Clinical Findings on Chromosome 1 Copy Number Variations

Filipa Leitão[1]   Ana Grangeia[1,2,3]   Joel Pinto[1,3]   Armanda Passas[4]   Sofia Dória[1,3]

[1] Department of Pathology, Genetics Service, Faculty of Medicine, University of Porto, Porto, Portugal
[2] Medical Genetics Service, Centro Hospitalar Universitário de São João, Porto, Portugal
[3] I3S-Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal
[4] Neurodevelopment Unit- UNIA, Centro Hospitalar Vila Nova de Gaia/Espinho – CHVNG, Vila Nova de Gaia, Portugal

Address for correspondence Sofia Dória, PhD, Faculty of Medicine, University of Porto, Al. Prof. Hernâni Monteiro, 4200 - 319 Porto, Portugal (e-mail: sdoria@med.up.pt).

Abstract

Copy number variants (CNVs) are a major contribution to genome variability, and the presence of CNVs on chromosome 1 is a known cause of morbidity. The main objective of this study was to contribute to chromosome 1 disease map, through the analysis of patients with chromosome 1 CNVs.

A cross-sectional study was performed using the array comparative genomic hybridization database of the Genetic Department of the Faculty of Medicine. Patients with pathogenic (P) or likely pathogenic (LP) CNVs on chromosome 1 were selected for the study. Clinical information was collected for all patients. Databases and related literature were used for genotype–phenotype correlation.

From a total of 2,516 patients included in the database we identified 24 patients (0.95%) with P (9 patients) or LP (15 patients) CNVs on chromosome 1. These CNVs account for 6.1% (24/392 CNVs) of the total P/LP CNVs in the database. Most common CNVs found were in the 1q21.1–1q21.2 region.

This study reinforces the association between chromosome 1 CNV and neurodevelopmental disorders and craniofacial dysmorphisms. Additionally, it also strengthened the idea that CNVs interpretation is not always a linear task due to the broad spectrum of variants that can be identified between benign and clearly pathogenic CNVs.

Keywords
► chromosome 1
► copy number variant
► array comparative genomic hybridization
► neurodevelopmental disorders
► 1q21.1 syndrome

Introduction

Copy number variant (CNV) is a term used to describe deletions and duplications of deoxyribonucleic acid (DNA) segments that can range in size from 50 base pairs (bps) to megabases (Mbs). Approximately 9.5% of our genome may correspond to CNVs. Even though these variants might be responsible for adaptive traits, they might also be maladaptive and lead to disease, particularly neurodevelopmental disorders.[1]

The presence of CNVs on chromosome 1 is a known cause of morbidity, which is not surprising since it is the largest human chromosome corresponding to approximately 8% of our genome.[2] Despite 9.6% of this chromosome not having been sequenced yet, (http://www.cshlp.org/ghg5_all/section/dna.shtml), it contains 4,495 genes, 2,033 protein-coding (http://vega.archive.ensembl.org/Homo_sapiens/Location/ Chromosome?r = 1), and 53,206,540 short variants (https://www.ensembl.org/ Homo_sapiens/Location/Chromosome ?r = 1).

As stated before, chromosome 1 allelic variation is an established cause of a wide range of human diseases including neurodevelopmental and Mendelian diseases, as well as cancer.[2] Until now, several disease susceptibility regions have been identified being some associated with known genetic syndromes, such as the 1p36 region, the most common terminal deletion syndrome in humans. The severity of the phenotype is variable, nonetheless, it usually causes developmental delay (DD), intellectual disability (ID), and dysmorphic features.[3,4]

The 1q21.1 region is a hotspot for deletions and duplications since it contains extensive and complex low-copy repeats.[5,6] CNVs in this region may cause 1q21.1 deletion and duplication syndromes, or thrombocytopenia-absent radius (TAR) syndrome.[6] The 1q21.1 syndromes were initially associated with CNVs in distal 1.35-Mb region of 1q21.1, which included a minimum of 7 genes.[7] Additional CNVs including the TAR critical region were recognized, extending the interval to a total of approximately 2 Mb.[5] Both syndromes can lead to phenotypes of DD, ID, autism spectrum disorder (ASD), psychiatric conditions, epilepsy, craniofacial dysmorphism (CFD), and multisystemic congenital abnormalities,[8] although with incomplete penetrance and variable expressivity.[5] More centromeric proximal microdeletions/microduplications have been less extensively studied and are associated with a more highly variable phenotype and lack of consistent dysmorphisms.[9] The exception are individuals with TAR syndrome, characterized by hypomegakaryocytic thrombocytopenia with bilateral radius aplasia with present thumbs. This very rare disorder has an autosomal recessive (AR) inheritance and is associated with a 1q21.1 microdeletion.[10,11]

