Application of high-resolution array comparative genomic hybridization in children with unknown syndromic microcephaly

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BACKROUND: Microcephaly can either be isolated or it may coexist with other neurological entities and/or multiple congenital anomalies, known as syndromic microcephaly. Although many syndromic cases can be classified based on the characteristic phenotype, some others remain uncertain and require further investigation. The present study describes the application of array-comparative genomic hybridization (array-CGH) as a diagnostic tool for the study of patients with clinically unknown syndromic microcephaly.

METHODS: From a cohort of 210 unrelated patients referred with syndromic microcephaly, we applied array-CGH analysis in 53 undiagnosed cases. In all the 53 cases except one, previous standard karyotype was negative. High-resolution 4 × 180K and 1 × 244K Agilent arrays were used in this study.

RESULTS: In 25 out of the 53 patients with microcephaly among other phenotypic anomalies, array-CGH revealed copy number variations (CNVs) ranging in size between 15 kb and 31.6 Mb. The identified CNVs were definitely causal for microcephaly in 11/53, probably causal in 7/53, and not causal for microcephaly in 7/53 patients. Genes potentially contributing to brain deficit were revealed in 16/53 patients.

CONCLUSIONS: Array-CGH contributes to the elucidation of undefined syndromic microcephalic cases by permitting the discovery of novel microdeletions and/or microduplications. It also allows a more precise genotype–phenotype correlation by the accurate definition of the breakpoints in the deleted/duplicated regions.

Microcephaly is defined as an occipitofrontal head circumference, which is 2 or more SDs below the mean (−2 SD) for age and sex. It results from the premature fusion of the cranial sutures due to impaired neurogenesis. Most countries have created their own standard growth curves for term and preterm infants according to sex and age. Many classifications regarding microcephaly have been proposed by experts. The most accurate classification is the one that is based on the time of onset, and it defines microcephaly as congenital or postnatal (1). Both categories can be due to genetic or non-genetic factors. Nevertheless, genetic factors are more frequently responsible for congenital microcephaly, whereas non-genetic factors often cause postnatal microcephaly.

Non-genetic factors, such as radiation, smoking, alcohol, intrauterine hypoxemia, antiepileptic drugs, poorly controlled maternal phenylketonuria or diabetes, and congenital infections due to rubella, cytomegalovirus, toxoplasmosis, and herpes virus, may affect the infant prenatally. Other causes including respiratory distress, hypoxic-ischemic encephalopathy, hyperthermia, central nervous system infections, and intracranial bleeding can potentially influence the infant perinatally, whereas malnutrition, congenital heart diseases, and chronic diseases can cause microcephaly, which presents postnatally (2).

Microcephaly due to genetic causes includes isolated and syndromic microcephaly, various metabolic diseases, Fanconi anemia, primary craniosynosteosis, and brain malformations. Syndromic microcephaly is associated with other neurological entities and/or dysmorphic features or multiple congenital anomalies. It may be due to an autosomal-dominant or -recessive disorder, an X-linked-dominant or -recessive defect, numerical chromosomal abnormalities, structural chromosomal abnormalities of >5–10 Mb detected by conventional Karyotype, and submicroscopic genomic rearrangements identified by high-throughput molecular technologies (2). In the present study, we describe the extensive application of array comparative genomic hybridization (array-CGH) in patients with unexplained syndromic microcephaly and discuss the clinical interpretation of genotype–phenotype correlation.

