Institute of Mother and Child

Rekomendacje Europejskiego Towarzystwa Genetyki Człowieka w badaniach cytogenetycznych

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POLICY

European guidelines for constitutional cytogenomic analysis

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Abstract

With advancing technology and the consequent shift towards an increasing application of molecular genetic techniques (e.g., microarrays, next-generation sequencing) with the potential for higher resolution in specific contexts, as well as the application of combined testing strategies for the diagnosis of chromosomal disorders, it is crucial that cytogenetic/cytogenomic services keep up to date with technology and have documents that provide guidance in this constantly evolving scenario. These new guidelines therefore aim to provide an updated, practical and easily available document that will enable genetic laboratories to operate within acceptable standards and to maintain a quality service.

The following standards **should be considered as minimum acceptable criteria for constitutional chromosomal/cytogenomic analysis** and therefore, any laboratory consistently operating below the minimum standard may be at risk of failing to maintain a quality service. S

Main clinical indications for prenatal diagnosis

- Abnormal foetal ultrasound;
- High-risk maternal serum screening/NIPT result indicating an increased risk of a chromosomally abnormal foetus;
- Parental chromosome rearrangement, mosaicism or previous aneuploidy;
- Previous livebirth/stillbirth with a chromosome abnormality;
- Possible foetal mosaicism detected by prior prenatal study;
- Familial monogenic disorder (i.e., CF, Noonan syndrome, etc).

Main clinical indications for postnatal diagnosis

- Abnormal clinical phenotype;
- Multiple congenital abnormalities;
- Clinically significant abnormal growth – short stature, excessive growth, microcephaly, macrocephaly;
- Primary or secondary amenorrhoea or ovarian insufficiency;
- Ambiguous genitalia;
- Infertility of unknown aetiology;
- Sperm abnormalities – azoospermia or severe oligospermia;
- A malformed foetus or stillbirth of unknown aetiology;
- Third and subsequent miscarriage(s): products of conception;
- Significant familial history of chromosome rearrangements;
- Significant familial history of intellectual disability (ID) of possible chromosomal origin where it is not possible to study the affected individual;
- Carriership for an X-linked recessive disorder in a female.

Table 1 Methods used in cytogenomic analysis, their resolution and limitations

Method	Resolution	Limitations
Karyotyping	5–10 Mb	Cannot detect: small rearrangements below the resolution; nucleotide variants; mosaicism < 10%[a]; UPD[b]
FISH	~100 kb	Limited to the probes used (targeted analysis); Cannot detect: nucleotide variants; mosaicism < 10%[c]; UPD
Array-based techniques chromosomal microarray SNP-based array	~20–200 kb	Cannot detect: balanced rearrangement; mosaicism < 10%[d]; nucleotide variants; the nature of a structural aberration; independent cell lines; heterochromatic markers; triploidy (exception SNP array); UPD (exception SNP array)
CNV detection in whole-exome sequencing	100 bp (exonic regions) – ~150 kb (genome wide)[e]	Cannot detect: balanced rearrangement; mosaicism < 18%[f]; the nature of the structural aberration; independent clones/cell lines

[a]Hsu and Benn [7]; Hook [36]

[b]Uniparental disomy

[c]Wiktor and van Dyke [37]; Ballif et al. [38]; Mascarello et al. [39]

[d]Vermeesch et al. [23]; Ballif et al. [38]

[e]Pfundt et al. [32]

[f]Pagnamenta et al. [40]

Zasady ogólne:

- Analiza powinna być wykonana prze wykwalifikowanego pracownika, po odpowiednim treningu.
- Każde badanie powinno być sprawdzone niezależnie przez drugą wykwalifikowaną osobę.
- Wynik badania powinien być jednoznaczny i zrozumiały dla niespecjalisty.
- Wynik badania musi zawierać interpretację w odniesieniu do wskazania do badania.
- Wynik badania powinien zawierać zapis zgodny z najnowszą wersją ISCN (Inetrnational System for Human Cytogenomic Nomenclature)
- Nieprawidłowości stwierdzone w pojedynczych komórkach oraz polimorfizmy nie powinny być zawarte w wyniku badania

