Microdeletion and Microduplication Syndromes

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Summary

The widespread use of whole genome analysis based on array comparative genomic hybridization in diagnostics and research has led to a continuously growing number of microdeletion and microduplication syndromes (MMSs) connected to certain phenotypes. These MMSs also include increasing instances in which the critical region can be reciprocally deleted or duplicated. This review catalogues the currently known MMSs and the corresponding critical regions including phenotypic consequences. Besides the pathogenic pathways leading to such rearrangements, the different detection methods and their limitations are discussed. Finally, the databases available for distinguishing between reported benign or pathogenic copy number alterations are highlighted. Overall, a review of MMSs that previously were also denoted “genomic disorders” or “contiguous gene syndromes” is given. (J Histochem Cytochem 60:346–358, 2012)

Keywords

microdeletion syndrome, microduplication syndrome, contiguous gene syndromes, non-allelic homologous recombination, array comparative genomic hybridization, fluorescence in situ hybridization, multiplex ligation-dependent probe amplification, quantitative polymerase chain reaction

Intellectual disability in humans is characterized by significantly impaired cognitive functioning in skills such as communicating, taking care of oneself, and social interactions. Factors such as maternal drug abuse during pregnancy, perinatal oxygen distress, or postnatal infections can be reasons for intellectual disability; however, causative genetic alterations can often be identified. These genetic reasons for intellectual disability in human can be studied by different approaches, related to the assumed underlying genetic defect. Nowadays, banding cytogenetics is still the most widely used initial test in routine diagnostics. If a normal karyotype is observed, further tests may include molecular cytogenetics, to exclude cryptic rearrangements, or molecular genetics. During the past few years an increasing number of so-called contiguous gene syndromes (CGSs) have been identified, mainly in patients with intellectual disability along with a limited number of other syndromes or diseases. CGSs are caused by an aberrant copy number (gain or loss) of a specific subchromosomal region. Originally, CGSs were considered to have critical regions of two or more genes, located in close proximity to each other.

Meanwhile, it is known for some of these syndromes that many genes may be involved in the usually duplicated or deleted region, but only one of them is gene-dosage sensitive and causative for the specific clinical signs. Thus, the denomination CGS has been replaced by the designation microdeletion or microduplication syndromes (MMSs).

Emerging Numbers of MMSs

One model system for MMSs is the Charcot–Marie–Tooth syndrome type 1A (CMT1A) caused by a duplication of the

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Figure 1. Growing numbers of publication per year found by searching for the terms *microdeletion syndrome new* and *microduplication syndrome new* in Pub Med (<http://www.ncbi.nlm.nih.gov/pubmed>).

PMP22 gene and its reciprocal microdeletion syndrome hereditary neuropathy with liability to pressure palsies (HNPPs) (Lupski, 1999). Although before the year 2000 only some dozens of MMSs were known, more and more MMSs have been identified with the availability of new technologies for high-resolution analyses of entire genomes. In particular, the array comparative genomic hybridization (aCGH) technique, which entered the human genome research and diagnostic fields in the end of the last century (Pinkel et al. 1998), was groundbreaking. The hallmark of aCGH is the identification of submicroscopic gains or losses of euchromatic material, recently called benign or pathogenic copy number variations (CNVs). Although for a pathogenic CNV, correlation with a certain syndrome/phenotype is possible, no such relation is known (yet) for benign CNVs. A pathogenic effect of a CNV is suggested if it is *de novo* (e.g., Alesi et al. 2011) or if a specific CNV cannot be found in numerous unrelated healthy individuals studied in parallel (e.g., Willat et al. 2005).

Meanwhile, the common use of aCGH in research and diagnostics has led to an increase of known benign as well as disease-causing pathogenic CNVs, which is especially reflected in the still-growing numbers of publications. From 1990 to 2011, 200 papers concerning new MMSs were published (Fig. 1). Keeping in mind that a common cause for recurrent microdeletions and microduplications is the structure of the human genome, which has countless repeats that can result in non-allelic homologue recombination (NAHR), the expected total number of reciprocal MMSs should be much higher (Gu et al. 2008). Theoretically, for every microdeletion syndrome there should be a reciprocal microduplication syndrome. However, there are at present 211 microdeletion syndromes versus only 79 microduplication syndromes reported (Table 1, Suppl. Table 1, Figure 2). This is a 2.5:1 ratio for a total of 267 different genomic loci with MMSs. Only for 56 of these, loci are reported as reciprocal/colocalizing MMSs, that is, 21%. This is due

to several reasons; for instance, meiotic errors leading to duplication and deletion should occur at equal frequencies, but recent studies indicate that early selection during gametogenesis favors either one or the other (Turner et al. 2008). In fact, it is a general observation that microduplications appear to result in a milder or no clinical phenotype compared with the reciprocal microdeletion. This is even the case on the chromosomal level: trisomies of whole chromosomes or supernumerary marker chromosomes are better tolerated (Liehr et al. 2011) than are autosomal monosomies.