METHODS

Subjects

From a cohort of 210 patients with syndromic microcephaly who were referred for genetic evaluation during a 4-year period, we applied array-CGH in 53 undiagnosed cases in order to identify

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Received 10 October 2016; accepted 4 March 2017; advance online publication 24 May 2017. doi:10.1038/pr.2017.65

Table 1a. CNVs in patients with syndromic microcephaly and suspect clinical diagnosis of a microdeletion/microduplication syndrome

Patients	Phenotype	Brain MRI	CNVs: chromosomal region/size (Mb)/ genome coordinates/inheritance	Known syndromes	Genes contributing to small brain size
Patient 1 (f, 6mo)	cMIC, ID, DF, MCA, E	Delay of myelin formation	DEL 4p16.3-p16.1 (7.39 Mb; 62,447–7,455,153), hg18, *de novo*	Wolf-Hirschhorn syndrome	*WHSC1, WHSC2, SLBP*
Patient 2 (f, 2y)	cMIC, ID, DF, MCA, E	Abnormal myelin formation of the white matter. Thin corpus callosum	DEL 4p16.3 (2.1 Mb; 71,552-2,159,567), hg18, *de novo*	Wolf-Hirschhorn syndrome	*WHSC1, WHSC2, SLBP*
Patient 3 (f, 5y)	cMIC, ID, DF, MCA, E	Periventricular leukomalacia (PVL)	DEL 4p16.3 (2.4 Mb; 71,552–2,468,797), hg18, *de novo*/ DUP 4p16.3 (3.02 Mb) 2,488,607–5,510,109), hg18, *de novo*	Wolf-Hirschhorn syndrome	*WHSC1, WHSC2, SLBP*
Patient 4 (m, 18mo)	pMIC, ID, DF, E	Thin corpus callosum. Dilatation of brain ventricles	DEL 4p16.3p16.1 (5.2 Mb; 1,333,133–6,505,273), hg19, *de novo*	Wolf-Hirschhorn syndrome	*WHSC1, WHSC2, SLBP*
Patient 5 (f, 1y)	pMIC, ID, DF, CHD	N	DEL 22q11.21 (2.5 Mb; 17,299,942–19,794,119) hg18, *de novo*	DiGeorge syndrome	RANBP1

genetic causes of microcephaly. The study is both retrospective and prospective and has received approval from the Ethical Committee of "Aghia Sophia" Children's Hospital.

The selection of the 53 patients was based on a well-standardized five-step protocol:

Step 1: (i) Detailed personal history including obstetric and perinatal history. (ii) Detailed parental and family history.

Step 2: Detailed physical examination and genetic evaluation carried out by experienced clinical geneticists.

Step 3: Developmental, neurological—including brain MRI and cardiological, opthalmological, and audiological evaluations in all patients.

Step 4: (i) In all patients, clinical and/or laboratory exclusion of the following medical conditions: non-genetic causes of microcephaly, craniosynosteosis, and metabolic diseases. Patients with the three most common numerical chromosomal aberrations (trisomy 21, trisomy 13, and trisomy 18) were not included in the present study. In all cases, except one, previous standard karyotype was normal. (ii) In a number of patients, according to their phenotype, targeted genetic investigation for monogenic microcephaly syndromes, such as Angelman, Rett, and Cornelia de Lange syndrome, was performed.

Step 5: Further investigation by array-CGH was performed: (i) in five patients with characteristic phenotype who were clinically suspected of having a well-known syndrome such as Wolf Hircsh-horn syndrome and DiGeorge syndrome and (ii) in 53 undiagnosed cases in order to identify genetic causes of microcephaly.

We define microcephaly, the occipitofrontal head circumference, which is 2 or more SDs below the mean (-2SD) for age and sex.

Informed written consent was obtained from the parents of all the patients participating in the study.

Array-CGH Analysis

Genomic DNA from 58 patients and their parents was obtained from 3 ml of peripheral blood using the BioRobot M48 System (Qiagen, Hilden, Germany) and the commercially available kit MagAttract DNA Blood Midi M48 Kit (Qiagen). The quality and quantity of the DNA samples was determined using a NanoDrop ND-1000 UV–VIS spectrophotometer. High-resolution 4×180 K and 1×244 K Agilent SurePrint G3 arrays ($>170{,}000$ and $>236{,}000$ probes, respectively, average resolution >8.9 kb) were used in this study (Agilent Technologies, Santa Clara, CA, www.agilent.com). Labeling, hybridization, and data processing were carried out according to the manufacturer's recommendations and as previously described (3). The benign CNVs (or copy number polymorphisms) reported in the Database for Genomic Variants (http://dgv.tcag.ca/dgv/app/home) were removed from the results.