Table 2 Recommended reporting times (calendar days) for 90% of the referrals

Prenatal aneuploidy testing by FISH /QF-PCR/MLPA	4 days
Amniotic fluid and CVS analysis on cultured or uncultured cells by karyotyping and/or genome-wide array analysis	14 days
Lymphocyte cultures	28 days
Products of conception/foetal skin (where pregnancy is not ongoing)	28 days
Urgent[a] lymphocyte, cord blood cultures	7 days
Postnatal array analysis	10 days (urgent) 28 days (other)

These apply in the absence of specific National Guidelines

[a]URGENT – referrals where the result will have immediate implications for patient management

Table 3 Minimum G-banding quality according to the reason for referral

Reason for referral	Minimum G-banding quality (QAS) [a]
Confirmation of aneuploidy	QAS 2[a] ⇔ < 300 bphs
Exclusion of known large structural rearrangements	QAS 3 ⇔ 300 bphs
Identification and exclusion of small expected structural rearrangements; routine prenatal specimen preparations	QAS 4 ⇔ 400 bphs[b]
Prenatal specimen abnormal ultrasound referrals (in the absence of array-based analysis)	QAS 5 ⇔ 550 bphs[b]
Routine postnatal specimen preparations	QAS 6 ⇔ 550 bphs[b]

bphs bands per haploid set

[a]QAS score – see ACC Professional guidelines for clinical cytogenetics v1.04 (2007; http://www.acgs.uk.com/media/765607/acc_general_bp_mar2007_1.04.pdf)

[b]SNP-based array or other molecular techniques/SNP-based array or other molecular techniques may be more applicable for some of these referral categories

Table 4 Minimum number of metaphases to be analysed according to tissue type

Sample	Referral/result	Minimum analysed[a]	Additional cells counted[b]
Prenatal	Routine	2 (2 cultures)[c]	0[d]
Postnatal	Routine	2[e]	0
Pre- and postnatal	Mosaicism exclusion or single-cell anomaly detection	2	28[f]

[a]Analysed (banded metaphases where every set of homologues without any crossovers are evaluated in their entirety). (In practice more metaphase are analysed to clear any crossovers – see section Karyotyping)

[b]Counted (metaphases where the number of centric chromosomes and/or the presence/absence of a specific cytogenetic feature is evaluated)

[c]QF-PCR and one culture in case of aneuploidy testing

[d]Extra cells may be counted to exclude mosaicism or a single-cell anomaly

[e]ACC Professional guidelines for clinical cytogenetics v1.04 (2007; http://www.acgs.uk.com/media/765607/acc_general_bp_mar2007_1.04.pdf) and Hastings et al. [1]

[f]Hsu and Benn [7] and Hook [36]

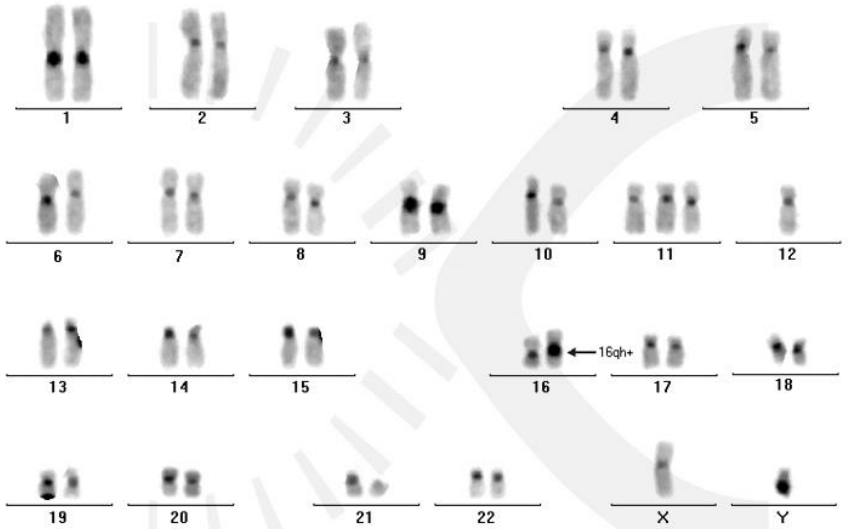
W diagnostyce prenatalnej:

- Co najmniej dwie niezależne hodowle
- Jeśli występuje podejrzenie zanieczyszczenia materiału komórkami matczynymi należy wykonać badania molekularne z krwi matki

Warianty polimorficzne

Warianty polimorficzne, które wcześniej były uwzględniane w wyniku jako „łagodne”, nie powinny być obecnie wpisywane.