Causes for Recurrence of MMSs

The basis of recurrent genomic rearrangements such as deletions, duplications, insertions, inversions, and translocations is the architecture of the primate/human genome. Innumerable repetitive elements serve as substrates for illegitimate intra- or interchromosomal/chromatide recombination during meiosis as well as in mitosis. This human specific genomic instability is not only causative, for example, for MMSs, but also has a great impact on genome evolution and flexibility in terms of gaining new gene functions or direct gene dosage effects by euchromatic copy number alterations (Marques-Bonet and Eichler, 2009; Gazave et al. 2011). Most recurrent genomic rearrangements are mediated by sequences such as segmental duplications (Bailey and Eichler, 2006) and low copy repeats flanking a certain region and allow NAHR leading to a high number of same-size *de novo* rearrangements (Stankiewicz and Lupski 2002) or SINEs/LINEs/LTRs (Korbel et al. 2007; Kidd et al. 2008). The second, rarer cause of genomic rearrangements is non-homologous end joining (NHEJ) following double stranded breaks, which is a cell repair mechanism and not directly mediated by specific sequence features (Lieber 2008). NHEJ can affect the same region but not the exact breakpoint in a group of patients covering a dosage-sensitive gene in the shortest region of overlap

Table 1. All Known Microdeletions and/or Reciprocal Microduplications up to January 2012 by Chromosomes from pter to qter That Are Reported at Least in Two Different Studies or in More Than One Individual

Microdeletion Syndrome	Microduplication Syndrome	OMIM	Cytoband	Start Position (kb) [hg18]	End Position (kb) [hg18]
microdeletion 1p36	—	607872	1pter-p36.31	0	5.309
microdeletion 1p36 (GABRD)	microduplication 1p36 (GABRD)	613060	1pter-p36.3	0	10.000
—	microduplication 1p34.1	—	1p34.1	45.591	46.808
microdeletion 1p32.2	—	613735	1p32.2	55.500	60.900
microdeletion 1p21.3	—	—	1p21.3	97.320	99.250
microdeletion 1q21.1	microduplication 1q21.1	612475	1q21.1	144.980	146.343
thrombocytopenia-absent radius syndrome/TAR	—	274000	1q21.1	144.150	144.427
deletion 1q21.1 (GJA5)	duplication 1q21.1 (GJA5)	121013	1q21.1	145.040	145.860
microdeletion 1q24q25	—	—	1q24.3q25.1	170.135	172.099
microdeletion 1q24.3	—	—	1q24.3	170.000	170.600
Van der Waude syndrome/VWSI	—	119300	1q32.2-q41	207.709	208.277
microdeletion 1q41-q42	—	612530	1q41-q42	221.135	221.775
corpus callosum agenesis microdeletion	—	612337	1q44	242.576	242.936
—	microduplication 2p25.3	—	2p25.3	3.250	3.450
Feingold syndrome/FS	—	164280	2p24.3	15.999	16.005
hypotonia-cystinuria syndrome/HCS	—	606407	2p21	44.384	44.442
holoprosencephaly 2/HPE2	—	157170	2p21	45.022	45.026
—	microduplication 2p21	—	2p21	45.200	45.900
NRXN1 microdeletion	NRXN1 microduplication	600565	2p16.3	50.011	50.437
microdeletion 2p15-16.1	—	612513	2p15-16.1	57.537	61.534
microdeletion 2p14-p15	—	612513	2p14-15	63.756	65.377
microdeletion 2p11.2-p12	—	613564	2p11.2-p12	77.597	87.091
microdeletion 2q11.2 (LMAN2L, ARID5A)	—	—	2q11.2	96.090	97.040
mesomelic dysplasia/MMD	—	605274	2q11.2	99.530	100.125
microdeletion 2q11.2q13 (NCK2, FHL2)	microduplication 2q11.2q13 (NCK2, FHL2)	602633/604930	2q11.2q13	100.060	107.810
nephronophthisis 1/NPHP1	microduplication 2q11.2q13	256100	2q13	110.293	110.320
microdeletion 2q13	microduplication 2q13	—	2q13	111.050	112.950
autism-dyslexia microdeletion 2q14.3	microduplication 2q14.3 (own case)	—	2q14.3	124.500	125.500
Mowat-Wilson syndrome/MWS	—	235730	2q22.3	144.900	144.994
microdeletion 2q23.1	—	156200	2q23.1	148.964	149.150
microdeletion 2q23.3q24.1	—	156200	2q23.3-q24.1	153.150	156.930
microdeletion 2q24.3	neonatal epilepsy microduplication	607208/604403	2q24.2-q24.3	165.133	166.562
synpolydactyly 1/SPD1	microduplication 2q31.1	613681	2q31.1	176.659	177.679
microdeletion 2q31.2-q32.3	—	612345	2q31.2-q32.2	177.640	191.380
microdeletion 2q33.1	—	612313	2q33.1	196.538	204.915
brachydactyly-mental retardation syndrome/BDMR	—	600430	2q37	239.620	242.951
distal 3p deletion	—	613792	3p25-p26	0	6.995
Von Hippel Lindau disease/VHL	—	193300	3p25-p26	10.158	10.169
microdeletion 3p21.31	—	—	3p21.31	49.120	52.220
microdeletion 3p14.1p13	—	605515	3p14.1-p13	71.164	71.959
microdeletion 3p11.1p12.1	—	—	3p11.2-p12.1	87.069	87.408
proximal 3q microdeletion syndrome	—	—	3q13.11-q13.12	106.400	108.900
microdeletion 3q13.31	—	—	3q13.31	115.335	115.916

