Genetic research on the basis of Leonhard‘s differentiated psychopathology

Update November 2013

Leonhard's classification of the schizophrenic psychoses

	psychic system		
	psychomotility	affectivity	thought
cycloid psychoses	motility psychosis	anxiety-happiness psychosis	confusion psychosis
	good prognosis		
	poor prognosis		
unsystematic schizophrenias	periodic catatonia	affect-laden paraphrenia	cataphasia
systematic schizophrenias	systematic catatonias	hebephrenias	systematic paraphrenias

Replication study on loci genome-wide associated schizophrenia markers in a German population

				Allele frequency				Schizophrenia (SCZ) n = 936 Controls (CON) n = 585			Bipolars (BP) n = 633 Controls (CON) n = 585			SCZ + BP n = 1,569 CON n = 585		
Chr	Locus	SNP	Risk allele	SCZ	BP	SCZ +BP	CON	HWE	P	OR (CI)	HWE	P	OR (CI)	HWE	P	OR (CI)
2	ZNF804A	rs1344706	T	0.62	0.60	0.61	0.61	1.00	0.31	1.08 (0.93–1.26)	0.83	0.82	1.02 (0.86–1.20)	0.51	0.58	1.04 (0.90–1.20)
11	intergenic	rs1602565	C	0.13	0.13	0.13	0.12	0.55	0.87	1.02 (0.81–1.28)	0.16	0.66	1.06 (0.82–1.35)	0.42	0.75	1.03 (0.84–1.28)
11	OPCML	rs3016384	C	0.49	0.48	0.49	0.49	0.54	0.85	1.01 (0.87–1.18)	0.18	0.67	1.04 (0.88–1.22)	0.23	0.94	1.01 (0.88–1.15)
12	NOS1	rs6490121	G	0.31	0.34	0.32	0.32	0.58	0.67	1.04 (0.88–1.22)	0.72	0.29	1.10 (0.92–1.31)	0.82	0.81	1.02 (0.88–1.18)
16	intergenic	rs7192086	T	0.23	0.23	0.23	0.23	0.87	0.78	1.03 (0.86–1.23)	0.76	0.90	1.01 (0.83–1.23)	0.32	0.81	1.02 (0.87–1.20)
16	RPGRIP1L	rs9922369	A	0.03	0.03	0.03	0.02	0.69	0.39	1.23 (0.75–2.02)	0.85	0.35	1.27 (0.75–2.17)	0.40	0.32	1.25 (0.79–1.97)

Chr: Chromosome; HWE: Hardy-Weinberg-equilibrium; P: significance level; OR (CI): estimated Odds ratio (95% confidence interval).

Schanze et al. 2011

New genetic loci for periodic catatonia on chromosome 7p14.1 and 19p12 in a genome-wide association study with pooled DNA

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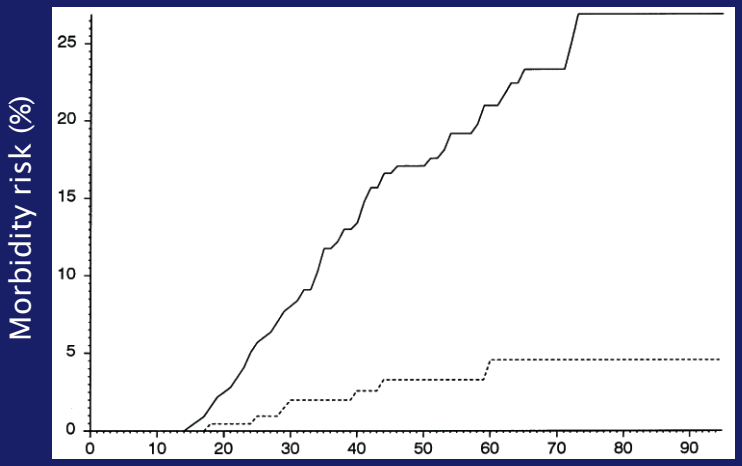
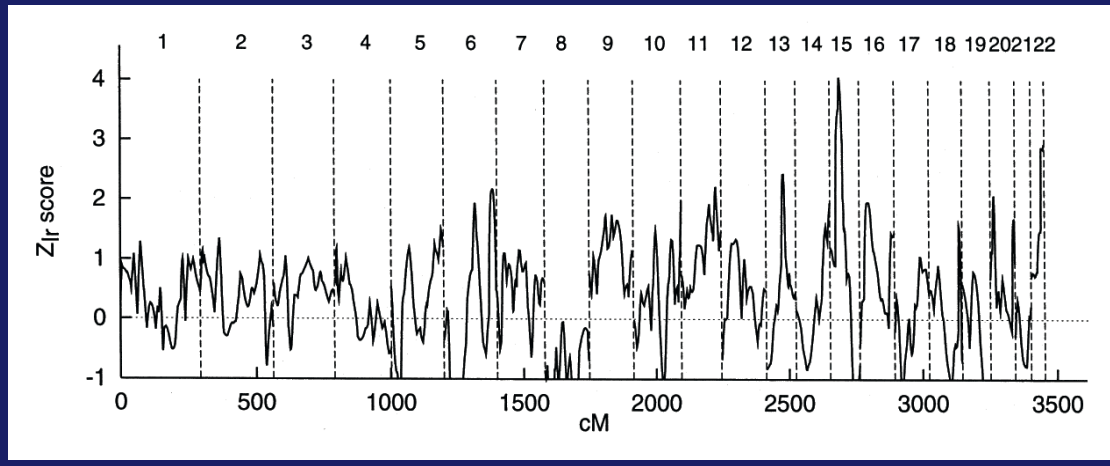
Berlin, September 27 2013

The Biological Basis of Periodic Catatonia



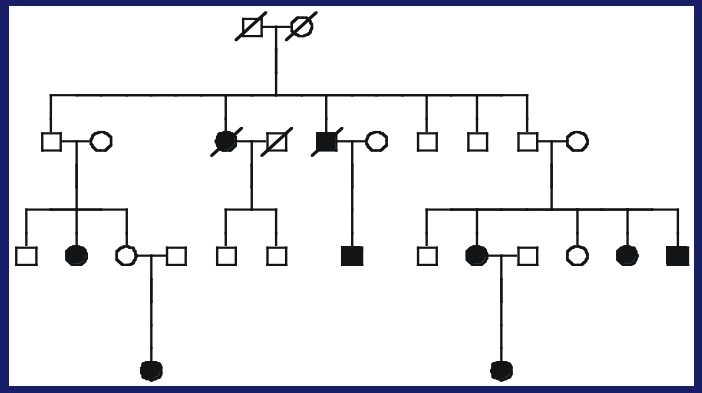
Periodic catatonia (83 cases; 323 first degree relatives)

