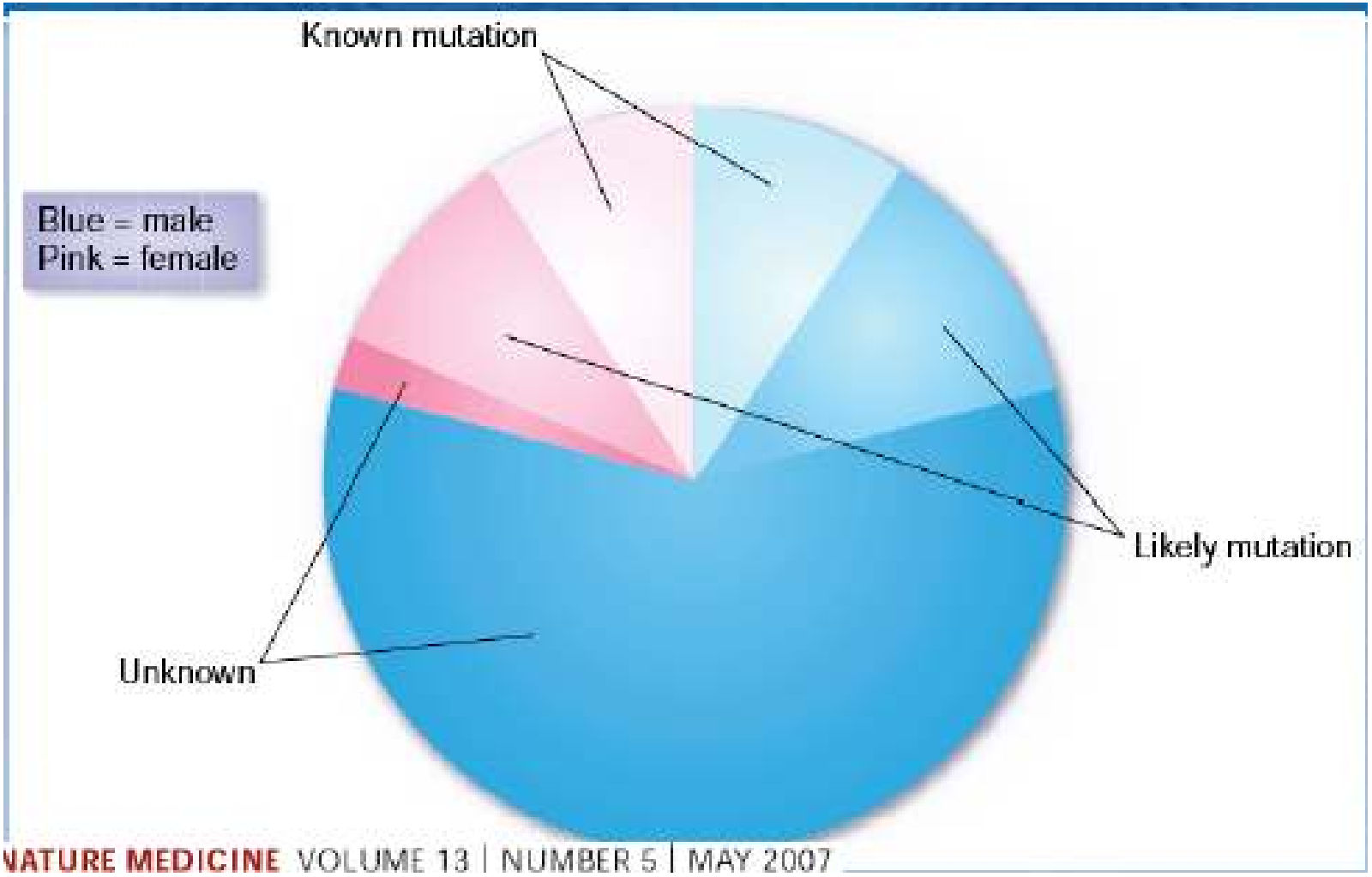


Dysmorphism in a clinical geneticist's view





Autism – diagnostic potential

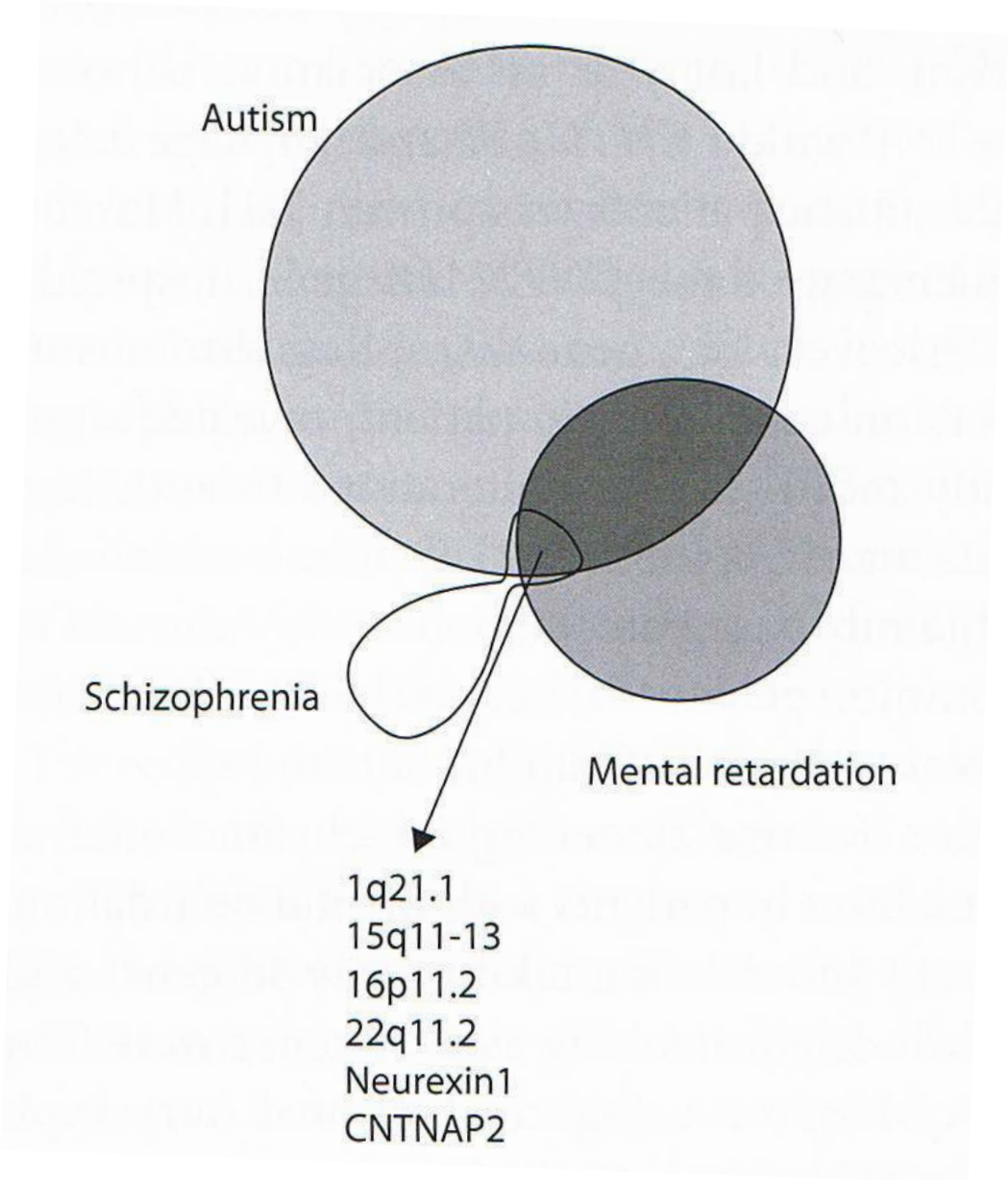


NATURE MEDICINE VOLUME 13 | NUMBER 5 | MAY 2007

Autism - diagnostics

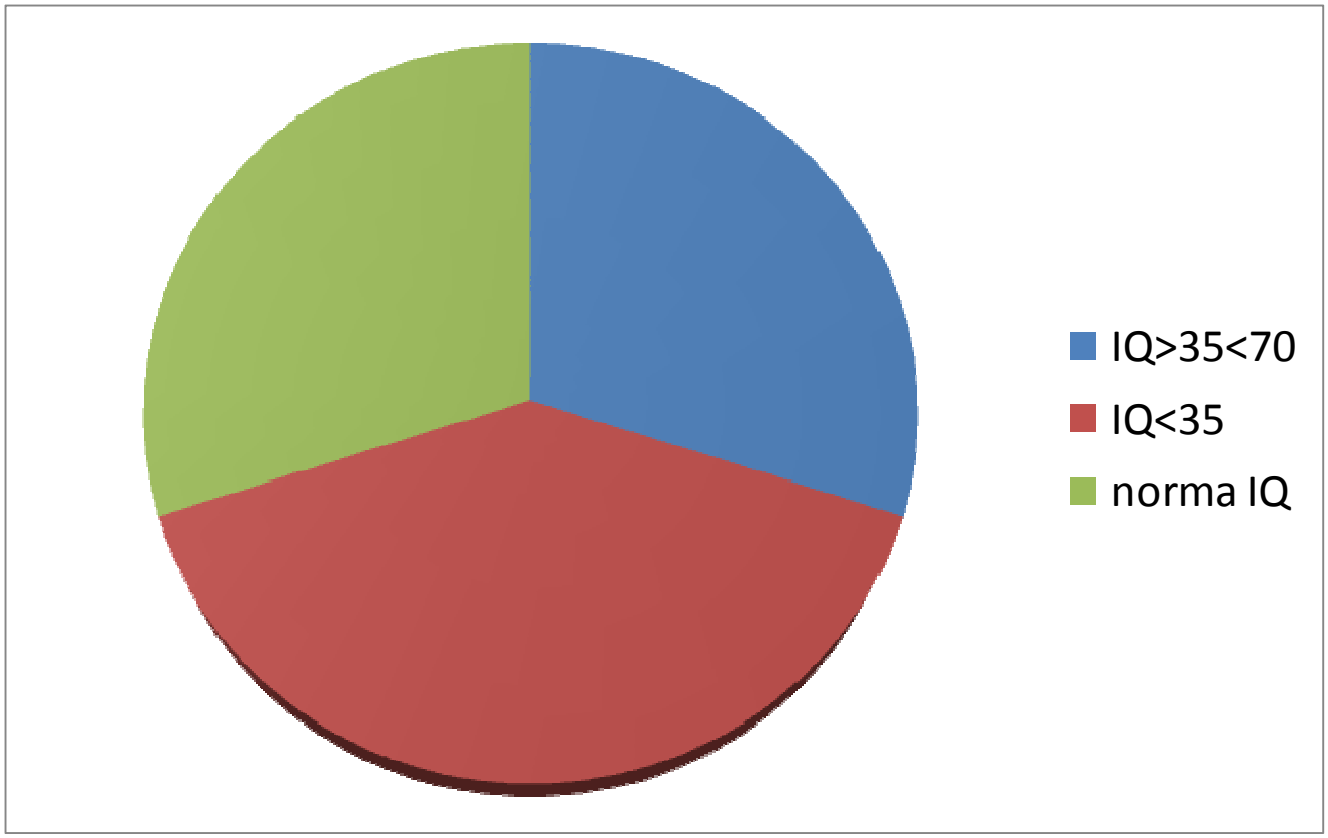
- 10-20% - known genetic basis
- Monogenic syndromes: Fragile X syndrome, Rett syndrome, Tuberous sclerosis, *PTEN*, *SHANK3*, *NLGN*, *NRXN*, Neurofibromatosis type 1, Angelman syndrome
- Chromosomal aberrations (in syndromic ASDs up to 30%!)

Autism and else…



Autism and… ID

Meta-analysis of 19 studies



