Cytogenetic study of a large cohort of patients with corpus callosum abnormalities

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Introduction
Corpus callosum (CC) is the main fiber bundle connecting the brain interhemispheric white matter tracts. Abnormalities of CC may be isolated, or occur in association with other brain anomalies as a part of monogenic syndrome, with some inborn errors of metabolism or due to chromosomal abnormalities.

Aim
Detecting the prevalence and characteristics of chromosomal aberrations among patients with CC abnormalities in addition to determining the subsequent imperative diagnostic steps in order to reach a precise diagnosis.

Patients and methods
A total of 105 patients have been enrolled into this study. All patients were subjected to full history taking, thorough clinical examination, neuroimaging studies, psychological assessments, and cytogenetic studies including G-banding karyotyping and fluorescence in-situ hybridization analysis.

Results
The vast majority of the studied patients presented with developmental delay (74%). Intellectual disability was demonstrated in 50.4%. Dysmorphic features and microcephaly have been detected in 34% and 28%, respectively. According to MRI findings, the patients have been categorized into four groups: agenesis, hypoplasia, dysplasia, and dysplasia with hypoplasia of the CC. The category of hypoplasia of CC comprised 54.3% of the entire patients. Parental consanguinity was detected among 40% of the studied patients, suggesting the role of autosomal recessive genes. Cytogenetic abnormalities have been detected in 22% of the studied cases and parental chromosomal abnormalities have been detected in 13% of them.

Conclusion
Detailed cytogenetic analysis of patients with CC abnormalities as well as their parents is mandatory in the plan of diagnosis and for setting appropriate genetic counseling for families of these patients. The use of chromosomal microarray analysis is an essential future step to precisely characterize areas of chromosomal gains and losses in addition to the detection of novel copy number variants.

Keywords:
chromosomal abnormalities, corpus callosum, developmental delay, fluorescence in-situ hybridization, intellectual disability

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Introduction

Corpus callosum (CC) is the main fiber bundle connecting brain interhemispheric white matter tracts. It interconnects homologous regions of the two hemispheres as a commissure and is consisted of 200 million topographically organized axons (Wahl *et al.*, 2007). CC has a fundamental contribution in moderating different cognitive functions (Paul *et al.*, 2007).

The size of the CC is determined by the number and size of its constituent axons, degree of myelination, packing density, vasculature, and extravascular fluid (Paul *et al.*, 2011). The number of callosal fibers is mainly determined at birth. However, structural changes continue throughout development and are marked through the childhood and adolescence periods (Luo and O'Leary, 2005; Luders *et al.*, 2010; Garel *et al.*, 2011).

Agenesis of the corpus callosum (ACC) is among the most frequent human brain anomalies. On the basis of neonatal and prenatal imaging studies, ACC occurs

in at least 1 : 4000 live births. Approximately 2–5% of patients assessed for neurodevelopmental disorders show ACC (Guillem *et al.*, 2003; Wang *et al.*, 2004).

CC abnormalities are clinically and genetically heterogeneous conditions which may be isolated or more commonly associated with genetic syndromes or metabolic–genetic disorders (Talisetti *et al.*, 2003; Schell-Apacik *et al.*, 2008; Tang *et al.*, 2009). Less commonly they can result from antenatal infections, vascular or toxic insults (Wozniak *et al.*, 2009).

Among the affected patients, there is a considerable variation in cognitive and neurological consequences, ranging from mild behavioral disturbance to a wide range of neurological deficit with variable degrees of severity (Lau *et al.*, 2013). Developmental delay and intellectual disability are usually prominent features associated with CC abnormalities (Doherty *et al.*, 2006; Schell-Apacik *et al.*, 2008). In addition, they have a major risk for developing autism spectrum disorders (Paul *et al.*, 2014).

The genetic causes of CC abnormalities include chromosomal rearrangements or recognizable genetic syndromes caused by single-gene mutations (Bedeschi *et al.*, 2006; Schell-Apacik *et al.*, 2008; Al Hashim *et al.*, 2016). Several consistent chromosomal rearrangements were previously documented including del(4p16) or Wolf-Hirschhorn syndrome, 22q11.2 deletion syndrome, supernumerary marker 15, ring chromosome 14, duplications and deletions involving 8p, del(6q25), 21q22.1-22.3 deletions, and deletions involving Xp22.3 and Xq13-q21 (Kleczkowska *et al.*, 1987; Pirola *et al.*, 1998; Gentile *et al.*, 2003; Ramocki *et al.*, 2003; Talisetti *et al.*, 2003; Yao *et al.*, 2006; Schell-Apacik *et al.*, 2008).

In the current research, we studied a large cohort of patients presented with various phenotypes associated with CC malformations in an attempt to determine the prevalence and characteristics of chromosomal abnormalities among these patients, in addition to determining the subsequent imperative diagnostic steps to reach a precise diagnosis which allows for proper management and proper genetic counseling.

Patients and methods

Patients

A group of 105 patients have been enrolled into the current study through a period of 3 years from 2016 to 2019.

Inclusion criteria was based on the results of MRI as the study comprised patients presented with various manifestations associated with CC malformations. Patients with secondary causes for CC abnormalities as a result of degeneration, atrophy, or metabolic cause have been excluded from the study.

Cases were selected from the Clinical Genetics Department Clinics, The National Research Centre. An informed consent was taken from all legal guardians according to the guidelines and approval of the Medical Research Ethics Committee of the National Research Centre.

Methods

All patients were subjected to the following:

(1) Full history taking with laying stress on prenatal, natal, and postnatal history, including exposure to environmental agents, for example, infections, pesticides, cigarette smoking, or any maternal chronic medical condition as well as history of any previous child with similar condition. A family pedigree was constructed for each case with at least three generations. Neonatal and infancy history were taken with stressing on apneic spells, abnormal breathing pattern, feeding problems, abnormal eye movements, dyskinesia, and seizures including onset type and medications for control. Developmental history included physical and mental milestones
(2) Clinical examination: a thorough clinical examination has been done particularly neurological evaluation including gait, eye movement (oculomotor apraxia), nystagmus, abnormal movements (such as fasciculation, extrapyramidal manifestations), wasting, muscle power, tone, reflexes, and sensation.

Neuroimaging studies

MRI for the brain was our clue for selecting the patients. Indications for imaging are delayed physical and mental milestones, epilepsy, and microcephaly. Interpretation of all MRI studies have been reviewed with particular attention to the degree of callosal abnormality, presence of the interhemispheric cyst, Probst bundle, other commissural fibers, ventriculomegaly, and the presence and type of associated malformations.

Abnormalities of CC have been categorized according to Hanna *et al.* (2011) into total agenesis, hypoplasia, dysplasia, and dysplasia with hypoplasia. The association with central nervous system malformations was registered.

Other investigations, for example, echocardiography, abdominal ultrasound, neurophysiological studies, and laboratory tests have been done as indicated for each individual case.