Another well-described susceptibility region is the 1q43–1q44. CNVs in these regions can be responsible for neurological impairment and structural brain disorders.[12]

The main objective of this study was to contribute to the chromosome 1 disease map, through the analysis of patients with rearrangements in this susceptibility loci for disease, as well as highlight the clinical usefulness of array comparative genomic hybridization (array-CGH) as a first evaluation method for patients with neurodevelopmental disorders and polymalformative syndromes.

Materials and Methods

We performed a cross-sectional study using the Genetics Department array-CGH database of the Faculty of Medicine, University of Porto, Porto, Portugal. All the patients were from Centro Hospitalar Universitário de São João, Porto (CHUSJ) and Centro Hospitalar Vila Nova de Gaia (CHVNG) and performed array-CGH from 2013 to 2020. From the general database (2,516 patients) 24 patients were included. Patients were selected (DNA extracted from peripheral blood, amniotic fluid, or chorionic villus) if chromosome 1 pathogenic/probably pathogenic CNVs were identified.

Array-CGH were performed using Agilent SurePrint G3 Human Genome 4×180K or 8×60K according to the manufacturer's recommendation, Version 7.5, 2016 (Agilent Technologies, Santa Clara, United States). Results were ana-

lyzed using Cytogenomics software (Agilent Technologies) with versions from 2.0 to 4.0.3.12. Familiar studies were performed by quantitative polymerase chain reaction (qPCR) or by array-CGH. Unfortunately, not all the progenitors were available for study, largely due to the COVID pandemic situation or because they refused the study.

The arrays nomenclature was described according to International System for Human Cytogenomic Nomenclature 2020[13] and using Human Genome build Genome Assembly hg19 (GRCh37).

For CNVs analysis and classification, national and international databases were used, namely: DGV (http://dgv.tcag.ca), DECIPHER (https://decipher.sanger.ac.uk/), OMIM (https://www.omim.org), ClinGen (https://www.clinicalgenome.org/), data from the literature, and PubMed (https://www.ncbi.nlm.nih.gov/). CNVs were classified as pathogenic (P), benign (B), variant of unknown significance (VUS), and VUS likely pathogenic (LP) or likely benign (LB), in accordance with the American College of Medical Genetics Standards and Guidelines.[14]

To establish a genotype–phenotype correlation, we collected information from patient's clinical records, including clinical data and diagnostic exams (such as magnetic resonance imaging, electroencephalogram, karyotype, qPCR, or multiplex ligation-dependent probe amplification) and an exhaustive literature search was done, looking for similar CNVs.

This study was conducted with the formal approval of the CHUSJ Ethical Committee, authorization request CE 357–20. After collection all the clinical information, database anonymization was performed by giving each patient a random number, following the guidelines of the CHUSJ Ethical Committee.

Results

A total of 2,516 patients with different clinical indications were studied by array-CGH and in approximately 15.6% (392/2,516) P/LP CNVs were detected. Within these 6.1% (24/392 CNVs) were located on chromosome 1. From these 24 CNVs, 15 (62.5%) were classified as LP and the remaining 9 (37.5%) as P.

The most common CNV found were on 1q21.1 (either deletions or duplications), with some of them also spanning the 1q21.2 region. Four patients presented additional CNVs that were not related with chromosome 1. Patient's clinical and genetic data are shown in ►**Table 1**. More detailed data are available in ►**Supplementary Table S1** (available in the online version).

CNVs from 1p31.1 to 1p36.32
Four CNVs (deletions ranging from 41 to 3,880 Kb) were found, 3 classified as LP and 1 as P (patients 1–4). Patient 1 had an additional P CNV on chromosome 17. Patient's phenotypes shared several clinical features including ID, ASD, CFD, and epilepsy.