Journal of Autism and Developmental Disorders 2003; 33(4)

Deletion 22q11.2 syndrome – behavioural phenotype

- Impulsiveness, backing out (Swillen)
- Anxiety, perseverations, ASDs (Niklasson, Swillen, Fine)
- ADHD (Swillen, Gerdes)
- Schizophrenia, Bipolar disorder
- Psychoses (20-30%)

Rett syndrome

Responsible gene: *MECP2* **Cytogenetic locus:** Xq28
Inheritance: XLD
Clinical Features and Diagnostic Criteria: ID, developmental regression (especially language and hand use), acquired microcephaly, stereotypical wringing hand movements, hyperventilation, bruxism, paroxysmal laughing, prolonged QT, scoliosis
Clinical Tests: EEG (nonspecific for Rett), ECG
Molecular Tests: *MECP2* sequencing (>80%), Need to test parents if a novel variant found. *MECP2* MLPA or quantitative PCR testing for deletion (~16%).
Disease Mechanism: Decreased function of loss-of-function of MECP2. Normally MECP2 binds methylated CpG islands.
Treatment/Prognosis: Seizures are often difficult to manage SSRI's for agitation, monitor for scoliosis, periodic ECG to monitor for long QT. Small subset have *CDKL5* mutations and present atypically with early onset seizures.