Systematic catatonia (56 cases; 220 first degree relatives)



Non-parametric multipoint analysis and parametric analysis point to a major disease locus on chromosome 15q15 (Genehunter-Plus); 12 Pedigrees

F 11



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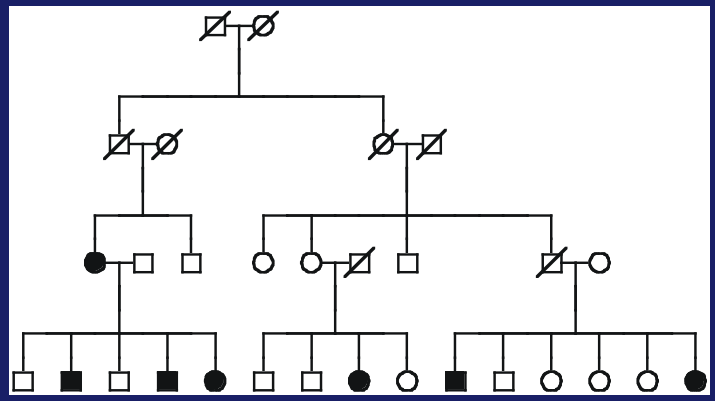
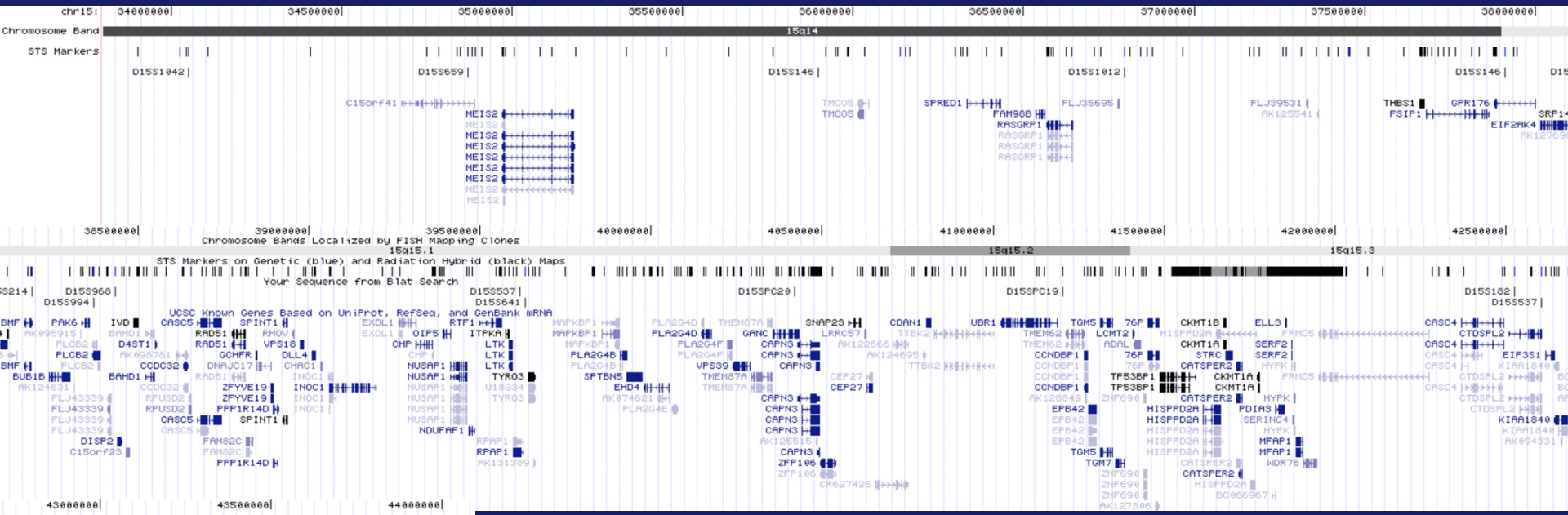
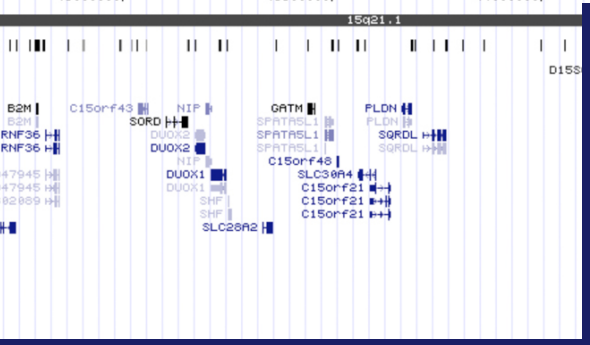
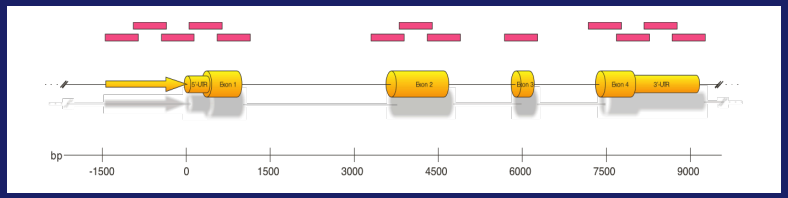