CNVs on 1q21.1-q21.2
Fifteen CNVs were found, 10 deletions and 5 duplications (patients 5–19). From these, 8 were classified as P and 7 as LP.

Table 1 Clinical findings in patients presenting chromosome 1 CNVs

Patient	Age (y)	Sex	Clinical findings	a-CGH result	Genes involved	CNV type	Dimension (Kb)	Inheritance
1	22	M	ID; epilepsy; craniofacial dysmorphism (including dolichocephaly)	arr[GRCh37]1p31.3p31.2(65394582_69274113)x1,17q12(34450405_36243028)x1	DNAJC6, LEPR, SLC35D1, IL23R, RPE65 +25 other genes and LHX1, LOC28400 +23 other genes	P and P	3880 del and 1793 del	Unknown
2	8	M	ASD;	arr[GRCh37] 1p32.2(58527830_58678806)x1	DAB1	LP	151 del	Paternal
3	8	M	ID; with language impairment; craniofacial dysmorphism (including macrocephaly)	arr[GRCh37] 1p34.234.1(43748505_44331125)x1	MPL, PTPRF, KIAA0467, ST3GAL3 +9 other genes	LP	583 del	De novo
4	6	M	ASD	arr[GRCh37] 1p36.32(3149613_3190921)x1	PRDM16	LP	41 del	Paternal
5	27	F	Fetus with right radius agenesis; severe left radius hypoplasia, bilateral ulna hypoplasia; presence of thumbs	arr[GRCh37]1q21.1(145291711_145833054)x1	RBM8A, PEX11B +19 other genes	P	541 del	Maternal
6	4	M	ASD; ID; epilepsy	arr[GRCh37] 1q21.1(145388355_145833054)x1	RBM8A, PEX11B +19 genes	P	444 del	Maternal
7	21	M	ID; craniofacial dysmorphism; retinitis pigmentosa	arr[GRCh37] 1q21.1(145568116_145609227)x1	NBPF20, NBPF10, PIAS3 +4 other genes	LP	41 del	Unknown
8	9	F	ID, craniofacial dysmorphism	arr[GRCh37] 1q21.1(145632334_145833054)x1, 8p21.3(222222050_22370282)x3	NBPF20, NBPF10, CD160, PDZK1, GPR89A, RNF115, NBPF25P and PPP3CC, SLC39A14	LP and LP	201 del and 148 dup	Unknown
9	8	F	ID; ADHD	arr[GRCh37] 1q21.1(146506310_146769928)x1	PRKAB2, FMO5, CHD1L +4 other genes	LP	264 del	Unknown
10	6	M	ASD; ID; ADHD; craniofacial dysmorphism (including macrocephaly); hereditary spherocytosis	arr[GRCh37] 1q21.1(146542843_146628179)x1	PRKAB2, RNVU1-8, NBPF13P	LP	85 del	Unknown
11	42	M	ID; craniofacial dysmorphism	arr[GRCh37] 1q21.1q21.2(145818702_149378266)x1,16p11.2(29652999_30198600)x1	GJA5, GJA8 +26 other genes and SPN +28 other genes	P and P	3560 del and 545 del	Unknown
12	4	F	DD; craniofacial dysmorphism	arr[GRCh37] 1q21.1q21.2(146506310_147824207)x1	GJA5, GJA8, +12 other genes	P	1318 del	De novo
13	7	M	ID with language impairment; ADHD	arr[GRCh37] 1q21.1q21.2(146506310_147824207)x1, 16p11.2(28824794_29042118)x1	GJA5, GJA8, +12 other genes and ATXN2L +9 other genes	P and LP	1318 del and 217 del	Unknown

(Continued)

Table 1 (*Continued*)