Rett syndrome
behavioural phenotype

- Eye-to-eye contact
- Delayed response to stimuli
-„…they understand more than doctors think”
- Body language and body contact
- Intensive hand stereotypies
- Brain-pain-crying
- Sleep disturbances

Metabolic screen, US

UNIFORM PANEL				
MS/MS				
Acylcarnitines		Amino acids		
(9) OA	(5) FAO	(6) AA	(3) Hematology	(6) Others
IVA	MCAD	PKU	Hb SS	CH
GA-I	VLCAD	MSUD	Hb S/ß Th	BIOT
HMG	LCHAD	HCY	Hb S/C	CAH
MCD	TFP	TYR I		GALT
MUT	CUD	ASA		HEAR
Cbl A,B		CIT		CF
3MCC				
PROP				
BKT				

Diagnostics of IEM – some indications

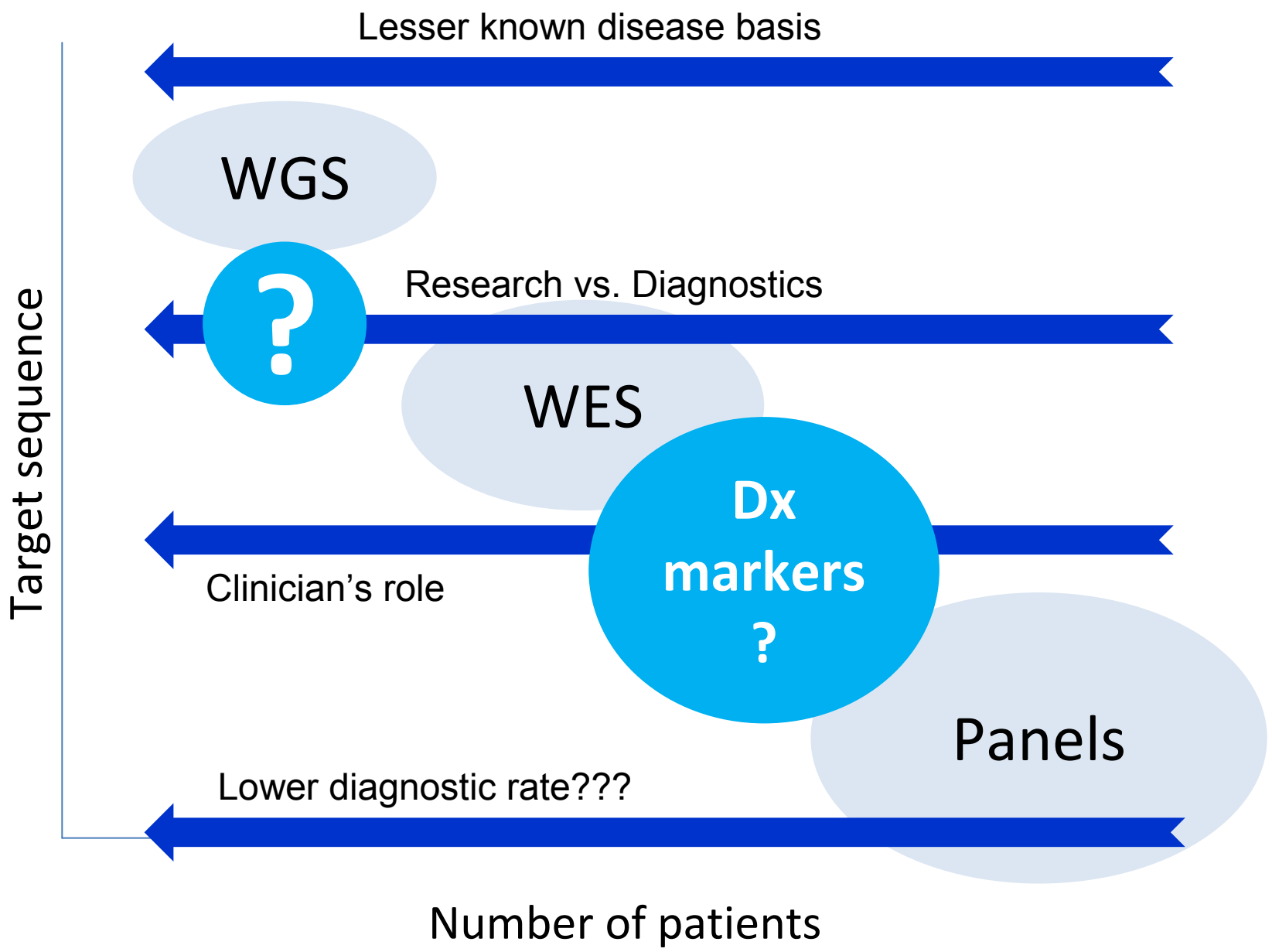
- macrocephaly, seizures resistant to therapy, coma, ID, cardiomyopathy
- hypoglycemia, hyperammonemia, metabolic aciduria
- Energy-deficit symptoms
- Reye syndrome
- SIDS, ALTE in a family!!!