Psychological assessment

Assessment of intellectual function and social adaptation: using Wechsler Intelligence Scales for Preschool Children (Wechsler, 2002). The scale comprised subtests for verbal and performance intelligence.

Assessment of behavioral disorders: using Revised Behavioral Problem Checklist for Children (Quay and Peterson, 1993). The six Revised Behavioral Problem Checklist subscales measure conduct disorder, socialized aggression, attention problems – immaturity, anxiety – withdrawal, psychotic behavior, and motor tension-excess.

Cytogenetic studies

A sample of venous blood (3–3.5 ml) was taken from the family trio as well as the siblings whenever required under aseptic conditions into a sterile heparin-coated vacutainer.

Conventional cytogenetic analysis using the G-banding technique have been performed according to Verma and Babu (1995). Twenty-five metaphases have been analyzed and karyotyped for each enrolled individual. Karyotype description followed the International System for Human Cytogenetic Nomenclature (ISCN, 2016) recommendations. ISCN,2016 is an abbreviation that should be remain as it is, and the reference should be added after the word recommendations (Mcgowan-Jordan *et al*., 2016)

Further characterization of cytogenetic abnormalities was done using the fluorescence in-situ hybridization (FISH) technique for precise detection of breakpoints, translocations, marker chromosomes, and identification of microdeletions. The technique was carried out according to the modification of Pinkle *et al.* (1986) on peripheral blood metaphases and interphase lymphocytes. According to each case, specific DNA probes in addition to DAPI II counterstain (4,6-diamidino-2-phenylindole dihydrochloride) have been used. The DNA probes were used according to the manufacturer's instructions and included centromere-specific probes, locus-specific and whole chromosome painting probes (Cytocell FISH probes; Oxford Gene Technology, The Molecular Genetics Company, Cambridge, UK). At least 100 cells were scanned in every case and analyzed using Zeiss Axio Plan Microscope (Zeiss, Le Pecq, France). Images acquisitions were performed using a CCD camera and analyzed using the In Situ Imaging System program (MetaSystems, Altlussheim, Germany).

Results

A total of 105 patients have been studied throughout a 3-year period from 2016 to 2019. They were 56 men and 49 women with a percentage of 53.3 and 46.7%, respectively. Their ages ranged from 8 days to 17 years with a mean age of 4.5 years. History taking revealed 40% prevalence of consanguinity among patients' parents.

Positive family history has been detected in 29.5% of the studied cases. The majority of our patients presented with developmental delay representing 74%. Assessment of intellectual function and social adaptation revealed the presence of intellectual disability in 50.4% and dysmorphic features have been detected in 34%. Abnormal head circumference in the form of microcephaly has been detected in 28% of all cases. Epilepsy has been clinically diagnosed in 11 (10.4%) of the cases. Among patients who underwent EEG evaluation, 39% showed epileptogenic abnormalities, while 46% showed other brain anomalies in addition to CC abnormalities. Other associated signs including macrocephaly, nystagmus, short stature, premature closure of cranial sutures, precocious puberty, and cyanosis have been detected in sporadic cases (Tables 1 and 2). According to MRI findings our patients have been categorized into four groups based on the CC morphological abnormalities previously verified in the MRI. These groups comprise complete ACC, hypoplastic corpus callosum (HCC), dysplastic CC, and hypoplasia with dysplastic CC.

Fifty-seven (54.3%) cases are confined to the category of HCC, while the other forms represent collectively 53 (45.7%) cases (Fig. 1 and Table 3). Cytogenetic studies revealed chromosomal abnormality in 23 cases (Figs 2–7), which represents 22% of the entire studied cases. Among cases that exhibited chromosomal abnormalities 15 (68%) cases showed HCC, six (27.2%) cases showed ACC, and one (4.5%) case was HCC with dysplasia of the CC. G-banded karyotype revealed add chromatin material in six cases, supernumerary marker chromosome in six

Table 1 Collective data from the history and clinical examination of the entire 105 patients

Finding	Family history [*n* (%)]	Consanguinity [*n* (%)]	Developmental delay [*n* (%)]	Intellectual disability [*n* (%)]	Dysmorphic features [*n* (%)]	Abnormal head circumference [*n* (%)]	Congenital heart disease [*n* (%)]	Seizures [*n* (%)]	Autistic behavior [*n* (%)]
Value and percentage of positive cases	31 (29.5)	42 (40)	78 (74)	53 (50.4)	36 (34)	29 (28) Microcephaly	11 (10.4)	11 (10.4)	9 (8.5)

Table 2 Clinical data and investigations of the patients who showed cytogenetic abnormalities

Patient no.	Age (Y:M)	Sex	Cardinal clinical features	MRI findings	Other relevant investing
1	3:00	F	Premature closure of cranial sutures, precocious puberty, MIC	ACC	EEG: generalized epileptogenic dysfunction
2	2:5	M	Developmental delay, dysmorphic features, MIC	ACC	EEG: normal
3	1:2	M	Developmental delay, MIC, dysmorphic features, autistic features	HCC	EEG: epileptogenic focus
4	1:00	M	Developmental delay	HCC, mild cortical atrophy	EEG: epileptogenic focus
5	3:5	F	Dysmorphic features, fits	ACC	EEG: epileptogenic focus
6	2:00	F	ID, Developmental delay	ACC	-
7	00:9	M	GDD, preauricular tag, plagiocephaly	HCC, cerebellar hypoplasia, CVH	-
8	00:10	F	GDD, short stature, CHD	HCC, DWM	Echo: pulmonary stenosis
9	4:00	F	ID, psychomotor delay , dysmorphic features , autistic behavior, fits	HCC, WM changes	-
10	00:6	F	GDD, bilateral coloboma, microphthalmia	HCC	Echo: congenital bicuspid aortic valve, supra aortic aneurysm
11	1:3	F	GDD, glaucoma, dysmorphic features	HCC, CVH, abnormal cortex	Echo: mild cardiomyopathy
12	2:5	M	GDD, CHD	HCC	-
13	00:4	M	GDD, dysmorphic features	HCC	-
14	00:3	M	Dysmorphic features , MCA, GDD, CHD, MIC	HCC	EEG: normal
15	7:6	F	Psychomotor delay, dysmorphic features	HCC	-
16	2:3	F	GDD, ID	ACC	-
17	5:6	M	ID, developmental delay	HCC	EEG: generalized foci
18	2:5	M	GDD, dysmorphic features , bilateral cleft lip, arachnodactyly	ACC	-
19	2:5	M	GDD, dysmorphic features	HCC, cortical atrophy	-
20	00:10	M	Developmental delay, autistic features, dysmorphic features, MIC	HCC	EEG: epileptogenic focus
21	3:5	M	Psychomotor delay, autistic features, ID, dysmorphic features	HCC	-
22	6:5	F	Psychomotor delay, dysmorphic features	HCC	-
23	2:00	F	Developmental delay, webbing of neck	ACC	-

ACC, agenesis of the corpus callosum; CHD, congenital heart disease; CVH, cerebellar vermian hypoplasia; DWM, demyelinating white matter disease; EEG, electroencephalogram; F, female; GDD, global developmental disability; HCC, hypoplasia of the corpus callosum; ID, intellectual disability; M, male; MCA, multiple congenital anomalies; MIC, microcephaly; WM, white matter; Y : M, year : month.