Patient	Age (y)	Sex	Clinical findings	a-CGH result	Genes involved	CNV type	Dimension (Kb)	Inheritance
14	11	M	ASD, DD	arr[GRCh37] 1q21.2 (147404212_149378266)x1	GPR89B, NBPF15, NBPF11, PPIAL4A, NBPF14 + 12 other genes	LP	1974 del	Paternal
15	10	F	ID; epilepsy	arr[GRCh37] 1q21.1 (144994947_145833054)x3	RBM8A, PEX11B+ 25 other genes	LP	838 dup	De novo
16	7	M	ASD; ID; ADHD	arr[GRCh37] 1q21.1 (145212590_146542902)x3	RBM8A, PEX11B + 27 other genes	P	1330 dup	Maternal
17	5	M	DD; epilepsy	arr[GRCh37] 1q21.1 (145264630_145833054)x3	RBM8A, PEX11B + 17 other genes	LP	568 dup	Paternal
18	9	M	ASD; ID; with language impairment; epilepsy; thin corpus callosum	arr[GRCh37] 1q21.1 (146506310_147824207)x3	GJA5, GJA8+ 12 other genes	P	1318 dup	Inherited (present in the brother)
19	6	M	ASD	arr[GRCh37] 1q21.1q21.2 (146506310_149243967)x3	GJA5, GJA8 + 22 other genes	P	2738 dup	Paternal
20	5	M	DD; craniofacial dysmorphism (including dolichocephaly)	arr[GRCh37] 1q22–1q23.1 (156132786_157120342)x3	SEMA4A, PRCC, NTRK1 +36 other genes	LP	988 dup	De novo
21	1	M	DD; *delayed myelination*	arr[GRCh37] 1q23.1 (161967426_162280549)x3	NOS1AP, OLFML2B	LP	313 dup	Maternal
22	26	M	ID; epilepsy; craniofacial dysmorphism (including microcephaly)	arr[GRCh37] 1q43 (2373381873_237497031)x1	RYR2	LP	115 del	De novo or paternal
23	11	M	ID; with language impairment	arr[GRCh37] 1q43 (239855264_239912160)x1	CHRM3	LP	57 del	Unknown
24	4	M	DD; with language impairment	arr[GRCh37] 1q43q44 (243977880_244609719)x1	AKT3	LP	102 del	Unknown

Abbreviations: a-CGH, array comparative genomic hybridization; ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; CNV, copy number variation; DD, developmental delay; Del, deletion; Dup, duplication; F, female; ID, intellectual disability; LP, likely pathogenic; M, male; P, pathogenic.

Additionally, other 3 CNVs were identified on chromosomes 8 and 16 (patients 8, 11, 13). Patients showed a phenotype that includes mainly ASD, ID, DD, CFD, and epilepsy.

Deletions ranged between 41 to 3,560 Kb. Out of 10 patients, 7 had ID, 6 CFD, 3 ASD, 3 attention deficit hyperactivity disorder (ADHD), 2 DD, and 1 epilepsy. In one case at prenatal diagnosis stage, a heterozygous deletion including *RBM8A* gene was identified in a fetus with a TAR syndrome phenotype.

Duplications ranged between 568 to 2,738 Kb and patients shared some phenotypic features, namely 3 had ASD, 3 ID, 3 epilepsy, and 1 DD.

CNV from 1q22 to 1q23.1
Two CNVs (duplications spanning 313 and 988 Kb) were found, both classified as LP (patients 20–21). These patients shared several characteristics including DD, CFD, strabismus, and neuromuscular symptoms.

CNVs on 1q43-q44
Three CNVs (deletions ranging from 57 to 115 Kb) were found, all classified as LP (patients 22–24). Patients showed a variable phenotype including neurodevelopmental disorders, epilepsy, and CFD. Two of the patients had ID with hypotonia and other two had marked language impairment.

Discussion

Presently, the only recurrent CNVs curated in ClinGen on chromosome 1 are on 1q21.1, proximal (BP2-BP3 including RBM8A) and distal (BP3-BP4, including GJA5) regions. In this study, we described CNVs on chromosome 1 and the phenotype findings in 24 patients which could be a good reference for clinicians to refer to when they have patients with CNVs overlapping these regions.