OTC deficit

- Late form – after puberty (lack of appetite, nausea, vomiting, psychoses, sleepiness, coma, Reye's syndrome), don't want to eat proteins – disturbances of consciousness, MRI – generalized atrophy
- dgn.: ammonia, aminoacidogram, orotic acid

Storage disorders – broad diagnostic spectrum

- Blood count incl leukocytes
- X-ray studies
- Ophth exam (fundoscopy)
- MPS and glycoproteins in urine
- Lysosomal enzymes in plasma
- Consider bone marrow biopsy
- Biochemical studies in fibroblasts +/- leukocytes
- Other – depending on clinical suspicion


Lesser known disease basis
WGS
?
Research vs. Diagnostics
WES
Dx markers ?
Clinician's role
Panels
Lower diagnostic rate???
Target sequence
Number of patients

Greenwood Genetic Center

Next Generation Sequencing Syndromic Autism Panel

83 genes

ADNP	DYRK1A	MECP2	PTEN	TBR1
ALDH5A1	EHMT1	MED12	PTPN11	TCF4
AMT	FGD1	MEF2C	RAB39B	TMEM231
AP1S2	FMR1	MID1	RAD21	TMLHE
ARID1B	FOLR1	NHS	RAI1	TSC1
ARX	FOXG1	NIPBL	RELN	TSC2
ATRX	FOXP1	NLGN3	SCN1A	TUBA1A
BCKDK	FOXP2	NLGN4X	SCN2A	UBE3A
BRAF	GABRB3	NRXN1	SETBP1	UBE3C
CACNA1C	GRIN2B	NSD1	SETD2	VPS13B
CASK	HDAC8	NTNG1	SHANK3	ZEB2
CDKL5	HOXA1	OPHN1	SLC2A1 (GLUT1)	
CHD7	HPRT1	PAFAH1B1 (LIS1)	SLC9A6	
CHD8	KDM5C (JARID1C)	PCDH19	SMC1A	
CNTNAP2	KIRREL3	PHF6	SMC3	
CREBBP	L1CAM	PNKP	STXBP1	
CTNNB1	LAMC3	PQBP1	SYNE1	
DHCR7	MBD5	PTCHD1	TBL1XR1	

Exome sequencing in ASDs (1)

- **Sporadic** cases: 11/20 likely pathogenic, incl 4 novel candidate genes [O'Roak, 2011]
- Simons Simplex Collection [O'Roak, 2012]: **sporadic**, more severe spectrum (phenotype overlap with ID) - 248 *de novo* in 60 genes (120 nonsynonymous and LOF)
- Neale, 2012: **oligo- or polygenic model** – incomplete penetrance, only few more muts than in ctrls, limited importance of *de novo* variants
- Cukier, 2014: **familial cases (multiplex)** – 36 SNVs segregating in 40 families
- Chahrour, 2012: **pathogenic AR variants** in 4 of 16 nonconsanguineous families

Exome seq in ASDs (2) – conclusions and limitations

- Significant effect of clinical overlap with ASDs: ID, epilepsy, schizophrenia, depression
- List of so called *recurrent genes* and *recurrent hits*!
- Protein interactions – modela with proteins p53, FMR1, WNT and chromatin modelling – genic functional modelling
- Bamshad, 2011: „Significant genetic variance is observed in exomes of healthy people. This way, each individual with/without ASDs is *per se* a carrier of about one *de novo* protein-shortening variant."
- Gratten, 2013: „Lifting the power of the future studies by increasing sample sizes to thousands of families should identify candidate genes only if three or more *de novo* disruptive mutations in these genes are present in probands"