Table 3 Classification of all patients according to the corpus callosum abnormalities based on MRI findings

Group	Corpus callosum abnormality	Number and percentage of affected patients
Group I	Agenesis of the corpus callosum	24 (22.8%) patients
Group II	Hypoplasia of the corpus callosum	57 (54.3%) patients
Group III	Dysplasia of the corpus callosum	14 (13.3%) patients
Group IV	Hypoplasia with dysplasia of the corpus callosum	10 (9.5%) patients

cases, partial monosomy in three cases, chromosomal translocation in five cases, chromosomal aneuploidy in two cases, in addition to ring chromosome in a single case. FISH results were conclusive for some cases, while others require further delineation using different FISH probes or other molecular cytogenetic tools. Parental chromosomal abnormalities have been detected in 13% of patients who revealed cytogenetic abnormalities (patients no. 6, 9, 17) in the form of balanced translocation (Figs 3, 4 and Table 4).

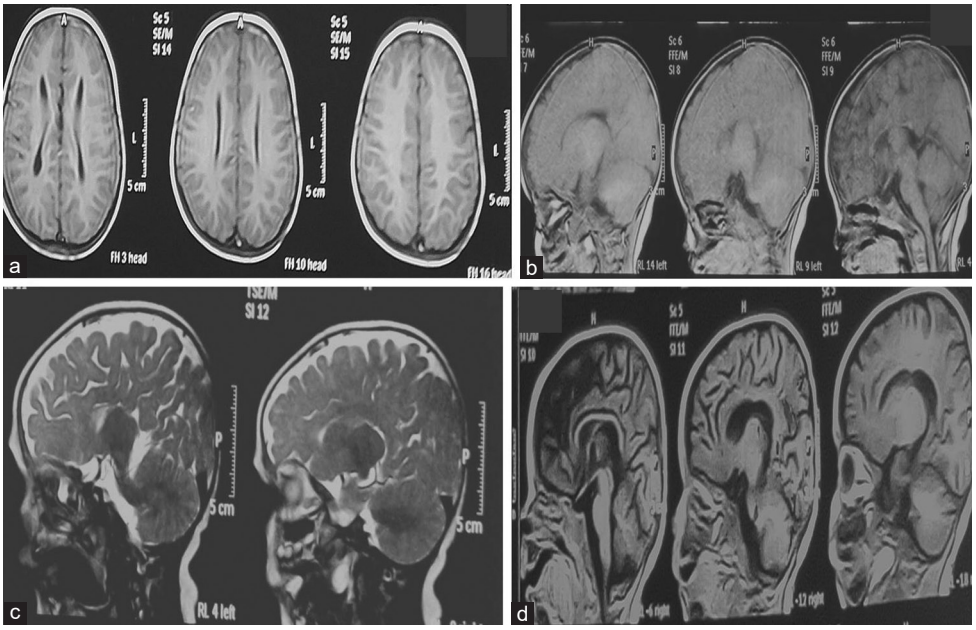
Discussion

Abnormalities of the CC may be either an isolated anomaly or occur in association with other neuroanatomical lesions and/or congenital anomalies, and have been recorded with different genetic causes. Neuropsychological outcome varies considerably from normal to profound intellectual disability depending on the etiology (Palmer and Mowat, 2014).

Detection of CC abnormalities should be carefully clinically assessed in order to determine and manage the underlying condition. It is clearly recognizable that genetic factors contribute to anomalies of the CC in the majority of cases (Vigdorovich *et al.*, 2020).

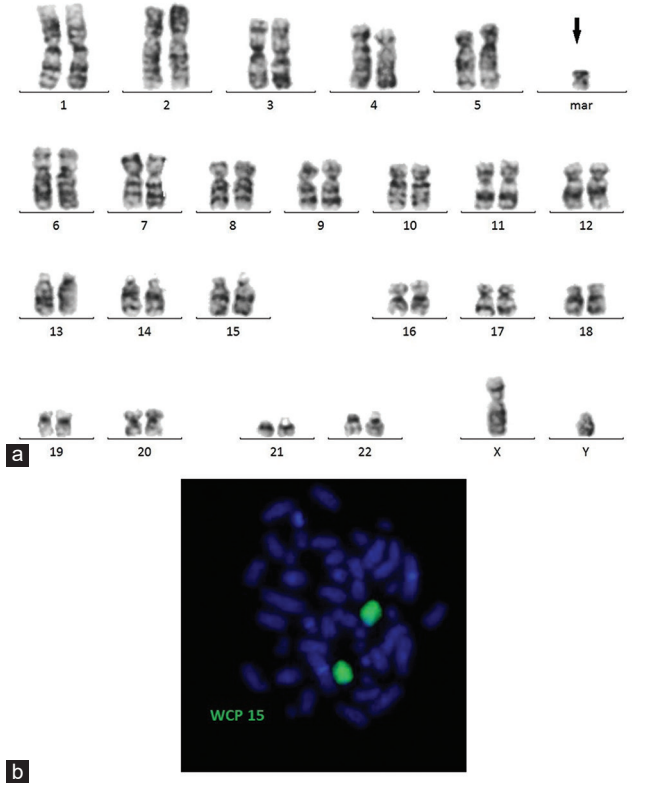
In this study we have studied 105 patients with different forms of CC abnormalities based on neuroradiological

Figure 1



MRI of the brain of different patients showing: (a) parallel lateral ventricles denoting agenesis of the corpus callosum (ACC) (patient no. 5); (b and c) ACC (patient no. 36, 47, respectively); (d) hypoplasia of the corpus callosum (HCC) (patient no. 59).

Figure 2



(a) G-banded karyotype of male patient no. 4 showing 47,XY,+mar. (b) FISH on a blood metaphase of the same patient using whole chromosome painting probe for chromosome 15. The figure showed that the marker is not derived from chromosome 15. FISH, fluorescence in-situ hybridization.

findings. They were 56 men and 49 women, their ages ranged from 8 days to 17 years with a mean age of 4.5 years.