CNVs from 1p31.1 to 1p36.32
Regarding 1p31.3p31.2 region, the DECIPHER database describes patients presenting several phenotypes such as ID, ASD, CFD, and obesity. The majority of CNVs published are distal deletions including *NFIA* gene (score = 3 for haploinsufficiency, ClinGen). The CNV found in patient 1 do not included this gene, nevertheless, is a large deletion that includes other possible relevant genes. In ClinVar it was reported a patient (#RCV000140906.4) with a 2.6-Mb CNV that significantly overlaps our patient CNV, presenting ID, short statute, and panhypopituitarism. Additionally, in patient 1 it was also detected a chromosome 17 deletion, which partially overlaps 17q12 microdeletion syndrome (OMIM #614527) region. Nagamani et al studied 4 patients with deletions between 1.06 and 2.46 Mb, including *LHX1* and *LOC28400* genes, that were also deleted in our patient.[15] The phenotype included from moderate language impairment to severe ID, seizures, short statute, and renal anomalies. Therefore, it may be expectable that both CNVs might contribute to the phenotype and a confounding factor may be present. According to the clinical records the patient's

mother has mild ID and the father had epilepsy, unfortunately, they were not available for study.

Patient 2 has a deletion on 1p32.2 including the *DAB1* gene (disabled-1 gene, OMIM #603448). The *DAB1* protein participates in the reelin glycoprotein signaling pathway, playing an important role in the correct positioning of neurons within the developing cerebral cortex and cerebellum, contributing to the maintenance of synaptic function.[16,17] Moreover, the reduced expression of reelin is an established cause of autism.[18] This patient was previously described by our group in other study that evaluated CNVs found in a ASD cohort.[19] There are still few cases reported in the literature with *DAB*1 gene deletion and the DECIPHER database only includes nine patients with CNVs bellow 1 Mb overlapping this region.[20] In ClinVar it is reported one patient (#VCV000148218) presenting chorea features with a deletion of 329 Kb affecting only *DAB1* gene. The qPCR analysis performed on the proband's progenitors revealed that the father is a carrier raising the possibility of incomplete penetrance. It is not yet completely clear if this CNV could fully explain the ASD, nevertheless there is enough evidence to classify this CNV as LP.

Concerning patient 3 (deletion on 1p34.2–34.1), the literature describes that mutations on *KIAA0467* (*SZT2*) gene are related to epileptic encephalopathy (OMIM #615463). Deletions are usually classified as VUS since the relation with nonsyndromic ID has not yet been proven. Falcone et al reported three brothers with moderate ID and a homozygous deletion of 3 bp in *SZT2* gene.[21] *ST3GAL* gene homozygous mutations have also been associated with epileptic encephalopathy (OMIM #615006). Edvardson et al described a Palestinian family with four members with epileptic encephalopathy, DD, and severe ID.[22] The DECIPHER database describes several patients with deletions that partially overlap with our patient CNV and sharing several features with our patient, including ID, language impairment, muscular hypotonia, and macrocephaly.[20] Array-CGH, patient progenitor's study revealed a de novo deletion. Taking into account that there is not yet a recognized syndrome and it is not still clear if this deletion is the cause of the phenotype (although very likely), this CNV was classified as LP.

In patient 4, a deletion on 1p36.32 that includes the *PRDM16* gene (PR Domain-Containing Protein 6, OMIM #605557) was found. This gene has several functions, namely as transcriptional regulator of DNA or repressor of transforming growth factor-β signaling. Heterozygous pathogenic variants and deletions in the *PRDM6* gene cause left ventricular noncompaction-8 and dilated cardiomyopathy-1LL in an autosomal-dominant (AD) heritance (OMIM#615373). According to previous reports these diseases are mainly associated when exon 4 and 17 (chr1:3224674_3354772) are implicated.[23] The deletion present in our patient involved only exon 3 which may explain absence of cardiac disease. The DECIPHER database describes several patients with deletions including *PRDM16* gene presenting with similar phenotypes to our patient, mostly, electroencephalogram abnormalities (19 patients) and ASD (patients 317660 and 398407). Notwithstanding, the majority of the deletions

including *PRDM16* gene were larger with other genes also affected (87% had between 1 and 10 Mb). Therefore, the phenotypes described in those patients are more compatible with the classic 1p36 deletion syndrome. The qPCR analysis performed on patient's parents revealed that the microdeletion was inherited from the father suggesting an incomplete penetrance. Considering the presented information, the asymptomatic progenitor, and the size, this CNV was classified as LP.