Stöber et al. 1996, 2000, 2001

Positional cloning approach on chromosome 15q15: systematic mutation screening

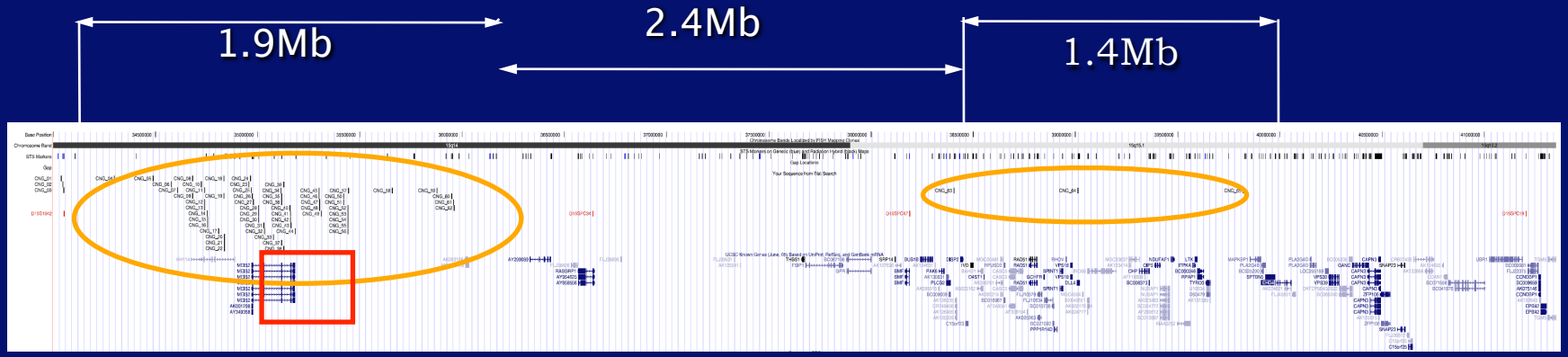






Gene	Potential Function	Position (Mb)	Number of SNPs
Meis2	hox-DNA binding cofactor (mouse) homolog 2	30079195 – 30286139	2
KIAA0945	large brain expressed protein of unknown function	33627659 – 33656167	3
KIAA0252	large brain expressed protein of unknown function	34605175 – 34671002	10
KIAA1403	large brain expressed protein of unknown function	34705197 – 34732287	23
Tyro 3	receptor protein tyrosine kinase	34747219 – 34767358	7
β-spectrin V	beta V spectrin	35036168 – 35082098	51
EHD4	EH-domain containing protein	35087461 – 35160567	12
SNAP 23	synaptosomal- associated protein	35683658 – 35721079	6

Conserved non-genic sequences (CNGs) and ultra-conserved elements at chromosome 15q15



MEIS2 locus
62 CNGs within 1.9Mb
(⌀ 1x each 31.5kb)
7 ultra-CNGs

3 CNGs
within 1.4Mb
0 ultra-CNGs

UCSC Genome Browser

Sequencing of CNGs in 8 independent cases (multiplex pedigrees) and 8 controls:

34 variants in 22 CNGs: **1x 9 bp-duplication**

9 SNPs within CNG, 24 SNPs in flanking regions

1 SNP appeared in controls only

3 SNPs appeared in cases only, 1 SNP cosegregated in the corresponding pedigree (Ped. 14)

analysis in 240 cases and controls each failed association

Schanze et al. 2012

Phase I: Pooling-based genomewide SNP association study

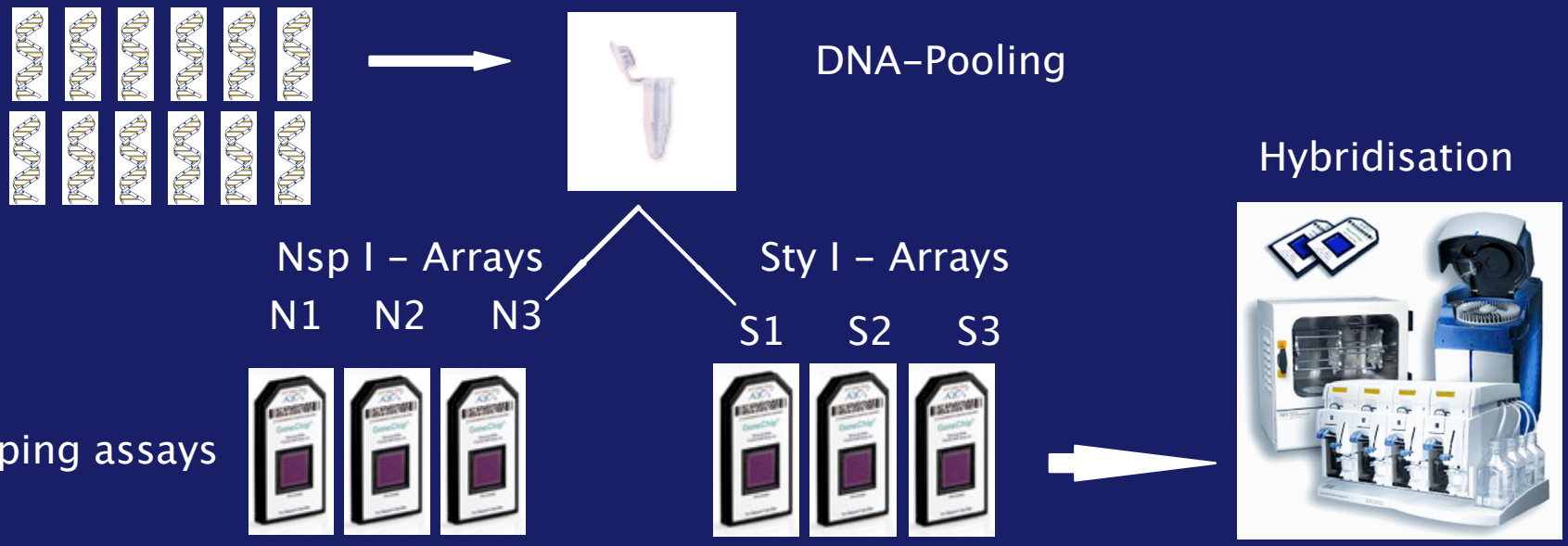
Affymetrix: GeneChip® 500K – SNP-Array

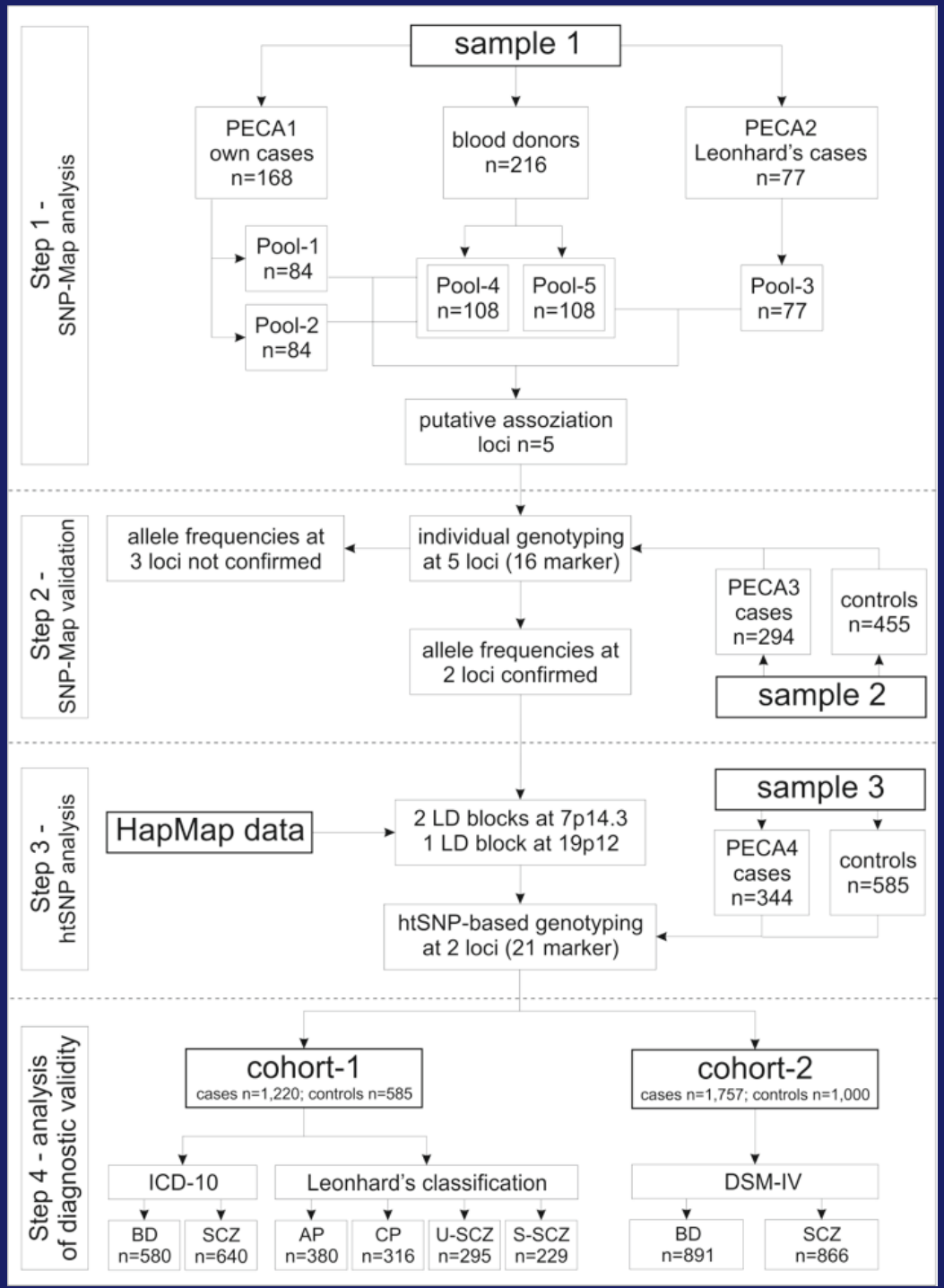
500k Chip – 500.568 SNPs

incl. 1242 SNPs at chromosome 15q15

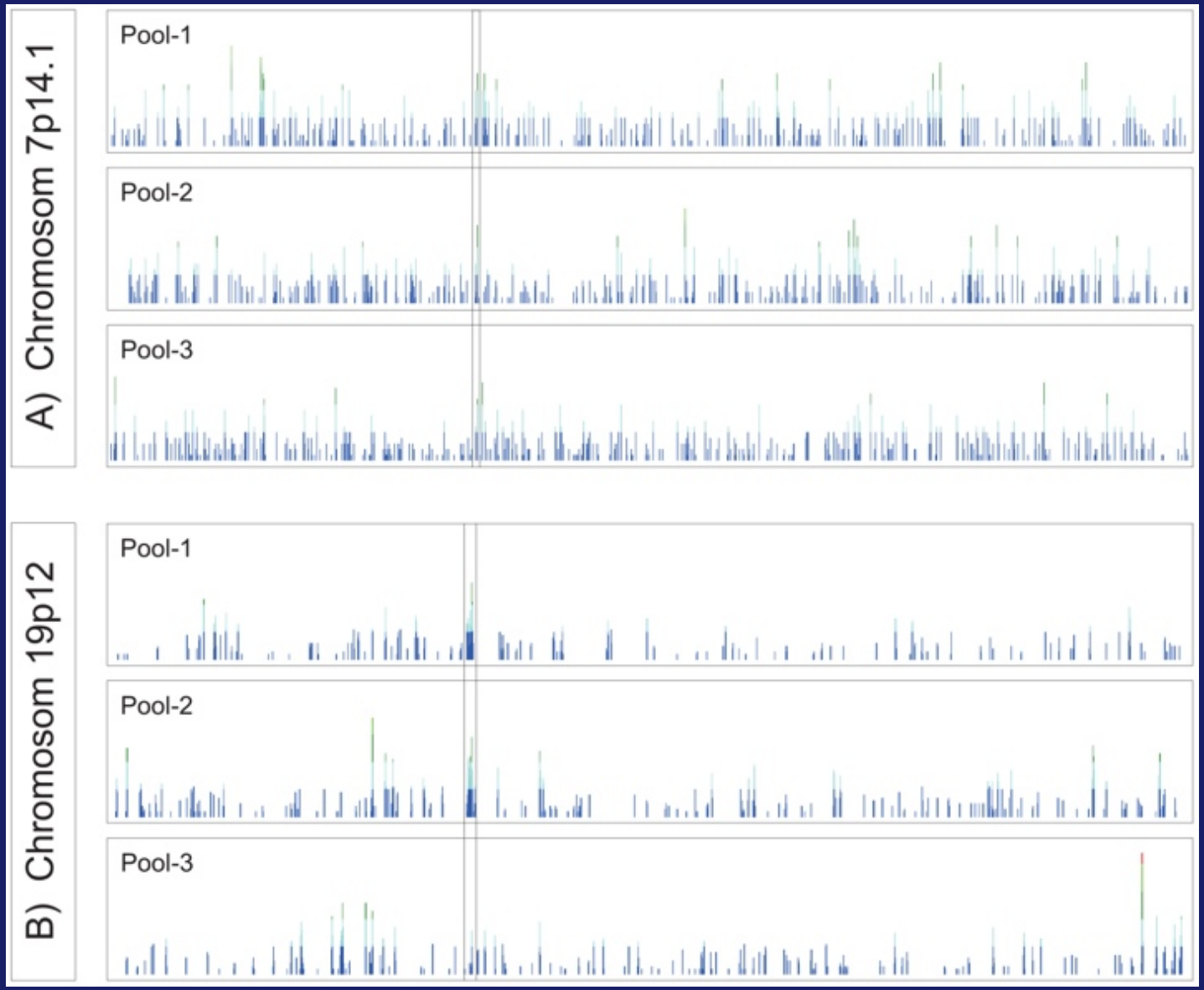
pooling of DNAs (checking probe intensity and quantifying DNA, placed into subpools)