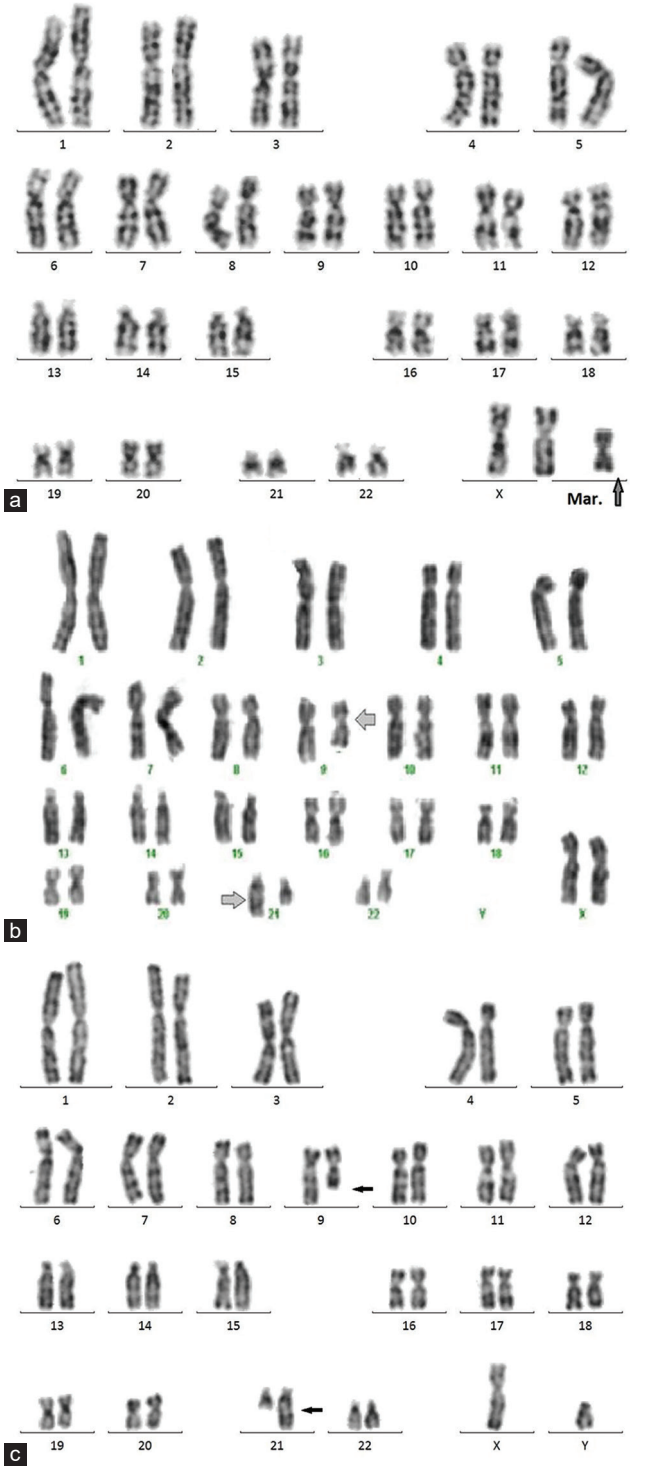
Parental consanguinity has been detected in 37% of our patients apart from patients with chromosomal abnormalities, which suggests an autosomal recessive pattern of inheritance in some cases. This was in accordance to Issa *et al.* (2018) who reported positive consanguinity among 53.1% of the 64 studied patients with CC abnormalities. According to Temtamy and Aglan (2012), consanguinity rate in the general Egyptian population was 30% throughout the last four decades. Hence, we have reported a higher parental consanguinity rate among patients with CC abnormalities compared with the general population in Egypt.

In accordance to Hanna *et al.* (2011), our patients have been categorized into four groups based on the CC morphological abnormalities previously verified in the MRI. These groups comprise complete ACC, HCC, dysplastic CC, and hypoplasia with dysplasia of the CC (Fig. 1).

The present study revealed that developmental delay was the prevailing presentation among 75% of the category of ACC and 74% of all the studied cases. These results were in conformity with the research study of Doherty *et al.* (2006) who reported that 80.7% of patients with ACC had a significant developmental delay.

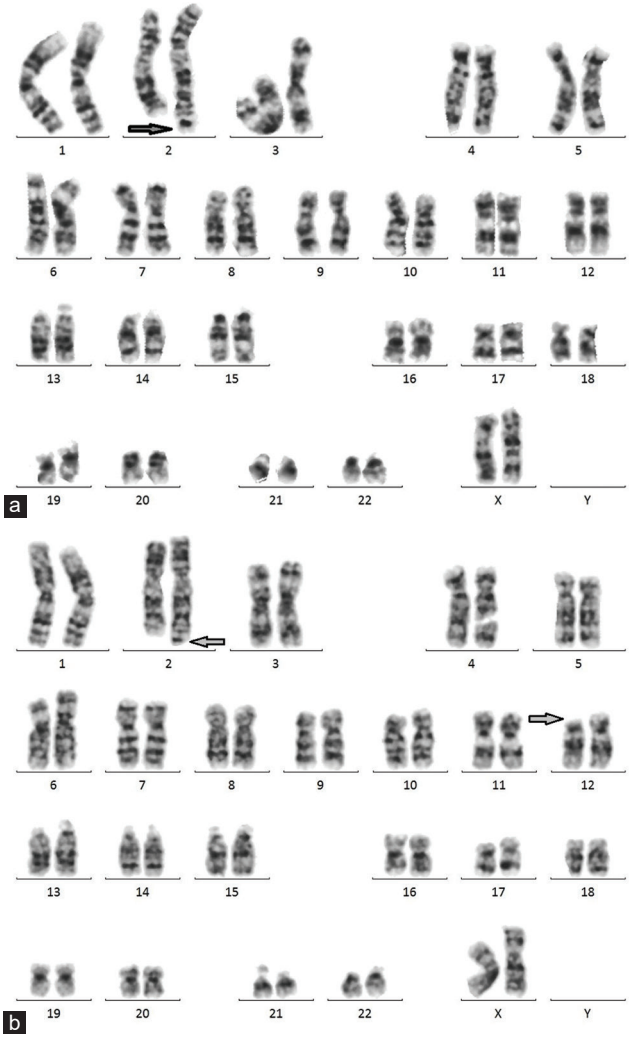
Schell-Apacik *et al.* (2008) reported intellectual disability in 60% of patients in their study and emphasized that even in the absence of intellectual disability, mild behavioral or social problems have

Figure 3



(a) G-banded karyotype of female patient no. 6 showing 47,XX,+mar; (b) G-banded karyotype of the mother of patient no. 6 showing 46,XX, t(9;21)(q22;q22); and (c) G-banded karyotype of the normal male sibling showing 46,XY,t(9;21)(q22;q22).

Figure 4



(a) G-banded karyotype of female patient no. 9 showing 46,XX,add(2)(q); (b) G-banded karyotype of the mother of patient no. 9 showing balanced translocation; 46,XX,t(2;12)(q37;p11).

been described. Results of our study were analogous to this research and showed a percentage of 50.4% in all patients.