Sekwencjonowanie genomowe w ASDs

- Jiang, 2013: 6 wariantów *de novo* w 32 rodzinach
- Michaelson, 2012: pary bliźniąt MZ - 668 mutacji: 565 (87%) *de novo* i 53 (9%) prawdziwie dodatnich(!!!) SNVs odziedziczonych od jednego z rodziców
- Nie wiadomo jak znacząco zastosowanie WGS w porównaniu z WES w zaburzeniach spektrum autyzmu wpływa na zwiększenie efektywności diagnostycznej
- Ograniczenia finansowe tego typu badań

aCGH and WES – diagnostic effectiveness [JAMA, 2015]

	aCGH	WES
Examined group	258	95 trios
Dx (%)	24 (9.3%)	8(8.45%)
Essential autism	6.3%	
Complex autism (Autism+)	37.5%	
Summed up	**15/95 (15.8%)**	

Diagnostically significant phenotypic variables (% - in ASDs population)

- Physical: dysmorphism 15-20%, macrocephaly 35%, microcephaly 5-15%, CNS anomaly 20%
- Neurologic: seizures 25%, abnormal EEG 50%, sleep disturbances 65%
- Clinical: onset, developmental regression 30%
- Family history: ASDs 25%, alcohol 30%, Bipolar disorder 30%

Genet Med 13(4); 2011

Autism vs Autism+

	Autism = 187 (80%)	**Autism+ = 46 (20%)**
Sex M/F	**6.5:1 (162:25)**	**3.2:1 (35:11)**
Mean IQ/IR (SD)	**70.4 (25.4)**	**53.1 (22.8)**
Seizures	17% (31/187)	39% (18/46)
Abnormal EEG	30% (32/107)	46% (12/26)
CNS anomaly	13% (15/122)	28% (8/29)
Syndromic dx (before aCGH)	0% (0/187)	24% (11/46)
Recurrence risk	increased	*de novo*

Am J Med Genet 135A, 2005

Autistic child in practice

- **Autism+** (*complex*) vs. **Autism** (*essential*)
- **Autism+** 20-30% ASDs, biomarkers (e.g. dysmorphism, microcephaly), worse prognosis, lower M/F proportion, lower recurrence risk, no family history

Dgn effectiveness in autism+ group 25%

ASDs – genetic diagnostics (Baylor, TCM 2008 recommendations)

- kariotyp – all affecteds
- aCGH – all affecteds
- FRAX – all affecteds
- *PTEN* – consider if head circumference > 2.5SD
- *MECP2* – consider in females

Who's supposed to do it?

ASDs – genetic diagnostics (ACMG 2008 recommendations)

- karyotype (5%)
- aCGH (10% - 20% - 30%!) **[IMiD=12/146]**
- FRAX (5%)
- *PTEN* (3%, when head circumference >2.5SD)
- *MECP2* (5% - only in females)
- other (10%)

American College of Medical Genetics 2008
www.acmg.net

Clinical practice(1)

- 24/12 boy referred from early intervention with **F84.9**
- markers: pedigree (-), anomalies (-), dysmorphism (-), growth delay (-), **dev delay (+)**, seizures (-), macro-/microcephaly (-)
- Autism vs. Autism+
- karyotype/aCGH ⇒ observation (F84.0?F70?) ⇒ FRAX? monogenic syndrome? unknown?

Clinical practice (2)

- 24/12 boy referred from early intervention with **F84.9**
- markers: pedigree (-), **anomalies (+) (renal hypo-/dysplasia, bilateral ing hernia)**, **dysmorphism (+)**, growth delay (-), **dev delay (+)**, seizures (-), macro-/microcephaly (-)
- Autism vs. Autism+
- karyotype/aCGH → monogenic disorder? (Pallister-Killian s.?) → clinical recommendations, genetic counselling

ASDs – referral

- + dysmorphism
- + congenital anomaly (-ies)
- + ID / cev delay
- + macrocephaly >3SD / microcephaly <3SD
- + ASDs family history or of other neuropsychiatric disease in 1st / 2nd degree relatives
- + abnormal genetic test result
- + dev regression

Genet Med 10(4), 2008
Texas Children's Hosp guidelines 2011