(continued)

Table 1. (continued)

Microdeletion Syndrome	Microduplication Syndrome	OMIM	Cytoband	Start Position (kb) [hg18]	End Position (kb) [hg18]
blepharophimosis, ptosis, and epicanthus inversus syndrome/BPES	—	110100	3q23	140.146	140.148
Dandy–Walker syndrome/DWS	—	220200	3q24	148.610	148.617
microdeletion 3q27.3q29	—	—	3q27.3-q29	188.870	198.080
microdeletion 3q29	microduplication 3q29	609425/611936	3q29	197.126	198.982
Wolf–Hirschhorn syndrome/WHS	microduplication 4p16.3	194190	4pter-p16.3	0	2.043
—	microduplication 4p16.1	—	4p16.1	9.450	10.450
microdeletion 4p15.3	—	—	4p15.3	16.583	20.747
microdeletion 4q21.21q21.22	—	613509	4q21.21q21.22	81.950	83.350
microdeletion 4q21	—	613509	4q21	82.228	83.601
microdeletion 4q21.2q21.3	—	—	4q21.2-q21.3	89.148	89.218
—	Parkinson disease/PARK1	163890/168601	4q22.1	90.747	91.018
Rieger type 1/RIEGL	—	180500	4q25	111.758	111.779
—	4q32.1-q32.2 Triple/ Duplication syndrome	613603	4q32.1q32.2	157.356	161.615
Cri–du–Chat syndrome/CdCS	—	123450	5p15.2-p15.33	0	11.777
Cornelia de Lange syndrome/CDLS	NIPBL microduplication	613174	5p13.2	36.997	37.033
spinal muscular atrophy/SMA	—	253300	5q13.2	70.278	70.286
microdeletion 5q14.3	—	600662	5q14.3	86.142	86.413
microdeletion 5q14.3-q15	—	612881	5q14.3-q15	88.400	90.090
familial adenomatous polyposis/FAP	—	175100	5q22.2	112.129	112.249
—	adult-onset autosomal dominant leukodystrophy/ ADLD	169500	5q23.2	126.046	126.233
PITX1 microdeletion	—	602149	5q31.1	134.222	134.463
microdeletion 5q31.3	—	—	5q31.3	139.117	141.682
—	Pseudo trisomy 13 syndrome	264480	5q35.1	170.222	171.584
microdeletion 5q35.1	—	—	5q35.1	172.592	172.595
parietal foramina/PFM	—	168500	5q35.2	174.084	174.091
Sotos syndrome	microduplication 5q35	117550	5q35.2-q35.3	175.063	177.389
microdeletion 6p	—	612582	6p25	0	—
microdeletion 6p22.3	—	—	6p22.3	20.850	21.250
adrenal hyperplasia/AH	—	201910	6p21.32	32.114	32.117
microdeletion 6p21.31	—	—	6p21.31	33.273	34.086
microdeletion 6q13–14	—	613544	6q13–14	72.650	76.310
Prader–Willi like	—	176270	6q16.2	100.943	101.018
—	transient neonatal diabetes mellitus 1/TNMD1	601410	6q24.2	144.303	144.427
microdeletion 6q25.2-q25.3	—	612863	6q25.2-q25.3	155.500	158.853
PARK2 microdeletion	PARK2 microduplication	602544	6q26	161.688	162.784
microdeletion 6q27 anosmia	Chondroma/CHDM	215400	6q27	165.554	170.762
Saethre–Chotzen syndrome/SCS	—	101400	7p21.1	19.121	—
Greig cephalopolysyndactyly/GCPS	—	175700	7p14.1	41.967	42.243
Williams–Beuren syndrome/WBS	microduplication 7q11.23	609757/194050	7q11.23	71.971	74.255
WBS-distal deletion (RHBDD2, HIP1)	—	613729	7q11.23	74.800	76.500
split hand/foot malformation 1/ SHFM1	—	183600/220600	7q21.3	95.370	96.619
microdeletion 7q22.1-q22.3	—	—	7q22.1-q22.3	101.040	104.560
autism/dyslexia microdeletion 7q31.1	—	—	7q31.1	110.654	111.266

(continued)

Table 1. (continued)