CNVs on 1q21.1-q21.2

The proximal region of chromosome 1q21.1 is related to TAR syndrome, caused by loss of function of the *RBM8A* gene (ribonucleic acid binding motif protein 8 or Y14 protein, OMIM#605313), usually by compound/biallelic inheritance.[24] Typically, one *RBM8A null allele* is necessary, usually a 200-kb microdeletion at the proximal 1q21.1 region, that can be inherited or occur de novo.[10] Associated, other *RBM8A* hypomorphic allele should be present,[25] consisting in one of two low-frequency single-nucleotide polymorphisms in *RBM8A* noncoding regulatory regions, either at the 5′ untranslated region or within the first intron.[24] The *RBM8A* protein is essential for several basic cellular functions, being present in all hematopoietic lineages, including platelets. The levels of this protein are decreased in this syndrome which may justify the characteristic thrombocytopenia.[24] In patient 5 we identified a heterozygous deletion inherited from the mother that included the *RBM8A* gene. Due to TAR syndrome AR inheritance other pathogenic variant should be detected. However, *RBM8A* gene sequencing revealed no pathogenic coding variants, so further studies should be performed to identify a second variant (intronic or in a promoter region) that could explain the phenotype.

The 1q21.1 microdeletion/microduplication syndromes can occur de novo or be inherited from a parent in an AD manner, with incomplete penetrance and variable expressivity.[5,8] In our series, seven CNVs were inherited and two were de novo. All the inherited CNVs were heterozygous, and the majority of the cases were inherited from apparently asymptomatic parents. This reinforces the possibility of incomplete penetrance associated with this region. A highly variable clinical phenotype, including ID, DD, ASD, ADHD, CFD, epilepsy, and congenital anomalies, is also described. According to literature, the 1q21.1 microdeletion syndrome's most recognizable features are microcephaly, facial dysmorphisms, DD, mild ID, and schizophrenia, whereas chromosome 1q21.1 microduplication syndrome is more prone to be associated with macrocephaly, frontal bossing, hypertelorism, DD, ID, and ASD.[5,6,8,26] In our study, we identified two patients with the same genomic interval on 1q21.1 (patients 12 and 18). Patient 12 (1q21.1 deletion) presents with DD and dysmorphic features whereas patient 18 (1q21.1 duplication) was diagnosed with ASD, ID with language impairment, and epilepsy, and revealed a thin corpus callosum in the neuroimaging. This highlights the fact that both loss and gain of function of the genes included in these regions may contribute to the phenotype, as indicated by the haploinsufficiency (HI = 3) and triplosensitivity scores (TS = 3) in the ClinGen database.

Furthermore, we identified three patients with 1q21.1 deletions (patients 6, 10, and 14) with ASD. Most patients described in the DECIPHER database with autism have 1q21.1 duplications and not deletions.[20] In patient 14 the deletion was more distal and corresponded only partially to the interval described in the 1q21.1 microdeletion syndrome, consequently, it was not clear if the deletion could explain the phenotype and was classified as LP. The progenitors analysis performed by array-CGH showed that the microdeletion was inherited from the father.

Additionally to ASD, patient 10 also had macrocephaly, which is typically described in 1q21.1 duplications. A whole-exome sequencing (WES) was also performed in this patient and a heterozygous variant c.4873C > T [p. (Arg1625Ter)] on the *SPTB* gene was identified. This variant inherited from his father has already been associated with hereditary spherocytosis type 2 as presented also in the patient.