Np. 1qh+/qh-, 9qh+/qh-, 16qh+/qh-, akrocentryczne p+ or p-, Yqh+/qh-, inv(9)(p11q13), inv(2)(p11.2q13) oraz warianty euchromatynowe, takie jak: 4p16, 8p23.1, 9p12, 9q13-q21.12, 15q11.2, 16p11.2



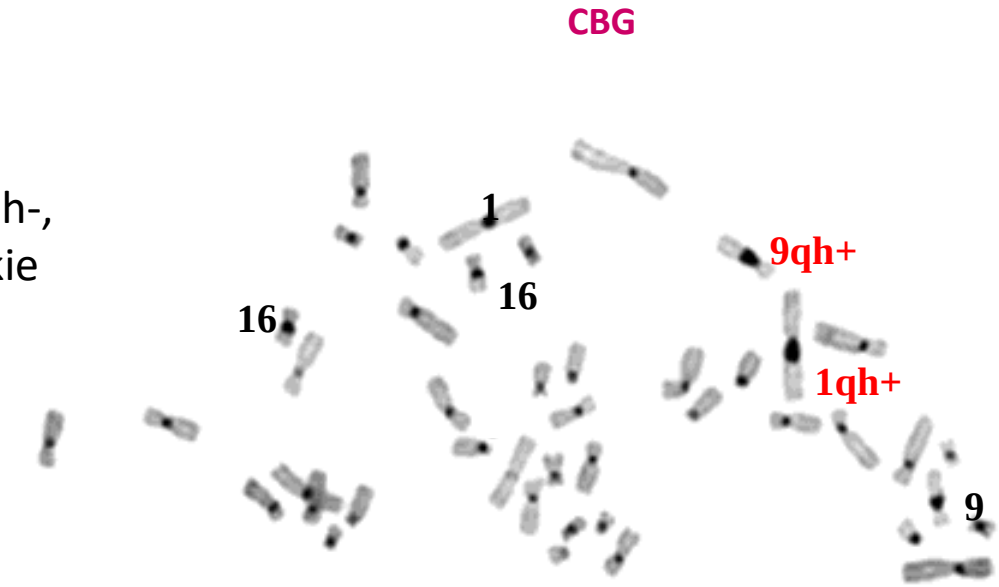


Table 5 Applications of FISH analysis

Type of FISH analysis	Applications of FISH analysis
Rapid prenatal FISH	High risk of chromosome aneuploidy or recurrent microdeletion (e.g., abnormal ultrasound); late gestation referral.
Interphase FISH	Evaluation/characterisation of Numerical abnormalities; Duplications; Deletions; Sex chromosome constitution; Mosaicism; Gene amplification.
Metaphase FISH	Marker chromosome; Unknown material attached to a chromosome; Rearranged chromosome(s); Suspected gain or loss of a chromosome segment; Mosaicism.

Table 6 Minimum criteria for analysing FISH results according with probe type

Probe type	Analysis	Additional comments
Locus-specific identifier (LSI) probes	5 metaphases	Score to confirm or exclude an abnormality (e.g., in case of suspected microdeletion syndrome or identification of a marker chromosome)
Multiprobe analysis	3 metaphases	Per probe. Scored to confirm a normal signal pattern. Confirmation is advisable for abnormal signal patterns if no control probe is present
Interphase analysis for aneuploidy testing	≥50 cells	For each probe set
Interphase analysis to detect mosaicism	≥100 cells	For each probe set

Mikromacierze:

- Rozdzielczość dla badań prenatalnych – przynajmniej 400-600 kpz
- Rozdzielczość dla badań postnatalnych – przynajmniej 200-400 kpz
- Platformy BAC nie są wystarczające do badań diagnostycznych.