Further genetic analysis of genes involved in autosomal-recessive primary microcephaly (microcephaly primary hereditary) was not performed.

RESULTS

Array comparative genomic hybridization (array-CGH) was carried out in the following way:

(a) In five patients with distinguishable phenotype for clinical confirmation (**Table 1a**—Patients 1–5). In all these cases, array-CGH confirmed the clinical diagnosis and revealed syndromes that are known to cause microcephaly. In patient No 5, fluorescence *in situ* hybridization analysis was pathogenic, but because of the severe phenotype, array-CGH was performed to exclude the coexistence of other pathogenic copy number variations (CNVs).

(b) In 53 patients who presented with syndromic microcephaly of unknown etiology. In 25 out of the 53 patients (10 male/15 female patients) various chromosomal aberrations (a total of 23 microdeletions and 12 microduplications) ranging in size between 15 kb and 31.6 Mb were detected (**Table 1b**). Complex rearrangements contributed to the overall clinical phenotype in 10 out of the 25 patients. In all the 25 patients, microcephaly was accompanied by dysmorphic features and developmental delay/intellectual disability (mild to severe; 100%). Other associated symptoms included the following: epilepsy in 5/25, hypotonia in 9/25, congenital heart disease in 4/25, and other congenital anomalies in 3/25 cases. Brain MRI was pathogenic in 12 out of the 25 patients.