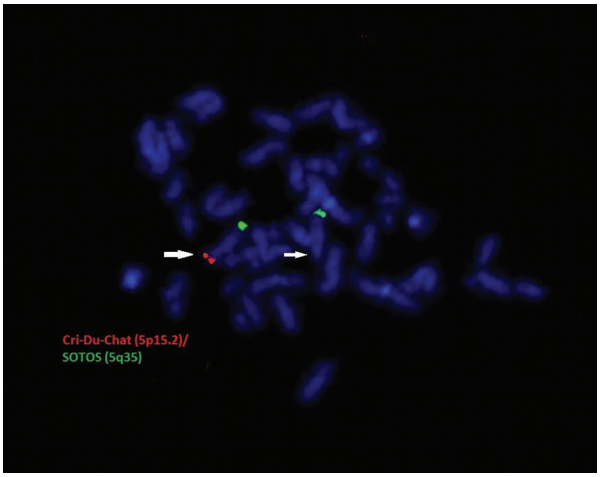
Our study showed autistic behavior in nine patients which represents 8.5% of all cases with CC abnormality and 38% of cases with ACC. These findings are in accordance with Paul *et al.* (2007) who found that individuals with ACC exhibit several cognitive and clinical characteristics that are similar to the ones observed in autism.

Al Hashim *et al.* (2016) found that dysmorphic features were present in 61% of the studied patients and this is nearly similar to our study that reported a percentage of 74% of the studied patients.

It is clearly obvious in our study that patients with CC abnormalities mainly presented with developmental delay and intellectual disability followed by dysmorphic features.

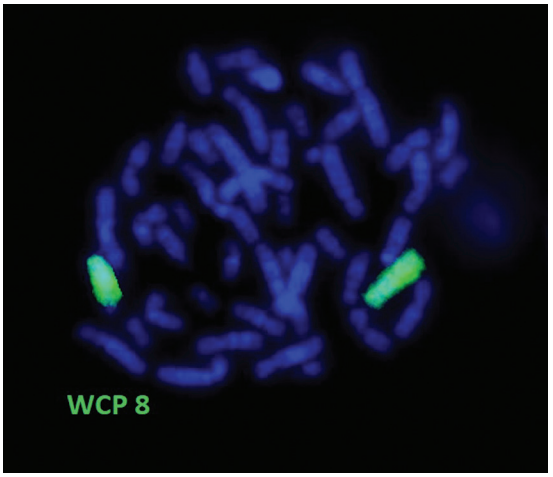
Regarding cytogenetic studies, chromosomal abnormalities have been detected in 23 patients representing 22% of all cases, while they were 11.3 and 15.8% in the studies done by Santos *et al.* (2002) and Bedeschi *et al.* (2006), respectively. Another

Figure 5



FISH on a blood metaphase of patient no. 11 shows deletion of the cri-du-chat syndrome locus (red signal). SOTOS locus (green signals) is used as control. FISH, fluorescence in-situ hybridization.

Figure 6



FISH on a blood metaphase of patient no. 12 using whole chromosome painting probe for chromosome 8. The figure showed an added chromatin material which is derived from chromosome 8. FISH, fluorescence in-situ hybridization.

recent study performed by Stoll *et al.* (2019) showed cytogenetic abnormalities in 16.2% of cases. These variances could be attributed to the difference in samples size as our study included 105 cases while the previously mentioned studies were confined to 20, 63, and 99 cases, respectively. Hence, we could notice that the percentage of chromosomal abnormalities increases proportionally with increasing the number of the studied cases.

We have studied a female infant who revealed 47,XX,+13 by G-banded karyotype (case 10, Tables 2 and 4). Trisomy 13, 18, and 21 are the only known autosomal trisomies where the fetus can survive postnatal, which can be ascribed to the lower gene dosage of these three chromosomes compared with other autosomes (Nussbaum *et al.*, 2016). Although patients with trisomy 21 could survive thereafter, patients with trisomy 13 and 18 rarely do so (Alberman *et al.*, 2012). Our patient was 6-month old and has been presented with global developmental disability, bilateral coloboma, and microphthalmia, and had shown HCC by MRI, while echocardiography revealed congenital bicuspid aortic valve and supra aortic aneurysm. Trisomy 13 patients exhibit multiple malformations affecting various systems as the central nervous system, including the CC, cardiac, and urogenital system. It was reported that about 90% of live-born patients could not survive beyond the first year of life (Rasmussen *et al.*, 2003). Longer survival could be ascribed to the presence of mosaicism or in patients with trisomy 13 translocations; however, no clear association was noticed between the phenotypic severity and mosaicism level (Rasmussen *et al.*, 2003; Hsu and Hou, 2007).

Peroos *et al.* (2012) reported an 8-year-old girl child with nonmosaic trisomy 13 and severe phenotypic abnormalities including brain anomalies with ACC and epilepsy. They attributed her survival to the normal cardiac development, revealed by echocardiography.

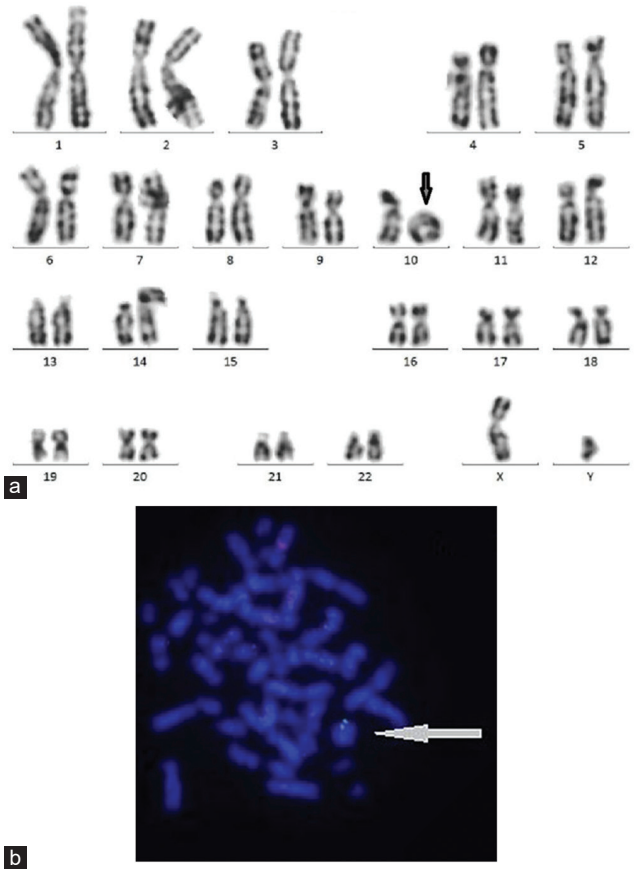
Although our patient did not exhibit blood mosaicism, tissue-specific mosaicism or a cryptic deletion in the extra copy of chromosome 13 could be a potential cause of postnatal survival.

The latter assumption could be verified through performing chromosomal microarray study to detect the existence of chromosomal gains and losses.

The present study detected a supernumerary marker chromosome in six patients, four men (47,XY,+mar) (Fig. 2) and two women (47,XX,+mar). MRI showed HCC in patients no. 4 and 7, while patients no. 2, 5, and 6 exhibited ACC (Fig. 1). Patient no. 3 showed hypoplasia with dysplasia of the CC. FISH analysis of patients no. 2, 5, and 7 revealed that the marker was derived from chromosome 15. Cytogenetic results of these three cases were the most prevalent among other cases with cytogenetic abnormalities in our study and this could enhance the same postulation of Jovanović-Privrodski *et al.* (2009), who correlate marker chromosome 15 with CC abnormalities. The results of their study augmented the hypotheses that extra copies of different regions of proximal 15q are related to malformations of the CC. It was demonstrated that the origin of the supernumerary marker chromosome originates from acrocentric chromosomes, especially chromosome 15 (in 65%), while in only 7% it is derived from chromosomes 13, 14, 21, or 22 (Buckton *et al.*, 1985; Liehr *et al.*, 2006).