Microdeletion Syndrome	Microduplication Syndrome	OMIM	Cytoband	Start Position (kb) [hg18]	End Position (kb) [hg18]
speech-language-disorder 1/SPCH1	—	602081	7q31	114.085	114.090
holoprosencephaly 3/HPE3	—	142945	7q36.3	155.288	155.298
—	triphalangeal thumb polysyndactyly syndrome/TPTS	174500	7q36.3	155.836	156.425
Currarino syndrome/CS	—	176450	7q36.3	156.490	156.496
microdeletion 8p23.1	microduplication 8p23.1	179613	8p23.1	8.156	11.803
microdeletion 8p21.2	—	—	8p21.2	20.750	24.390
microdeletion 8p12p21	—	—	8p12p21	24.500	31.300
—	microduplication 8q11.23	610928	8q11.23	53.450	54.050
CHARGE syndrome	microduplication 8q12	214800	8q12.2	61.754	61.942
microdeletion 8q12.3q13.2	—	—	8q12.3-q13.2	65.450	69.020
mesomelia-synostoses syndrome/MSS	—	600383	8q13	70.541	70.908
microdeletion 8q21.11	—	614230	8q21.11	77.389	77.929
nablus mask-like facial syndrome/NMLFS	—	608156	8q21.3-q22.1	93.210	97.940
microdeletion 8q22.2q22.3	—	—	8q22.2-q22.3	100.690	104.560
Langer-Giedion syndrome/LGS	—	150230	8q24.11	118.881	119.193
sex reversal syndrome 4/SRXY4	—	154230	9p24.3	0	1.048
monosomy 9p syndrome	—	158170	9pter-p22.3	0	16.168
—	microduplication 9q21.11	613558	9q21.11	71.051	71.197
microdeletion 9q22.3	PTCH1 microduplication	601309	9q22.3	94.420	99.100
holoprosencephaly 7/HPE7	—	610828	9q22.32	97.284	97.319
nail-patella syndrome/NPS	—	161200	9q33.3	128.417	128.499
early infantile epileptic encephalopathy 4/EIEE4	—	612164	9q34.11	129.414	129.495
microdeletion 9q34 (EHMT1)	microduplication 9q34 (EHMT1)	607001	9q34.3	136.950	140.200
subtelomere deletion 9q	—	610253	9q34.3	139.473	140.273
hypoparathyroidism, sensorineural deafness, and renal disease/HDRS	—	146255	10p15	8.137	8.157
Di George syndrome 2/DGS2	—	601362	10p12.31	21.144	21.170
microdeletion 10q22-q23 (NRG3, GRID1)	—	—	10q22-q23	81.655	88.984
juvenile polyposis syndrome/JPS	—	612242	10q23.2-q23.3	88.675	89.613
—	Split-Hand/Foot Malformation 3/SHFM3	246560	10q24.32	102.977	103.445
microdeletion 10q25q26	—	609625	10q25q26	117.098	qter
Beckwith-Wiedemann syndrome/BWS—Silver Russell syndrome/SRS microdeletion	Beckwith-Wiedemann syndrome/BWS—Silver Russell syndrome/SRS microduplication	130650	11p15.5	2.861	2.864
WAGR syndrome	microduplication 11p13	194072/612469	11p13	31.767	32.467
Potocki-Shaffer syndrome/PSS	—	601224	11p11.2	43.905	46.080
—	spinocerebellar ataxia type 20/SCA20	608687	11q12.2q12.3	61.210	61.503
microdeletion 11q14.1	—	—	11q14.1-q14.2	86.334	86.344
Jacobsen syndrome/JBS	—	147791/188025	11q23.3-qter	115.400	134.452
—	microduplication 12p13.31	—	12p13.31	8.050	8.250
microdeletion 12q14	—	—	12q14	63.356	66.932
nasal speech-hypothyroidism microdeletion/NSH	—	—	12q15-q21.1	68.802	701.392

(continued)

Table 1. (continued)