triplicate technical replicates (total of 30 arrays)




Step 1 - SNP-Map analysis
sample 1
PECA1 own cases n=168
blood donors n=216
PECA2 Leonhard's cases n=77
Pool-1 n=84
Pool-2 n=84
Pool-4 n=108
Pool-5 n=108
Pool-3 n=77
putative assoziation loci n=5
Step 2 - SNP-Map validation
allele frequencies at 3 loci not confirmed
individual genotyping at 5 loci (16 marker)
PECA3 cases n=294
controls n=455
allele frequencies at 2 loci confirmed
sample 2
Step 3 - htSNP analysis
HapMap data
2 LD blocks at 7p14.3
1 LD block at 19p12
sample 3
PECA4 cases n=344
controls n=585
htSNP-based genotyping at 2 loci (21 marker)
Step 4 - analysis of diagnostic validity
cohort-1
cases n=1,220; controls n=585
cohort-2
cases n=1,757; controls n=1,000
ICD-10
Leonhard's classification
DSM-IV
BD n=580
SCZ n=640
AP n=380
CP n=316
U-SCZ n=295
S-SCZ n=229
BD n=891
SCZ n=866

Genomewide association study with pooled-DNA



Validation of the five top ranking loci from SNP-MaP analysis: individual genotyping in periodic catatonia samples (step 2)

permutation test (10.000 replicates) using Haploview 4.0

			frequency				
locus	marker	allele/ haplotype	cases (n=294)	controls (n=455)	P	Pc	OR (95 % CI)
4p12	rs17573717	T	0.460	0.423	0.1656	–	–
	rs7694104	T	0,677	0.591	**0.0008**	**0.0097**	**1.45 (1.16-1.81)**
	rs13146880	G	0.654	0.585	**0.0079**	0.0987	–
		TTG	0.290	0.255	0.1283	–	–
5p15.1	rs2929723	T	0.608	0.563	0.0849	–	–
	rs2929726	G	0.629	0.571	**0.0257**	0.2884	–
	rs2929710	A	0.627	0.572	**0.0350**	0.3666	–
		TGA	0.440	0.388	**0.0453**	0.5290	–
7p14.1	rs6943380	A	0.699	0.605	**0.0003**	**0.0037**	**1.51 (1.21-1.90)**
	rs4724159	G	0.782	0.729	**0.0222**	0.2532	–
	rs10274944	A	0.588	0.559	0.2835	–	–
	rs12690832	T	0.589	0.540	0.0668	–	–
		AGAT	0.377	0.289	**0.0003**	**0.0031**	**1.49 (1.20-1.86)**
10q26.3	rs2637630	G	0.802	0.770	0.1405	–	–
	rs2814154	C	0.901	0.883	0.2762	–	–
	rs2035804	G	0.799	0.776	0.2947	–	–
		GCG	0.778	0.751	0.2383	–	–
19p12	rs16999009	G	0.894	0.853	**0.0222**	0.2510	–
	rs398945	A	0.881	0.840	**0.0292**	0.3155	–
	rs4932803	G	0.753	0.658	**0.0001**	**0.0012**	**1.59 (1.25-2.01)**
		GAG	0.714	0.625	**0.0004**	**0.0037**	**1.50 (1.20-1.87)**

Haplotype-tagging SNP association analysis at chromosomes 7p14.1 and 19p12 in periodic catatonia (step 3)

			frequency				
locus	marker	allele/ haplotype	cases (n=344)	controls (n=585)	P	Pc	OR (95 % CI)
7p14.1	rs6943380	A	0.687	0.628	**0.0109**	0.0958	–
LD	rs4724159	G	0.775	0.748	0.1907	–	–
block-1	rs996791	T	0.427	0.379	**0.0426**	0.3162	–
	rs10273427	T	0.494	0.494	0.9949	–	–
	rs10243751	A	0.684	0.684	0.9857	–	–
		AGTCA	0.140	0.19	**9.3949^{-25}**	**1.88^{-24}**	**8.34 (7.87-8.81)**
7p14.1	rs6960577	A	0.645	0.636	0.6899	–	–
LD	rs1006108	C	0.287	0.260	0.2029	–	–
block-2	rs10274944	A	0.566	0.546	0.4158	–	–
	rs17708960	A	0.813	0.790	0.2375	–	–
	rs12690832	T	0.553	0.531	0.3503	–	–
	rs7782118	C	0.483	0.457	0.2890	–	–
	rs1029479	A	0.278	0.248	0.1531	–	–
		ACAATCA	0.213	0.189	0.2265	–	–
19p12	rs874843	A	0.714	0.655	**0.0084**	0.0621	–
	rs4933016	C	0.740	0.668	**0.0012**	**0.0086**	**1.42 (1.21-1.63)**
	rs8100273	T	0.726	0.654	**0.0013**	**0.0090**	**1.41 (1.20-1.61)**
	rs16999009	G	0.885	0.857	0.0806	–	–
	rs398945	A	0.878	0.845	0.0512	–	–
	rs11085548	T	0.845	0.801	**0.0163**	0.1197	–
	rs433830	T	0.708	0.635	**0.0013**	**0.0089**	**1.40 (1.19-1.60)**
	rs4932803	G	0.757	0.673	**0.0001**	**0.0015**	**1.51 (1.30-1.73)**
	rs8101516	C	0.756	0.674	**0.0002**	**0.0020**	**1.50 (1.28-1.71)**
		ACTGATTGC	0.655	0.588	**0.0042**	**0.0384**	**1.33 (1.14-1.53)**