The study of the family of patient no. 6 showed that the affected female sibling had the same marker

Figure 7



(a) G-banded karyotype of patient no. 19 showing ring chromosome 10. (b) FISH on a blood metaphase of the same patient using subtelomere 10p (green) and 10q (red) mix showing ring 10 with deletion of 10q and duplicated signal of 10p subtelomeres. FISH, fluorescence in-situ hybridization.

chromosome, while the mother showed a balanced translocation: 46,XX,t(9;21)(q22;q22). The same balanced translocation was also detected in the clinically normal male sibling: 46,XY,t(9;21)(q22;q22). By performing FISH analysis for both affected female patients, the marker was confirmed to be derived from translocation between chromosomes 9 and 21. This occurred as a result of maternal meiotic segregation with one of the two derivates of the translocation chromosome and the other normal chromosomes being passed to the same gamete resulting in trisomy for the chromosomal segments in the marker (Fig. 3). These findings reflect the significance of cytogenetic analysis for parents of patients with chromosomal abnormalities and its valuable role in genetic counseling. FISH of patient no. 3 and 4 showed that the marker was not derived from chromosome 15 (Table 4). The marker in both patients was de novo as the parent's karyotypes were normal. Supernumerary marker chromosomes are de novo in about 70% of the cases, while it is either inherited from the mother in 20% or the father in 10% (Jafari-Ghahfarokhi *et al.*, 2015).

Patient no. 9 was a female patient presenting with intellectual disability, dysmorphic features, and psychomotor delay. GTG banded karyotype showed 46,XX,add(2)(q37) (Fig. 4a). FISH analysis showed that the added chromatin material was not derived from chromosome 2. Maternal karyotype showed a balanced translocation: 46,XX,t(2;12)(q37;p11) (Fig. 4b), demonstrating that abnormal chromosome 2 in the proband's karyotype was derived from 2q37;12p11 translocation, leading to 12p trisomy. 12p duplication could arise as a de novo abnormality or more frequently as a result of parental balanced translocation (Oliveira *et al.*, 2020). However, it is a rare finding characterized phenotypically by dysmorphic features, multiple congenital anomalies, intellectual disability with variable degrees of psychomotor retardation (Hung *et al.*, 2012; Poirsier *et al.*, 2014). Besides, our patient exhibited frequent fits and showed HCC by MRI. Few patients with 12p duplication may present with neurodevelopmental abnormalities (Oliveira *et al.*, 2020). Despite resemblance of our patient phenotype with that previously described with cases of 12p duplication, we could not construct the final diagnosis before performing chromosomal microarray study.

Partial monosomy 9p is a rare condition, which is characterized by trigonocephaly, facial dysmorphism, and developmental delay. Neuroradiological aspects of this syndrome have not yet been fully described. A HCC or dysplastic CC and a diffuse white matter hypoplasia were present in more than half of patients of 9p deletion syndrome studied by Spazzapan *et al.* (2016). Similarly, we have perceived two patients (no. 15 and 22) who showed 9p deletion by both G-banding and FISH techniques. MRI for the two patients showed HCC.

A female patient (no. 11) with cri-du-chat syndrome has been enrolled in our study and the diagnosis was confirmed by both G-banding and FISH techniques (Fig. 5). The patient was presenting with global developmental delay and dysmorphic features and the brain MRI showed HCC. Similarly, Nandhagopal and Udayakumar (2014) studied a female patient with cri-du-chat syndrome, demonstrating ACC and pontine hypoplasia. Although pontine hypoplasia is a common finding in cases of cri-du-chat syndrome, CC abnormalities have been rarely reported in these cases.

No. 12 was a male patient presenting with developmental delay, congenital heart disease, and showed HCC by brain MRI. Karyotype analysis revealed 46,XY,add(8)(p). FISH analysis confirmed that the additive material was derived from chromosome 8 (Fig. 6). García-Santiago *et al.* (2015) reported seven patients with inversion duplication 8p associated with deletion of the

Table 4 Abnormal cytogenetic findings in relation to corpus callosum abnormalities

Patient no.	Corpus callosum abnormality	Abnormal karyotype	FISH
1	ACC	46,XX,add(4)(q35)	The added chromatin material is not derived from chromosome 4
2	ACC	47,XY,+mar	The marker is derived from chromosome 15
3	HCC with dysplasia of corpus callosum	47,XY,+mar	The marker is not derived from chromosome 15
4	HCC	47,XY,+mar	The marker is not derived from chromosome 15
5	ACC	47,XX,+mar	The marker is derived from chromosome 15
6	ACC	47,XX,+mar	The marker is derived from chromosome 9 and 21 as a result of maternal balanced translocation between the two chromosomes
7	HCC	47,XY,+mar	The marker is derived from chromosome 15
8	HCC	46,XX,add(15)(q)	The added chromatin material is not derived from chromosome 15
9	HCC	46,XX,add(2)(q)	The added chromatin material is not derived from chromosome 2. Maternal karyotype showed balanced translocation; 46,XX,t(2;12)
10	HCC	47,XX,+13	NP
11	HCC	46,XX, del (5)(p13)	Deletion of cri-du-chat locus
12	HCC	46,XY, add (8)(p)	The added chromatin material is derived from chromosome 8
13	HCC	46,XY,t(1;4)	NP
14	HCC	46,XY, add (14)(q).	The added chromatin material is not derived from chromosome 14
15	HCC	46,XX, del (9) (p24.3p22.17)	Deletion of 9p subtelomere
16	ACC	46,XX,add(1)(q44)	The added chromatin material is not derived from chromosome 1 and there is a deletion of 1q subtelomere
17	HCC	46,XY,t(1p; 9q)	Translocation between chromosomes 1 and 9 has been confirmed and maternal karyotype showed a balanced translocation between the two chromosomes
18	ACC	46,XY,t(2;6)(p24;p15)	NP
19	HCC	46,XY,r(10)	Deletion of 10q subtelomere and duplication of 10p subtelomere
20	HCC	46,XY,t(1q;13;22)	NP
21	HCC	46,XY,t(7;15)(q21;q21)	Translocation between Ch. 7 and 15 has been confirmed
22	HCC	46,XX, del (9)(p24.3p21)	Deletion of 9p subtelomere
23	ACC	45,X	NP

ACC, agenesis of corpus callosum; FISH, fluorescence in-situ hybridization; HCC, hypoplasia of corpus callosum; NP, FISH has not been performed.

short arm of chromosome 8p11.2-p22 region. Among the studied patients, three cases had ACC and a single case had HCC. They reported that most of these cases are associated with CNS malformations and structural cardiac abnormalities. These findings were similar to ours which suggest a strong genotype–phenotype correlation.

