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Neuropsychiatric features and genetic aspects in 22q11.2 deletion syndrome

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1. INTRODUCTION

22q11.2 Deletion Syndrome (22q11.2DS; MIM #192430, MIM #188400) is the most common recurrent microdeletion in humans, affecting 1/4000 live births, with an estimated incidence ranging from 1/2000 to 1/6395 (Devriendt K et al., 1998; Botto LD et al., 2003; McDonald-McGinn DM and Sullivan KE, 2011).

Screening of fetuses for 22q11.2DS through prenatal procedures reveals an even higher frequency of ~1/1000, suggesting a high mortality *in utero* (Wapner RJ et al., 2012; Grati FR et al., 2015). Males and females are equally affected and there is no evidence that the deletion is more frequently associated with any specific ethnic origin (Kruszka P et al., 2017; McDonald-McGinn DM et al., 2015).

1.1. 22q11.2 Deletion Syndrome: clinical features

The long arm deletion of chromosome 22 can present with a variety of phenotypes, including DiGeorge Syndrome (DGS; MIM #188400), Velocardiofacial Syndrome (MIM #192430), conotruncal anomaly face syndrome or Takao syndrome (MIM #217095), and Opitz GBBB Syndrome type II (MIM #145410).

The term 22q11.2 deletion syndrome is currently considered as an umbrella term used to describe all the different presentations of a common genetic etiology (Hacihamdioglu B et al., 2015), characterized by a constellation of clinical signs, that has expanded within the last decade. In this context, a collective acronym for those phenotypes is used: CATCH22 (Cardiac Abnormality/ abnormal facies, T-cell deficit due to thymic hypoplasia, Cleft palate, Hypocalcemia due to hypoparathyroidism resulting from 22q11 deletion).

The phenotypic spectrum of this syndrome is very wide and includes (Figure 1, Table 1): congenital heart defects, palatal abnormalities, hypocalcemia, severe feeding/ gastrointestinal problems, immunodeficiency and dysmorphic facial features (McDonald-McGinn DM and Sullivan KE, 2011; Philip N and Bassett A, 2011). In details, congenital heart defects are present in 74% of

deleted cases and are the major cause of mortality (>90% of all deaths). The most frequent anomalies are the conotruncal defects of the outflow tract, including tetralogy of Fallot (20%), interrupted aortic arch (12%), and ventricular septal defect (21%).

Sixty-nine percent of patients have palatal abnormalities, most frequently velopharyngeal incompetence. Hypocalcemia due to hypoparathyroidism is present in 17-60% of cases. About 36% of children have significant feeding difficulties due to dysmotility in the pharyngoesophageal area, which is derived from the third and fourth pharyngeal pouches. The immune deficits, whose prevalence and severity are highly variable, occur because of the thymic hypoplasia. Among the alterations of the immune system alteration, impaired T-cell production is the primary defect, but autoimmune disorders are also observed, including polyarticular juvenile rheumatoid arthritis, idiopathic thrombocytopenia purpura, Grave's disease, hypothyroidism, vitiligo, hemolytic anemia, autoimmune neutropenia, aplastic anemia and celiac disease.

Finally, typical craniofacial findings include auricular abnormalities, nasal abnormalities, ocular hypertelorism, cleft lip and palate and craniosynostosis (Figure 2). The severity of symptoms is variable, ranging from quite severe to near-normal life conditions (Bassett AS et al., 2011). Not surprisingly given the numerous affected organ systems, 22q11.2DS patients have a diminished life expectancy (Repetto et al., 2014), with a median life expectancy of 42 years, compared with 60–70 years of age of normal siblings (Bassett AS et al., 2009; Bassett AS et al., 2011; Repetto GM et al., 2014).

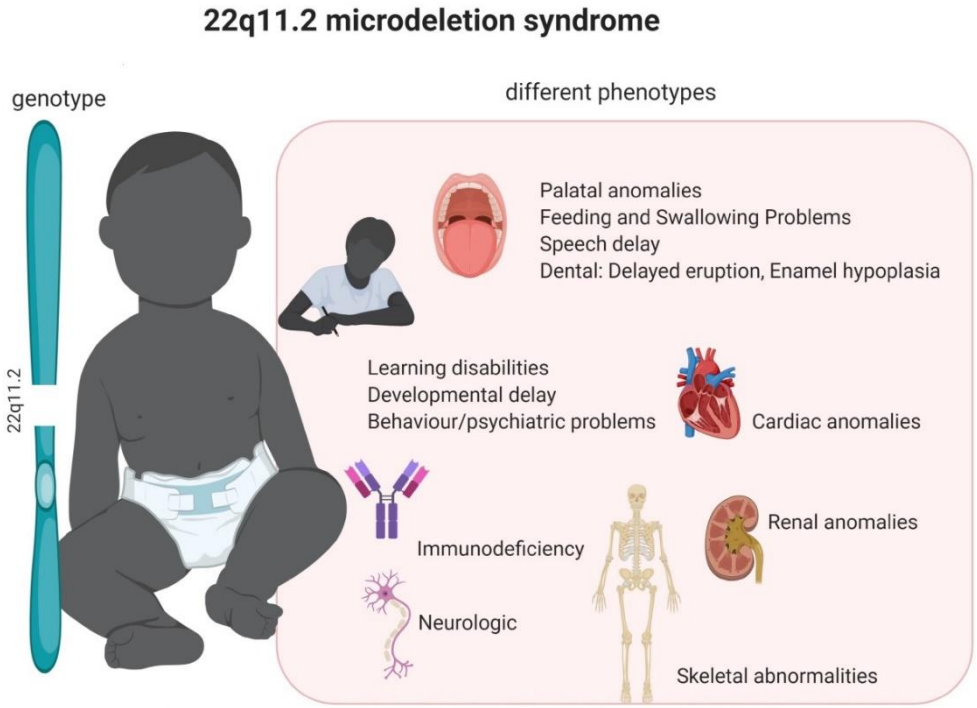


Figure 1. Organ and system involvement in 22q11.2DS. 22q11.2DS leads to significant morbidity (and some premature mortality), with frequent multi-organ system involvement, such as heart, palate, brain and immune, endocrine, genitourinary and gastrointestinal systems (Karbarz M, 2020).

Table 1. Spectrum of clinical features and frequency in 22q11.2DS

Major phenotypic features	Frequency (%)
Dysmorphia	
Hooded eyelid	25
Bulbous nasal tip	60
Nasal dimple	10
Micrognathia	21
Microtia	12
Posteriorly rotated ears	13
Palatal defects	
Velopharyngeal insufficiency	42
Submucous cleft palate	16
Overt cleft palate	11
Cleft lip and palate	2
Immune deficiency-Endocrine	77
T-cell lymphopenia	67
Delayed IgG production	10
Thymic aplasia with absent T cells	<0.5
Hypothyroidism*	
Growth hormone deficiency*	
Cardiac anomaly	74
Tetralogy of Fallot	20
Ventriculoseptal defect	21
Interrupted aortic arch	12
Truncus arteriosus	6
Vascular ring	6
Ophthalmic anomaly	
Posterior embryotoxon	49
Tortuous retinal vessels	34
Strabismus	18
Ptosis	4
Hearing loss	
Conductive hearing loss	31
Sensorineural hearing loss	2
Others features	
Renal anomaly	36
Esophageal dysmotility	36
Feeding and swallowing issues	35
Dental caries	32
Postaxial polydactyly	6
Intestinal malrotation*	
Hirschprung*	
Tracheoesophageal fistula*	
Esophageal atresia*	

* Infrequent but Medically Significant Issues
(modified from McDonald-McGinn DM and Sullivan KE, 2011)

Figure 2. Craniofacial features associated with 22q11.2DS. **a**) The patients shown here, from infancy through adulthood, demonstrate the variability of 22q11.2DS craniofacial features — most with few recognizable dysmorphia; **b** and **c**) In these unrelated families (daughter and father (**b**) and son and mother (**c**), respectively), adults only come to attention following the diagnosis in a child with suggestive features; **d**) microstomia and asymmetric crying facies and **e**) malar flatness and micrognathia provide important clues to the diagnosis; **f**) External eye findings may include upslanting palpebral fissures and hypertelorism (**1**), hooded eyelids and/or ptosis (**2**) and mild epicanthal folds (**3**); **g**) Nasal features may include a bulbous nasal tip with hypoplastic alae nasi (**4**) often with a nasal dimple or crease with or without a faint haemangioma (**5**); **h**) Auricular peculiarities frequently include thick overfolded, squared-off and crumpled helices, microtic, cupped or posteriorly rotated ears, attached lobes and preauricular pits or tags (arrows) (McDonald-McGinn DM et al., 2015).

The psychiatric and neurocognitive features of 22q11.2DS have received an increasing attention over the last years. Indeed, 22q11.2DS has been recently associated with a high risk of neuropsychiatric disorders, including intellectual disability, schizophrenia (SCZ), attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), anxiety disorders, seizures and epilepsy, and early-onset Parkinson's disease (EOPD) (Table 2).

Table 2. Schematic spectrum of neuro-psychiatric features and frequency

Neuropsychiatric features	**Frequency (%)**
Weschler IQ	
Average	18
Low average	20
Borderline	32
Mentally retarded	30
Developmental delay in infancy	75
Behavioral/psychiatric issues	
ADHD	12-68
Schizophrenia	30
Autism	14-50
Generalized anxiety	40-46
Phobias	42
Depression	9-35
Central nervous system	
Unprovoked seizures*	
Provoked seizures*	
EOPD*	
Structural CNS anomaly	
Polymicrogyria	1
Craniosynostosis	1
Cerebellar hypoplasia	<1
Myelomeningocele	<1

* new peculiar neurologic aspects; frequency is not known, yet
IQ: Intelligence Quotient; EOPD: Early Onset Parkinson Disease; ADHD: Attention Deficit Hyperactivity Disorder; CNS: Central Nervous System
(modified from McDonald-McGinn DM and Sullivan KE, 2011)

1.1.1. Cognitive and psychiatric features in 22q11.2DS

Although the syndrome has been known since 1978 (Shprintzen RJ et al., 1978), the behavioral and neurocognitive developmental phenotypes have only been studied since 1992 (Shprintzen RJ et al., 1992).

As regards the neurocognitive profile, individuals with typical 22q11.2 deletion present with significant inter- and intra-familiar variability and across lifespan. Motor delays, language deficits and learning difficulties are common during infancy and school age (Swillen A et al., 2000). Attention, working memory, math skills, visual space and executive functions are the most compromised neurocognitive abilities (De Smedt B et al., 2006; Bearden CE et al., 2001; Zinkstok J and van Amelsvoort T, 2005). Most patients have a borderline intellectual level (IQ between 70 and 84), while a severe intellectual disability is diagnosed in about 30% of cases (Chow EW et al., 2006; Evers LG et al., 2009).

The psychiatric phenotype of 22q11.2DS is well defined (Baker K and Vorstman JA, 2012). The incidence of psychiatric diseases is higher than in the general population (Murphy KC, 2005) with up to 30% of adolescents/young adults developing schizophrenic spectrum disorders (Murphy KC, 2002).

Schizophrenia occurs in about one out of four individuals with 22q11.2DS, a proportion more than 30-fold higher compared to the general population, whose risk is around 1% (Van OJ and Kapur S, 2009).

Several pieces of evidence prove that 22q11.2DS represents a clinically relevant cause of schizophrenia, confirming that this syndrome is the greatest known genetic risk factor for schizophrenia. In some patients it is also possible to recognize the prodromal symptoms (Swillen A et al., 2000; Gothelf D et al., 2008), and subjects with anxiety or mood disorder have an increased risk of developing a schizophrenic spectrum disorder (Odds Ratio 6.07%). The median age of schizophrenia onset is 17.7 years (Gothelf D et al., 2013), without differences in age at onset, positive and negative symptoms, and overall functioning compared to schizophrenic patients in the general population (Murphy KC, 2002; Bassett AS et al., 2003). Importantly, the brain phenotype of patients with 22q11.2DS and psychosis is substantially similar to that of idiopathic schizophrenia, suggesting that genetic subtypes of psychosis can provide insights into brain mechanisms more broadly associated with psychosis (Feinstein C et al., 2002). Other common psychiatric diseases are: anxiety (40-

46%), depression (9-35%), ADHD (12-68%) and ASD (14-50%) (Fung WL et al., 2010; Antshel KM et al., 2006; Antshel KM et al., 2007).
A correlation has been described between the spectrum of psychiatric disorders and the patients' age; indeed, schizophrenia increases in a linear manner with age, mood disorders are predominant in early adulthood, anxiety disorders can occur at any time in life, whereas ADHD decreases with age (Green T et al., 2009).

1.1.2. Seizures and epilepsy in 22q11.2DS

The neurological manifestations of 22q11.2DS have yet to be fully clarified. Patients may be more prone to present either provoked seizures (*e.g.*, by hypocalcaemia, fever, neuroleptic drugs) or unprovoked ones than the general population (Kao A et al., 2004; Cheung EN et al., 2014). Patients between 6 months and 5 years of age have a high risk of febrile seizures due to their predisposition to recurring infections. A proportion of patients, between 1% and 14.5%, have hypocalcemia-induced seizures. In adulthood, 17.6% of 22q11.2DS subjects exposed to psychotropic drugs have epileptic seizures, suggesting a reduced seizure threshold (Eaton CB et al., 2019).
In two pediatric populations of children diagnosed with 22q11.2DS, the estimated prevalence of epilepsy was 6.9% and 15.2%, respectively (Kao A et al., 2004; Kim EH et al., 2016); indeed, when considering generalized genetic epilepsy alone, the prevalence was estimated around 2.2% and 8.3%.
Few studies have examined seizures in adults with 22q11.2DS. Strehlow V et al. (2016) performed a revision of literature and identified 53 patients with epilepsy. According to published data, among 22q11.2DS subjects diagnosed with epilepsy, about 40% had genetic generalized epilepsy (GGE); in some of those cases, recurrent myoclonia and other electroclinical features suggestive of juvenile myoclonic epilepsy (JME) were detected (Roubertie A et al., 2001; El Tahir MO et al., 2004; Kao A et al., 2004; Bernhard MK et al., 2007; Lemke JR et al., 2009; De Kovel CG et al., 2010; Lal D et al., 2015; Kim EH et al., 2016). Overall, twenty-five cases (47%) have also been diagnosed with focal epilepsy (Coppola G et al., 2001; Roubertie A et al., 2001; Kao A et al., 2004; Bernhard MK et al., 2007; De Kovel CG et al., 2010; Boot E et al., 2015, Lal D et al., 2015; Kim EH et al., 2016). Wither RG et al. (2017) reported a history of seizures in

15.8% (32 cases) of 202 patients with 22q11.2DS (age range: 18–63 years): 23 out of 32 had acute symptomatic seizures due to antipsychotic drugs and/or hypocalcemia, while the remaining 9 patients had a diagnosis of epilepsy.
In summary, between 4.4% and 36.8% of 22q11.2 deletion patients have a diagnosis of epilepsy. GGE represents about 1%-6.9% of cases. The most reported structural brain abnormalities are diffuse cerebral atrophy (18.8%), polymicrogyria (13.9%), hippocampal malrotation (10.9%), gray and white matter heterotopia (5.9%) and focal cortical dysplasia (2%) (Eaton CB et al., 2019).

1.1.3. Motor disorders in 22q11.2DS

Recently, 22q11.2DS has been considered a genetic risk factor for early-onset Parkinson's disease, accounting for approximately 0.5% of the cases (Butcher NJ et al., 2013).
Though associations between this genetic syndrome and parkinsonian features have been identified since 1998 (Krahn L et al., 1998), today there are only few case reports or small populations described in literature (Zaleski C et al., 2009; Booij J et al., 2010; Butcher NJ et al., 2013; Rehman AF et al., 2015; Oki M et al., 2016; Pollard R et al., 2016; Dufournet B et al., 2017; Butcher NJ et al., 2017a,b).
Sometimes parkinsonism or early-onset Parkinson's disease precedes the diagnosis of 22q11.2DS (Pollard R et al., 2016; Rehman AF et al., 2015). In a recent article, EOPD risk was estimated to be increased by approximately 20 times (Mok KY et al., 2016). The main features of these patients were early onset of symptoms, absence of family history for Parkinson's disease and no mutations in known genes causing early-onset Parkinson's disease. Lateralization (asymmetrical) of the parkinsonian signs, disease course and response to medications (L-Dopa and dopamine agonists) in 22q11.2DS are all similar to those observed in typical Parkinson's disease (Boot E et al., 2018). Data from Boot E et al. (2020) have recently suggested that parkinsonian signs could be more common and more prominent in 22q11.2DS than in controls, more severe in those with a history of psychotic illness and positively correlated with increasing age in individuals with 22q11.2DS.

Moreover, the few neuropathologic studies published to date have reported a loss of midbrain dopaminergic neurons and, less frequently, the presence of Lewy bodies. Recently, neuroimaging studies have documented an increase of striatal echogenicity with transcranial sonography and an elevated striatal 11C-dihydrotetrabenazine (11C- DTBZ) binding detected through PET, in patients at risk for Parkinson's disease compared to the healthy control group. Based on these data, the authors hypothesized that in patients with 22q11.2DS at risk of PD a hyperdopaminergic state precedes the onset of dopaminergic denervation due to the chronic neurotoxic exposure to dopamine and its metabolites (Butcher NJ et al., 2017b).

For the first time Sumitomo A et al. (2018) presented evidence that the mouse model for 22q11.2 deletion had motor deficits and molecular features, such as elevated alfa-synuclein expression, relevant to PD. Moreover, they observed that enhanced mTOR activity caused both motor and non-motor deficits in mouse, suggesting a common biological disturbance that could underlie both schizophrenia and early-onset Parkinson's disease.