- Platformy wzbogacone o sondy SNP, identyfikujące utratę heterozygotyczności:

- w wyniku powinien być uwzględniany region >10 Mpz – może być wskazaniem do disomii jednorodzicielskiej (UPD)

- >1% homozygotycznych regionów autosomów – wskazanie do pokrewieństwa pomiędzy rodzicami.

- Class 1: Benign;
- Class 2: Likely benign;
- **Class 3: Uncertain clinical relevance (uncertain significance);**
- **Class 4: Likely pathogenic;**
- **Class 5: Pathogenic**

Klasa 1 i 2 mogą być uwzględnione w wyniku tylko jeśli aberracja zawiera geny choroby recesywnej, której objawy są obecne u pacjenta. Np. delecja regionu 2q13 zawierająca gen *NPHP1* (130 kpz).

Table 3
ACMG recommended categories for defining the clinical significance of a CNV

Category	Definition	Example
Pathogenic	CNV is documented as clinically significant consistently and in multiple publications and/or case databases	22q11.2 deletion syndrome (DiGeorge/velocardiofacial syndrome)
Uncertain (no subclassification)	Clinical significance not know at time of reporting but CNV meets the laboratory reporting criteria; expected that CNVs in this category will shift toward pathogenic or benign over time	500-kb deletion of chromosome 3 that contains 5 genes but the inheritance of the deletion is not known and there is nothing known about the 5 genes in the deleted interval
Subclassifications		
Uncertain: likely pathogenic	Some evidence to increase the likelihood that the CNV is pathogenic	500-kb *de novo* deletion of chromosome 3 that contains 5 genes and there is a single case report in the literature that has similar breakpoints and shares a distinct phenotype
Uncertain: likely benign	Some evidence to increase the likelihood that the CNV is benign	500-kb deletion of chromosome 3 that does not contain any genes but is not found in any control databases
Benign	CNV is documented as benign consistently and in multiple publications and/or control databases or represents a common polymorphism (present in >1% of the population)	2q37.3 telomere polymorphism (~200 kb in size and well documented as benign in multiple studies)

Adapted from Kearney HM, Thorland EC, Brown KK, et al. Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. Genet Med 2011;13(7):680–85.

Diagnostyka prenatalna

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ESHG

POLICY

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Prenatally a CNV should be reported if it is consistent with the ultrasound findings as it will possibly affect the management of the pregnancy or a future individual or the family and is actionable. However, care should be taken when interpreting the identification of known pathogenic CNVs in a prenatal context in the absence of ultrasound anomalies as there is clinical ascertainment bias within the postnatal population [30].

Europejskie Rekomendacje

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Review

Implementation of genomic arrays in prenatal diagnosis: The Belgian approach to meet the challenges

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Europejskie Rekomendacje
Aspekty techniczne

- Wykonywane u wszystkich pacjentek, nawet dla ciąż o niskim ryzyku (1.7%)

Technical aspects
Use 60k arrays (or comparable resolution)
Always test for maternal cell contamination – to be evaluated after 6 months
Always obtain a parental blood sample (can be stored) – to be evaluated after 6 months
6 ml amniotic fluid is the minimum for direct DNA isolation to obtain reliable results on 60k array
Always have at least 1 backup flask in culture
Testing for triploidy is done by SNP array or a short tandem repeat (STR) multiplex system
A rapid aneuploidy test is not necessary if the turnaround time of the array is less than one week