Another 3-month-old male patient with HCC (no. 14) showed 46,XY,add(14)(q) karyotype. He presented with dysmorphic features, global developmental disability, microcephaly, and congenital heart disease, while EEG showed normal results. FISH revealed that the added material was not derived from chromosome 14. Unfortunately, we could not get a sample from the parents to perform karyotyping. When feasible, chromosomal microarray study would be of great help to allow precise detection of copy number deletion or duplication.

A male patient presenting with developmental delay and dysmorphic features (no. 19) showed a karyotype with ring chromosome 10 (Fig. 7). FISH analysis revealed deletion of 10q subtelomere and duplication of 10p subtelomere. The data from the literature shows that there are no specific clinical findings to define r(10) syndrome. However, distal deletion of the long arm of chromosome 10 is associated with craniofacial dysmorphism, microcephaly, delayed developmental milestones, intellectual disability, behavioral abnormalities, ocular, urogenital, and limb abnormalities (Guilherme *et al.*, 2013).

Among our studied cases, we have encountered a female patient (no. 23) who presented at the age of 2 years with developmental delay and webbed neck. ACC was evident in the MRI and chromosomal analysis in addition to clinical picture showed Turner syndrome: 45,X. Lee *et al.* (2008) have reported a case of a female patient with Turner syndrome who showed

hypertelorism, small jaw, short webbed neck as well as ACC by MRI. They declared that ACC is rarely associated with cases of Turner syndrome.

Conclusion

CC abnormalities could be simple or serious neurological insult that has many behavioral, cognitive, and neurological consequences. Autosomal recessive pattern of inheritance among patients with CC abnormalities is suggested as a cause since parental consanguinity has been detected in a relatively high percentage in the studied cohort. Developmental delay, intellectual disability, and dysmorphic features are common manifestations associated with CC abnormalities followed by other somatic abnormalities. MRI showed HCC in most of our patients (54.3%) that may be mild and missed in computed tomography brain, so MRI is the cornerstone for diagnosing and delineating CC abnormalities. Chromosomal analysis should be done on a routine basis during investigating these cases as our study found that chromosomal aberrations were prevailing in 22%. When feasible, the use of chromosomal microarray analysis can significantly increase the diagnostic yield in patients with different brain anomalies and allows for the detection of novel copy number variants and novel associated genes throughout the genome.

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Conflicts of interest

There are no conflicts of interest.

References

Al Hashim AH, Blaser S, Raybaud C, MacGregor D (2016). Corpus callosum abnormalities: neuroradiological and clinical correlations. *Dev Med Child Neurol* **58**:475–484.

Alberman E, Mutton D, Morris JK (2012). Cytological and epidemiological findings in trisomies 13, 18, and 21: England and Wales 2004-2009. *Am J Med Genet A* **158A**:1145–50.

Bedeschi MF, Bonaglia MC, Grasso R, Pellegri A, Garghentino RR, Battaglia MA, *et al.* (2006). Agenesis of the corpus callosum: clinical and genetic study in 63 young patients. *Pediatr Neurol* **34**:186–193.

Buckton KE, Spowart G, Newton MS , Evans HJ (1985). Forty-four probands with an additional 'marker' chromosome. *Hum Genet* **69**:353–370.

Doherty D, Tu S, Schilmoeller K, Schilmoeller G (2006). Health-related issues in individuals with agenesis of the corpus callosum. *Child Care Health Dev* **32**:333–342.

García-Santiago FA, MartínezGlez V, Santos F, GarcíaMiñaur S, Mansilla E, Meneses AG, *et al.* (2015). Analysis of invdupdel (8p) rearrangement: clinical, cytogenetic and molecular characterization. *Am J Med Genet* **A167**:1018–1025.

Garel C, Cont I, Alberti C, Josserand E, Moutard ML, le Pointe HD (2011). Biometry of the corpus callosum in children: MR imaging reference data. *Am J Neuroradiol* **32**:1436–1443.

Gentile M, Di Carlo A, Volpe P, Pansini A, Nanna P, Valenzano MC, *et al.* (2003). FISH and cytogenetic characterization of a terminal chromosome 1q deletion: clinical case report and phenotypic implications. *Am J Med Genet* **A117**:251–254.

Guilherme RS, Kim CA, Alonso LG, Honjo RS, Meloni VA, Christofolini DM, *et al.* (2013). Ring chromosome 10: report on two patients and review of the literature. *J Appl Genet* **54**:35–41.

Guilherme RS, Kim CA, Alonso LG, Honjo RS, Meloni VA, Christofolini DM, *et al.* (2013). Ring chromosome 10: report on two patients and review of the literature. *J Appl Genet* **54**:35–41.

Guillem P, Fabre B, Cans C, Robert Gnansia E, Jouk PS (2003). Trends in elective terminations of pregnancy between 1989 and 2000 in a French county (the Isere). *Prenat Diagn* **23**:877–883.

Hanna RM, Marsh SE, Swistun D, Al-Gazali L, Zaki MS, Abdel-Salam GM, *et al.* (2011). Distinguishing 3 classes of corpus callosal abnormalities in consanguineous families. *Neurology* **76**:373–382.

Hsu HF, Hou JW (2007). Variable expressivity in Patau syndrome is not all related to trisomy 13 mosaicism. *Am J Med Genet A* **143A**:1739–1748.

Hung CC, Lin CH, Lin SY, Shin JC, Lee CN, Su YN (2012) Prenatal diagnosis of a fetus with a *de novo* trisomy 12p by array-comparative genomic hybridization (array-CGH). Gene **495**:178–182.

Issa MY, Ismail S, Helmy N, Kamel AK, Amin SK, Baky OMA, *et al.* (2018). Corpus callosum abnormalities in 64 Egyptian patients: Neuropsychological and genetic studies. *Middle East J Med Genet.***7**:88.

Jafari-Ghahfarokhi H, Moradi-Chaleshtori M, Liehr T, Hashemzadeh-Chaleshtori M, Teimori H, Ghasemi-Dehkordi P (2015). Small supernumerary marker chromosomes and their correlation with specific syndromes. *Adv Biomed Res.***4**:140

Jovanović-Privrodski JD, Kavečan II, Obrenović MR, Buonadonna LA, Bukvić NM (2009). Autism and hypoplastic corpus callosum in a case of monocentric marker chromosome 15. *Pediatr Neurol* **41**:65–67.

Kleczkowska A, Fryns JP, Moerman PH, Vandenberghe K, Berghe HVD (1987). Trisomy of the short arm of chromosome 5: autopsy data in a malformed newborn with inv dup (5)(p13. 1 p15. 3). *Clin Genet* **32**:49–56.

Lau YC, Hinkley LB, Bukshpun P, Strominger ZA, Wakahiro ML, Baron-Cohen S, *et al.* (2013). Autism traits in individuals with agenesis of the corpus callosum. *J Autism Dev Disord* **43**:1106–1118.