Microdeletion Syndrome	Microduplication Syndrome	OMIM	Cytoband	Start Position (kb) [hg18]	End Position (kb) [hg18]
Noonan syndrome 1/NS1	—	163950	12q24.1	111.341	111.432
microdeletion 13q12 (CRYL1)	microduplication 13q12 (CRYL1)	—	13q12.11	19.710	19.910
spastic ataxia Charlevoix–Saguenay/SACS	—	270550	13q12.12	22.336	23.807
microdeletion 13q12.3–q13.1	—	600185	13q12.3–q13.1	31.137	31.871
retinoblastoma/RBI	—	613884	13q14.2	47.776	47.954
Hirschsprung disease 2/HSCR2	—	600155	13q22	77.369	77.391
holoprosencephaly5/HPE5	—	609637	13q32.3	99.432	99.437
microdeletion 14q11.2	—	613457	14q11.2	20.920	20.947
congenital Rett variant/CRV	microduplication 14q12	613454	14q12	28.300	30.000
microdeletion 14q22–q23	—	607932	14q22–q23	53.486	60.261
autism spherocytosis microdeletion/ASC	—	—	14q23.2–q23.3	63.924	64.471
microdeletion 14q32.2	—	—	14q32.2	99.463	100.574
microdeletion 15q11.2 (NIPA1)	microduplication 15q11.2 (NIPA1)	608145	15q11.2	20.350	20.640
Angelman syndrome Typ1/AS1	microduplication 15	105830	15q11.2–q13.1	20.405	26.231
Angelman syndrome Typ2/AS2	microduplication 15	105830	15q11.2–q13.1	21.309	26.231
Prader–Willi syndrome Typ 1/PWS1	microduplication 15	176270	15q11.2–q13.1	20.405	26.231
Prader–Willi syndrome Typ 2/PWS2	microduplication 15	176270	15q11.2–q13.1	21.309	26.231
microdeletion 15q13.3 (CHRNA7)	microduplication 15q13.3 (CHRNA7)	612001	15q13.3	28.525	30.489
microdeletion 15q14	—	—	15q14	33.471	35.072
deafness and male infertility syndrome/DMIS	—	611102	15q15.3	41.613	41.747
microdeletion 15q21	—	—	15q21	48.382	48.565
microdeletion 15q24 (BBS4,NPTN, NE01)	—	601907	15q24	70.700	72.200
microdeletion 15q24	microduplication 15q24	613406	15q24	72.158	73.949
orofacial clefting/OC	—	614294	15q24.3–q25.2	76.080	80.338
microdeletion 15q25	—	614294	15q25	82.900	83.600
microdeletion 15q26.1	—	—	15q26.1	91.100	91.600
Fryns syndrome/FNS	—	229850	15q26.2	92.238	96.520
microdeletion 15q26.2–qter	—	—	15q26.2–qter	95.600	100.339
ATR–16–syndrome	—	141750	16p13.3	0	774
tuberous sclerosis microdeletion syndrome/PKDTS	tuberous sclerosis microduplication	600273	16p13.3	2.038	2.079
Rubinstein–Taybi syndrome 1/RSTS1	Rubinstein–Taybi–microduplication	610543/613458	16p13.3	3.762	3.801
microdeletion 16p13.1 (MYH11)	microduplication 16p13.1 (MYH11)	132900	16p13.1	14.789	16.281
microdeletion 16p11.2–p12.2	microduplication 16p11.2–p12.2	613604	16p11.2–p12.2	21.521	28.950
microdeletion 16p12.1 (EEF2K,CDR2)	microduplication 16p12.2 (EEF2K,CDR2)	117340/606968	16p12.1	21.850	22.370
16q11.2 distal microdeletion (SH2B1)	16q11.2 distal microduplication (SH2B1)	—	16q11.2	28.680	29.020
microdeletion 16p11.2 (TBX6)	microduplication 16p11.2 (TBX6)	602427/611913	16p11.2	29.551	30.059

(continued)

Table 1. (continued)

Microdeletion Syndrome	Microduplication Syndrome	OMIM	Cytoband	Start Position (kb) [hg18]	End Position (kb) [hg18]
microdeletion 16q11.2-q12.1	—	—	16q11.2-q12.1	45.401	45.579
microdeletion 16q21-q22	—	—	16q21-q22	65.621	65.692
microdeletion 16q12.1-q12.2	—	—	16q12.1-q12.2	48.018	52.726
microdeletion 16q24.1	—	601089	16q24.1	82.908	85.153
FANCA deletion	—	227650	16q24.3	88.392	88.411
Miller–Dieker syndrome/MDLS	Miller–Dieker microduplication	247200/613215	17p13.3	0	2.492
microdeletion 17p13.3 (YWHAE)	microduplication 17p13.3 (YWHAE)	247200/613215	17p13.3	2–310	2.870
microdeletion 17p13.1	—	613776	17p13.1	7.429	7.937
hereditary liability to pressure palsies/HNPP	Charcot–Marie–Tooth 1A/CMT1A	162500/118220	17p12	13.855	15.375
Smith–Magenis syndrome/SMS	Potocki–Lupski syndrome/PTLS	610883	17p11.2	16.527	20.423
neurofibromatosis 1/NF1	microduplication NF1	613675	17q11	26.102	27.243
microdeletion 17q11.2-q12	—	—	17q11.2-q12	26.280	31.030
microdeletion 17q12a	—	—	17q12	31.977	33.150
renal cysts and diabetes syndrome/RCAD	microduplication 17q12b	137920	17q12	31.830	33.350
Van Buchem disease/VBCH	—	239100	17q12-q21	39.187	39.192
microdeletion 17q21.3 (MAPT)	microduplication 17q21.31 (MAPT)	610443/613533	17q21.3	40.988	41.566
microdeletion 17q21.31-q21.32	—	—	17q21.31-q21.32	41.769	43.113
microdeletion 17q22-q23.2	—	—	17q22–23.2	48.300	54.200
—	microduplication 17q23.1–23.2	613355/613618	17q23.1–23.2	55.457	57.693
microdeletion 17q24.2-q24.3	—	—	17q24.2-q24.3	61.730	65.690
carney complex syndrome 1/CNC1	—	160980	17q24.2-q24.3	63.260	65.594
—	microduplication 17q24.3	278850	17q24.3	65.642	66.847
holoprosencephaly 4/HPE4	—	146390	18p11.31	3.445	3.448
proximal 18q microdeletion	—	601808	18q12.3-q21.1	37.500	42.500
Pitt–Hopkins syndrome/PTHS	—	610954	18q21.1	51.083	51.282
microdeletion 18q22.3-q23	—	607842	18q22.3-q23	70.474	73.111
—	Sotos-like microduplication 19p13.2	—	19p13.2	9.107	11.094
microdeletion 19p13.13	microduplication 19p13.13	613638	19p13.13	12.793	13.104
microdeletion 19p13.12	—	—	19p13.12	14.119	14.439
microdeletion 19p13.11	—	—	19p13.11	16.485	17.554
microdeletion 19q13.11	—	613026	19q13.11	37.300	40.200
Diamond–Blackfan anemia/DBA	—	105650	19q13.2	47.056	47.067
microdeletion 20p12.3	—	112261	20p12.3	6.907	7.012
Alagille syndrome 1/ALGS1	—	118450	20p12	10.478	10.669
microdeletion 20q13.13-q13.2	—	—	20q13.13-q13.2	49.760	50.840
Albright hereditary osteodystrophy/AHO	—	103580	20q13.32	56.900	56.92
microdeletion 20q13.33	—	—	20q13.33	61.246	62.376
microdeletion 21q21.1	—	—	21q21.1	19.950	20.250
—	microduplication 21q21.3	—	21q21.3	25.960	26.470
platelet disorder/PD	—	601399	21q22.12	34.743	35.343
—	Down syndrome/DS	190685	21q22.13	37.300	38.502
—	Cat-Eye syndrome/CES	115470	22p11.1-q11.21	0	16.977