Evaluation of htSNP-based association results in bipolar and schizophrenic disorders according to ICD-10 and DSM-IV (step 4)

SNP	allele	cohort-1 (ICD-10) controls (n=585) vs		cohort-2 Bonn (DSM-IV) controls (n=1000) vs		comparison of allele frequencies between control groups	
		bipolar disorder (n=580)	schizophrenia (n=640)	bipolar disorder (n=891)	schizophrenia (n=866)		
		P	*P*	*P*	*P*	P	Δf [%]
rs6943380	A	0.5924	0.8579	0.8402	0.9826	0.3700	1.6
rs4724159	G	0.8105	0.6647	0.4495	0.3589	0.6810	0.7
rs996791	T	0.8627	0.5959	0.5278	0.7834	0.5534	1.1
rs10273427	T	0.6992	0.9430	n.a.	n.a.	n.a.	n.a.
rs10243751	A	0.1033	0.2559	0.6178	0.6653	0.8577	0.3
rs6960577	A	0.7087	0.0987	0.2553	0.5234	0.6280	0.9
rs1006108	C	0.4943	0.7509	0.3777	0.5971	0.7760	0.5
rs10274944	A	0.8973	0.3463	0.1343	0.8970	0.7044	0.6
rs17708960	A	0.3522	0.6934	**0.0368**	**0.0296**	0.2445	1.8
rs12690832	T	0.3395	0.4766	0.1484	0.8260	0.3715	1.7
rs7782118	C	0.2800	0.2140	**0.0484**	0.7737	0.7419	0.6
rs1029479	A	0.2012	0.3949	0.5176	0.0846	0.2849	1.6
rs874843	A	**0.0121**	0.1313	0.7164	0.1659	**0.0011**	5.5
rs4933016	C	**0.0104**	0.1584	0.7675	0.2594	**0.0142**	4.1
rs8100273	T	**0.0051**	0.1913	0.8008	0.2062	**0.0024**	5.2
rs16999009	G	0.1716	0.4264	0.5783	0.8020	**0.0162**	2.9
rs398945	A	0.1760	0.3217	0.5363	0.3821	**0.0092**	3.3
rs11085548	T	0.1514	0.6428	0.2513	0.2152	0.1432	2.1
rs433830	T	0.0504	0.2278	0.5915	0.1502	**0.0045**	4.9
rs4932803	G	**0.0174**	0.1420	0.6435	0.1344	**0.0020**	5.2
rs8101516	C	**0.0144**	0.1580	0.6200	0.1581	**0.0054**	4.7

Conclusion: Periodic Catatonia

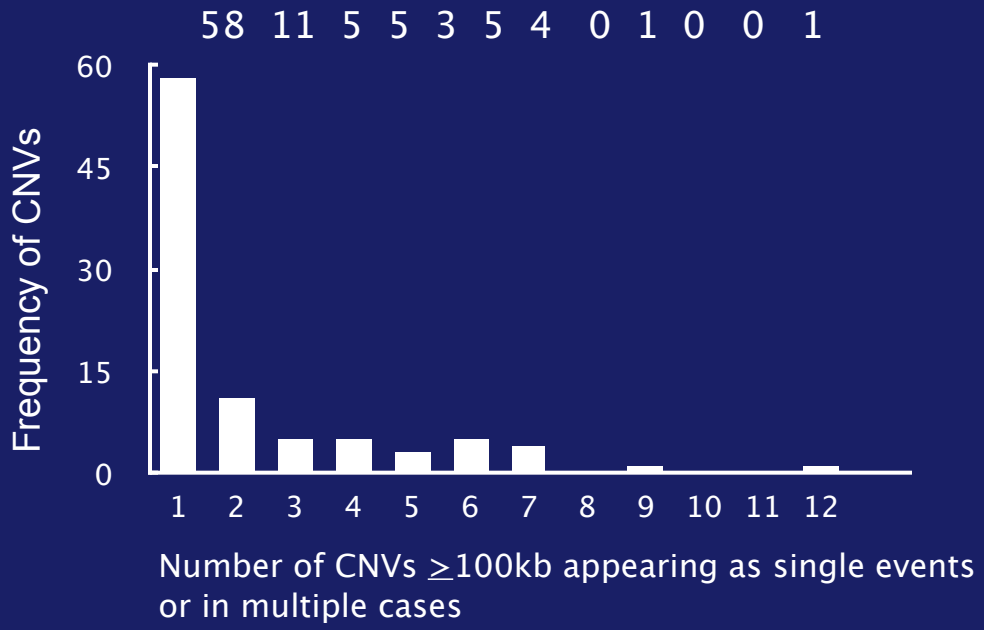
- complex disorder with genetic heterogeneity
- chromosome 15q15 as major disease locus:
  lack of relative allele frequency differences in sub-pools
  lack of founder mutation in coding regions
  lack of common ancestral haplotypes
- SNP-MaP genome-wide association study and individual genotyping of SNPs: associated loci at chromosome 7 and 19
- no association of CNVs with early-onset cases

Why study de novo CNVs?

- CNVs that increase risk to develop schizophrenia are under strong selection pressure and should be filtered out from the population by selection.
- Pathogenic CNVs must maintain prevalence by de novo mutations.
- CNVs with large effects on disease risk should be enriched among de novo mutations.
- Rare de novo CNVs may highlight particular genes/ pathways that are disrupted in wider SZ population.