1.2. Brain neuroimaging features in 22q11.2DS

Neuroimaging studies in 22q11.2DS are of great importance since they help to delineate the brain alterations associated with the deletion. Although multiple types of brain malformations have been reported in patients with 22q11.2DS, the prevalence of each of them is difficult to define as only case reports and small case series have been reported.

The most common abnormalities range from benign malformations such as cavum septum pellucidum and nonspecific white matter abnormalities (Schmitt JE et al., 2014) to polymicrogyria, pachygiria, Chiari malformation and gray matter heterotopia (Bohm LA et al.,2017; Hopkins SE et al., 2018). The first case of polymicrogyria involving the right middle cerebral artery territory was described in 1996 (Cramer SC et al., 1996). Since then, several cases including hemispheric polymicrogyria have been published (Bingham PM et al., 1998; Kawame H et al., 2000). Polymicrogyria is mostly unilateral with prevalent involvement of the perisylvian region and the right hemisphere (Robin NH et al., 2006). It is known that perisylvian polymicrogyria causes oromotor apraxia and dysarthria and represents a remarkable risk factor for epilepsy and cognitive

impairment (Barkovich AJ et al., 1999; Kuzniecky R et al., 1993). Moreover, some 22q11.2DS patients could have complex structural brain abnormalities that often denote a more severe clinical phenotype. In these patients, other genetic factors, within or outside the 22q11.2 deleted region, could play a key role. For example, a mutation in the non-deleted allele in 22q11.2 region may unmask an autosomal recessive syndrome. For instance, mutations in *SNAP29* (Small Nuclear RNA-Activating Protein complex 29) may cause the autosomal recessive CEDNIK Syndrome (MIM #609528) (cerebral dysgenesis, neuropathy, ichthyosis, and keratoderma), and be responsible for some of the phenotype variations observed in patients with 22q11.2DS such as hypertrophic neuropathy (McDonald-McGinn DM et al., 2015).

1.3. 22q11.2DS: molecular bases

The 22q11.2DS is the most common chromosomal microdeletion disorder in humans. The 22q11.2 microdeletion involves approximately 50 Mb in length and represents 1.4-6% of the whole chromosome 22 (Figure 3).

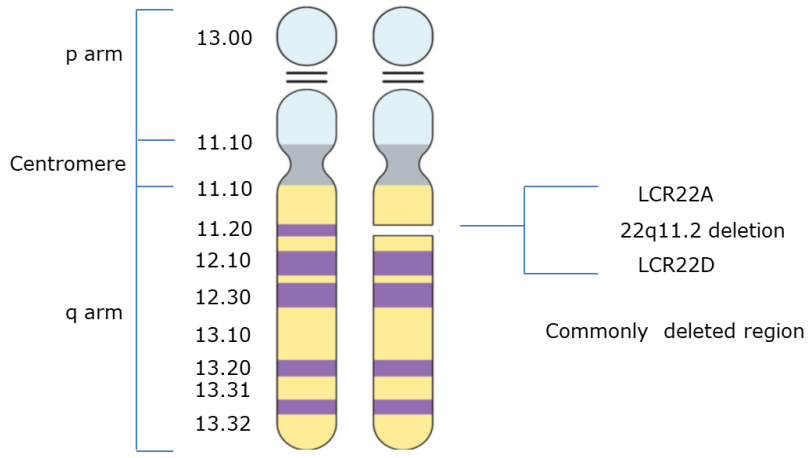


Figure 3. Cytogenetic representation of the human chromosome 22 showing the short (p) and long (q) arms along with the centromere. Chromosome 22 is an acrocentric chromosome, as indicated by the two horizontal lines in the p arm. The 22q11.2 deletion occurs on the long arm of one of the two chromosomes, depicted by dashed lines in the 22q11.2 band. The position of the two low copy repeats (LCRs) on 22q11.2 (LCR22-A and LCR22-D), which flank the typical 3-Mb deletion, is indicated (Zinkstok JR et al., 2019).

This region is one of the most structurally complex areas in the human genome, mainly due to several low copy number repeats (LCRs), also known as segmental duplications (SD's). LCRs are region-specific DNA blocks that share 95%–97% similarity with each other and usually sized 5–10 kb. The proximal region of 22q11.2 is enriched with LCRs (LCR22-A to LCR22-H).

The regions where segmental duplications occur are prone to nonallelic homologous recombination (NAHR) due to their high degree of sequence identity. Indeed, NAHR represents one of the major genomic rearrangement mechanisms occurring between two lengths of DNA that share high sequence similarity but are not alleles (Figure 4).

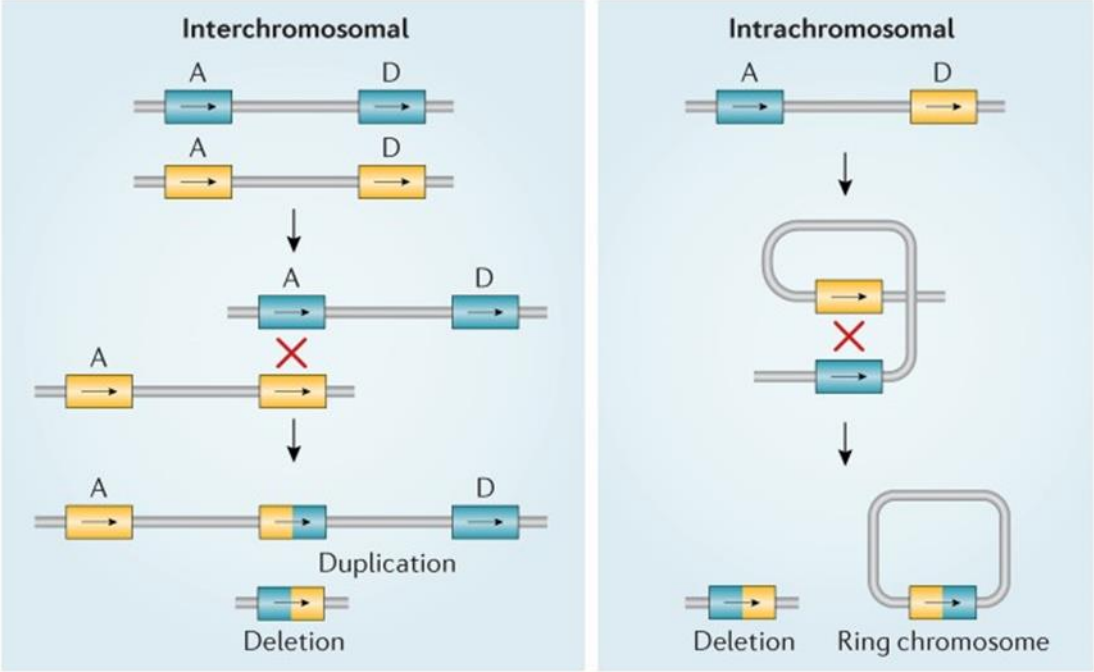


Figure 4. 22q11.2 non-allelic homologous recombination. Diagram of two different types of meiotic non-allelic homologous recombination events that can occur between low copy repeats on chromosome 22 (LCR22s). Rearrangements between LCR22-A and LCR22-D are indicated (A and D) on each allele (blue versus yellow). Interchromosomal events (left) occur between paralogous LCR22s (A and D) in two different alleles owing to >99% sequence identity of direct repeats ('X' shows the crossing over of the two chromosomes). The hybrid LCR22 is shown as half yellow and half blue. This process results in a duplication or deletion of intervening genes in resulting gametes. Intrachromosomal recombination events (right) result from crossing over (indicated by 'X') within one allele, resulting in a deletion (left) or a ring chromosome (right); the ring chromosome is not viable (McDonald-McGinn DM et al., 2015).

Nonallelic homologous recombination can result in deletions, duplications, and inversions (Shaffer LG and Lupski JR, 2000; Feuk L et al., 2006). LCRs in chromosome 22 contain a palindromic sequence characterized by AT-rich repeats. These sequences are highly unstable and undergo extensive point mutations, insertions, deletions and represent a hotspot for several recombination events. Indeed, recent findings suggest that palindromic AT-rich sequences are the breakpoint of a number of translocations involving 22q11 as well as other chromosomes (Li T et al., 1996; Kurahashi H et al., 2000a,b; Edelmann L et al., 2001; Nimmakayalu MA et al., 2003; Spiteri E et al., 2003; Gotter AL et al., 2004, 2007; Emanuel BS, 2008).

Eight long intergenic noncoding RNAs (lncRNAs) are distributed within or around the LCRs. These lncRNAs are part of the FAM230 lncRNA gene family and contain Translocation Breakpoint Type A (TBTA) sequences. At the molecular level, the TBTA harbors palindromic AT-rich repeat sequences (PATRR) and AT-rich region #2 that form a very long stem loop. These sequences have loop breakpoint sites directly associated with translocations that can result in genetic disorders involving 22q11.2 (Figure 5) (Delihas N, 2018).

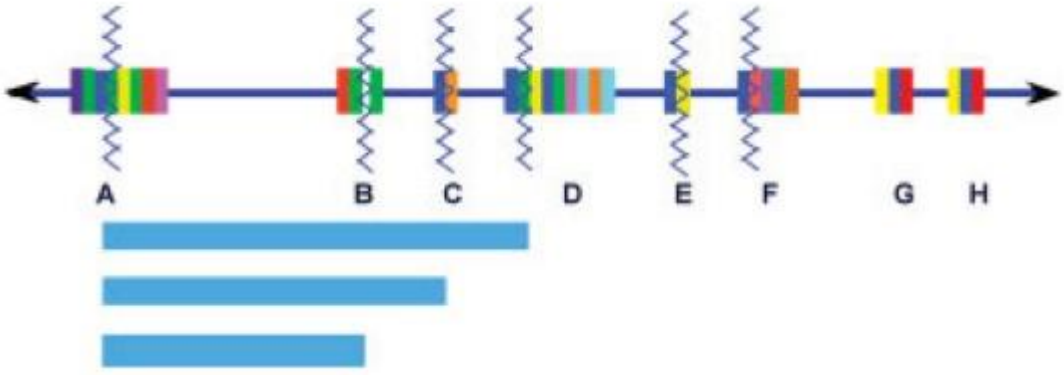


Figure 5. A schematic depiction of LCRs that mediate genomic instability on chromosome 22. The 22q11 region is enlarged, represented by the blue line with arrows at both ends, and is drawn from centromere (left) to telomere (right). The complex modular LCRs that characterize this region are represented by the multicolored vertical rectangular boxes, each color representing a stretch of DNA sequence repeated in multiple LCRs. Zigzag lines represent the locations of multiple recurrent breakpoints. Light blue horizontal rectangles on the lower portion of the diagram indicate the extent of the prevalent, recurrent LCR-mediated 22q deletions seen in the 22q11.2DS. It has been demonstrated that palindromic AT-rich repeats in the white "gap" indicated in LCR-B of 22q11 can form hairpins and cruciforms that mediate translocations between chromosome 22 and several other partner chromosomes (Emanuel BS, 2008).

The 22q11.2 deletion results from variable-sized deleted regions, ranging from small deletions (the minimum size is not known yet) to a typically deleted region (TDR) of ~3 Mb. All kinds of microdeletion are caused by incorrect rearrangement of chromosomes during meiosis.

The 22q11 deletion interval contains at least four large blocks of duplicated DNA sequence, which appear to coincide with the common recurrent deletion endpoints. The typical 3-Mb deletion (accounting for 90% of cases) occurs between the most proximal (LCR22-A) and the most distal (LCR22-D) units, whereas a 1.5 Mb deletion could be an LCR22 A-B (the second most common deletion type) or LCR22 A-C deletion (least frequent) (Hacihamdioglu B et al., 2015). These are referred to as proximal deletions and are causal to most clinical characteristics.

A limited number of individuals have deletions between LCR22 B-D or LCR22 C-D, which are referred to as central deletions (Saitta SC et al., 1999; Burnside RD, 2015). Deletions involving the telomeric LCR22s (LCR22-E, -F, -G and H) are less frequently observed and are associated with a heterogeneous phenotype, often not characteristic of 22q11.2DS (Saitta SC et al., 1999; Shaikh TH et al., 2007; Burnside RD, 2015; Guna A et al., 2015) (Figure 6).

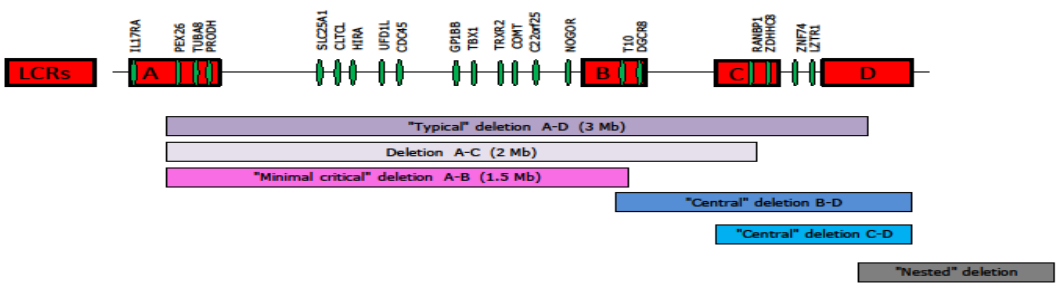


Figure 6. The region affected by the 22q11.2 chromosomal deletions spans approximately 4 Mb, with 8 low copy repeats (LCR22-A to LCR22-D are shown). The recombination of these highly homologous sequences results in different deletions. Each deletion is represented in the figure with differently colored rectangular boxes.

About 90% of patients has a *de novo* deletion, while in 6-28% of cases the syndrome is inherited in an autosomal dominant manner. Parental age and parental origin of *de novo* deletions seem to have no discernible phenotypic effect (McDonald-McGinn DM et al., 2015). The genetic counseling is crucial when there is a parent with 22q11.2DS, because the recurrence risk is about 50% and the phenotype in progeny is more serious. However, anticipation has never been observed (Sandrin-Garcia P et al., 2002). The 3 Mb chromosomal region contains approximately 90 genes (LCR22-A to LCR22-D) and the smaller proximal ~1.5 Mb deletion encompasses 55 of them. Just over half (n= 46, 51.1%) of the 90 genes are protein-coding, and most (n=41, 89.1%) are expressed in the human brain. For the proximal nested deletion, there are 30 protein-coding genes, 27 (90%) expressed in the brain.

The LCR22-A to LCR22-D interval contains 27 pseudogenes, 10 non-coding RNA (ncRNA) genes and 7 microRNAs (miRNAs) (Figure 7) (Fernandez A ET AL., 2015; Guna A et al., 2015; Meechan DW et al., 2015).

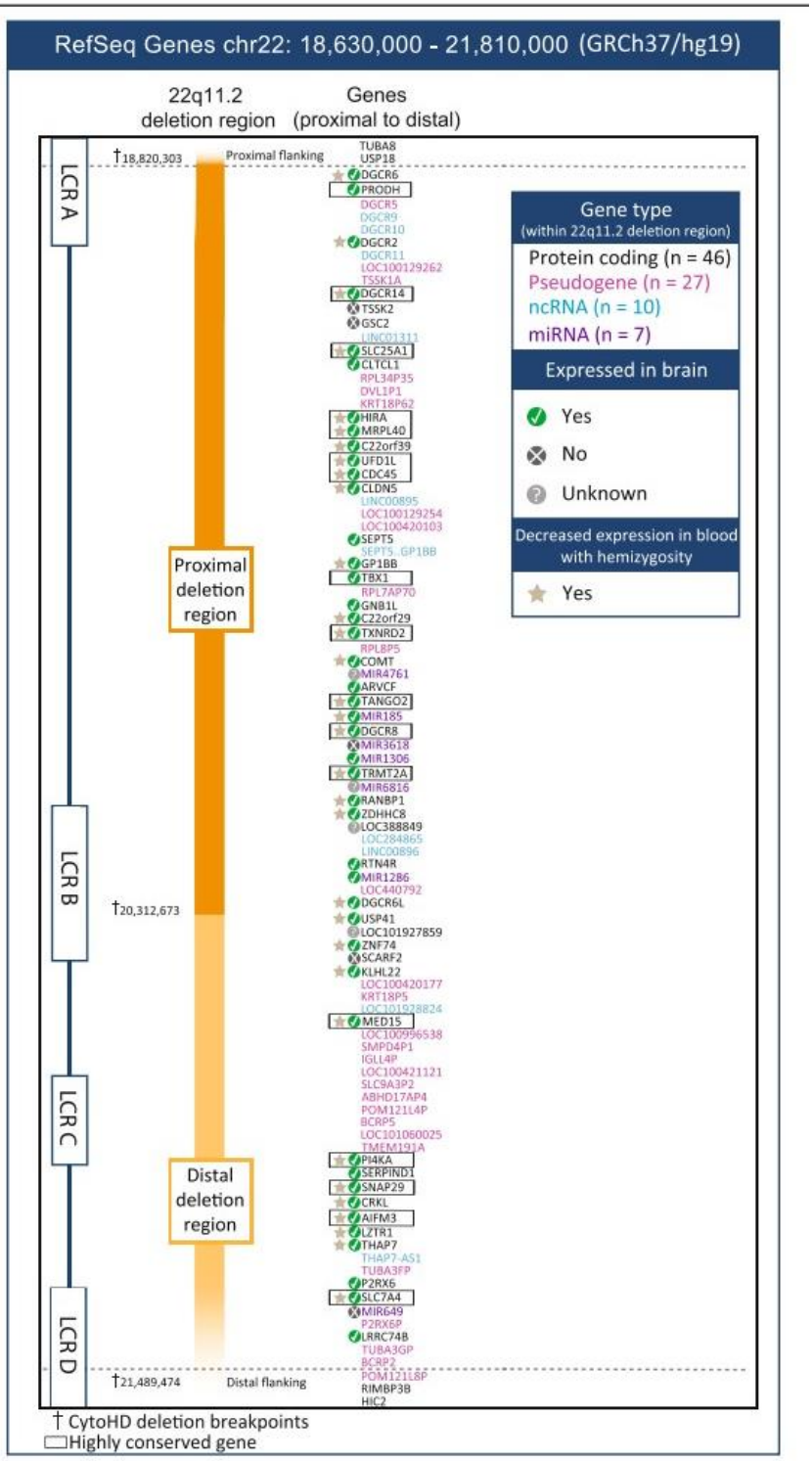


Figure 7. Genetic landscape of the human 22q11.2 region. The typical ~3 Mb 22q11.2DS deletion spans 90 RefSeq genes. Region breakpoints are mediated by four chromosome specific low-copy repeats (LCR-A to LCR-D; approximate locations shown). Gene expression, indicated by a green circled check mark, was established using The Human Brain Transcriptome. Data for decreased expression with hemizygosity were collated from experimentally demonstrated reductions in gene expression in blood cells from patients with 22q11.2DS. Gene names within a rectangle denote the 17 genes conserved across the mouse, zebrafish, fruit fly, and worm (Guna A et al., 2015).