CNVs described in 1q21.1 deletion syndrome usually range between 1.35 and 2 Mb.[5,7] In our study, some of the deletions detected (patients 7–10) were smaller (from 41 to 264 kb). In the DECIPHER database several patients presented with small deletions overlapping our cases, most of them with ID. Patient 7 CNV corresponds to a 41-kb deletion, and it was not clear if this CNV could explain the phenotype. Considering absence of enough causative evidence, WES in trio was performed and a homozygous variant was detected on the exon 7 of the *DHDDS* gene, c.565G > C [p. (As189His)]. This variant classified as VUS has not yet been reported in the literature and the bioinformatics analysis was not conclusive. Both progenitors are asymptomatic carriers for the variant and the proband's twin sister with a similar phenotype plus epilepsy was also homozygous for the same variant. The *DHDDS* gene (dehydrodolichyl diphosphate synthase, OMIM# 608172) is located in 1p36.11 and encodes for an enzyme required for the biosynthesis of several classes of glycoproteins. Pathogenic variants have been described in patients with retinitis pigmentosa type 59 and congenital disorder of glycosylation type 1bb, with an AR pattern.[27] Patients with DD and seizures, with or without movement abnormalities, have also been reported.[28] This homozygous variant was considered to better explain the phenotype rather than the 1q21.1 deletion.

Beyond the 1q21.1 deletion we found in three patients (patients 8, 11, and 13) additional CNVs. In patient 8, a duplication in the short arm of chromosome 8 involving the *PPP3CC* and the *SLC39A14* genes was identified. Both CNVs present in this patient were classified as LP, and it is not clear which of them could justify the phenotype or if they could have an additive effect. In patients 11 and 13, the array-CGH identified additional CNVs on chromosome 16. Even though these two patients had deletions compatible with 1q21.1 microdeletion syndrome that may explain the phenotype, the chromosome 16 deletions overlap the 16q11.2 microdeletion syndrome region, previously reported in several patients with ID and ASD.[29,30] Since both regions (1q21.1 and 16q11.2) are associated with recognized syndromes

associated with neurodevelopment diseases, it is difficult to establish a direct correlation between the phenotypes and the causative effect of each CNV individually. Unfortunately, in both patients it was not possible to test the parents, which would be important for a better understanding of the pathogenicity of the microdeletions.

Regarding 1q21.2 duplications we found five variants spanning different sizes (patients 15–19). ClinGen and DECIPHER databases describe CNVs with similar sizes or even smaller, classified as VUS or LP CNVs. Patients features included, among others, ID, seizures, language difficulties, behavior and psychiatric abnormalities, congenital heart disease, and cleft palate. Our patients present mainly ASD, ID, epilepsy, or other neurodevelopment disorders. A heterozygous variant c.91G > A [p. (Ala31Thr)] (classified as VUS and inherited from the father) in the *KCNMA1* gene exon 1 was also found by WES in case 17. Pathogenic variants in this gene have been reported in patients with epilepsy and paroxysmal dyskinesia, which may justify at partially the presence of epilepsy in patient 17.

CNVs from 1q22 to 1q23.1
Patient 20 showed a duplication that includes 39 genes, some of them already associated with disease. For example, *SEMA4A* gene (semaphorin 4A, OMIM #607292) belongs to a family of soluble transmembrane proteins that are responsible for guiding axonal migration during neuronal development, and for immune responses. The *NTRK1* gene (neurotrophic tyrosine kinase receptor type 1, OMIM# 191315) is a member of a family of nerve growth factor receptors whose ligands include neurotrophins, proteins that play an important role in regulating development of both the central and the peripheral nervous systems. The DECIPHER database describes several patients with overlapping duplications, ranging between 165 Kb and 108 Mb. These patients share several features with our patient, including DD (5 patients), scoliosis (4 patients), strabismus, and hypotonia (2 patients). The qPCR study performed in the patient 20 parents revealed that this deletion was de novo. This CNV was classified as LP considering the size, the involved genes, and data from the literature.

Patient 21 revealed a duplication involving the *NOSA1P* and *OLFM2B* genes. The *NOS1AP* gene (nitric oxide synthase 1 adaptor protein, OMIM #605551) is also known as *CAPON* and is involved in neuronal nitric oxide synthesis regulation. *NOS1AP-L*, the long isoform of this gene, has an important role in initiation, growth, and maturation of dendritic cells.[31] In the DECIPHER database a few CNVs overlapping this duplication have been reported, one of those shared the phenotype of mitral regurgitation, and another of neuromuscular symptoms (251161 and 289199 patients ID). The qPCR study performed in the parents, showed that this CNV was maternally inherited raising the possibility of incomplete penetrance. This variant was classified as LP.