Table 1b. CNVs in patients with Unknown Syndromic Microcephaly

Patient	Phenotype	Brain MRI	CNVs: chromosomal region/size (Mb or kb)/genome coordinates/inheritance	Known syndrome	Genes contributing to the small brain size
Group A: cases with CNVs related with microcephaly					
Patient 1 (m, 2y)	pMIC, ID, DF	Corpus callosum atrophy	DEL 4q35.1q35.2 (5.2 Mb) (185,592,747–190,790,881), hg18, *de novo* DUP 9p24.3p21.1 (31.6 Mb), (204,193–31,367,372), hg18, *de novo*	**9p duplication syndrome** **4q deletion syndrome**	***FREM1*** *DOCK8*
Patient 2 (f, 22mo)	cMIC, ID, DF, CHD	Thin corpus callosum, Dilatation of brain ventricles, White matter edema	DUP 9p24.3-p22.1 (19.01 Mb; 194,193–19,203,881), hg18, *de novo*/DEL 13q33.1-q34 (13.6 Mb; 100,510, 439–114,114,568), hg18, *de novo*	**9p duplication syndrome** **13q33-q34 deletion syndrome**	***FREM1***, *DOCK8*, ***SOX1, ARHGEF7***
Patient 3 (f, 15mo)	pMIC, ID, DF, CHD, MCA	Periventricular leukomalacia (PVL) Pontine atrophy cerebral atrophy	DEL 3p13 (0.083 Mb) (71,506,966–71,589,967), hg18, *de novo*/DEL 9q34.3 (3.12 Mb) (137,027,481–140,145,742), hg18, *de novo*	**9q subtelomeric syndrome (Kleefstra syndrome)**	*FOXP1* ***EHMT1***
Patient 4 (f, 28mo)	pMIC, ID, DF, A, HY, FT	N	DEL 16q21q22.1 (2.03 Mb; 64,772,843–66,806,006), hg18, *de novo*	**Interstitial 16q21q22.1 microdeletion**	
Patient 5 (f, 14mo)	cMIC, ID, DF, HY, CHD,E	Dilatation of brain ventricles	DEL 1p36.33-p36.32 (2.778 Mb; 554,268–3,332,604), hg18, *de novo/* DUP 21q21.2-q21.3 (0.58 Mb; 25,718, 751–26,303,088), hg18, *de novo*	**1p36 microdeletion syndrome**	*PLCH2, SKI*
Patient 6 (f, 15mo)	cMIC, ID, DF	N	DEL 15q13.2 (0.071 Mb; 28,731,195–28,801,859), hg18, *de novo*	**15q13.2 microdeletion syndrome**	*ARHGAP11B*
Patient 7 (f, 3,5y)	pMIC, ID, DF	N	DEL 1p36.33-q36.31 (5.7 Mb; 554,287–6,286,347), hg18, *de novo* DEL 9q34.2-q34.3 (4.5 Mb; 134,921,595–139,498,580), hg18, *de novo*	**1p36 microdeletion syndrome**	*PLCH2, SKI,* ***CHD5***
Patient 8 (m, 26mo)	MIC, ID, DF, SST	N	DEL 1q25.1-q25.3 (9.04 Mb; 173,679,845–182,719,617) hg18, *de novo/* DUP 5p13.1p12 (1.4 Mb; 42,164,723–43,548,219, hg18, *pat*	**Intermediate 1q syndrome**	*ASTN1*
Patient 9 (f, 7mo)	MIC, ID, DF, MCA, HY	Dysplastic right brain hemisphere-pachygyria	DEL 1p36.33p36.22 (10.3 Mb; 564,424–10,892,722), hg19, *de novo* DUP 16p11.2 (0.401 Mb; 28,629,244–29,031,059) hg19 *de novo*	**1p36 microdeletion syndrome**	*PLCH2, SKI,* ***CHD5***, ***RE(RE)***
Patient 10 (m, 27mo)	MIC, ID, DF, E, HY	Brain desgenesis (lissencephaly, type 1 and pachygyria	DEL 1p36.33-p36.32 (1.46 Mb; 852,863–2,310,801), hg18, *de novo*	**1p36 microdeletion syndrome**	*SKI*
Patient 11 (m, 4y)	pMIC, ID, DF, HY	Dilation of brain ventricles	DUP 4p16.3p15.33 (11.6 Mb; 13,226–11,612,448), hg19, *de novo/* DEL 22q13.31q13.33 (4.4 Mb; 46,766,785–51,193,680) hg19, *de novo*	Distal partial 4p trisomy 22q13 microdeletion syndrome (Phelan McDermid syndrome)	
Group B: cases with CNVS probably related with microcephaly					
Patient 12 (m, 9y)	cMIC, ID, DF,	Coloid cyst	DEL 17q25.3 (1.38 Mb; 77,257,422–78,633,678), hg18, *de novo/*DUP 12q24.32q24.33 (7.076 Mb) (125,213,381–132,289,149), hg18, *de novo*		*RAC3, FOXK2*