T-box protein 1 (*TBX1*) represents the most important candidate gene for a major role in determining the clinical features of 22q11.2DS. It encodes a T-box-containing transcription factor that belongs to a large family of transcription factors, with various roles ranging from embryonic differentiation to response pathway. The precise transcriptional role of *TBX1* in the patterning of the pharyngeal arches and pouches has been investigated in several works (Yagi H et al., 2003; Zemble R et al., 2010; Bassett et al., 2011; Fung WL et al., 2015; Mcdonald-Mcginn DM et al., 2015; Meechan DW et al., 2015; Baldini A et al., 2017; Sullivan KE, 2019). In mouse models, inactivation of one allele resulted in mild cardiovascular defects, and inactivation of both alleles caused a worse cardiovascular phenotype associated with cleft palate, thymic and parathyroid gland aplasia (Jerome LA and Papaioannou VE, 2001; Lindsay EA et al., 2001; Merscher S et al., 2001). As well as in humans, in animal models a series of alleles with varying expression levels of *Tbx1* confirmed that this gene is very sensitive to copy number changes (Baldini A, 2006; Zhang Z and Baldini A, 2008), but with a sensitivity that is not the same in all tissues and organs. The different degrees of *Tbx1* dosage could explain the phenotypic variability observed in 22q11.2DS patients (Zhang Z and Baldini A, 2008).

Direct downstream transcriptional target genes of TBX1 protein have been recently discovered, and TBX1 effect on chromatin has been identified (Fulcoli FG et al., 2016; Chen L et al., 2012). Small fluctuations in the levels of *TBX1* could modulate the expression of thousands of transcripts, in a stochastic way from cell to cell (Baldini A et al., 2017; Fulcoli FG et al., 2016). Thus, variations in TBX1 levels could underlie the severity of the malformations in the pharyngeal region.

Another gene that acts in the same genetic pathway of *TBX1* and may play a crucial role in the development of thymus, parathyroid glands, aortic arch and heart is *CRKL* (CRK like proto-oncogene, adaptor protein). *CRKL* maps to the LCR22 C-D region and encodes a cytoplasmic adaptor protein involved in growth factor signaling (Guris DL et al., 2006; Guris DL et al., 2001; Moon AM et al., 2006). *Tbx1* and *Crkl* null mutant mice show similar phenotype (Guris DL et al., 2006; Guris DL et al., 2001). Unlike *Tbx1*, whose expression is restricted to specific cells and tissues, *Crkl* is ubiquitously expressed in all cells (Guris DL et al., 2001). It is hypothesized that *Tbx1* acts upstream of the *Fgf8* (Fibroblast

growth factor 8) gene, and this activates *CRKL* in neural crest cells and leads to the activation of the downstream signaling (Moon AM et al., 2006).

Besides coding genes, non-coding genes, such as miRNAs might also contribute to the etiology of 22q11.2DS. The miRNAs are small noncoding RNAs that regulate the expression of target genes by binding to specific sites in messenger RNAs, causing repression of translation or degradation. miRNAs regulate cellular homeostasis in almost all tissues, are very stress responsive and facilitate tissue repair and regeneration (Mendell JT and Olson EN, 2012).

Another gene of note required for both cardiovascular and brain development or function is *DGCR8* (DiGeorge Critical Region 8) (Chapnik E et al., 2012; Earls LR et al., 2012; Sellier C et al., 2014). It encodes a subunit of the microprocessor complex that mediates the biogenesis of miRNAs (Gregory RI et al., 2004), so that a 50% reduction in DGCR8 expression, as observed in 22q11.2DS, is expected to modulate the expression of hundreds of miRNAs (Rao PK et al., 2009; Huang ZP et al., 2010; Du Q et al., 2020). Accordingly, 22q11.2DS patients have a reduced expression of numerous miRNAs in peripheral blood compared with normal controls (de La Morena MT et al., 2013; Sellier C et al., 2014). Moreover, in mouse models the haploinsufficiency of *Dgcr8* causes a 30-50% reduction in the overall expression of miRNAs screened in neurons (Stark KL et al., 2008), and the deficiencies of neuronal miRNA in *Dgcr8+/-* mice are similar to those observed in mouse models of 22q11.2DS. These findings suggest that a 50% decrease in *Dgcr8* is involved in the reduction expression of miRNAs (Earls LR et al., 2012).

Another potentially relevant gene is *HIC2* (Hypermethylated in Cancer 2), which is deleted in individuals with atypical longer deletions in LCR22-D (Dykes IM et al., 2014).

HIRA (Histone Cell Cycle Regulator) is also a gene of interest, since it encodes a component of a protein complex that deposits the variant histone H3.3 at gene regulatory regions, thereby modulating gene expression (Dilg D et al., 2016; Farrell MJ et al., 1999; Majumder A et al., 2015; Zhang X et al., 2017). Chen C. et al. demonstrated that HIRA establishes a proper epigenetic state in chromatin modifying the accessibility to important sites (Chen C et al., 2020). It has been shown that HIRA gives a crucial contribution to diverse biological processes due to its role in development and function of many cell types, such as germ, muscle

and endothelial cells and neurons. The conditional inactivation of *Hira* in mouse mesoderm cells causes cardiac defects (Manchineella S et al., 2016).

Further complexity to 22q11.2DS is added by the 12 lncRNAs embedded in the frequently deleted segment. LncRNAs can act as scaffold RNAs, transcriptional assembly hubs, regulators of chromatin accessibility and genome stability (Angrand PO et al., 2015; Lee S et al., 2016; Quinn JJ and Chang HY, 2016; Du Q et al., 2019). They exhibit limited evolutionary sequence conservation and are generally expressed at much lower levels than protein coding genes (Cesana M et al., 2011; Keniry A et al., 2012; Dey BK et al., 2014; Yang L et al., 2014; Zhou J et al., 2015). The significance of these lncRNAs remains unclear in 22q11.2DS patients but their role in some medical conditions such as lung adenocarcinoma (Sui et al., 2016; Chen EG et al., 2017), hepatocellular cancer (Zhang J et al., 2015) and multiple myeloma (Ronchetti et al., 2018) suggest that haploinsufficiency could functionally contribute to determining the clinical phenotypes.

It has been recently suggested that also regulatory variants in the non-deleted allele could play a role in the pathogenesis of 22q11.2DS. Zhao Y et al. (2020) found a common variant in a 350 kb region on the non-deleted allele, within the LCR22 C-D interval, that is associated with moderate increased risk for conotruncal heart defects (CTDs) in individuals with the typical 3 Mb 22q11.2 deletion. According to mouse genetic studies, the authors identified *CRKL*, one of the four known protein coding genes that map to LCR22 C-D interval, as gene for which altered expression by non-coding variants on the remaining allele of 22q11.2 might influence risk for CTDs.

Moreover, the presence of genetic modifiers outside the deleted region, as well as the altered expression of genes mapping in the deleted regions or outside could contribute to the variable phenotype of this syndrome (Mlynarski EE et al. 2015; Taddei I et al. 2001).

Recently, Dantas AG et al. (2019) performed a gene expression study and observed a downregulation of the genes *TUBA8* (Tubulin Alpha 8) and *GNAZ* (G Protein Subunit Alpha Z), which flank the deleted region, and are implicated in neurological and psychiatric diseases. They also found a downregulation of *JAM3* (Junctional Adhesion Molecule 3), located on chromosome 11 and previously described as a candidate gene for the cardiac phenotype in patients with 11q23-

qter deletion. These data suggest that haploinsufficiency of this region could contribute to the cardiac phenotype in 22q11.2DS, also (Phillips et al., 2002).
Emerging research suggest that also additional rare copy number variations (CNVs) elsewhere in the genome may shape the expression of cardiac phenotypes associated with 22q11.2DS. A higher rare CNV burden was observed in 22q11.2DS patients with congenital heart diseases (CHDs) than in 22q11.2DS individuals with normal hearts. When rare CNVs were carefully examined with regard to gene interactions, specific cardiac networks, such as Wnt signaling appeared to be overrepresented in 22q11.2DS CHD cases but not in 22q11.2DS controls with no cardiac phenotype (Mlynarski et al., 2015, 2016).
Finally, personal genomics approaches to understand an individual's susceptibility to disease are now possible with next-generation sequencing methods. Whole genome sequencing (WGS) provides the potential to directly identify causative coding and non-coding variants. A WGS study in 22q11.2DS patients with tetralogy of Fallot showed a significant locus on 5q14.3 which could contain potential genetic risk factors. Several genes located in this locus, including *MEF2C* (Myocyte enhancer factor 2C) that is a known gene for tetralogia of Fallot in animal models, could act as modifiers in 22q11.2DS (Guo T et al., 2017).

1.3.1. Role of 22q11.2 region in neurological phenotype

Currently, the precise genetic mechanisms underlying disrupted cortical circuit formation in 22q11.2DS are unknown. Most of the protein-coding genes within the deleted region are highly brain-expressed (Guna A et al., 2015), and several of them are involved in early neurodevelopment.
The knowledge of 22q11.2 deleted genes role in brain development offered to neuroscientists a unique insight into the neurobiology of common developmental and neurodegenerative diseases.

Genes mapping in the 22q deleted region and neuropsychiatric phenotype
A fundamental role in determining the behavioral, neurocognitive and psychopathological phenotype is certainly played by the reduced dose of 22q11 genes normally expressed in the brain from early development through maturity (Sivagnanasundaram S et al., 2007; Philip N and Bassett A, 2011). The expression of most 22q11 genes in the fetal and adolescent brain could contribute to neuronal proliferation, migration and circuit differentiation. Thus,

heterozygous deletion of 22q11.2 may alter the expression of genes required for proper development and function of neuronal circuits in the Central Nervous System (CNS) (Maynard TM et al.,2003) (Figure 8).

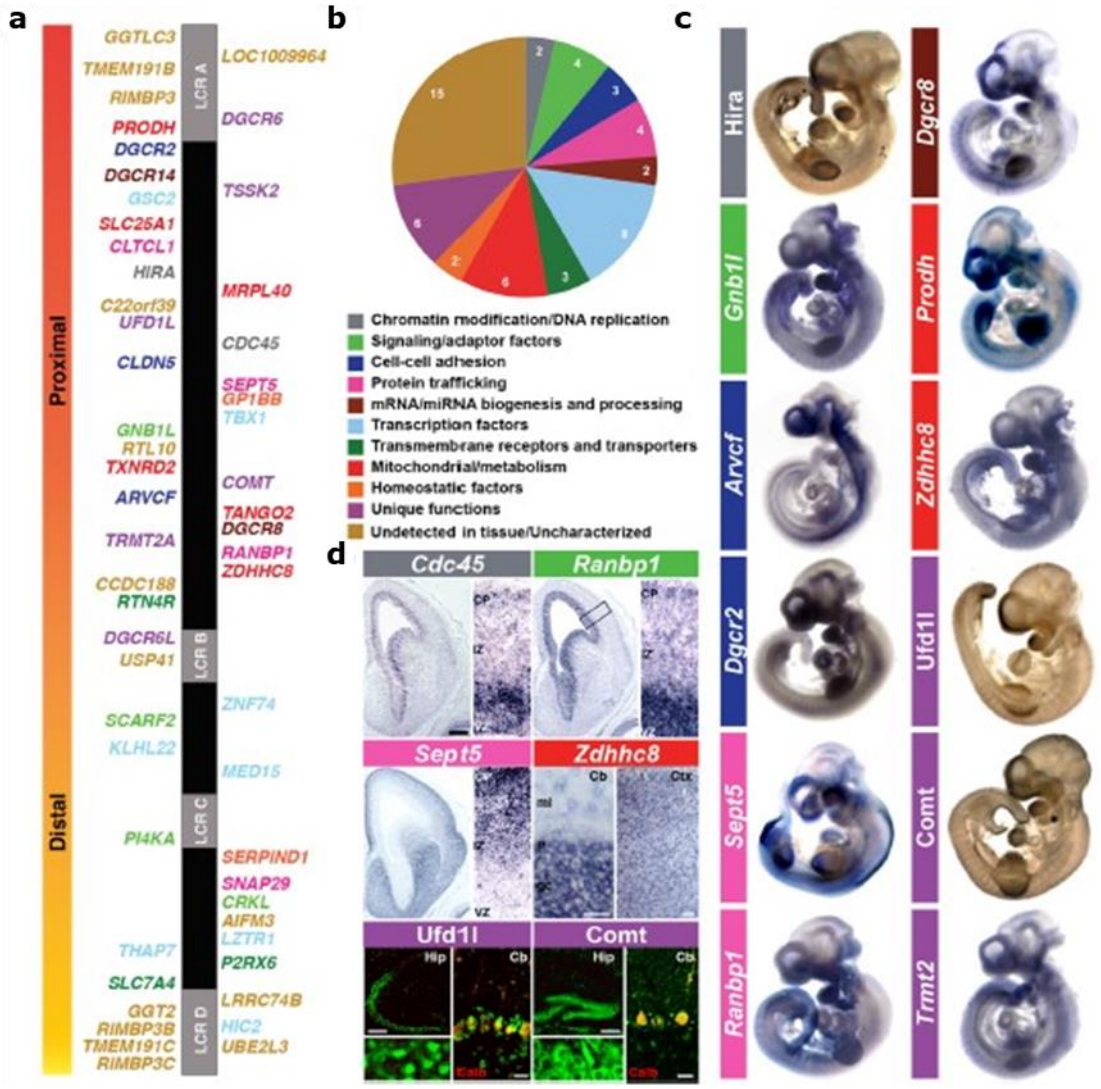


Figure 8. Genes deleted in 22q11.2DS. **a**) Schematic view of the 22q11.2 region. LCRs are shown as gray boxes: LCR A-D (not to scale). **b**) Protein-coding genes (n = 56) are color-coded based on primary, putative, or family member functions as eleven groups. **c**) mRNA (*Prodh, Zdhhc8, Sept5, Gnb1l, Ranbp1, Dgcr8, Arvcf, Dgcr2, and Trmt2a*) or protein (*Ufd1l, Hira, Comt*) expression of selected genes at mouse embryonic stage E10.5. **d**) Expression localization of *Cdc45, Ranbp1,* and *Sept5* in the entire cortical hemisphere of E14.5 embryos (left) and in a higher magnification (right). Expression pattern of *Zdhhc8* is shown in the adult cerebellum (left) and the cortex (right). Immunolocalization of *Ufd1l* and *Comt* proteins in the hippocampus (left) and cerebellum (right) of the adult mouse brain are shown. *VZ* ventricular zone, *IZ* intermediate zone, *CP* cortical plate, *gc* granular cell layer, *P* purkinje cell layer, *ml* molecular layer, *Cb* cerebellum, *Ctx* cortex. *Hip* hippocampus, *Calb* calbindin. Scale bars: Cdc45, *Ranbp1*, *Sept5* =250 μm, insets = 6.6x, *Zdhhc8* = 50 μm (left) and 100 μm (right). *Ufd/Comt*: hippocampus (upper left) = 250 μm, insets (lower left) = 10x, cerebellum (right) = 25 μm (Motahari Z et al., 2019).

Numerous genes for which individuals with 22q11.2DS are hemizygous have been linked to cognitive and behavioral problems (Vorstman JA et al., 2009). Several commonly diagnosed disorders are apparently more frequent in LCR22-A to LCR22-B deleted individuals. Furthermore, various genes from LCR22-A to LCR22-D including *COMT* (Catechol-O-methyltransferase)*, PRODH* (Proline dehydrogenase), *GNB1L* (Guanine nucleotide-binding protein subunit beta-like protein 1), *TBX1, SEPT5* (Septin 5)-*GP1BB* (Glycoprotein Ib platelet, beta polypeptide), *ZDHHC8* (Zinc finger DHHC-type containing 8), *PI4KA* (Phosphatidylinositol 4-kinase alpha), and *ARVCF* (Armadillo Repeat gene deleted in Velo-Cardio-Facial syndrome) have been individually associated with SCZ, ASD, ADHD, and other disorders frequently diagnosed in individuals with 22q11.2DS (Hiroi N et al., 2013).

In humans, the most studied gene for schizophrenia is *COMT,* that is located in the 1.5 Mb critical deletion region. Its protein product is the COMT enzyme, that has a critical role in the metabolism of catecholamines dopamine, norepinephrine, and epinephrine. The *COMT* gene encodes both a membrane-bound and a soluble protein, with the former being the predominant form in the brain (Bertocci B et al., 1991; Chen J et al., 2004). Along with monoamine oxidase A (MAOA), the COMT enzyme has a particularly strong influence on dopamine metabolism, especially in the prefrontal cortex (Tunbridge EM et al., 2004; Tunbridge EM et al., 2006), where there is a relatively low concentration of dopamine transporters and thus a need for an alternate way to clear dopamine from synapses. While some studies support a role of the reduced enzyme activity of COMT in determining cognitive and/or behavioral deficits, others do not, therefore the importance of this *COMT* functional variation remains controversial (Armando M et al., 2012; Franconi CP et al., 2016).