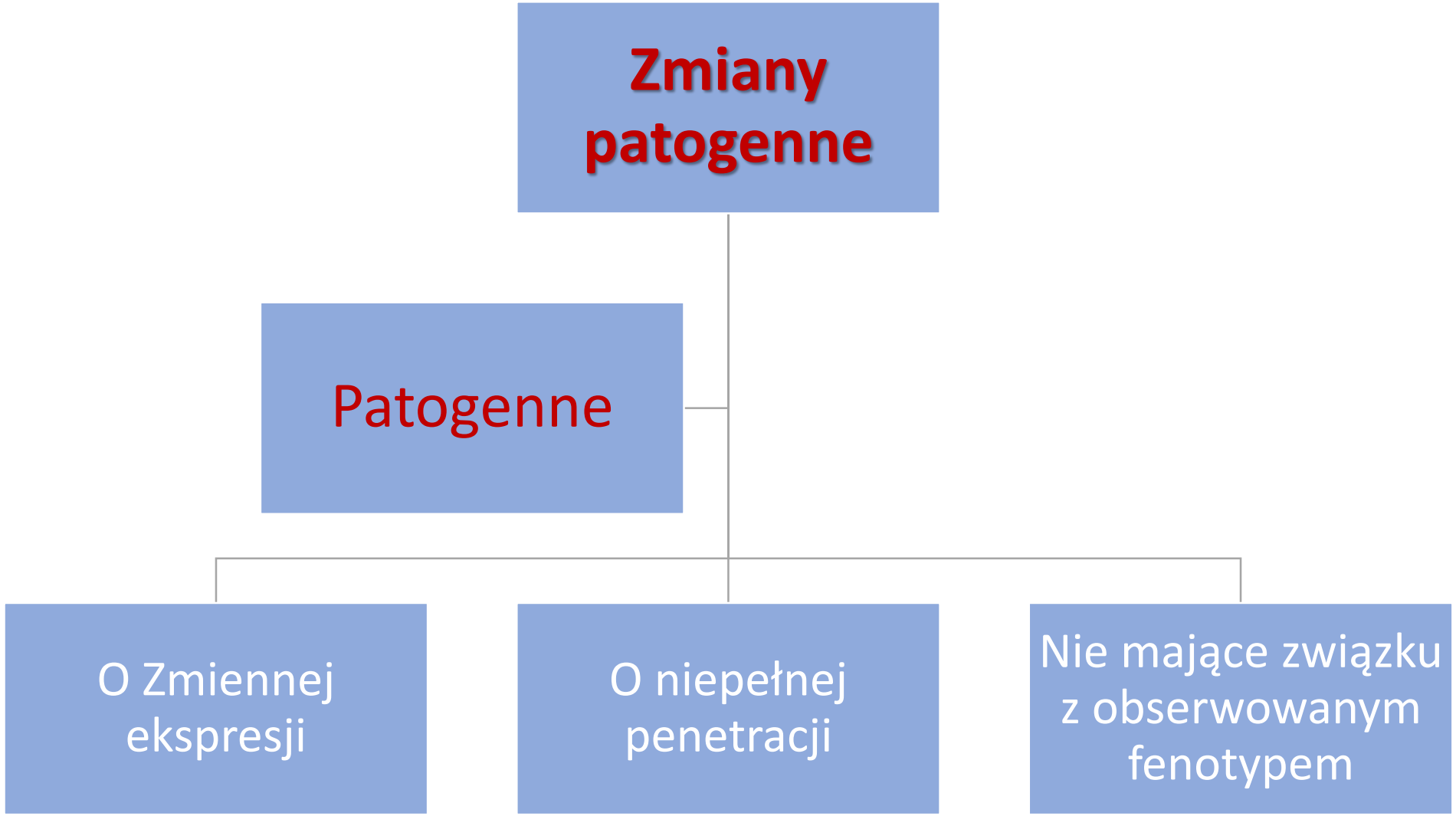
Europejskie Rekomendacje

Porada genetyczna przed wykonaniem badania

- Poinformowanie przyszłych rodziców o możliwościach i ograniczeniach technicznych metody
- Poinformowanie o możliwości stwierdzenia zmiany która NIE zostanie uwzględniona w wyniku (VOUS, CNV o zmiennej ekspresji, penetracji, zmiany patogenne które nie mają wpływu na nieprawidłowy obraz USG)
- Nie można wymagać od rodziców podjęcia natychmiastowej decyzji - ulotki

Kryteria oceny patogenności stwierdzonej zmiany:

- Zmiana *de novo lub* odziedziczona od chorego rodzica (>99% dziedziczonych CNV to CNV łagodne)
- Region delecji/duplikacji zawiera geny lub cały region opisany jest jako patogenny w bazie DECIPHER
- Region delecji/duplikacji nie znajduje się na liście wariantów polimorficznych (baza DGV)
- **TYLKO ZMIANY O ZNANEJ PATOGENNOŚCI!**


Zmiany patogenne
Patogenne
O Zmiennej ekspresji
O niepełnej penetracji
Nie mające związku z obserwowanym fenotypem

Zmiany nie mające związku z obserwowanym fenotypem/wskazaniem do badania

- Highly penetrant monogenic disorder which do not have a direct consequences for the fetus itself, but may have implication later for the individual or his/her relatives.

➢ Late-oneset disorders – których rokowanie zależy od wczesnego włączenia leczenia/ wpływ na członków rodziny/ leczenie może pojawić się w przyszłości (Lista genów i regionów opisana przez American College of Medical Genetics)(głównie onkologia)

➢ Stwierdzenie u płodu żeńskiego aberracji związanej z zespołem sprzężonym z chromosomem X

➢ Choroby autosomalnie recesywne, tylko jeśli mutacje występują w ogólnej populacji częściej niż 1:50

Zmienna expresja/niepełna penetracja

Table 2
Characteristics of susceptibility CNV's that are communicated.

CNV	Size	Gene	OMIM	p-value[a]	Ctrls (1/)[b,c]	Penetrance[d]	% de novo[b,d]	% 2nd hit[d]	Neurodevelop-mental phenotype	Ultrasound anomaly	References
Distal del 1q21.1	1.35 Mb	GJA5	613474	3.28E-07	3821	36.9 (23–55)	18-20%	8	DD, ID, ASD, E, ADHD	Microcephaly, CHD, Eye	Haldeman-Englert et al. [2011]
Distal dup 1q21.1	1.35 Mb	GJA5	612475	0.0002	4367	28.1 (163.9–46.8)	16.7–20%	7	DD, ID, ASD, SCZ	Macrocephaly, CHD,Eye	Haldeman-Englert et al. [2011]
Proximal del 1q21.1	200 kb	HFE2	274000	0.0859	10,191	17.3 (10.8–27.4)	0%	ND		Absent radius (TAR - recessive phenotype)	Toriello et al. [2012]
Distal del 16p11.2	220 kb	SH2B1	613444	0.0107	15,287	62.4 (26.8–64.4)	30–33.3%	13	DD, ID		Bachmann, et al. [2010]
Del 16p11.2	550 kb	TBX6	611919	3.39E-08	3387	46.8 (31.5–64.2)	65–70.2%	8	DD, ID, ASD, E	(CHD)	Wat et al. [2011]; Miller et al. [2011]
Del 17q12	1.4 Mb	TCF2	614527	0.0484	7643	34 (13.7–70)	55.6–62%	12	DD, ID, ASD, (SCZ)	CAKUT	Moreno-De-Luca et al. [2010]; Mefford et al. [2007]; Decramer et al. [2007]; Ulinski et al. [2006]
Dup 22q11.2	1.5-Mb/3 Mb	TBX1	608363	1.26E-05	1798	21.9 (14.7–31.8)	7–25.5%	8	DD, ID	Bladder extrophy, (CHD), (CP)	Firth et al. [2009]; Draaken et al. [2010]; Lundin et al. [2010]; Portnoï et al. [2009]

ADHD: attention deficit hyperactivity disorder, ASD: autism spectrum disorder, CAKUT: congenital anomalies of kidney and urogenital tract, CDH: congenital diaphragmatic hernia, CHD: congenital heart defect, CP: cleft palate Ctrls: controls; DD: developmental delay, E: epilepsy, ID: intellectual disability, SCZ: schizophrenia, TAR: thrombocytopenia absent radius.
( ): suggestive based on a few reports.
[a] association of CNV with developmental disorders, p value [taken from Cooper et al., 2011].
[b] Cooper et al. [2011].
[c] Rosenfeld et al. [2013].
[d] Girirajan et al. [2012].