Table 1 Continued

Patient	Phenotype	Brain MRI	CNVs: chromosomal region/size (Mb or kb)/genome coordinates/inheritance	Known syndrome	Genes contributing to the small brain size
Patient 13 (f, 10d)	cMIC, ID, DF, HY, CHD, PS		DEL 16p13.2 (0.093 Mb; 6,768,161–6,860,972), hg18, *de novo*		*FOX1 (A2BP1)*
Patient 14 (m, 2y)	cMIC, ID, DF, E	N	DEL 19p13.3 (0.075 Mb; 1,223,651–1,280,683), hg18, *de novo*		*EFNA2*
Patient 15 (f, 28mo)	pMIC, ID, DF, HY	Dilatation of brain ventricles Thin corpus callosum Reduced white matter volume Regions with small cysts	DEL 16q24.2-q24.3 (3.0 Mb; 85,681,658–88,690,615), hg18, *de novo*	16q24.3 microdeletion syndrome	*MAP1LC3B, FBX031*
Patient 16 (m, 10mo)	pMIC, ID, DF, MCA	Delay of myelin formation	DUP 1p36.33 (1.7 Mb; 564,424–2,290,021), hg18, *de novo*/DEL 22q11.22-q11.23 (655.9 Kb; 22,998, 284–23,654,222), hg18, *de novo*	22q11.2 microdeletion syndrome dup 1p36.33 syndrome	*RAB36*
Patient 17 (f, 3y)	MIC, ID, DF		DUP 9p24.3 (67.12 Kb; 204193–271,316), hg19, *de novo*		*DOCK8*
Patient 18 (f, 3y)	pMIC, ID, DF	N	DEL 19q13.43 (0.107 Mb) (63,676,549–63,784,382), hg18, *de novo*		*TRIM28* *CHMP2A*
Group C: cases with CNVS not related with microcephaly					
Patient 19 (f, 20mo)	pMIC,ID, DF, E	Reduced white matter volume	DUP 16p12.1 (0.593 Mb; 21,744,993–22,338,093), hg18, *de novo*		
Patient20 (f, 2,5y)	pMIC, ID, DF, HY	N	DEL 22q13.31q13.33 (4,7 Mb; 46,553,521–51,219,009), hg19, *de novo*	22q13 deletion syndrome (Phelan McDermit syndrome)	
Patient 21 (m, 5y)	pMIC, ID, DF	N	DEL 12p12.2 (0.386 Mb; 20,908,843–21,295,433), hg18, *de novo*		
Patient 22 (f, 8,5y)	cMIC, ID, DF, A, SST	N	DEL 17q23.3 (0.015 Mb; 59,307,904–59,323,512), hg18, *de novo*		
Patient 23 (m, 1,5y)	pMIC, ID, DF, E	N	DUP 14q24.2 (0.031Mb; 69,304,703–69,335,731), hg18, *de novo*		
Patient 24 (f, 4y)	MIC, ID, DF, SST	N	DUP Xp22.31 (1.648 Mb; 6,477,006–8,091,810), hg18, *de novo*		
Patient 25 (m, 1y)	pMIC, ID, DF, HY	*Ex vacuo* dilatation of brain ventricles	DEL 17q21.31q21.32 (0.472 Mb; 41,367,183–42,049,599), hg18, *de novo*	Koolen de vries syndrome	

A, autism; CD, celiac disease; CHD, congenital heart disease; cMIC, congenital microcephaly; CNV, copy number variation; d, days; DF, dysmorphic feature; E, epilepsy; f, female; FT, failure to thrive; HY, hypotonia; ID, intellectual disability; m, male; MCA, multiple congenital anomaly; MIC, microcephaly of unknown onset; mo, months; N, normal; pMIC, postnatal microcephaly; PS, pyloric stenosis; SST, short stature; y, year.
In bold are the known microdeletion or microduplication syndromes that have been associated with syndromic microcephaly and the genes that have already been recognized as having a significant role in the brain deficit and microcephaly.

Table 2. Protein function and role of the genes that may contribute to the small brain size