(continued)

Table 1. (continued)

Microdeletion Syndrome	Microduplication Syndrome	OMIM	Cytoband	Start Position (kb) [hg18]	End Position (kb) [hg18]
Di George syndrome/CATCH22/DGS	microduplication 22q11.2	608363/145410	22q11.21-q11.23	16.932	20.672
distal microdeletion 22q11.2 (BCR, MAPK1)	distal microduplication 22q11.2 (BCR, MAPK1)	611867	22q11.2	20.446	22.026
neurofibromatosis 2 microdeletion syndrome	—	101000	22q12.2	28.330	28.425
Phelan–McDermid syndrome	microduplication 22q13 (SHANK3)	606232	22q13	49.449	49.691
Leri–Weill dyschondrosteosis/LWDS	—	127300	Xp22.33	0	724
X-Linked autism-2/AUTSX2	—	300495	Xp22.32-p22.31	5.818	6.157
Steroid sulphatase deficiency/STS	—	308100	Xp22.31	6.452	8.128
Kallmann syndrome 1/KALI	—	308700	Xp22.31	8.457	8.660
MIDAS syndrome	—	309801	Xp22.2	11.039	11.659
Nance–Horan syndrome/NHS	—	302350	Xp22.13	16.853	17.768
microdeletion Xp22.11	—	300830	Xp22.11	22.928	23.309
X-linked congenital adrenal hypoplasia/AHC	DAX1 microduplication	300679	Xp21.2	30.233	30.237
complex glycerol kinase/CGK	—	300679	Xp21.2	30.233	30.659
muscular dystrophy Duchenne/DMD	—	310200	Xp21.2	32.445	33.268
Xp11.3 deletion syndrome	—	300578	Xp11.3	46.193	46.627
Goltz syndrome/GS	—	305600	Xp11.23	48.252	48.264
—	17-beta-hydroxysteroid dehydrogenase X/HSD	300801	Xp11.22	53.467	53.730
—	microduplication Xq12q13.1	300127	Xq12-q13.1	67.435	68.633
X inactivation specific transcript/XIST	—	314670	Xq13.2	72.863	73.063
Bruton agammaglobulinemia/XLA	—	300755	Xq22.1	100.490	100.497
microdeletion Xq22.2	Pelizaeus–Merzbacher microduplication/PMD	312080	Xq22.2	102.609	103.098
microdeletion Xq22.3q23	—	300194/303631	Xq22.3-q23	107.214	110.239
lymphoproliferative syndrome 1/XLPI	—	308240	Xq25	123.308	123.335
—	X-linked hypopituitarism/SRXX3	300833	Xq27.1	139.413	139.415
fragile site mental retardation 1/FMRI	—	309550	Xq27.3	146.801	146.840
microdeletion Xq28	—	—	Xq28	147.043	147.543
Rett syndrome/RS	MECP2 microduplication	300475/300815/300845	Xq28	152.535	153.044
sex-determining region Y/SRY	—	480000	Yp11.31	2.715	2.716
AZFα microdeletion	—	415000	Yq11.21	12.934	13.664
AZFβ microdeletion	—	415000	Yq11.221-q11.223	18.698	24.475
AZFβ+c microdeletion	—	415000	Yq11.221-q11.23	18.474	26.203
AZFγ microdeletion	—	415000	Yq11.223-q11.23	23.387	26.203

Reported genes in certain MMSs are given in *italics* in brackets; abbreviations that were also used in Fig. 2 are given in capital letters after the slash. If known, the OMIM number is reported as well as the cytogenetic localization and start and end positions in kilobasepairs (kb), human genome version 18 [hg18]. Additional information on locus specific bacterial artificial chromosomes for every region, as well as the reference are given in a supplementary table. Dash/empty stands for no known reciprocal MMS up to January 2012.