Another gene that has been extensively studied in humans is *PRODH*, located within the 1.5 critical deletion region. *PRODH* encodes proline dehydrogenase that converts proline to glutamate and is involved in neurotransmission (Squarcione C et al., 2013). Proline is an intermediate in the biosynthesis of glutamate (Phang JM et al., 2001), and proline dehydrogenase catalyzes the rate-limiting step in the conversion of proline to glutamate (Bender H et al., 2005). The PRODH reduction causes an increase in proline, which interacts with the glutamate pathway and indirectly with the dopaminergic one. As for *COMT*,

the relevance of *PRODH* has not been definitively ascertained (Carmel M et al., 2014; Radoeva PD et al., 2014; Zarchi O et al., 2013).

Among the candidate genes for schizophrenia there is *PIK4CA* (Phosphatidyl-Inositol 4-Kinase, Catalytic, Alpha polypeptide). It is expressed in the gray matter in both adult and fetal brain, and it is potentially involved in neurodevelopmental processes. *PIK4CA* is associated with the development of schizophrenia in individuals with and without 22q11.2DS (Vorstman JA et al., 2009).

Furthermore, other genes of interest required for brain or behavioral function in mouse models are *ZDHHC8* (Mukai J et al., 2004), *RANBP1* (Paronett EM 2015), *RTN4R* (Reticulon 4 receptor)(Hsu R et al., 2007; Kimura H et al., 2017) and *DGCR8* (Diamantopoulou A et al., 2017; Eom TY et al.,2017; Fenelon K et al., 2011).

The alteration of GABA (γ-aminobutyric acid) -ergic neurotransmission is another mechanism that could increase the risk of schizophrenia. The haploinsufficiency of *DGCR6* seems to compromise the expression of GABAB receptors on the cell surface (Zunner D et al., 2010). Moreover, the reduced expression of the *CXCR4* gene (CXC motif Chemokine Receptor 4) could modify the number, migration and distribution of GABA interneurons, with consequent dysfunction of the cortical inhibitory circuits (Meechan DW et al., 2012).

Important genes for mitochondrial function have been recently found to play a role in the etiology of schizophrenia in mouse models (Devaraju P and Zakharenko SS, 2017; Meechan DW et al., 2011). Alterations in mitochondrial functioning could compromise the survival of neurons and the integrity of neuronal circuits (Ben-Shachar D, 2002; Ben-Schachar D and Laifenfeld D, 2004; Bubber P et al., 2004). Within the 22q11.2 region, 6 mitochondrial genes expressed in the brain have been identified (*MRPL40*- Mitochondrial Ribosomal Protein L40, *PRODH*, *SLC25A1*- Solute Carrier family 25 member 1, *TXNRD2*- Thioredoxin Reductase 2, *TANGO2*- Transport And Golgi Organization 2, *ZDHHC8*) (Maynard TM et al., 2008).

Moreover, as well as suggested for other phenotype aspects, it could be hypothesized the occurrence of genetic modifiers outside the deleted region that could play a crucial role in schizophrenia. The application of new genetic diagnostic techniques has allowed to study those modifying factors such as CNVs and genes mutations. Some array comparative genome hybridization (a-CGH)

studied showed that 22q11.2DS patients with psychosis had a higher rare CNV burden compared with 22q11.2DS without psychiatric disorders (Williams HJ et al., 2013; Bassett AS et al., 2017). Functional network analysis of these rare CNVs highlighted four interaction clusters with neurological genes involved in axon guidance, neuronal adhesion, nerve growth factor signaling, purinergic receptors, calcium channels, glutamatergic and adenosine receptors, and synaptic trafficking (Williams HJ et al., 2013; Bassett AS et al., 2017).
Finally, some studies that used whole genome sequencing (WGS) approach indicated that, despite its high impact, the risk conferred by the 22q11.2 deletion is even greater when associated with the genome-wide risk factors for schizophrenia in the general population (Cleynen I et al., 2020).

Genes mapping in the 22q deleted region and seizures and epilepsy
Since 1999, a potential seizure locus has been identified at 22q11.2 band and some of the genes involved in genetic generalized epilepsy (GGE) have been identified in the "critical" 22q11.2 region (Gong S et al., 2003; Blumenfeld H, 2003). Recently, Piccione M et al. (2013) highlighted a possible role of *RAB36* (Ras-associated protein 36) as a candidate gene within the 22q11.2 region. Rab36, localized at the Golgi body and seems to be involved, like some other Rab family proteins, in vesicular transport (Zhou J et al., 2000). The data reported by Chen L et al. (2010) suggest that both haploinsufficiency and overexpression of Rab36 may compromise neurotransmitter processing with an erroneous trafficking mechanism of secretion.
Another gene that could play a key role in epileptogenesis is *PRODH*. The *PRODH* reduction causes an increase in proline, which interacts with the amino acid glutamate, the major excitatory neurotransmitter. A moderate to severe reduction (>50%) in *PRODH* activity resulting from recessive deletions and/or missense mutations has been shown to cause type 1 or type 2 hyperprolinemia (HPI-HPII).
Patients with these conditions could present both mild and severe clinical phenotypes including neuropsychiatric disorders such as mental retardation, autism spectrum disorder, schizophrenia and epileptic syndromes (from GGE with myoclonic features to epileptic encephalopathies) (Afenjar A et al., 2007; Di Rosa G et al., 2008). Recently HP has been observed in association with heterozygous mutations of *PRODH* gene, including a series of patients with 22q11.2DS. These

data suggest that high proline levels may facilitate cortical hyperexcitability mechanisms involved in epileptogenesis. As well as excitatory neurotransmission, the reduction of the gabaergic inhibitory pathway is involved in epileptogenesis, also. According to these mechanisms, haploinsufficiency of *DGCR6* and *CXCR4* genes, involved in cortical inhibitory circuits (Zunner D et al., 2010; Meechan DW et al., 2012) could explain the epileptic phenotype.

Genes mapping in the 22q deleted region and motor disorders

The pathogenic mechanisms that determine the increased risk of Parkinson's disease in 22q11.2DS are not fully understood. Haploinsufficiency of one or more dose-sensitive genes within the deleted region has probably a crucial role in PD. Indeed, the proximal 22q11.2 deletion region does contain plausible candidate genes implicated in PD-related pathways. These include microRNA miR-185, predicted to target *LRRK2* (Leucine-Rich Repeat kinase 2), and *DGCR8*, a key gene in the biogenesis of brain miRNAs. Other possible candidate genes in this 22q11.2 deletion region include *SEPT5*, encoding septin-5-protein that functionally interacts with the product of *PARK2* (Parkin2) and inhibits exocytosis of dopamine and glutamate; *COMT*, essential to dopamine level regulation, and 6 mitochondrial genes (Butcher NJ et al., 2015). Some evidence from simple organism models shows that *PRODH* and *TXNRD2* could be involved in motor functioning (Guna A et al., 2015).

Recently, a pilot study of WGS suggest that the cumulative burden of genome-wide sequence variants may contribute to expression of early-onset PD in the presence of threshold-lowering dosage effects of a 22q11.2 deletion. Therefore, these data suggest that hemizygosity of the 22q11.2 deletion region is not sufficient for EOPD (Butcher NJ et al., 2017a).

1.4. Clinical and genetic approaches for 22q11.2DS diagnosis

There are several molecular approaches that are used to identify the 22q11.2 deletion. The diagnostic procedure most often used for the detection of deletions and duplications is the chromosomal analysis (karyotype) coupled with fluorescent in situ hybridization (FISH). FISH is one of the most used methodologies for the deletion size analysis (Carlson C et al., 1997; Saitta SC et

al., 1999; Shaikh TH et al., 2000), and is usually based on two commercial probes such as ARSA and TUPLE1 (www.molecular.abbott/us/en/chromosome/22). FISH probes located between LCR22-A and LCR22-B identify typical deletions involving the *HIRA* gene, but miss atypical nested deletions that occur outside of the LCR22 A-B region.

Recently, Multiplex Ligation-dependent Probe Amplification (MLPA) has been considered a cost-effective, rapid, and sensitive method for the detection of the typical and atypical deletions (Fernandez L et al., 2005; Vorstman JA et al., 2006). MLPA is most useful to confirm a suspected diagnosis because it only probes the 22q11.2 region.

The approaches for diagnostic testing of 22q11.2DS are an important discussion point in the context of potential dual diagnoses or second-hit factors, since the use of FISH will neither identify atypical deletions outside of the LCR22 A-B region not involving the *HIRA* gene nor CNVs on other chromosomes. Also MLPA do not detect CNVs, deletions and duplications, or gene mutations in genes not related to the 22q11.2 deletion site (Vorstman JA et al., 2006; Sivertsen A et al., 2007).

Therefore, a high-density MLPA (HDMLPA) probe set, incorporating all LCRs, has been developed and successfully tested (Jalali GR et al., 2008).

Recently, numerous findings support the hypothesis that haploinsufficiency of different genes mapping in the 22q11.2 deleted region do not explain the wide phenotype variability. In this context, among the various mechanisms that could play a crucial role it has been suggested the occurrence of stochastic modifying multigenic interactions, environmental processes as well as genetics modifiers (Dantas AG et al., 2019; Cohen JL et al., 2018; Du Q et al., 2020).

Patients with atypical features should prompt consideration of coexisting diagnoses due to mutations/CNVs on the remaining allele, and additional mutations/CNVs outside the 22q deleted region. The hemizygous deletion could unmask an autosomal recessive disorder due to a deletion/mutation in an important gene on the other allele (dual diagnosis or second-hit variant) (McDonald-McGinn DM et al., 2015). Indeed, several genes within the deleted region have been demonstrated to cause autosomal recessive conditions. It is therefore crucial to give special consideration to these known conditions, including Bernard-Soulier syndrome type B, a coagulopathy due to a mutation in *GP1BB* (MIM #231200), CEDNIK syndrome due to a mutation in *SNAP29* (MIM

#609528), and a condition with severe contractural arachnodactyly and skeletal anomalies known as van den Ende-Gupta syndrome (VDEGS), related to a *SCARF2* (Scavenger Receptor Class F Member 2) mutation (MIM #600920).

Other interesting genomic mechanisms, mainly involving factors outside the deleted region, might be responsible for the phenotypic heterogeneity among patients with 22q11.2DS. In particular, large CNVs might regulate long-range chromosome interactions (between distant regions on a single chromosome or between different chromosomes), chromatin organization, epigenetic profiles and gene expression (Zhang X et al., 2018).

In this context, genome-wide microarray testing, as single nucleotide polymorphism array (SNP-array) and a-CGH are efficient tools for identifying chromosomal imbalances such as deletions and duplications of 22q11.2 (Krepischi-Santos AC et al., 2006; Urban AE et al., 2006; Tokuyasu TA et al., 2007). Similar to MLPA, a-CGH allows simultaneous interrogation of several DNA probes. Thus, it offers an efficient and high-throughput alternative for detecting microdeletions and duplications. Several studies demonstrated that array measurements were in complete concordance with FISH analysis, supporting the diagnostic utility of this approach (Tokuyasu TA et al., 2007; Krepischi-Santos AC et al., 2006).

One of the unexpected discoveries of genome-wide testing is the identification of atypical nested 22q11.2 deletions such as the LCR22 B-D or C-D deletions. These deletions are associated with some similar clinical features of the typical deletions which occur with less severe phenotype (Racedo SE et al., 2015; Rump P et al., 2014). Moreover, the application of this approach disclosed the occurrence of several distal deletions, interstitial duplications and it has facilitated the localization of different breakpoints in several proximal 22q11.2 deletions as well (Urban AE et al., 2006; Jalali GR et al., 2008). This approach led to the recognition of a broader spectrum of features for the 22q11.2DS, uncovering 22q11.2 deletions that were not suspected based on phenotype.

Therefore, MLPA should be considered as a cost-effective diagnostic screening test, whereas a-CGH should be regarded as the ultimate test as it is able to detect 22q11 copy number changes in patients with non classic phenotypes.

Finally, the advent of next-generation sequencing (NGS), a new technology used for DNA and RNA sequencing, has allowed the analysis of large panels of genes and even whole genome in a short period of time. Massive parallel sequencing

offers new opportunities for understanding an individual's susceptibility to disease.

NGS can be performed at different levels. It can be used to identify coding and non-coding variants such as single nucleotide variants (SNV) and small insertions/deletions (INDEL). WGS is used less commonly in clinical and more often in research settings.

In this context, WGS approach were applied to analyzed cohorts of 22q11.2DS cases with the aim of finding genetic modifiers, as CNV or single nucleotide variants both on the non deleted allele and outside the deleted region (Chung HC et al., 2015; Guo T et al., 2017; Butcher NJ et al., 2017a). Although WGS provides the potential to directly identify causative coding and non-coding variants, the interpretation challenge of an enormous amount of raw data and the risk of identification of incidental (secondary) findings are the main limiting factors of this approach.

2. THE AIMS OF THE WORK

22q11.2DS is a multi-systemic disease, with variable severity and penetrance. The phenotypic spectrum of this syndrome encompasses a heterogeneous range of manifestations, including congenital heart diseases, palatal abnormalities, facial features, immune deficiency, hypocalcemia and gastrointestinal problems, which have been widely described and investigated. However, less is known about other more recently recognized aspects, such as neurodevelopmental, cognitive, behavioral and psychiatric symptoms. Among these, the psychiatric features have certainly received more attention: indeed, numerous studies suggested that the risk of schizophrenia for a patient with a 22q11.2 microdeletion may be approximately 30 times that of the general population risk (1%), and several works confirmed such unequivocal association between this chromosomal abnormality and schizophrenia. Conversely, the neurological manifestations are far from being fully clarified: only single case reports or small case studies dealing with the neurological aspects have been published to date. However, a more thorough knowledge of the neuropsychiatric features of 22q11.2DS could promote patients' management and follow-up. Besides, the exhaustive phenotypic characterization could facilitate the identification of the molecular mechanisms underlying the wide clinical variability of 22q11.2DS.

In light of these considerations, one of the main scopes of our study is to outline the neurologic and psychiatric characteristics of a large cohort of Italian adult 22q11.2DS patients, especially with regard to peculiar neurological features. By means of an extensive clinical and instrumental evaluation, we particularly focused on two main aspects, *i.e.* the predisposition to develop provoked and unprovoked seizures and Parkinson's disease. In fact, previous papers dealing with the neurological manifestations of 22q11.2DS mainly reported either epileptic or parkinsonian features, though no correlation between these features was detected. Besides, none of these papers investigated the possible relationships between the neurological, psychiatric and systemic features of this peculiar syndrome, which we have carried out in the present study.

The second focus of our work consisted in the search for possible correlations between genotypic characteristics and neurological features of 22q11.2DS.

Indeed, genotype-phenotype correlations are crucial to predict the patient's clinical evolution (with future potential therapeutic impact) and also to achieve a better knowledge of the molecular aspects underlying distinct clinical features of this complex syndrome. Although the link between genotypic characteristics and psychiatric disorders - in particular, schizophrenia - has been extensively investigated (despite controversial results), no previous work has actually attempted to analyze genotypic-phenotypic correlations with regard to the peculiar neurologic manifestations of 22q11.2DS.

It is well known by now that the deletion alone is insufficient to cause the syndrome clinical variability, and that many factors, *e.g.* genetic modifiers on the non deleted 22q11.2 allele or elsewhere in the genome (second-hit), genome-wide mutation burden, stochastic events during embryogenesis and gene-environment exposures during pregnancy, could play a crucial role in phenotype expression. Based on these considerations, in our study we selected an adult population of patients with an established molecular diagnosis of 22q11.2DS and we used a-CGH to detect DNA copy number changes. First of all, we identified specific characteristics of the 22q deletion region, including chromosome position and deleted region length. Then, we classified the patients into subgroups, accordingly, and analyzed the different expression of neuropsychiatric features.

3. MATERIAL AND METHODS

3.1. Patients' cohort recruitment

Subjects were enrolled at the Department of Human Neurosciences of Policlinico Umberto I of Rome ("Sapienza" University of Rome) from January 2015 to December 2019. The AIDEL22 (Associazione Italiana Delezione 22) contributed to the identification of patients, who were then referred to our institution for centralized care management.

The patients' family and personal medical history was collected by a multi-disciplinary team of pediatricians, cardiologist, neurologists and psychiatrists at Policlinico Umberto I of Rome ("Sapienza" University of Rome).

All subjects were followed at the Department of Pediatric for general medical comorbidities such as immune deficiency, endocrine diseases, heart malformations, electrolyte disorders and orthopedic deformities.

In all patients physiological anamnesis focusing on the perinatal anoxia around the time of birth have been acquired.

All subjects underwent ECG, echocardiography, urines' analysis and blood laboratory exams including complete blood count, electrolytes, parameters of liver, kidneys and thyroid functionality, glucose, lipid profile (cholesterol and triglyceride levels), and vitamins (B12, D, folic acid). Further investigations according to the specific individual's conditions were carried out, such as radiography to study spinal deformity and other skeletal abnormalities, bone mineral density scan in patients with vitamin D deficiency to diagnose osteoporosis, thyroid and parathyroid ultrasound exams.

All patients were evaluated for neurologic and psychiatric features at the Department of Human Neurosciences. Psychiatric evaluation, neurological examination and video-EEG monitoring were performed in all cases. A subgroup of compliant patients underwent also a neuropsychological assessment and neuroimaging exams.

3.2. Cytogenomic analysis

In all patients, 22q11.2DS was clinically diagnosed after birth at different stages of life. A genetic diagnosis, through FISH or a-CGH tests, was available only in few cases. A-CGH was performed in patients without a genetic diagnosis and in those cases that had only underwent FISH. The genetic tests were performed in different Italian laboratories, including Laboratory of Medical Genetics of CSS-Mendel Institute and Laboratory of Medical Genetics of Bambino Gesù Hospital of Rome.