CNVs on 1q43-q44
In patient 22 the array-CGH identified a deletion that includes the *RYR2* gene (ryanodine receptor 2 gene, OMIM #180902) that encodes for a calcium channel playing a key role in triggering cardiac muscle contraction. Defects in this gene may cause aberrant channel activation, leading to arrhythmia. *RYR2* gene is also expressed in the cerebral cortex and about half of *RYR2* variants carriers develop epileptic seizures, which are not secondary to arrhythmogenic cardiac dysfunction. *RYR2* deleterious variants have also been sporadically reported in individuals with early-onset schizophrenia or ID.[32] The DECIPHER database describes patients with larger deletions that partially overlap with our patient CNV and presenting DD, CFD, and multiple systemic anomalies. The qPCR study performed in the patient's mother was normal, and it was not possible to study his father since he passed away. Although very likely, it is not completely clear if this CNV is the cause of the phenotype in our patient. Despite being smaller, it spans intron 1 to intron 3 (including deletion of the exons 2 and 3 of the gene), and therefore it was classified as LP.

In patient 23 the array-CGH identified a deletion that included the *CHRM3* gene (cholinergic receptor muscarinic 3, OMIM#118494), that mediates multiple important cellular responses, including inhibition of adenylate cyclase, breakdown of phosphoinositides, and modulation of potassium channels. The DECIPHER database describes several patients with overlapping deletions, with a variable phenotype including ID, DD, ASD, hypotonia, and CFD, among many other. Petersen et al described a 473-kb deletion involving only the *CHRM3* gene, associated with ASD, language impairment, and ADHD.[33] The deletion present in our patient is smaller (only 57 kb) affecting only intron 3; however, it is not possible to exclude the possibility that can still affect gene function. Unfortunately, it was not possible to study the progenitors. This deletion was classified as LP.

Patient 24 showed a deletion including the *AKT3* gene (AKT serine/threonine kinase 3, OMIM#611223). The *AKT3* is one of three closely related serine/threonine-protein kinases which regulate many biologic processes including metabolism, proliferation, cell survival, growth, apoptosis, and angiogenesis.[34] *AKT3* is the least studied *AKT* isoform, but it appears to play an important role in brain development. Specific *AKT3* deletions have been particularly linked to microcephaly and ID, although with incomplete penetrance and variable expression.[35] Gai et al suggested that in contrast to the larger 1q43q44 deletions, which occur mostly de novo, a pure *AKT3* deletion is more likely to be inherited and it seems to have no consistent or characteristic dysmorphism associated.[35] It would have been relevant to test the patient's parents for a better understanding of this phenotype, but unfortunately, they were not available. Considering the gene function and the literature, this variant was classified as LP.

Conclusion

This study summarizes phenotype findings in 24 patients with CNVs on chromosome 1 identified by array-CGH. The clinical interpretation of this molecular test is extremely relevant, as it might lead the clinician to confirm or exclude specific diagnosis, therefore influencing the patient's management and

counseling. The interpretation of the CNVs was made using national and international databases, guidelines, and related literature. Nevertheless, the CNVs classification may change over time since genomic information is continuously being updated. Therefore, an annual reassessment of their clinical relevance may be justified, namely in variants not classified as clearly pathogenic. This interpretation remains particularly difficult when small, rare, or nonrecurrent CNVs are found. Additionally, it should be stressed that many of these regions are associated with variable expression and incomplete penetrance. So, reporting these variants is important to update databases and literature, allowing to improve the diagnosis and better genetic counseling.

This study reinforced the association between chromosome 1 CNVs and neurodevelopmental disorders and CFDs. These disorders seem to have a complex etiology, indicating that multiple rare CNVs may be involved, becoming a great challenge to analyze and interpret these variants. Even if in a few of our cases it was possible to easily establish a phenotype–genotype correlation due to the identification of a single compromised gene as the cause of the phenotype, in the majority, haploinsufficiency or triplosensitivity of several genes or regulatory factors may lead to a variable spectrum of phenotypes. Either way, the comprehension of the function of these genes and identification of new patients with CNVs seems to be the key for the improvement of our knowledge of these susceptibility regions, some of which are already associated with clinically recognized syndromes.

Conflict of Interest
None declared.

Acknowledgments

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