Frequency and length of CNVs in juvenile periodic catatonia

Index	Gender males	Age at first hospitalisation /yrs	Age at assessment /yrs	Index	Gender females	Age at first hospitalisation /yrs	Age at assessment /yrs
1–12	m = 12	11.9 SD ±2.6	37.4 SD ±19.0	13–24	f = 12	14.1 SD ±2.2	38.9 SD ±11.6
	range	7 – 16	18 – 62		range	8 – 17	23 – 55
total (n = 24)		13.2 SD ±2.6	38.2 SD ±15.4				

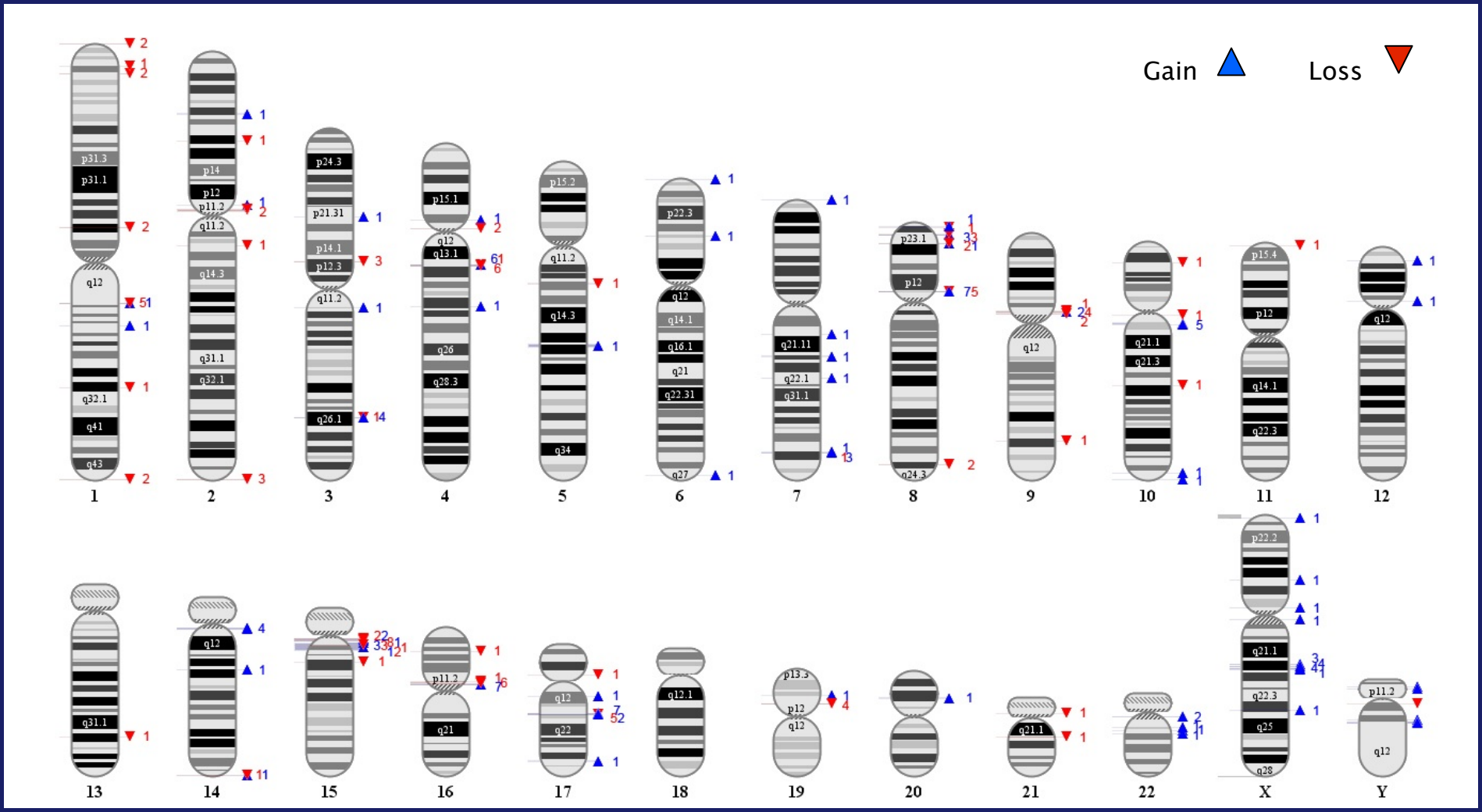


Number of CNVs ≥100kb in the total sample (n= 24)

93 individual CNVs with a total of 209 observations

62% of CNVs observed only once

Frequency and distribution of CNVs in juvenile periodic catatonia (n = 24)
SNP Microarrays – GTC software



Karyogram Genotype Console (GTC) v3.2; with segments >100kb, gain/loss ≥20 markers

Chromosomal deletion hot-spots in schizophrenia

Authors	Chromosome	Position (Mb)	juvenile Periodic Catatonia (n = 24)
International Schizophrenia Consortium 2008 Stefansson et al. 2008 Kirov et al. 2009	1q21.1	144.964403-146.281318	-
Vrijenhoek et al. 2008 Kirov et al.2008 Rujescu et al. 2008 Need et al. 2009	2p16.3 (NRXN1)	51.10-51.35	-
Bassett et al. 2008 Stefansson et al. 2008	15q11.2	20.3-20.8	-
International Schizophrenia Consortium 2008 Stefansson et al. 2008 Kirov et al. 2009	15q13.3	28.7-30.3	-
Walsh et al. 2008	16p22.1	69.395935-69.748827	-
Bassett et al. 2008 International Schizophrenia Consortium 2008 Kirov et al. 2009	22q11.21	17.275227-19.791017	-

Conclusions:

- We identified a total of 209 copy number variations (gains and losses) with ≥100kb and loss of ≥20 markers (without Y chromosome) in a sample of 24 individuals with juvenile periodic catatonia (average of 9 aberrations per individual).
- The CNVs were distributed over the whole genome.
- All CNVs were tested for their appearance in the Database of Genomic Variants (DGV, Iafrate et al. 2004).
  - 197 out of 209 CNVs (94.3%) were contained in the DGV.
  - 4 aberrations (1.9%) on chromosomes 8, 9, 10 and 13 (all losses) and 8 aberrations (3.8%) on the X chromosome (all gains) showed no overlaps with known CNVs from DGV,
  - but were contained in a CNV database at the Institute of Human Genetics, University of Erlangen, obtained from 1.488 controls from studies on psoriasis vulgaris or pseudoexfoliation syndrome (PEX).
- We identified no overlap with CNVs associated with schizophrenia and no enrichment of larger aberrations (>100kb) in the 24 individuals with juvenile periodic catatonia compared to published data.
- We were unable to identify associated CNVs in early onset periodic catatonia, a population one might expect to be enriched for genetic rather than environmental factors.