Gene	Genetic locus	Protein function/role
WHSC1	4p16.3	DNA damage and DNA replication stress response in early development (6)
WHSC2	4p16.3	Regulation of gene expression in both a negative and positive manner. Important for DNA synthesis and S-phase progression (4)
SLBP	4p16.3	Regulation of histone synthesis and availability during S phase. Important for DNA replication (4)
RANBP1	22q11.2	Ran GTPase-binding protein implicated in nuclear/cytoplasmic trafficking that participates in the regulation of the cell cycle (7)
FREM1	9p22.3	Basement membrane protein that may have a role in craniofacial development regarding duplication 9p (8)
DOCK8	9p24.3	Interaction with Rho-GTPase (14,15)
SOX1	13q34	Protein sharing a DNA-binding domain known as the HMG box, expressed in neural progenitor cells (10)
ARHGEF7	13q34	Rho-GTPase-activating protein (10)
FOXP1	3p13	Neural transcription factor (25)
EHMT1	9q34.3	Histone methyltransferase that represses transcription (11)
PLCH2	1p36.32	Promotes cellular calcium mobilization in the brain after birth, contributing to axon growth, growth cone guidance, and synapsis formation (26)
SKI	1p36.33	Transcriptional regulator with a dynamic expression pattern during cortical development. *SKI*-deficient cortices' phenotype includes disturbed timing of neurogenesis, misspecification of projection neurons, and altered cell cycle of neural progenitors (27)
ARHGAP11B	15q13.2	Rho-GTPase-activating protein 11B with an essential role in neuronal development (28)
CHD5	1p36.31`	Role in chromatin remodeling and gene transcription. Highly expressed in neurons (12)
ASTN1	1q25.2	Neuronal adhesion molecule important for CNS glial-guided migration of post-mitotic neuroblasts in cortical regions of the developing brain (29)
RE(RE)	1p36.23	Nuclear receptor coregulator that has a critical role in embryonic development (13)
RAC3	17q25.3	Rho-GTPase involved in the microtubule cytoskeletal dynamics, the activation of protein kinase, and the control of cell growth. High expression in cortical/hippocampal GABAergic interneurons (30)
FOXK2	17q25.3	Forkhead transcription factor crucial for cellular proliferation and survival (31)
FOX1 (A2BP1)	16p13.2	Positive or negative regulation of tissue-specific splicing. Highly expressed in the brain (32)
EFNA2	19p13.3	Regulation of the guidance of axon growth cones in the developing brain by binding with ephrin receptors via direct cell–cell interaction (33)
MAP1LC3B	16q24.3	Microtubule-associated protein having a role in microtubule assembly, and, therefore, it is important for neurogenesis (34)
FBXO31	16q24.2	F-box protein regulating the neuronal development by a variety of aspects such as neuronal morphogenesis and axonal identity as well as neuronal migration and dendrite growth in the developing cerebellar cortex (34)
RAB36	22q11.2	Ras-related GTPase, one of the essential mediators for neurite outgrowth downstream of RAB35 (ref. (22))
TRIM28	19q13.4	Transcriptional co-repressor. TRIM28-mediated histone modification is used by NPCs to silence ERVs and regulate transcription (35)
CHMP2A	19q13.4	Chromatin-modifying protein/CHMP family. Component of the endosomal sorting complex required for transport III (ESCRT-III). Highly expressed in the brain (36)

CHMP, charged multivesicular body protein; CNS, central nervous system; ERV, endogenous retrovirus; NPC, neural progenitor cell.
In bold are the genes that have already been proposed to have a significant role in brain deficit and microcephaly (https://genome.ucsc.edu/).

On the basis of CNV findings, we subdivided the 25 patients into the following three groups:

Group A (**Table 1b**, patients: 1–11): cases with CNVs related with microcephaly. The identified CNVs are known microdeletion/microduplication syndromes that have already been associated with microcephaly.

Group B (**Table 1b**, patients 12–18): cases with CNVs probably related with microcephaly. The CNVs include genes that could potentially contribute to the small brain size.

Group C (**Table 1b**, patients 19–25): cases with CNVs not related with microcephaly. On the basis of the existing literature, these CNVs have been linked with the pathologic phenotype, but they cannot explain microcephaly.

In summary, CNVs related with microcephaly were identified in 11/53 (20.75%; GROUP A), CNVs probably related with microcephaly were revealed in 7/53 (13.2%; GROUP B), and CNVs not related with microcephaly were present in 7/53 (13.2%; GROUP C) patients. In the remaining 28 out of the 53 cases (52.83%), array-CGH revealed only copy number polymorphisms and they were considered as normal.

Parental DNA samples were examined by array-CGH for all patients.

DISCUSSION

The aim of our study was to determine the contribution of gene-dosage alterations in the etiology of unexplained microcephaly through genome-wide screening using array-CGH analysis in patients and their parents. Array-CGH is an advanced well-known high-throughput genetic diagnostic technology that allows the identification of microdeletions and microduplications not detected by G-banding karyotype and other conventional methods.