Only patients with a confirmatory genetic test at the time of the enrollment or performed during the study were included.

The study was conducted in accordance with the Helsinki Declaration (World Medical Association General Assembly, 2013). Informed consent was obtained from patients and/or relatives who agreed to participate after they were informed about the study content.

Genomic DNA was isolated from the peripheral blood according to standard protocols. Data on the 22q deleted region (*e.g.* length, genomic coordinates) and the inheritance pattern were collected for each patient. For some cases, also the occurrence of potential pathogenic CNVs outside the 22q deleted region was collected.

In a subgroup of patients, segregation analysis was performed on DNA from parental blood samples.

3.3. Handedness

Dominance in manual skills was established by using the Edinburgh Scale (Oldfield RC, 1971). This scale is a measurement scale of 10 items used to assess the dominance of a person's right or left hand in daily activities, sometimes referred to as "laterality". Depending on the severity of neuropsychiatric pathology of the subjects, each patient or caregiver was asked to indicate preferences in the use of hands, feet or eyes in the following activities: write, draw, throw with hands and feet, cut with scissors, use the

toothbrush, use the knife (without fork), use the spoon, use the broom (upper hand), light a matchstick, open the box (lid), look at the lens with one eye.

3.4. Neurological examination

All subjects underwent a thorough neurological evaluation by an expert neurologist. A comprehensive neurological examination including an assessment of cognition, cranial nerves, motor, sensory, cerebellar, gait, reflexes, and long tract signs was performed in all patients.

The Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (Goetz CG et al., 2008) was administered in all patients to improve the evaluation of motor aspects and their motor and non-motor features were investigated. UPDRS consists of 4 parts: part I, non-motor aspects of daily life experiences (13 items); part II, motor aspects of daily life experiences (13 items); part III, motor examination (18 items giving rise to 33 scores for localization and lateralization); and part IV, motor complications (6 items). Parts II and III of the scale were performed in all patients. In particular, we assessed patients' perceptions of their ability to carry out activities of daily living, including dressing, walking and eating (part II of scale) and evaluated the motor aspects of disability including tremor, slowness (bradykinesia), rigidity and balance (part III of scale). Each patient were investigated for febrile seizures in childhood, symptomatic seizures and/or unprovoked seizures in lifetime.

3.5. Neurophysiology tests and neuroimaging studies

Video-EEG monitoring

All the patients underwent video-EEG monitoring, including activation procedures (Hyperventilation and Intermittent Photic Stimulation). A Micromed, System Plus, 21-channel device was used. EEG electrodes were placed on the scalp according to the 10-20 International System and all the tracings were interpreted using a bipolar longitudinal montage. Patients were tested by trained neurophysiology technicians during the video-EEG recordings. The diagnosis of epilepsy and the classification of seizure and epilepsy type were based on the recent International

League Against Epilepsy (ILAE) criteria (Scheffer IE et al., 2017; Fisher RS et al., 2017).

Neuroimaging

A brain scan in a 1.5 Tesla magnet with standard head coil was assessed in compliant subjects alone. MR imaging was performed by Gyroscan Intera Philips 1.5 Tesla system. The MRI examination was performed with 5 mm slices thickness using T1-weighted spin-echo (TR/TE = 600/20 ms), Proton Density and T2-weighted turbo spin-echo (TR/TE = 2800/40–110 ms) Fluid Attenuated Inversion Recovery (FLAIR) (TR/TE/TI = 6000/100/2000 ms). Diffusion-weighted eho planar imaging (TR/TE = 3500/120 ms) images were obtained in the axial plane, and an additional T2-weighted turbo spin-echo image was obtained in the coronal plane (TR/TE = 3000/110 ms).

3.6. Psychiatric and neurocognitive evaluation

All patients were interviewed by an expert psychiatrist. The psychiatric diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) V criteria (American Psychiatric Association, 2013) after the patients had been evaluated by means of the Structured Clinical Interview for DSM Disorders I and II (Structured Clinical Interview - SCID I and SCID II) (First MB et al., 2002; First MB et al., 1997). SCID I and SCID II are considered to be the gold standard semi-structured assessment instruments for clinical disorders and personality disorders respectively. SCID I and SCID II interviews were administered and performed face-to-face by an expert psychiatrist.

General intelligence was assessed in compliant subjects by means of the Standard Raven Progressive Matrices (SPM). SPM consists of five nonverbal sets of items typically used in educational settings to measure "general cognitive ability" (Raven J et al., 1998). Within each set, the items become progressively more difficult. At the beginning of each set, the items, although easy again, follow a different logic. The sets in turn become progressively more difficult. All the questions on the Raven's progressives consist of visual geometric design with a missing piece.

All the caregivers were also interviewed on different issues such as patients' personal psychiatric history, types and severity of patient's behaviors and their

impact on family routine, current and previous psychiatric treatments, any psychiatric diagnosis ascertained in other hospitals.

3.7. Neuropsychiatric familiar history

All the caregivers were also interviewed on patients' family neurologic and psychiatric history.

3.8. Clinical features and genomics correlation analysis

Several clinical aspects as hypocalcaemia, heart malformations, neurological phenotypes (i.e. epilepsy and parkinsonian features), psychotic disorder, intellectual disability were considered in our analysis. All clinical data regarding patient's neuropsychiatric history and general medical comorbidities were collected and integrated with instrumental data. Patients were classified according to the main clinical aspects. We explored possible link between neurological and psychiatric manifestations in each group. The relationships between different clinical features were evaluated to identify phenotypic "clusters" including both neuropsychiatric aspects and others comorbidities.

As regards the genomics, for each patient with an a-CGH test, we evaluated the deletion length (GCRH37/ hg19 version of the human genome) and gene content, using the UCSC Genome Browser (Lee CM et al., 2020; https://genome.ucsc.edu).

For gene content analysis and genomic annotations we used the following resources: DGV (Database of Genomic Variants, MacDonald JR et al., 2014; http://dgv.tcag.ca), DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources, Bragin E et al., 2014; https://decipher.sanger.ac.uk), OMIM (Online Mendelian Inheritance in Man, Amberger JS and Hamosh A, 2017; OMIM.org), ClinVar (Clinical Variants, Landrum MG et al., 2020; https://www.ncbi.nlm.nih.gov/clinvar), literature (PubMed).

Cases were divided into different groups according to the 22q11.2 deletion length (Mikhall FM et al., 2014; Burnside RD, 2015):

- LCR22 A-D deletion ("typical" deletion)

- LCR22 A-B/LCR22 A-C deletion (“minimal critical” deletion)
- LCR22 B-D/LCR22 C-D deletion (“central” deletion)
- LCR22 D-E/LCR22 D-F/LCR22 E-F deletion (“distal” deletion)

For selected cases also CNVs outside the 22q deleted region were considered, and their occurrence was compared to already reported CNV in patients with 22q11.2DS.

The Chi-square test or Fisher’s exact test were used to evaluate any significant differences between the expected frequencies and the observed frequencies with respect to each clinical variable between patients with specific phenotypes. A p value of 0.05 was considered statistically significant.

4. RESULTS

Overall, 85 adult patients with genetic diagnosis of 22q11.2DS (55 males, 30 females, mean age 27 years) from different Italian regions were enrolled in this study. All subjects had several clinical features suggestive for 22q11.2DS, such as multiple congenital anomalies, developmental delay or dysmorphic features.

4.1. Cytogenomic analysis

We collected the genetic data for 85 subjects. The genetic diagnosis was established through FISH studies and/or a-CGH. The molecular diagnosis was made by using FISH approach, through the commercially available TUPLE1/ARSA1 probe, in 46 cases (54%). 39 patients (46%) were diagnosed via a-CGH.

In details, 13/39 a-CGH were available at the moment of the recruitment, and the remaining were performed during the enrollment to confirm FISH diagnosis (10/39) or clinical suspicion (16/39). 26/39 a-CGH were carried out by using the Agilent-California USA Human Genome CGH Microarray kit 4x180K (AMADID 022060), with an average resolution of 75 Kb (GRh37/hg19). For the remaining 13 cases, the details on kits were not available.

The five a-CGH reports with data referring to the 2009 International System for Chromosome Nomenclature (ISCN) (GRh36/hg18) have been converted into the latest 2016 ISCN (GRh37/hg19). All genomic coordinates were based on the February 2009 assembly of Genome Reference Consortium build 37 (GRh37/hg19).

The inheritance pattern was available for 52/85 patients. In most cases (46/52) 22q11.2 deletion was a *de novo* event, while in the remaining six patients the pattern of inheritance was autosomal dominant (paternal in two and maternal in four cases, respectively).

4.2. General medical comorbidities

In all patients physiological anamnesis focusing on the perinatal anoxia around the time of birth and general medical history focusing on aspects related to hypocalcemia and cardiac malformations have been acquired.

Perinatal brain injury was observed in 19 patients and neonatal hypocalcemia in 20 (2 of whom also had acute symptomatic epileptic seizures). Twelve subjects had calcium metabolism impairment in adulthood (namely, hypocalcemia and vitamin D deficiency secondary to hypoparathyroidism), whereas 50 patients had one or more heart malformations (including aortic arch anomalies in 17 cases, Fallot tetralogy in 13, interventricular or interatrial defect in 17, crossed pulmonary arteries in 11) (Table 3).

Table 3. General characteristics of 22q11.2DS population

	n (%)
Gender	
Male	55 (65)
Female	30 (35)
Age at recruitment, median [range], years	27 [12-65]
Perinatal anoxia	19 (22)
Neonatal hypocalcemia	20 (23)
Hypocalcemia in adulthood	12 (14)
Heart malformations (one or more defects)	50 (59)
Aortic arch anomalies	17
Fallot tetralogy	13
Interventricular or interatrial defect	17
Crossed pulmonary arteries	11

4.3. Handedness

The Edinburgh scale was performed in all patients. We observed that 24 of the 85 enrolled patients (28%) were left-handed (17 of whom also had left-handed relatives); moreover, 29/61 of the right-handed subjects had a family history of left-handedness.

To date, no data related to manual preference in 22q11.2DS patients have been reported. Considering that the percentage of left-handed subjects in the general population is approximately 10% (Ockelenburga S et al., 2013), our results disclosed a significantly higher prevalence of this feature in 22q11.2DS cases compared to the general population.

4.4. Neurological aspects

4.4.1. Seizures and epilepsy

In all patients, previous history of seizures and febrile seizures was investigated. Twenty-two of the 85 participants had experienced at least one seizure in their lifetime: 6/22 patients had had febrile seizures and 4/22 reported acute symptomatic seizures (related to hypocalcemia in 2 cases, to major surgery in 1 and not well defined in 1). The remaining 12/22 patients had a diagnosis of epilepsy (Table 4). The family history was positive for epilepsy in 5 of the 12 epileptic patients.

Brain MRI, which was available for only 7 of these 12 subjects, in 1 case resulted normal, in 5 cases revealed minor alterations (*e.g.* empty sella, gliotic foci in the white matter) and in 1 subject disclosed a left frontal focal cortical dysplasia (FCD). Five main clinical clusters, according to seizure type and semiology, were identified by reviewing the epileptic patients' history: i) primary generalized tonic-clonic seizures (pGTCS) (1/12), ii) pGTCS and myoclonic seizures (3/12, associated with aggravation by antipsychotics in 1), iii) pGTCS and absences with or without myoclonic seizures (4/12, associated with aggravation by antipsychotics in 2), iv) absences and myoclonic seizures (1/12), and v) focal seizures with or without secondary-GTCS (3/12, associated with aggravation by neuroleptics in 1). No epileptic patient was drug-resistant. On the basis of

electro-clinical features (video-EEG data are discussed below) and neuroimaging findings, 9 patients were diagnosed with GGE, classified as JME in 5, whereas 3 received a diagnosis of focal epilepsy (whose etiology was structural in 1 case and unknown in 2) (Table 5).

These results disclosed a higher prevalence of epilepsy in the 22q11.2DS cases (14%) compared to the general population (0.5-1%) (Kim EH et al.,2016).

Table 4. Clinical and instrumental features in 22q11.2DS epileptic patients

Pts	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Sex/age	F/27	M/28	M/32	M/25	F/38	F/20	M/22	M/23	M/25	M/32	M/24	F/12
Perinatal brain injury	yes	yes	no	no	no	no	no	yes	yes	no	no	no
Hypocalcemia	no	yes	no	no	no	no	yes	no	no	yes	no	no
Febrile seizures	no	no	no	no	no	no	yes	yes	no	no	no	no
Heart malformations	no	yes	no	no	no	no	no	no	no	no	no	no
Seizure's onset	12y	17y	10y	14y	22y	15y	10y	9y	2y	23y	18y	1y
Seizure's type	s-GTCS/FS	p-GTCS	p-GTCS/ A/MS	p-GTCS/ MS	p-GTCS/ MS	A/ MS	p-GTCS/ MS	p-GTCS/A	s-GTCS/FS	FS	p-GTCS/A	p-GTCS/A
Myoclonus on awakening	yes	yes	no	yes	yes	yes	yes	no	no	no	no	no
Video EEG	slow GSW	left T slow W	GSW	GSW	normal	GSW	GSW	normal	left TL slow W	left TL SW	G slow W	G slow W
Brain MRI	left hemisphere hypotrophy	white matter gliosis	NA	NA	empty sella	NA	NA	wide right F sulcus	left FL FCD	wide central sulcus	normal	NA
Epileptic syndrome	unknown FE	GGE	JME	JME	JME	JME	JME	GGE	SFE	unknown FE	GGE	GGE
AEDs	VPA-LEV	VPA	LMT	VPA-CLZ	LEV	CLZ	VPA-CLZ	LMT-LEV	VPA-LCS-TPM-CLZ	TPM	VPA	no
Drug resistance	no	no	no	no	no	no	no	no	no	no	no	no
Intellectual disability	severe	moderate	no	NA	NA	no	severe	mild	NA	severe	NA	NA
Psychiatric diagnosis	schizophrenia, ID	anxiety	schizophrenia	schizophrenia	schizophrenia	no	schizophrenia	anxiety	schizophrenia	schizophrenia	schizophrenia	schizophrenia
Seizures increased by antipsychotics	typic zuclopenthixol	no	atypic olanzapine	no	no	NA	atypic clozapine	NA	no	no	atypic olanzapine	no
Deletion's lenght (Mb)	2.480	NA	2.520	NA	3.029	NA	NA	2.883	NA	2.569	2.569	NA
Family epileptic history	no	yes	no	no	no	yes	no	yes	no	yes	yes	no

F: female, M: male, y: years, p: primary, s: secondary, GTCS: Generalized Tonic-Clonic Seizures, FS: Focal Seiures, MS: Myoclonic Seizures, A: Absences, G: Generalized, S: Sharp, W: Wave, T: Temporal, F: Frontal, L: Lobe, FCD: Focal Cortical Dysplasia, NA: Not Applicable, FE: Focal Epilepsy, SFE: Symptomatic Focal Epilepsy, GGE: Genetic Generalized Epilepsy, JME: Juvenile Myoclonic Epilepsy, AEDs: Antiepileptic Drugs, VPA: Valproate, LEV: Levetiracetam, LMT: Lamotrigine, CLZ: Clonazepam, TPM: Topiramate, LCS: Lacosamide, ID: Intellectual Disability

Table 5. Epilepsy types in 22q11.2DS patients	
	n (%)
Juvenile myoclonic epilepsy	5 (42)
Others genetic generalized epilepsy	4 (33)
Structural focal epilepsy	1 (8)
Unknown focal epilepsy	2 (17)

4.4.2. Motor disorders

In all patients, motor disorders were evaluated. Parkinsonism was observed in 34 of the 85 patients, 19 of whom (11 males, 8 females, mean age 28 years) were taking antipsychotics (which were typical in 3 cases) at the time of the evaluation. All but 3 of the 19 subjects on neuroleptics had psychotic disorders, 4/19 presented calcium metabolism alterations and 4/19 had a family history of parkinsonism. By contrast, among the 15 patients with parkinsonism who were not on neuroleptics (12 males and 3 females, mean age: 30 years), 6 had calcium metabolism alterations while none had a family history of parkinsonism (Table 6). The MDS-UPDRS yielded higher total scores and sub-scores in the patients who were taking antipsychotics (Table 7-8).