Genome-wide search for de novo copy-number variations (CNVs) with phenotype correlation

Sample:

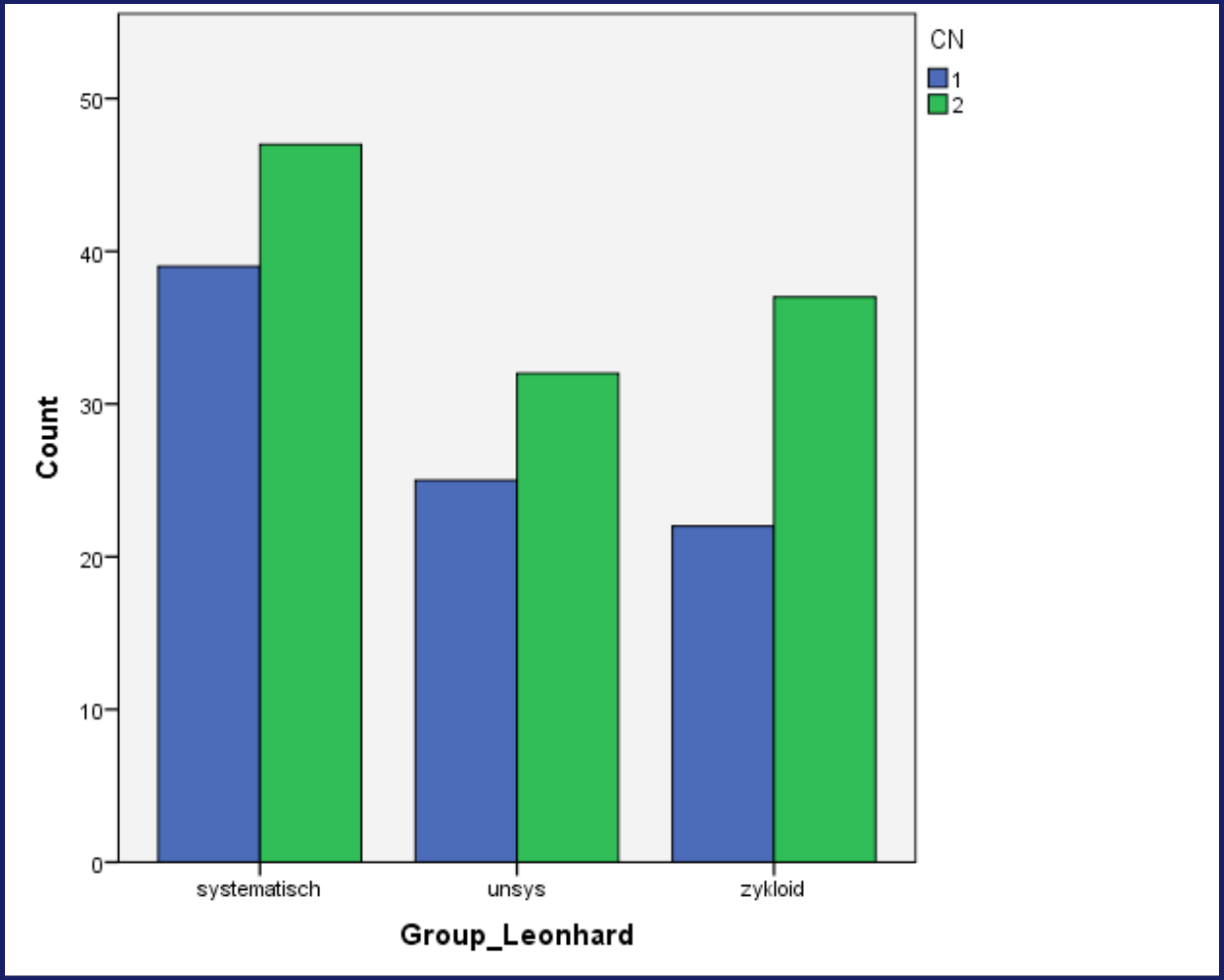
- Analysis of de novo CNV occurrence in 270 parent-proband trios with SZ according to ICD10.

- Phenotype characterisation
  - 105 cases with systematic schizophrenias.
  - 81 cases with cycloid psychoses.
  - 84 cases with unsystematic schizophrenias.
- Cases include 73% males; mean age at onset 24.4 years, mean age at assessment 31.5 years.

Control sample of 2000 German individuals (KORA).

CNV results:

- Mean size 284 kb, 73 probes, 11 CNVs > 1 MB
- 86 Deletions (blue, 43%) and 116 Duplications (green, 57%).



Recurrent CNVs in schizophrenia

Locus	CNV	Position (Mb)	Size (Mb)	Genes	Frequency		in 270 Trios
1q21.1	Del	144.6–146.3	1.67	11	0.00176		
2p16.3	Ex del	49.9–51.5	0.02–0.42	NRXN1	0.00182		
3q29	Del	197.2–198.8	0.84–1.6	19	0.0008		
3q29	Dup	196.8–196.9	0.05	2	0.00121		
7q36.3	Dup	158.5–158.8	0.12–0.36	VIPR2	0.00191		
15q11.2	Del	20.3–20.8	0.5	4	0.00551		
15q13.3	Del	28.7–30.3	1.5	8	0.00193		
15q11.2–13.1	Mat dup	20.3–26.4	4.1–9.0	13–24	0.00053		**2**
16p11.2	Dup	29.4–30.1	0.7	26	0.00313		
16p13.1	Dup	14.6–18.7	1.16	11	0.00299		**Del**
17p12	Del	14.1–15.4	0.93–1.31	15	0.00151		**2**
22q11.2	Del	17.1–20.2	1.4–2.5	29–43	0.00307		**Dup**

Transmission of CNVs:

Reported CNV de novo rate in SZ ~5%-10%:

- Walsh et al, 2008 (10%), Xu et al, 2009 (10%)
- Kirov et al, 2011 (5%), Malhotra et al, 2011 (5%)

	Trios	paternal	maternal	de novo CNVs	%
systematic	105	27	38	5	4.8
unsystematic	84	23	24	4	4.8
cycloid	81	18	20	2	2.4
controls	6316			115	1.8

Summary:

202 rare CNVs and 11 de novo CNVs were identified in 270 trios:

- Rate of deletions and double hits corresponded to Leonhard`s groups and might reflect course of disease.
- Early onset of disease correlated with CNV size.
- Early onset systematic catatonia showed highest burden (size).
- De novo rate in schizophrenias (4.8%) was similar to previous findings, but less in cycloid psychoses (2.4%).
- De novo CNVs point to genetic risk factors for systematic schizophrenias.