Moreover, recurrent MMSs can arise from an inversion polymorphism in one of the parental genomes (e.g., Gimelli et al. 2003; Koolen et al. 2006) or from a balanced translocation (Shaffer 2001). These inversion polymorphisms occur between inverted homologous sequences and are predisposed for NAHR in meiosis through inversion loop

Genomic microarrays refer to the principle of chromosome-based comparative genomic hybridization (CGH; Kallioniemi et al. 1992), where test DNA and control DNA are differentially labeled, mixed in a 1:1 ratio, and hybridized together with Cot 1 DNA to metaphase spreads of a normal donor. This special fluorescence in situ hybridization (FISH) procedure results in a yellow staining of regions unaffected by CNV. Green and red colors along the

Figure 3. (A) Example of microdeletion findings in array comparative genomic hybridization (aCGH) (Agilent Human Genome CGH Microarray 180k) in a female with karyotype 46,XX. The patient showed two de novo microdeletions (hg18): arr 16p12.1p11.2(22.665685–28.536945) x1/del(16)(p12.1p11.2)(5.871269–6.106456 Mb) arr 16q23.3q24.1(80.662135–84.286121)x1/del(16)(q23.3q24.1)(3.623986–3.648342 Mb) (B) The aCGH result was confirmed by fluorescence in situ hybridization with bacterial artificial chromosome clones located in the deletion regions. The microdeletions were confirmed on metaphase chromosomes and interphase nuclei.

chromosomes indicate genetic loss or gain in the DNA derived from the patient. Currently, hybridization is no longer done on metaphase spreads but on slides with spots of defined genomic sequences. This leads to a higher and better resolution compared with CGH depending on the number and the size of the probes used (Pinkel et al. 1998; Lee C et al. 2007). Besides copy number alterations, single nucleotide polymorphism (SNP)-based aCGH provides additional information on loss of heterozygosity, indicating a deletion or uniparental isodisomy.

Depending on the resolution, target size of the different platforms [bacterial artificial chromosomes (BACs), fosmids, oligonucleotides, SNPs], and the analysis criteria, MMSs as well as benign CNVs can be detected with varying accuracy. Thus, especially for benign CNVs, problems may arise when data from different platforms are compared by annotating them, for example, in the “database of genomic variants” (DGV). The exact sizes of the corresponding benign CNV might be available after the currently ongoing “1000 genomes project” (Sudmant et al. 2010) is finished.

In most cases, aCGH is used as a genome-wide screening method. Therefore, after all technical problems are solved, the main challenge is in the interpretation of all the genomic data. This step, together with the verification of an aberration (see below), is the most labor intensive. The most critical decision is whether a CNV is considered benign or disease causative, along with final interpretation.

Fluorescence In Situ Hybridization

Molecular cytogenetics, especially the FISH technique, is used for direct analysis of a certain suspected MMS critical region by applying locus-specific DNA probes. FISH is

used when a physician suspects a specific MMS or for verification of aCGH data. The advantage of the FISH approach is that single-cell information in the context of metaphase chromosomes becomes available. Besides the visualization of a balanced chromosomal aberration in a parent (leading to unbalanced situations in the index patient), mosaicism and complex rearrangements in a patient can also be detected (Mkrtchyan et al. 2010; Fig. 3).

One important limitation of the FISH approach is that the size of locus-specific probes should not be less than 10 kb (Liehr et al. 1997). Furthermore, duplications are harder to verify by FISH than deletions; however, this drawback can be overcome by analysis of interphase nuclei in addition to metaphase spreads and/or the use of software signal intensity and size measurements (Weise et al. 2008).

For classic, that is, well-known, MMSs, a panel of commercial available probes can be used. For all other genomic regions to be tested for CNVs, BACs and fosmids generated by the human genome project with annotated sequence/location/size information are good sources for directed microduplication and deletion testing (Weise et al. 2009). A list with suggested BAC probes for the currently known MMSs regions is given in Suppl. Table 1.

Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction (qPCR) was initially established as a method for quantifying different levels of gene expression. However, under the need to verify aCGH results, qPCR is now also routinely applied to detect CNVs (Weksberg et al. 2005). The qPCR technique provides a quantitative measurement of DNA CNVs of (nearly) any region of interest. The main limitations or problems are to find accurate primer pairs, to optimize the

PCR conditions, and to detect mosaicism. Additionally, when using the parents of an index patient for CNV verification, balanced rearrangements with a higher recurrence risk in offspring are not recognizable.

Multiplex Ligation-Dependent Probe Amplification

Multiplex ligation-dependent probe amplification (MLPA) is a directed multiplex PCR-based method. Only those primers that hybridize to the target sequences are amplified, and the resulting products can be analyzed by capillary electrophoresis. MLPA allows the detection of abnormal copy numbers of up to 50 different genomic sequences at the same time. Comparing the peak pattern obtained to that of reference samples indicates which sequences show aberrant copy numbers. The technique is commercially available for several sets of MMSs and therefore can serve as a time- and cost-reduced first or second screening step method (Jehee et al. 2011). Again, the limitations are the same as in aCGH and qPCR: no proper detection of mosaicism and no verification of balanced origin in one parent. In addition, MLPA has the disadvantage that it covers only a limited number of loci. Furthermore, for newly described MMSs, a specific MLPA test has to be designed.