Table 6. Clinical features and comorbidity in 22q11.2DS patients with motor signs

Pts	Motor signs	Sex/ age	Hypo calcemia	Heart malformations	Brain MRI	Other NP features	Anti-psychotics	PD family
#1	L	M/31	yes	yes	WMG	anxiety	no	no
#2	L	M/46	no	yes	WMG	depression	no	no
#3	L	M/24	no	no	normal	anxiety, ID	no	no
#4	L	F/20	yes	no	normal	anxiety, ID	no	no
#5	L	M/22	yes	yes	NA	no	no	no
#6	L	M/20	no	no	normal	anxiety	no	no
#7	Ax/B	M/49	yes	yes	WMG	anxiety, ID	no	no
#8	Ax/B	F/46	no	no	SP cyst, WMG	ID	no	no
#9	Ax/B	M/29	yes	no	NA	depression	no	no
#10	Ax/B	M/22	no	no	WMG	anxiety, ID	no	no
#11	Ax/B	M/25	no	yes	WMG	anxiety	no	no
#12	Ax/B	M/22	no	no	NA	no	no	no
#13	L-AT	M/23	no	no	NA	anxiety	no	no
#14	L-AT	F/34	yes	yes	WMG	depression	no	no
#15	Ax/B-AT	M/21	no	yes	NA	no	no	no
#16	L	F/27	no	no	hemispheric asymmetry	epilepsy, ID, schizophrenia	T	no
#17	L	M/28	no	yes	WMG	epilepsy, anxiety, ID	A	yes
#18	Ax/B	F/27	no	yes	WMG	schizophrenia, ID	A	no
#19	Ax/B	M/17	no	no	WMG	schizophrenia, ID	A	no
#20	Ax/B	F/49	no	no	CA	schizophrenia, ID	A	yes
#21	Ax/B	M/26	no	no	normal	NA	A	no
#22	Ax/B	M/32	yes	no	normal	ID	A	no
#23	AT	M/32	no	no	normal	epilepsy, schizophrenia	A	no
#24	AT	F/38	yes	no	CA	epilepsy, schizophrenia	A	no
#25	L-AT	M/22	yes	no	NA	epilepsy, schizophrenia, ID	A	no
#26	L-AT	M/30	no	yes	SA cyst	schizophrenia, ID	A	yes
#27	Ax/B-AT	M/17	no	no	SP cyst	schizophrenia, ID	A	no
#28	Ax/B-AT	M/31	no	yes	NA	schizophrenia	A	no
#29	Ax/B-AT	F/29	yes	no	NA	schizophrenia, anxiety, ID	A	no
#30	Ax/B-AT	M/25	no	no	left FCD	epilepsy, schizophrenia	T	yes
#31	Ax/B-AT	F/40	no	no	normal	schizophrenia	T	no
#32	L	F/16	no	yes	WMG	schizophrenia	A	no
#33	Ax/B	M/24	no	no	normal	epilepsy, schizophrenia	A	no
#34	AT	F/29	no	yes	NA	schizophrenia, ID	A	no

Ax: Axial, L: Lateral, B: Bilateral, AT: Action Tremor, WMG: White Matter Gliosis, NA: Not Applicable, SA: Subarachnoid, SP: Septum Pellucidum, CA: Cortical Atrophy, FCD: Focal Cortical Dysplasia, A: Atypic, T: Typic, NP: Neuro-Psychiatric, PD: Parkinson's Disease, ID: Intellectual Disability

Table 7. Subscore UPDRS-I-II-III in 22q11.2DS patients with motor signs taking anti-psychotics

UPDRS-I/II (subscore)	Sleep problems (0-4)	Daytime sleepiness (0-4)	Light headedness on standing (0-4)	Saliva and drooling (0-4)	Tremor (0-4)	Walking and balance (0-4)
^1	-	-	-	-	-	-
^2	0	0	0	0	0	0
^3	0	0	1	2	0	0
^4	0	0	0	0	0	0
^5	-	-	-	-	-	-
^6	0	1	1	3	0	1
^7	0	0	0	3	0	0
^8	1	2	0	0	o	0
^9	2	1	1	2	2	1
^10	0	0	0	0	3	0
^11	1	0	0	2	0	0
^12	2	1	0	0	0	0
^13	0	0	0	0	0	1
^14	4	0	0	0	0	0
^15	2	4	0	0	3	1
^16	2	2	0	3	2	1
^17	0	0	0	2	0	0
^18	0	0	0	3	1	0
^19	0	0	0	0	2	0
UPDRS-III (subscore)	**Facial expression (0-4)**	**Rigidity (0-20)**	**Body bradykinesia (0-4)**	**Postural tremor of the hands (0-4)**	**Kinetic tremor of the hands (0-4)**	**Total score (0-128)**
^1	-	-	-	-	-	-
^2	1	1	6	0	0	17*
^3	0	0	2	0	0	4
^4	3	3	1	0	0	28*
^5	-	-	-	-	-	-**
^6	2	3	7	0	0	24*
^7	2	5	3	0	0	12*
^8	1	1	1	1	0	6
^9	3	1	0	1	0	10
^10	2	1	9	1	2	33*
^11	3	1	3	1	0	17*
^12	0	0	1	2	0	14
^13	2	3	11	2	0	35*
^14	0	1	10	1	1	15*
^15	2	3	0	1	2	33
^16	2	1	9	1	0	15*
^17	1	1	3	0	0	8*
^18	3	1	0	1	0	10
^19	0	0	0	1	1	2
Media score	**1.6**	**1.5**	**4**	**0.8**	**0.4**	**16.6**

*Parkinsonism (Postuma RB et al., 2015); **L-Dopa inefficacy

Table 8. Subscore UPDRS-I-II-III in 22q11.2DS patients with motor signs no taking anti-psychotics						
UPDRS-I/II (subscore)	**Sleep problems (0-4)**	**Daytime sleepiness (0-4)**	**Light headedness on standing (0-4)**	**Saliva and drooling (0-4)**	**Tremor (0-4)**	**Walking and balance (0-4)**
#1	1	0	1	0	2	0
#2	0	0	2	0	0	0
#3	0	0	0	0	0	0
#4	-	-	-	-	-	-
#5	0	0	0	0	0	0
#6	0	0	0	0	0	0
#7	0	0	0	0	0	0
#8	0	0	0	0	0	1
#9	-	-	-	-	-	-
#10	-	-	-	-	-	-
#11	0	0	0	0	0	0
#12	0	0	0	0	0	0
#13	0	0	0	0	0	0
#14	0	1	0	2	0	0
#15	0	0	0	0	0	0
UPDRS-III (subscore)	**Facial expression (0-4)**	**Rigidity (0-20)**	**Body bradykinesia (0-4)**	**Postural tremor or the hands (0-4)**	**Kinetic tremor of the hands (0-4)**	**Total score (0-128)**
#1	0	0	1	0	0	3
#2	1	1	3	0	0	5*
#3	0	0	4	0	0	5
#4	-	-	-	-	-	-
#5	0	0	2	0	0	4
#6	0	0	3	0	0	6
#7	1	2	3	0	0	8*
#8	1	3	16	0	0	34*
#9	-	-	-	-	-	-
#10	-	-	-	-	-	-
#11	0	0	6	0	0	8
#12	1	1	4	0	0	12*
#13	0	0	5	1	0	10
#14	0	0	3	1	0	7
#15	0	0	2	1	0	3
Media score	**0.3**	**0.6**	**4.3**	**0.3**	**0**	**8.75**

***Parkinsonism (Postuma RB et al., 2015)

4.5. Instrumental findings

Video-EEG findings

All 85 patients underwent a video-EEG recording, which revealed abnormal findings in 10 of the 12 cases with a diagnosis of epilepsy: in particular, 7/10 had generalized abnormalities (which were clearly epileptiform in 4 patients, 1 of whom also presented cortical myoclonus) and 3/10 had focal ones (epileptiform in 2). The video-EEG performed in patients without epilepsy revealed generalized abnormalities in 26/73 cases (epileptiform in 3) and focal abnormalities in 4 (Figure 9). To our knowledge, this is the first study of EEG pattern of a 22q11.2DS cohort.

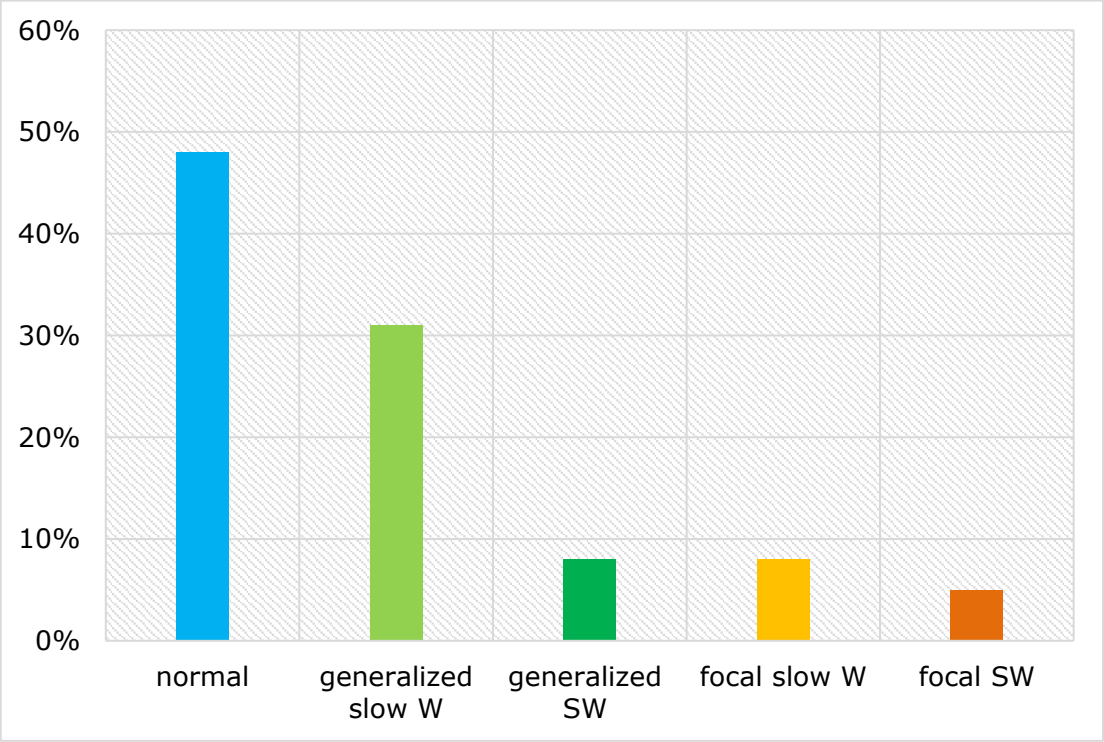


Figure 9. Spectrum of EEG patterns in patients with 22q11.2 deletion syndrome. W: wave SW: spike-and-wave

Brain Neuroimaging

52 patients performed a brain MRI. In most of cases (20/52) neuroimaging study showed white matter gliosis not associated with specific pathologic entity. 8/52 patients had focal or diffuse cortical atrophy, in 4/52 subjects were observed minor malformations (cavum vergae, cystic cavum septum pellucidum, subarachnoid cysts) (Figure 10), in two cases MRI showed major malformations (left frontal cortical dysplasia and right hemispheric polymicrogyria, respectively) (Figure 11) and in the remaining 18 patients MRI was unremarkable.

All these brain abnormalities (cavum septum pellucidum, cystic cavum septum, nonspecific white matter abnormalities (Schmitt JE et al., 2014), polymicrogyria and cortical dysplasia) have been described in patients with 22q11.2DS, yet (Bohm LA et al.,2017; Kuzniecky R et al., 1993; Hopkins SE et al., 2018).

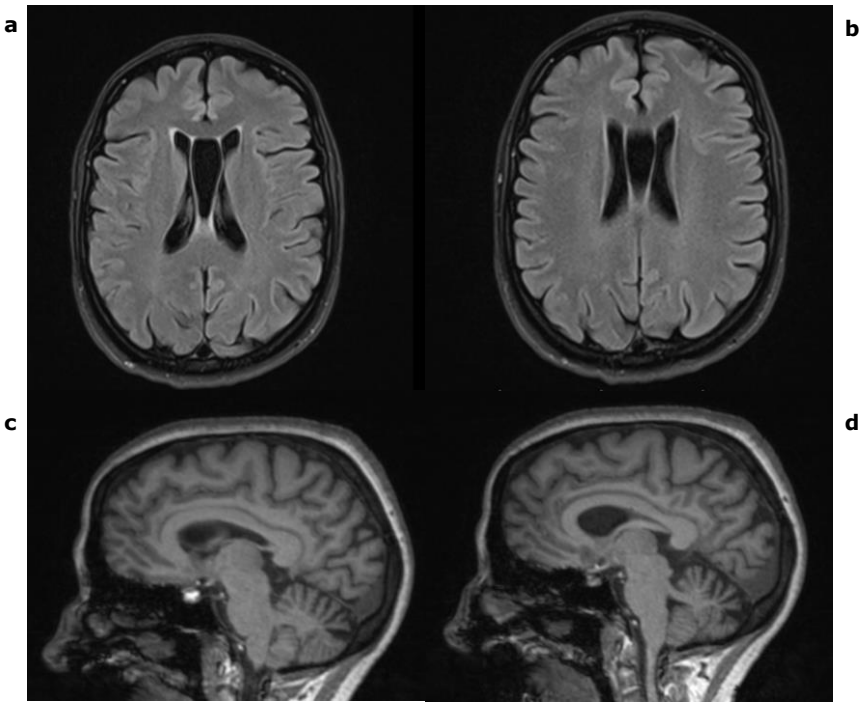


Figure 10. Brain MRI (axial FLAIR image **a**,**b**; sagittal T2-weighted image **c**,**d**) shows cavum septum pellucidum and vergae.

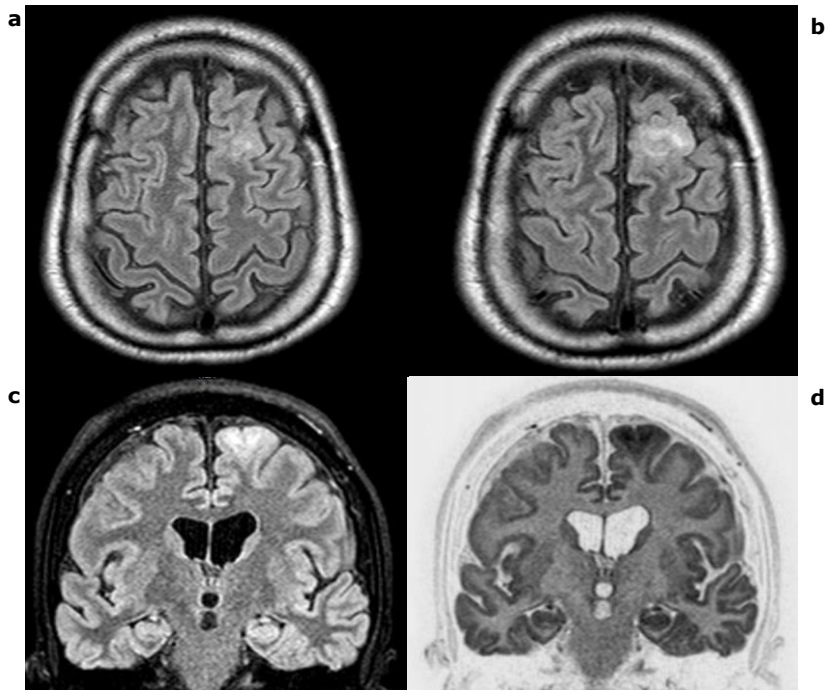


Figure 11. Brain MRI (axial FLAIR image **a**,**b**; coronal FLAIR image **c**; coronal IR image **d**) shows a left frontal cortical dysplasia.

4.6. Psychiatric and neurocognitive features

We performed a general intelligence evaluation of 48 patients. Twenty-eight subjects displayed a reduction in the Intelligence Quotient (IQ): mild (IQ from 50-55 to 70) in 13 cases, moderate (IQ from 35-40 to 55) in 5 and severe (IQ < 35) in 10. The IQ was normal in the remaining 20 patients (IQ >70).

As regards the psychiatric abnormalities, evaluated in all patients, we documented schizophrenia in 30/85 cases, depression in 3/85, anxiety in 14/85, personality disorders in 6/85, pervasive developmental disorders in 2/85, personality disorders and depression in 3/85, personality disorders and anxiety in 4/85 and personality and pervasive developmental disorders in 1/85 (Figure 12).

Our data are in line with those reported in the literature. In particular, as regards cognitive aspects moderate-to-severe intellectual disability was observed in about 31% of the cases, which is in accordance with other reports(Evers LJ et al., 2009). However, the proportion of psychotic disorders documented in our work (35%) is slightly higher than that reported in the literature (30%; Murphy

KC, 2002), whereas the percentage of anxiety (21%) and mood disorders (7%) is lower (Fung WL et al., 2010; Antstel KM et al., 2006).

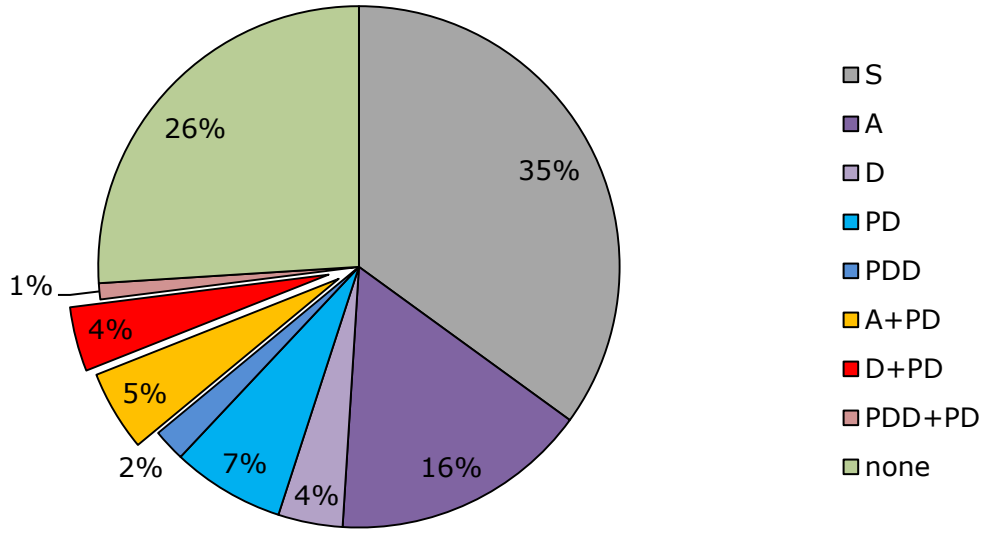


Figure 12. Psychopatology in 22q11.2DS population. S: Schizophrenia, A: Anxiety, D: Depression, PD: Personality Disorders, PDD: Pervasive Developmental Disorders.

4.7. Neuropsychiatric familiar history

We collected the familiar history of neurological/psychiatric disorders for all the patients and found that 46/85 subjects had at least one first and/or second-degree relative affected by one or more neuropsychiatric diseases. Specifically, we found the following associated phenotypes: intellectual disability (11 cases), psychiatric disorders (psychosis in 6 cases, depression in 5 and bipolar disorder in 1), epilepsy (9 cases), febrile seizures (2 cases), Alzheimer diseases (5 cases) and parkinsonism (7 cases) (Table 9).