In patients 1, 2, 9, and 11 (**Table 1b**, GROUP A), array-CGH revealed large chromosomal alterations. In patient 1, the previous standard karyotype revealed a derivative chromosome 9 (46, XY, der (9)) and further investigation by array-CGH was performed. In the other three patients, the karyotype was normal because of low band resolution that was applied. For patients 2 and 11, conventional karyotyping was carried out in chorionic villi sample, whereas in patient 9 it was carried out in amniotic fluid.

Defects in genes that are implicated in DNA repair, DNA damage response, DNA replication, and in S-phase progression or those disrupting the microtubule network have already been suspected of causing microcephaly (4). Seltzer *et al.* (5) highlighted specific biological pathways associated with postnatal microcephaly, with genes implicated in the regulation of gene expression in the developing brain, in histone modification, and in GTPase signal transduction.

In accordance with the literature, we identified candidate genes that may contribute to the microcephaly phenotype in all the patients in whom array-CGH testing was performed only for clinical confirmation. The four recognized genes, *WHSC1, WHSC2, SLBP,* and *RANBP1* (**Tables 1a** and **2**), have already been associated with microcephaly (4,6,7). In 16 out of the 25 patients with unknown microcephaly and CNV findings (**Table 2**), we identified genes that could potentially have a significant role in small head circumference. From a total of 21 candidate genes, only 6 (*FREM1, SOX1, ARHGEF7, EHMT1, CHD5,* and *RE(RE)*) have been previously reported to be involved in brain deficit causing microcephaly (**Tables 1b** and **2**) (8–13).

GROUP A: Cases with CNVs Related with Microcephaly

In 11 out of the 25 patients (No: 1–11; GROUP A, **Table 1b**), one or more of the identified CNVs are known microdeletion or microduplication syndromes that have already been associated with syndromic microcephaly—1p36 microdeletion, 9p duplication, 9q34.3 microdeletion (Kleefstra syndrome), 13q33-q34 microdeletion, 16q21-q22.1 microdeletion, 15q13.2 microdeletion, intermediate 1q microdeletion syndrome, and distal 4p trisomy. In patients 1 and 2, the probability of the existence of a balanced translocation in one parent was excluded, as the parental standard karyotype was normal. Genes probably contributing to microcephaly were identified in 9 out of the 11 patients with known syndromes.

9p partial duplication syndrome (patients Nos 1 and 2; GROUP A) is characterized by microcephaly, specific dysmorphic features, growth, and mental retardation. A critical region ranging from 9p22.3 to 9p22.2 is mainly responsible for the specific 9p partial trisomy phenotype (14). It is interesting that patient No 17 (GROUP B), a 3-year-old female with microcephaly of unknown onset, psychomotor retardation, and mild facial dysmorphic features has a 9p24.3 microduplication, 67.12 kb in size, containing the *DOCK8* gene. DOCK8 protein belongs to the Dock-C subgroup of the Dock family of guanine nucleotide exchange factors that interact with Rho GTPases and are components of intracellular signaling networks (15). Mutations in the *DOCK8* gene cause the autosomal-recessive hyper IgE recurrent infection syndrome (OMIM #243700), and *DOCK8* deficiency has been reported as the cause of autosomal-dominant mental retardation type 2 (OMIM #614113). Regarding the 9p partial duplication syndrome, it has been suggested that the *DOCK8* gene may contribute to the autistic spectrum disorder phenotype (14). We presume that, as the DOCK8 protein interacts with *Rho GTPases*, *DOCK8* duplication could also have a role in microcephaly and mental retardation regarding the 9p duplication syndrome.

GROUP B: Cases with CNVs Probably Related with Microcephaly

In seven patients (No: 12-18; GROUP B, **Table 1b**), the identified CNVs have not been associated with microcephaly so far; however, they include genes that are implicated in genetic pathways reported as crucial for the brain and head development (4,5).