Lee YY, Hung J, Chang TY, Huang CC (2008). Agenesis of the corpus callosum in Turner's syndrome: report of a case and review of the literature. *Acta Neurol Taiwan* **17**:194–198.

Liehr T, Mrasek K, Weise A, Dufke A, Rodríguez L, Guardia N *et al* (2006). Small supernumerary marker chromosomes - Progress towards a genotype-phenotype correlation. *Cytogenet Genome Res* **112**:23-34.

Luders E, Thompso PM, Toga AW (2010). The development of the corpus callosum in the healthy human brain. *J Neurosci* **30**:10985–10990.

Luo L, O'Leary DD (2005). Axon retraction and degeneration in development and disease. *Annu Rev Neurosci* **28**, 127–156.

Mcgowan-Jordan J, Simons A, Schmid M (2016). *An international system for human cytogenomic nomenclature (ISCN).* Basel: S. Karger.

Nandhagopal R, Udayakumar AM (2014) Cri-du-chat syndrome. *Indian J Med Res* **140**:570–571.

Nussbaum RL, Mcinnes RR, Willard HF (2016). Thompson & Thompson genetics in medicine. 8th ed. Philadelphia,PA: Elsevier Inc.

Oliveira JS, Joaquim TM, Silva RABD, Souza DH, Martelli LR, Moretti-Ferreira D (2020). Non-mosaic partial duplication 12p in a patient with dysmorphic characteristics and developmental delay. *Genet Mol Biol* **43**:e20180285.

Palmer EE, Mowat D (2014). Agenesis of the corpus callosum: a clinical approach to diagnosis. *Am J Med Genet C* **166**:184–197.

Paul LK (2011). Developmental malformation of the corpus callosum: a review of typical callosal development and examples of developmental disorders with callosal involvement. *J Neurodev Disord* **3**:3.

Paul LK, Brown WS, Adolphs R, Tyszka JM, Richards LJ, Mukherjee P, *et al.* (2007). Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity. *Nat Rev Neurosci* **8**:287–299.

Paul LK, Corsello C, Kennedy DP, Adolphs R (2014). Agenesis of the corpus callosum and autism: a comprehensive comparison. *Brain* **137**:1813–1829.

Peroos S, Forsythe E, Pugh JH, Arthur-Farraj P, Hodes D (2012). Longevity and patau syndrome: what determines survival?. *BMJ Case Rep* **2012**, bcr0620114381.

Pinkle D, Gray J, Trask B, van den Engh G, Fuscoe J, van Dekken H (1986). Cytogenetic analysis by *in situ* hybridization with fluorescently labeled nucleic acid probes. *Cold Spring Harb Symp Quant Biol* **51**:151–157.

Pirola B, Bortotto L, Giglio S, Piovan E, Janes A, Guerrini R *et al.* (1998). Agenesis of the corpus callosum with Probst bundles owing to haploinsufficiency for a gene in an 8 cM region of 6q25. *JMG* **35**:1031–1033.

Poirsier C, Landais E, Bednarek N, Nobecourt JM, Khoury M, Schmidt P, *et al.* (2014) Report on 3 patients with 12p duplication including GRIN2B. *Eur J Med Genet* **57**:185–194.

Quay HC, Peterson DR (1993). *The revised behavior problem checklist manual*. Odessa, FL: Psychological Assessment Resources.

Ramocki MB, Dowling J, Grinberg I, Kimonis VE, Cardoso C, Gross A, *et al.* (2003). Reciprocal fusion transcripts of two novel Zn-finger genes in a female with absence of the corpus callosum, ocular colobomas and a balanced translocation between chromosomes 2p24 and 9q32. *Eur J Hum Genet* **11**:527–534.

Rasmussen SA, Wong L-YC, Yang Q,, May KM, Friedman JM (2003). Population-based analyses of mortality in trisomy 13 and trisomy 18. *Pediatrics* **111**:777–784.

Santos ACED, Midleton SR, Fonseca RL, Santos SRD, Llerena Jr JC, Vargas FR (2002). Clinical, neuroimaging and cytogenetic findings in 20 patients with corpus callosum dysgenesis. *Arq Neuro-Psiquiatr* **60**:382–385.

Schell-Apacik CC, Wagner K, Bihler M, Ertl-Wagner B, Heinrich U, Klopocki E, *et al.* (2008). Agenesis and dysgenesis of the corpus callosum: clinical, genetic and neuroimaging findings in a series of 41 patients. *Am J Med Genet* **A146**:2501–2511.

Spazzapan P, Arnaud E, Baujat G, Nizon M, Malan V, Brunelle F, *et al.* (2016). Clinical and neuroradiological features of the 9p deletion syndrome. *Child Nerv Syst* **32**:327–335.

Stoll C, Dott B, Roth MP (2019). Associated anomalies in cases with agenesis of the corpus callosum. *Am J Med Genet* **A179**:2101–2111.

Talisetti A, Forrester SR, Gregory D, Johnson L, Schneider MC, Kimonis VE (2003). Temtamy-like syndrome associated with translocation of 2p24 and 9q32. *Clin Dysmorphol* **12**:175–177.

Tang PH, Bartha AI, Norton ME, Barkovich AJ, Sherr EH, Glenn OA (2009). Agenesis of the corpus callosum: an MR imaging analysis of associated abnormalities in the fetus. *Am J Neuroradiol* **30**:257–263.

Temtamy S, Aglan M (2012) Consanguinity and genetic disorders in Egypt. *Middle East J Med Genet* **1**:12–17.

Verma RS, Babu A (1995). *Human chromosomes: a manual of basic techniques*. New York, NY: McGraw-Hill.

Vigdorovich N, Ben-Sira L, Blumkin L, Precel R, Nezer I, Yosovich K, *et al.* (2020). Brain white matter abnormalities associated with copy number variants. *Am J Med Genet* **A182**:93–103.

Wahl M, Lauterbach-Soon B, Hattingen E, Jung P, Singer O, Volz S, *et al.* (2007). Human motor corpus callosum: topography, somatotopy, and link between microstructure and function. *J Neurosci* **27**:12132–12138.

Wang LW, Huang CC, Yeh TF (2004). Major brain lesions detected on sonographic screening of apparently normal term neonates. *Neuroradiology* **46**:368–373.

Wechsler D. (2002). The Wechsler Preschool and Primary Scale of Intelligence, (WPPSI-III). San Antonio, TX: The Psychological Corporation.

Wozniak JR, Muetzel RL, Mueller BA, McGee CL, Freerks MA, Ward EE, *et al.* (2009). Microstructural corpus callosum anomalies in children with prenatal alcohol exposure: an extension of previous diffusion tensor imaging findings. *Alcohol Clin Exp Res* **33**:1825–1835.

Yao G, Chen XN, Flores-Sarnat L, Barlow GM, Palka G, Moeschler JB, *et al.* (2006). Deletion of chromosome 21 disturbs human brain morphogenesis. *Genet Med* **8**:1–7.