Table 9. Neuropsychiatric familiar history of 22q11.2DS population	
	n (%)
Relatives with one or more neuropsychiatric diseases	46 (54)
Intellectual disability	11 (13)
Psychiatric disorders	
Psychosis	6 (7)
Depression	5 (6)
Bipolar disorder	1 (1)
Epilepsy	9 (11)
Febrile seizures	2 (2)
Parkinsonism diseases	7 (8)
Alzheimer diseases	5 (6)

4.8. Clinical features correlation analysis

We analyzed the recurrence of clinical aspects as hypocalcaemia and heart malformations, and several specific neurological and neuropsychiatric features (intellectual disability, epilepsy and parkinsonian features).

We documented a significantly higher prevalence of psychotic disorders among patients with severe intellectual disability than in those without cognitive impairment (p=0.005) (Figure 13). All patients with severe intellectual disability but one received a diagnosis of psychosis; in 5 other psychotic patients whose IQ was available, 3 had mild intellectual disability and one had normal intelligence. Moreover, we observed that non-psychotic subjects had more cardiac malformations than psychotic ones (34/55 versus 16/30).

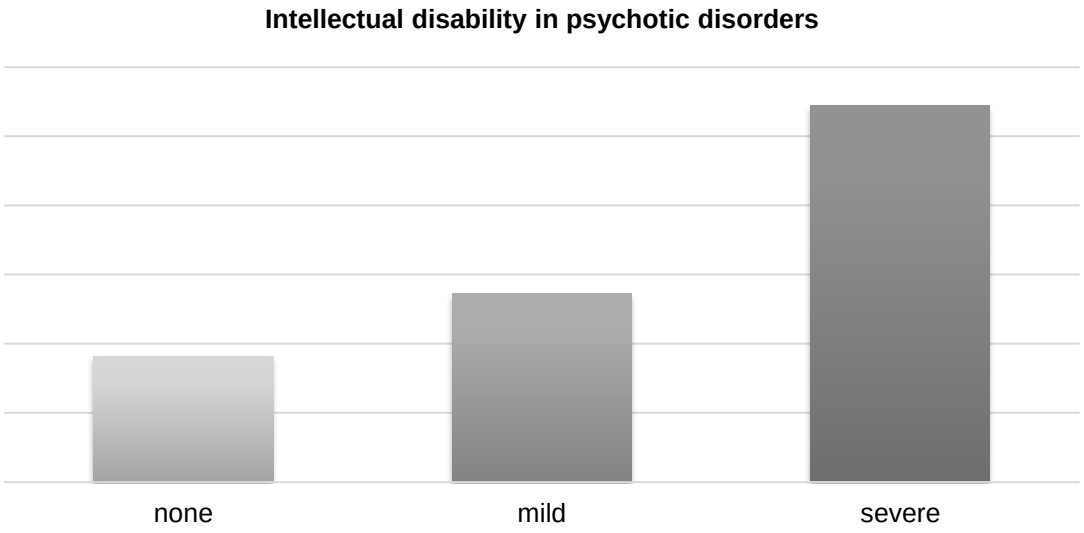
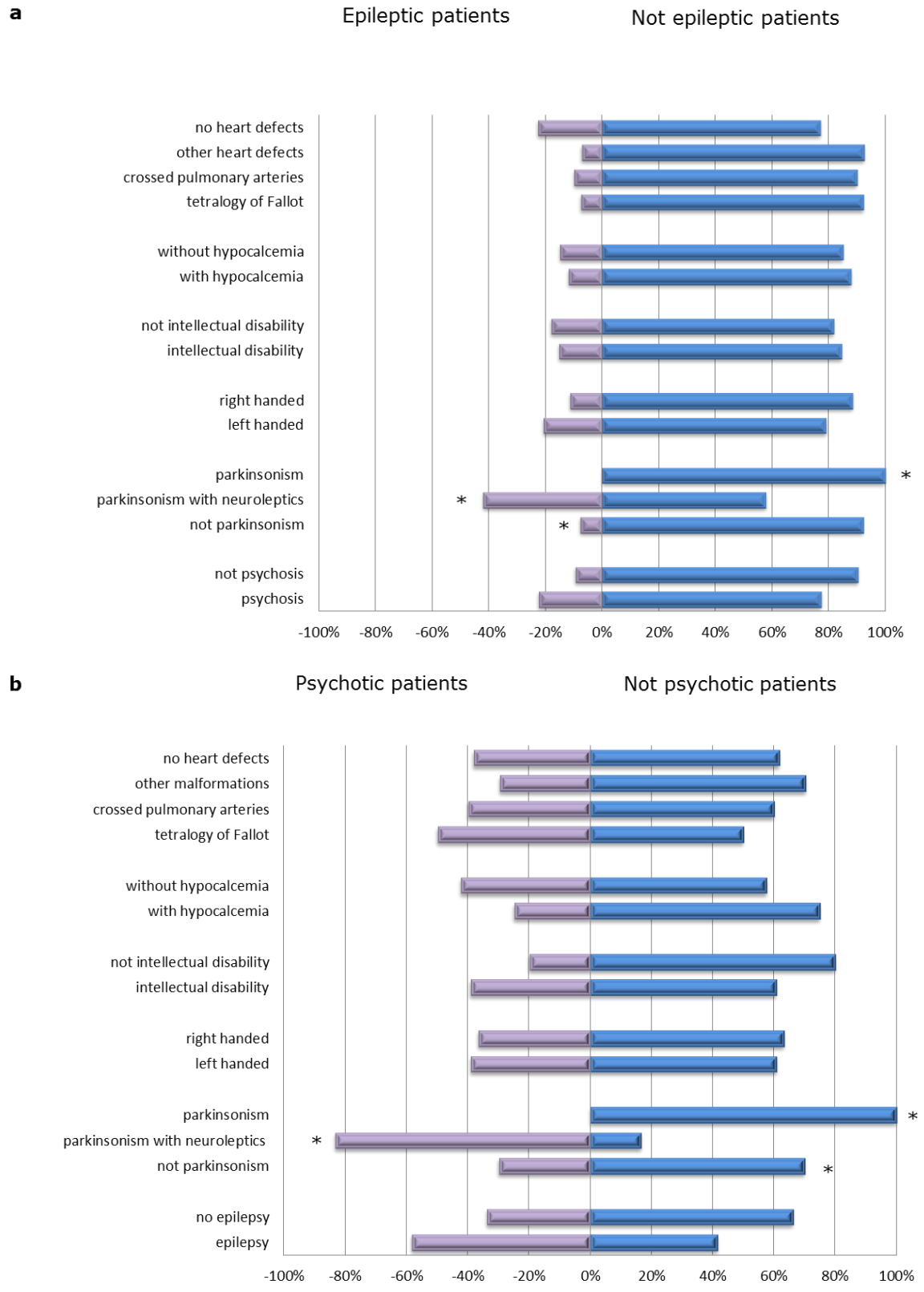


Figure 13. Intellectual disability in 22q11.2DS patients with psychotic disorders.

In addition, among 48 patients whose IQ was known, cardiac malformations were found to be more common in subjects with both mild intellectual disability (5/13) and a normal intelligence (7/20) than in those with a moderate-to-severe cognitive impairment (2/15). Besides, most patients with impaired calcium metabolism during the neonatal period and/or adulthood (26/85) did not have psychotic disorders (20/26).

All the epileptic patients but 1 had psychiatric comorbidities: 9 had schizophrenia while 2 suffered from anxiety. When 22q11.2DS subjects with epilepsy were compared with those without epilepsy, the former exhibited a higher prevalence of psychotic disorders and a lower prevalence of heart defects. Lastly, none of 15 patients with parkinsonism who were not taking neuroleptics were diagnosed with epilepsy, while 7 of the 19 patients with parkinsonism who were taking antipsychotics were diagnosed with epilepsy ($p<0.001$) (Figure 14).



* statistically significant results (p value is shown in the test)

Figure 14. Different clinical variables between epileptic/non-epileptic patients (**a**); psychotic/non-psychotic patients (**b**).

4.9. Genomics analysis

The analysis of the a-CGH data of 39 cases showed that 27 (69.2%) patients had a LCR22 A-D deletion, 5 (12.8%) LCR22 A-B, and 2 (5.1%) LCR22 A-C deletion. LCR22 A-B, LCR22 B-C, LCR22 C-D, LCR22 D-E, LCR22 E-F, LCR22 D-E, LCR22 D-F deletions have been observed in one case (4.3%) each (Figure 15, Table 10).

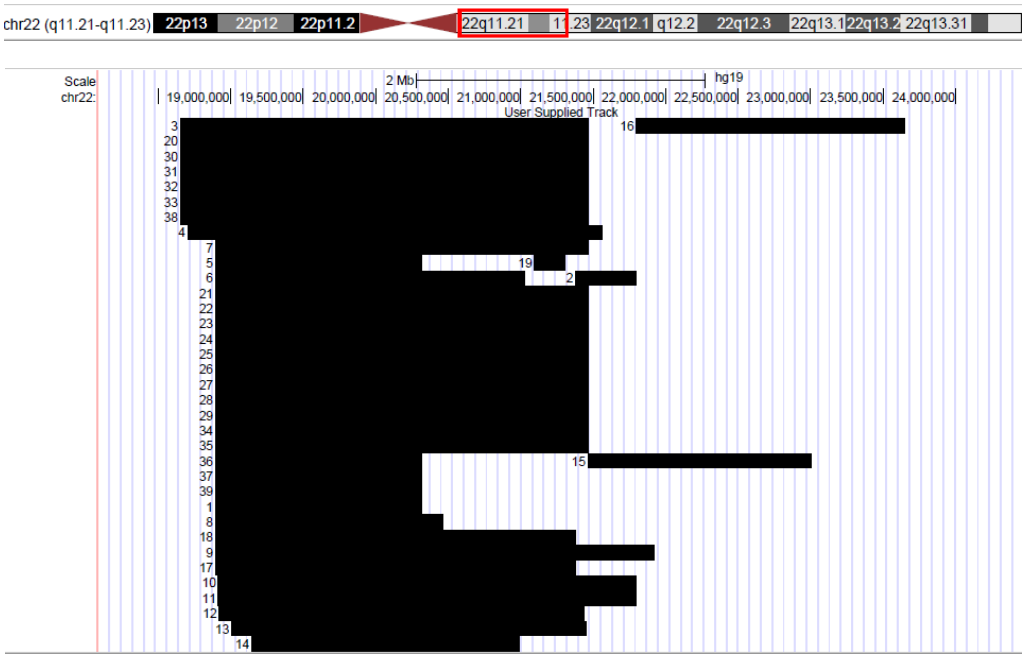


Figure 15. UCSC genome browser view of the 22q deleted region of the 39 22q11DS cases analyzed in this work.

Table 10. Genomic coordinates, 22q deletion class and inheritance pattern of 39 patients				
Pt	**Chr22 deletion (GRh37/hg19)**	**Deletion size (bp)**	**Deletion class**	**Inheritance**
#1	18894865-20311965 20733457-21461782	1.417.100 728.325	A-B; B-D	Not applicable
#2	21379903-21798705 22916612-22998331	418.802 81.719	D-E; E-F	Not applicable
#3	18651614-21464119	2.812.505	A-D	*de novo*
#4	18706023-21561492	2.855.469	A-D	*de novo*
#5	18894835-20311763	1.416.928	A-B	*de novo*
#6	18894835-21032298	2.137.463	A-C	*de novo*
#7	18894635-21464260	2.569.625	A-D	Not applicable
#8	18894865-20465977	1.571.112	A-B	*de novo*
#9	18896972-21926261	3.029.289	A-D	*de novo*
#10	18916842-21800471	2.883.629	A-D	*de novo*
#11	18916842-21800797	2.883.955	A-D	*de novo*
#12	18919942-21440514	2.520.572	A-D	*de novo*
#13	19009792-21452445	2.448.653	A-D	Not applicable
#14	19147404-20992700	1.845.296	A-C	*de novo*
#15	21465662-22997928	1.532.266	D-E	*de novo*
#16	21798705-23654222	1.855.517	D-F	*de novo*
#17	18897000-21380000	2.483.000	A-D	Father
#18	18895446-21375446	2.480.000	A-D	*de novo*
#19	21096874-21305776	208.902	C-D	Not applicable
#20	18651614-21464119	2.812.505	A-D	Not applicable
#21	18894835-21464119	2.569.284	A-D	*de novo*
#22	18894835-21464119	2.569.284	A-D	Mother
#23	18894835-21464119	2.569.284	A-D	*de novo*
#24	18894835-21464119	2.569.284	A-D	*de novo*
#25	18894835-21464119	2.569.284	A-D	Not applicable
#26	18894835-21464119	2.569.284	A-D	*de novo*
#27	18894835-21464119	2.569.284	A-D	Mother
#28	18894835-21464119	2.569.284	A-D	Not applicable
#29	18894835-21464119	2.569.284	A-D	Not applicable
#30	18651614-21464119	2.569.284	A-D	Not applicable
#31	18651614-21464119	2.569.284	A-D	*de novo*
#32	18651614-21464119	2.569.284	A-D	Mother
#33	18651614-21464119	2.569.284	A-D	Not applicable
#34	18894835-21464119	2.569.284	A-D	Not applicable
#35	18894835-21464119	2.569.284	A-D	*de novo*
#36	18894835-20311763	1.416.928	A-B	*de novo*
#37	18894835-20311763	1.416.928	A-B	*de novo*
#38	18651614-21464119	2.569.284	A-D	*de novo*
#39	18894835-20311763	1.416.928	A-B	*de novo*

Further potential pathogenic CNVs were analyzed in 26/39 patients, in the remaining 13 cases this information was not available as the genomic analysis was focused only on the 22q chromosome region.

Among those 26 patients, in 6 cases the a-CGH showed, besides the 22q11.2 deletion, the presence of additional CNVs. A total of 8 CNVs were detected and reported in Table 11.

CNVs were present on chromosome 5,11,12,16 and X, with a size ranging from 9 kb to 246 kb, and included five duplications and three deletions. Among the 8 CNVs three were inherited by the mother, two by the father and in the remaining three cases this data was unknown.

None of these CNVs were previously reported in patients with 22q11.2DS (Basset AS et al., 2017; Bertini V et al., 2017; Xie HM et al.,2019).

Table 11. Genomic coordinates and inheritance pattern of CNV outside the 22q deletion region

Pt	CNV type	Chr:position(bp)	Inheritance
#3	Dup	16:11719921-11827864	Father
#5	Del	16:77233587-77479804	Father
#11	Dup	X:52833688-52923471	Mother
#17	Del	16: 21518321-21756321	Mother
#21	Del	5:94786021-94856474	Father
#28	Dup	11:66046441-66055962	Not applicable
	Dup	12:27856722-27909127	Not applicable
	Dup	12:53433482-53465803	Not applicable

Patient's number is referred to Table 10

In details, additional CNVs on chromosome 16 were observed in three patients: one microduplication and two microdeletions. In patient #3 with a p13.13 microduplicated region of 108 kb in lenght, no OMIM gene mapped. In patient #5, the deletion involved the 16q23.1 region and was 246 kb long, including the OMIM gene *ADAMTS18* (MIM #607512) and the SYCE1L, with an unclear functional significance. Patients 17 had a deletion of 238 kb including the OMIM

gene *OTOA* (MIM #607038), and the genes METTL9 and IGSF6, and the non coding RNA LOC101927814.

In one patient (pt #11 in the table) a microduplication of 90 kb was detected on chromosome Xp, including the OMIM gene XAGE3 (MIM #300740) and the genes XAGE5 and FAM156B, of uncertain functional significance.

In one case (pt #21 in the table), a microdeletion of unclear functional significance was detected including the OMIM TTC37 gene (MIM #614589) and FAM81B gene.

Finally, in the patient with more than one CNVs (pt #28 in the table) were observed three microduplication: one on long arm of chromosome 11 and two on petit and long arms of chromosome 12, respectively. The CNV on chromosome 11 was 9 kb long and included the genes CNIH2 and Y1FA, those on chromosome 12 were 52 kb and 32 kb long, and included one gene MRPS35 and two genes TNS2 and SPRYD3, respectively. No morbid genes were contained in these microduplications (Table 12).

Table 12. Genomic features of CNVs outside the 22q deletion region

Pt	CNVs	CNV length (bp)	OMIM genes	Other genes	Non-coding genes
#3	Dup 16p13	108 Kb	none	none	none
#5	Del 16q23	246 Kb	ADAMTS18 (MIM #607512)	SYCE1L	none
#11	Dup Xp11	90 Kb	XAGE3 (MIM #300740)	XAGE5, FAM156B	none
#17	Del 16p12	238 Kb	OTOA (MIM #607038)	METTL9, IGSF6	LOC101927814
#21	Del 5q15	70 Kb	TTC37 (MIM #614589)	FAM81B	none
#28	Dup 11q13	9 Kb	none	CNIH2, Y1FA	none
	Dup 12p11	52 Kb	none	MRPS35	none
	Dup 12q11	32 Kb	none	TNS2, SPRYD3	none

Patient's number is referred to Table 10

As the limited number of cases with an available report of additional CNVs a genotype-phenotype correlation analysis among additional genomic variants that could influence the phenotype spectrum could not be performed.

4.10. Clinical and genomics' features correlations

We analyzed clinical features including left-handedness, epilepsy, schizophrenia and motor disorders in each group of 22q11.2 deletion class.

We observed that all patients with schizophrenia and epilepsy had a "typical" LCR22 A-D (median deletion lenght 2.320 Mb) or "minimal critical" LCR22 A-B (median deletion length 1.579 Mb) deletion (Table 13).