Databases for MMSs

To facilitate the analysis, especially of aCGH data, several databases are available summarizing genomic imbalances under different aspects. Before using these databases, one should check which version of the human genome sequence is used in one's own aCGH platform and the database, as the coordinates move in different versions. The currently used version is hg19 or Build 37.3, but not all databases listed below refer to this version and need to be updated or converted by the user.

The first step for analysis and interpretation of aCGH results is the classification of a detected CNV as benign or disease causative. This question can be answered best by the use of genome browsers showing both reported benign and pathological CNVs, such as the genome browser from the University of California, Santa Cruz (UCSC; <http://genome.ucsc.edu/cgi-bin/hgGateway>), the U.S. National Institutes of Health genome browser (NCBI; <http://www.ncbi.nlm.nih.gov>), or the Ensembl genome browser (http://www.ensembl.org/Homo_sapiens). These genome browsers also provide information on BAC or fosmid clones that can be used for FISH verification of the CNV. Furthermore, the direct sequence can be downloaded or BLASTed for qPCR and MLPA design. Some of the genome browsers' subdatabases are also directly available.

Copy Number Variation and Polymorphism

- Database of genomic variants (DGV): <http://projects.tcag.ca/variation>
Catalogue of structural and copy number variation in the human genome
- Chromosome Anomaly Collection: <http://www.ngrl.org.uk/Wessex/collection>
Collection of Unbalanced Chromosome Abnormalities (UCBAs) and Euchromatic Variants (EVs) visible by light microscope but without any phenotypic effect
- Small supernumerary marker chromosomes. <http://www.med.uni-jena.de/fish/sSMC/00START.htm>
Collection of all available case reports on small supernumerary marker chromosomes (sSMCs) with definition of critical regions for partial trisomies due to the presence of sSMCs

Catalogues of Pathological Imbalances

- Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER): <https://decipher.sanger.ac.uk/syndromes>
An interactive Web-based database that incorporates a suite of tools designed to aid the interpretation of submicroscopic chromosomal imbalance
- European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA): <http://umcecaruca01.extern.umcn.nl:8080/ecaruca/ecaruca.jsp>
Database that collects and provides cytogenetic and clinical information on rare chromosomal disorders, including microdeletions and microduplications
- The Chromosome Microdeletion/duplication Collection: http://www.ngrl.org.uk/wessex/microdel_collection.htm
Collection of MMS with the aim to interpret results of array CGH analysis

Literature and Educational Resources

- Chromosomal Variation in Man: <http://www.wiley.com/legacy/products/subject/life/borgaonkar/>
Interactive database and searching tool by chromosomes and regions on reviewed literature on all common and rare chromosomal alterations and abnormalities
- Online Mendelian inheritance in man (OMIM): <http://www.ncbi.nlm.nih.gov/omim>
Comprehensive, authoritative, and timely compendium of human genes and genetic phenotypes with several search options

- GeneReviews: <http://www.ncbi.nlm.nih.gov/books/NBK1116>
Expert-authored, peer-reviewed disease descriptions that apply genetic testing to the diagnosis, management, and genetic counseling of patients and families with specific inherited conditions
- Orpha net: <http://www.orpha.net>
Reference portal for information on rare diseases and orphan drugs for helping to improve the diagnosis, care, and treatment of patients with rare diseases

Conclusion and Outlook

Submicroscopic microdeletions and microduplications are only one aspect of variations taking place in the human genome. As most MMSs patients are unable to reproduce due to the severity of their intellectual disability, these kinds of diseases are more an expression of the structure of the human genome than inherited disorders. Due to recent progress in current diagnostic panels, MMSs can now be discovered during conventional diagnostics. Keeping in mind the limitations of every technique per se, clinicians should analyze patients with intellectual disability using careful stepwise analysis. Detection of whole chromosome trisomies and monosomies as well as gross chromosomal rearrangements should be studied via banding and molecular cytogenetic techniques. Such aberrations, especially when present in low mosaic levels, might be easily missed when only performing targeted molecular techniques such as qPCR or MLPA alone. Targeted FISH, qPCR or MLPA, and aCGH could be the next steps. It is noteworthy that rare MMSs will not be identified by any method other than array CGH due to the small size of the anomaly.

New techniques such as high throughput and fast next-generation sequencing (NGS) of whole genomes will produce much more data and will detect variations that will be difficult to interpret, which could be the highest limitation. Also, NGS will have limitations such as detection of copy numbers, low resolution in repetitive regions, and findings that need to be verified in the patient and also in the parent samples by aCGH, FISH, qPCR, MLPA, and even conventional cytogenetics. Overall, the older approaches like the aforementioned should not be neglected, as they have at least two important advantages: (1) in most cases results are relatively easy to interpret due to long-standing experience, and (2) large financial resources are not needed for their implementation.

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