In patient 14, a 2.5-year-old boy with severe congenital microcephaly, dysmorphic face, psychomotor retardation, hypertonia, and epilepsy, the 19p13.3 microdeletion encompasses only the following three genes: *CIRBP*, *C19orf24*, and *EFNA2*. The *EFNA2* gene encodes a membrane-bound protein, member of the ephrin family receptor-interacting protein. It binds with ephrin receptors, which are also membrane-bound proteins via direct cell–cell interaction. The signaling pathway regulates a variety of biological processes during embryonic development including the guidance of axon growth cones, which could contribute to the small head circumference. However, previous publications (16,17) do not support a causative role for microcephaly for these genes.

Patient 16, a 10-month-old male with postnatal microcephaly, delayed motor and cognitive skills, and multiple congenital anomalies, is the first case involving terminal 1p36 microduplication accompanied by a 22q11.2 distal deletion. 1p36 microduplications are very rare, and it is unknown whether there is any association with microcephaly (18,19). The 22q11.2 distal deletions are recurrent genomic rearrangements located in the region immediately telomeric to the ~3 Mb common DiGeorge and Velocardiofacial deleted region (20,21). In our patient, the small 22q11.2 microdeletion, between LCR22-5 and LCR22-6, 655.9 kb in size, encompasses the following four important genes: *BCR, RAB36, GNAZ,* and *RTDR1*. *RAB36* codes a protein that is one of the essential mediators for neurite outgrowth downstream of *RAB35* (ref. (22)) and may contribute to the

microcephalic phenotype. Haploinsufficiency of *BCR*, *GNAZ*, and *RTDR1* genes does not seem to have a role in microcephaly formation (23).

GROUP C: Cases with CNVs not Related with Microcephaly

In seven patients (Nos: 19–25; GROUP C, **Table 1b**), the identified CNVs include genes that could partially explain the patients' phenotype. These CNVs have not been associated with microcephaly nor do they include genes that could contribute to reduced head circumference.

Interstitial deletions of the short arm of chromosome 12 are very rare structural anomalies. Microcephaly has been described in a number of reported patients while important genes such as *SSPN*, *SOX5*, *Kras*, and *STK38L* have also been proposed (24). Patient No 21, a 5-year-old male, presented with a phenotype resembling Floating Harbor syndrome (extremely low birth weight, short stature, postnatal microcephaly, intellectual disability, and dysmorphic features, such as triangular face, prominent nasal bridge and collumella, short philtrum, thin upper lip, and cryptorchidism). The 12p12.2p12.1 microdeletion contained only the *SLCO1B3* and *SLCO1B1* genes. Both genes belong to the organic anion transporter family involved in the membrane transport of bile acids, conjugated steroids, and thyroid hormones. A case has been reported in DECIPHER with a *de novo* 12p12.2p12.1 deletion, with similar breakpoints as our patient and phenotypic features such as intellectual disability and microcephaly (DECIPHER ID: 251269). Another patient with a similar deletion of paternal origin presented with speech delay and macrocephaly (DECIPHER ID: 282690). The 12p12.2p12.1 deletion could contribute to our patient's clinical phenotype. However, these chromosomal aberrations are rare events.

The results of our study show that array-CGH may be considered as an invaluable diagnostic tool in patients with syndromic microcephaly. Array-CGH is a useful first-line diagnostic tool for the recognition of submicroscopic chromosomal aberrations in patients with syndromic microcephaly of unknown etiology. Further delineation of such genetic abnormalities, along with well-characterized clinical phenotypes, must be reported in order to identify new syndromes related with microcephaly and to recognize candidate genes, the haploinsufficiency of which could result in a small brain size.

In the future, the extensive application of whole-exome sequencing and whole-genome sequencing in patients with syndromic microcephaly of unknown etiology will provide more information regarding genes implicated in brain development.

ACKNOWLEDGMENTS

We thank the families of the patients for their contribution.

Disclosure: The authors declare no conflict of interest.

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