Motor disorders exhibited a significantly higher prevalence in the group of patients with "typical" deletion (p=0.019). We documented a significantly higher prevalence of febrile seizures in the group of patients with "distal" deletion (p=0.003). Left-handedness did not significantly associate with any of the 22q11.2 deletion groups.

All the four patients with autosomal dominant pattern of inheritance had a "typical" (LCR22 A-D) deletion.

All patients (n=6) with further CNVs had a "typical" LCR22 A-D (5/6) or "minimal critical" (LCR22 A-B) (1/6) deletion.

Table 13. Genotype and phenotype features of 39 patients with a-CGH results				
	LCR22 A-D ("typical" deletion)	**LCR22 A-B/C ("minimal critical" deletion)**	**LCR22 B/C-D (central deletion)**	**LCR22 D-E/ LCR22 D-F/ LCR22 E-F (distal deletion)**
Patient, n.	27	8	2 (1 pt nested deletion also)	3 (1 pt two distal deletions types)
Deletion lenght Mb(range)	2.320 (2.448-3.029)	1.579 (1.416-2.137)	468 (208-728)	1.296 (500-1.855)
Others CNVs n. (%)	5 (83%)	1 (17%)	0 (0%)	0 (0%)
AD inheritance n. (%)	4 (100%)	0 (0%)	0 (0%)	0 (0%)
Left handed n. (%)	6 (55%)	3 (27%)	0 (0%)	2 (18%)
Left-handed inheritance n. (%)	12 (60%)	6 (30%)	1(5%)	1 (5%)
Febrile seizures n. (%)	2 (40%)	0 (0%)	0 (0%)	3 (60%)**
Epilepsy n. (%)	6 (100%)	0 (0%)	0 (0%)	0 (0%)
Schizophrenia n. (%)	11 (73%)	4 (27%)	1 pt with "minimal critical" deletion also	0 (%)
Motor disorders	19 (86%) *	2 (9%)	0 (0%)	1 (5%)

* statistically significant association (p=0.019) between motor disorders and "typical" deletion

** statistically significant association (p=0.003) between febrile seizures and "distal" deletion

5. DISCUSSION

22q11.2DS is the most common recurrent microdeletion in humans. The phenotype is characterized by a constellation of clinical signs, whose characterization has become more and more accurate within the last decade. 22q11.2DS is currently considered the genetic model par excellence of schizophrenia. However, the growing interest for this syndrome has opened the gateway to the discovery of other neurologic and psychiatric manifestations, including epilepsy and movement disorders.

Moreover, the great phenotypic variability of 22q11.2DS, despite the same 3-Mb deletion harbored by most patients, supports the hypothesis that 22q11.2DS is a syndrome with variable expressivity and incomplete penetrance. Several mechanisms such as stochastic modifying multigenic interactions, environmental processes as well as genetic factors could play a crucial role in genotype-phenotype correlations. Some studies tried to explain the mechanisms underlying such a wide phenotypic variability, but to date data are not conclusive.

In this work, we described the neuropsychiatric features of an Italian adult population of 22q11.2DS subjects, focusing on the neurological manifestations and investigating their possible correlation with general clinical features. We also characterized the genomic aspects including inheritance pattern, length of the 22q11.2 deletion region, position of deleted region on chromosome 22 and potential causal CNV elsewhere in the genome in a subgroup of patients. Finally, we looked for a possible association between peculiar neuropsychiatric features and genetic findings.

As far as the cognitive profile is concerned, more than half of our patients (58%) had a reduced Intelligence Quotient, whereas moderate-to-severe intellectual disability was observed in about 31% of the cases, which is in accordance with reports by other authors (Evers LJ et al., 2009). The results of our study also confirm the higher prevalence of early onset psychotic disorders (30%) (Murphy KC, 2002), anxiety (40-46%) (Fung WL et al., 2010) and mood disorders (9-35%) in 22q11.2DS patients compared with the general population (Antstel KM et al., 2006). However, the proportion of psychotic disorders documented in our work (35%) is slightly higher than that reported in the literature, whereas the

percentage of anxiety (21%) and mood disorders (7%) is lower. We also observed a positive correlation between the patients' Intelligence Quotient and the severity of the psychiatric features: indeed, psychosis was significantly more frequent in subjects with severe intellectual disability, whereas anxiety, mood and personality disorders were only diagnosed in patients with either borderline or normal cognitive level. Interestingly, our results show that a more severe psychiatric phenotype is likely to be observed in subjects without general medical co-morbidities, such as heart malformations and calcium metabolism alterations. One of the best-described neurological feature in 22q11.2DS is the predisposition to present acute symptomatic epileptic seizures due to antipsychotic drug use, recurrent infections and hypocalcaemia (Kao A et al., 2004; Cheung EN et al., 2014). In our population, 26% of the patients had had at least one seizure in their lifetime. A specific triggering factor was identified in some of these subjects, in keeping with published reports on acute symptomatic seizures commonly occurring in 22q11.2DS subjects.

As regards unprovoked seizures, the published data mainly concern pediatric patients. In two large pediatric 22q11.2DS populations, the overall prevalence of epilepsy was estimated at 6.9% and 15.2%, respectively (Kao A et al., 2004; Kim EH et al., 2016), whereas the prevalence of generalized genetic forms was 2% and 8.3%. In one of the few studies performed in adults with 22q11.2DS, the authors reported seizures in 15.8% of subjects, 28% of whom received a diagnosis of epilepsy (Whiter RG et al., 2017).

In our population, 12 patients (14%) were diagnosed with epilepsy. Nine of them were classified as having genetic generalized epilepsy. More specifically, in 5/9 the electro-clinical features appeared to be consistent with the diagnosis of juvenile myoclonic epilepsy. The characterization of the specific epileptic syndrome may be quite challenging in patients suffering from epilepsy and either neuropsychiatric or non neurological comorbidities. This condition, which some authors have defined as "epilepsy plus", might be related to several pathogenic rearrangements, as recently demonstrated (Coppola a et al., 2019). However, in our population the epilepsy onset in adolescence/young adulthood, the coexistence of generalized tonic-clonic seizures and myoclonic seizures (occurring on awakening in 4/5 subjects), along with the EEG pattern of generalized spike-and-wave (documented in 4/5 cases) were in line with the typical phenotype of JME. None of our patients was drug-resistant, although

antipsychotic medications clearly worsened the seizures in 4 cases. Interestingly, psychotic disorders were associated with epilepsy, whereas heart malformations were less frequent in this subgroup.

In view of the current dearth of EEG data in 22q11.2DS, we also decided to perform video-EEG monitoring in all our patients, both epileptic and non-epileptic ones, with the aim to evaluate the prevalence of EEG abnormalities and identify the most common patterns. Abnormalities of varying degrees were detected in many cases (40/85). In keeping with the predominant clinical phenotype, *i.e.* generalized seizures (myoclonic, GTCS), bilateral and synchronous abnormalities were the most common EEG pattern, observed in 33 patients: such abnormalities were clearly epileptiform in 7/33 subjects, 3 of whom were not even diagnosed with epilepsy.

Another emerging neurological aspect of 22q11.2DS patients is their risk of developing early Parkinson's disease. Indeed, although only sporadic cases or small populations have been described to date, clinical symptoms and signs, disease course, therapy response and neuropathological data support the hypothesis that 22q11.2DS patients are at risk of developing a classic form of Parkinson disease (Mok KY et al., 2016; Dufornet B et al., 2017; Butcher NJ et al., 2017b). The estimated prevalence of Parkinson's disease in industrialized countries is 0.3% in the general population, 1.0% in people older than 60 years, 3.0% in those aged 80 years and older and 1-5/10.000 in those with early-onset (age <50 years) Parkinson's disease (Lee A et al., 2016).

The neurological evaluation revealed parkinsonism in 40% of our patients, regardless of the concomitant antipsychotic therapy. However, the clinical features differed according to the presence/absence of a concomitant antipsychotic treatment. Indeed, asymmetric motor parkinsonian signs were more common in subjects who were not taking neuroleptics, while axial involvement and action tremor were mainly observed in those receiving anti-psychotic medications. However, quite unexpectedly, a family history of parkinsonism was frequently reported in the latter group (accounting for about 21% of the subjects). This interesting finding might suggest that neuroleptic drugs could facilitate, rather than cause, the emergence of parkinsonian signs in subjects with genetic susceptibility.

By reviewing the overall neurological features of our 22q11.2DS population, we also found that none of the patients with parkinsonian features (who were not on

antipsychotics) received a diagnosis of epilepsy. The inverse relationship between these two traits supports the hypothesis of a biological "incompatibility" between the two diseases: different genetic substrates might explain the predominant involvement of either cortical or subcortical networks (De Angelis G and Vizioli R, 1984).

Finally, another extremely interesting and novel result is that about 28% of our patients were left-handed and that 54% had relatives with the same trait: this is a rather remarkable finding, if we consider that the percentage of left-handed subjects in the general population is approximately 10% (Ockelenburga S et al., 2013).

After a detailed characterization of the neuropsychiatric phenotype, we collected the genetic data of a proportion of patients in order to detect and analyze some genetic features, as 22q11.2 deleted region length and/or the occurrence of additional potential causal CNVs elsewhere in the genome, which may influence the variability of the neuropsychiatric manifestations.

It is known that about 90% of 22q11.2 deletion patients have *de novo* onset, while in 6-28% of cases the syndrome shows an autosomal dominant inheritance pattern (McDonald-McGinn DM et al., 2015). In accordance with literature data, in our population most patients (88%) had a *de novo* deletion.

In over 90% of 22q11.2 DS patients, the "typical" region between LCR22-A and LCR22-D is hemizygously deleted (Hacihamdioglu B et al., 2015). In line with published data, the results of a-CGH in our study confirm that the most commonly deleted region is the "typical" one (in 69% of cases).

The critical region contains approximately 90 genes within a "typical" 3-Mb region (LCR22-A to LCR22-D) and 55 genes within a proximal 1.5-Mb region (Fernandez A ET AL., 2015; Guna A et al., 2015; Meechan DW et al., 2015). Intense investigations about the causative genes within the deleted region have been undertaken to elucidate the etiology of the syndrome.

Individuals with the LCR22-A to LCR22-B deletion show the full phenotypic spectrum, also seen in association with the typical LCR22-A to LCR22-D deletion, supporting the hypothesis that the key 22q11.2DS features are largely due to diminished LCR22-A to LCR22-B gene dosage (Michaelovsky E et al. 2012).

Today, there are limited data available on any role for the 22q deletion size in neurological and psychiatric features and outcomes. Whereas for neuropsychiatric features such as schizophrenia there are some studies that

reported these aspects for all 22q11.2 deletion extents, including so-called atypical or distal nested deletions, there aren't data for emerging neurologic features and key knowledge gaps for a lot genetic aspects remain, yet.

The a-CGH results in our population suggest that LCR22-A to LCR22-B deletion could play a key role in determining the neuropsychiatric features. Indeed, we observed that all patients with schizophrenia and epilepsy had "typical" LCR22-A to LCR22-D deletion or "minimal critical" LCR22-A to LCR22-B deletion. It is known that several behavioral disorders, including schizophrenia, are apparently more frequent in cases harboring an LCR22-A to LCR22-B deletion (Burnside RD, 2015). Furthermore, several genes within this "minimal critical" region, such as *COMT, PRODH, GNB1L, TBX1, SEPT5/GP1BB, ZDHHC8, PI4KA* and *ARVCF*, have been associated with schizophrenia and other psychiatric conditions (Hiroi N et al., 2013).

Considering that all our epileptic patients that performed a-CGH had a deletion including this "minimal critical" region, we supposed that the genes located within this region are implicated in epileptic networks as well. Over the last years, some of the genes involved in cortico-subcortical networks associated with genetic generalized epilepsies have been identified in the "minimal critical" 22q11.2 region (Gong S et al., 2003; Blumenfeld H, 2003). Moreover, several studies have reported the association between microdeletions (including 22q11.2) and GGE, especially in patients with neurodevelopmental disorders, including intellectual disability and schizophrenia (Lal D et al., 2015; Perez-Palma E et al., 2017). One of the genes that could play a critical role in epileptogenesis is *PRODH*. Indeed, hyperprolinemia (HP) is associated with two autosomal recessive metabolic disorders (HPI-HPII), due to a mutation of the *PRODH* gene. Both mild clinical phenotypes and severe conditions including neuropsychiatric disorders such as mental retardation, schizophrenia and epileptic syndromes have been described (Afenjar A et al., 2007; Di Rosa G et al., 2008). Recently, hyperprolinemia has been associated to heterozygous mutations of the *PRODH* gene, including a series of patients with 22q11.2 deletion. These data suggest that high proline levels may facilitate cortical hyperexcitability mechanisms involved in epileptogenesis.

In addition to this, most of our patients with parkinsonism also showed "typical" LCR22-A to LCR22-D 22q11.2 deletion. The proximal 22q11.2 deletion region does contain plausible candidate genes implicated in PD-related pathways. These

include miRNA miR-185, predicted to target *LRRK2* and *DGCR8*, a key gene in the biogenesis miRNAs, *SEPT5* that functionally interacts with Parkin2 and inhibits exocytosis of dopamine and glutamate, *COMT* and 6 mitochondrial genes (Butcher N et al., 2015).

Some evidence from simple model organisms shows that *PRODH* and *TXNRD2* could be involved in motor functioning (Guna A et al., 2015). Among these genes, *COMT* is one of the strongest candidate for PD because it encodes an enzyme that degrades dopamine, a catecholamine of extraordinary relevance in this movement disorder. In 22q11.2DS, the enzymatic activity of COMT is decreased, although its overall activity in the brain is quite difficult to determine given the presence of the membrane-bound COMT isoform (Bialecka M et al., 2004-2005). Chronic exposure to the neurotoxic properties of dopamine and its metabolites has been proposed to contribute to the pathogenesis of Parkinson's disease (Goldstein DS et al., 2013). Besides, impaired mitochondrial function in 22q11.2DS might also increase oxidative stress and the vulnerability to dopaminergic cell death (Exner N et al., 2012; Butcher NJ et al., 2017a).

However, individuals carrying the LCR22-A to LCR22-B deletion appear significantly heterogeneous as observed even in siblings who inherit the same deletion from a 22q11.2 deleted parent (Vergaelen E, et al. 2015). Haploinsufficiency of different genes cannot fully explain such remarkable clinical variability. Numerous mechanisms, involving factors outside the 22q deleted region (second-hit), stochastic modifying multigenic interactions as well as environmental processes could be crucial.

According to the recent literature data, CNVs are a substantial risk factor for a variety of neurodevelopment and psychiatric disorders such as autism, ADHD, schizophrenia, epilepsy, and intellectual disability (Kirov G et al., 2009; Cooper GM and Mefford HC, 2011; Vaishnavi V et al., 2013). Some CNVs may act in the same genetic pathway as 22q11.2 genes, and either exacerbate or suppress individual phenotypes. In autism spectrum disorder, aside from 22q11.2, five additional CNV loci: 1q21.1, 3q29, 7q11.23, 16p11.2, and 15q11.2–13, as well as multiple *de novo* gene variants, have been identified as risk factors (Williams HJ et al., 2013; Bassett AS et al., 2017).

Moreover, large CNVs including 22q11.2 deletion could have an important role on several genomic and epigenomic levels during the embryonal development. Their network effect principally consisting of chromatin regulation and long-range

chromosome contact has been considered one of the main responsible for neuropsychiatric phenotypes such as schizophrenia and autism spectrum disorders (Zhang X et al., 2018).

All these factors could act alone or in combination to influence specific cellular mechanisms playing a role in 22q11.2DS pathogenesis. However, no single mechanism underlying this phenotypic variability has been identified so far, and the molecular substrates are still debated.

Among the limited number of patients that performed whole genome a-CGH, six had a rare CNVs outside 22q11.2 deletion region. In all cases, other CNVs were associated with "typical" LCR22-A to LCR22-D 22q11.2 deletion. The small number of patients with other and heterogeneous potential causal CNVs did not allow us to make specific correlations with neuro-psychiatric features.

The present work has some limitations, which include the relatively small size of the study population, the challenging characterization of all the different phenotypical aspects in such a complex, multisystem condition, and the small number of available a-CGH due to travel limitations related to COVID-19 pandemic. These limitations prevented us from accurately investigating the phenotype/genotype correlation including differences in the 22q deletion region and the occurrence of others CNVs elsewhere in the genome that might be responsible for the neurologic or psychiatric phenotypic heterogeneity.

6. CONCLUSION

Our study confirms that not only cognitive impairment and psychiatric features as schizophrenia but also epilepsy, parkinsonism and left-handedness are peculiar features of 22q11.2 deletion syndrome. The most interesting findings that emerge, and that were obtained by analyzing possible correlations between all the neuropsychiatric features and medical comorbidities, include an inverse correlation between lower IQ/psychosis/epilepsy and major cardiac diseases, a direct association between psychosis and both mental delay and epilepsy, and an inverse correlation between parkinsonism and epilepsy. These results have been published in a recent paper (Fanella M. et al., 2020)

Referring to array-CGH genetic results, neuropsychiatric aspects appear to be part of the phenotype associated with the "typical" LCR22-A to LCR22-D deletion or "minimal critical" LCR22-A to LCR22-B deletion. This suggests that the relevant genes for neuropsychiatric manifestations could be located within the "minimal critical" region. Nevertheless, it is necessary to extend the array-CGH analysis to our entire cohort of patients in order to analyze: 1) differences in the 22q deletion region and 2) the occurrence of other CNVs elsewhere in the genome that might be responsible for the neurologic or psychiatric phenotypic heterogeneity among patients with 22q11.2DS.

In conclusion, the co-existence of various neurological and psychiatric features, ranging from epilepsy to parkinsonism and psychosis, suggests that 22q11.2DS extensively impairs the central nervous system by disrupting separate complex neural networks, which might be attributed to the underlying involvement of several genes within and outside the 22q11.2 deleted region.

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