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Phenotypic and Genotypic Profile of an Egyptian Sample of Children with Neurodevelopmental Disorders; Two Case Reports of Microdeletion 22q11.2 Syndrome (DiGeorge/Velocardiofacial Syndrome)

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Short Title: Phenogenotypic Profile in Neurodevelopmental Disorders

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Abstract: ***Background:*** ***Psychiatric disorders in Pediatrics are often observed in association with other malformations and as a feature of well- defined genetic syndromes.*** ***Objectives:*** ***Exploration of the phenotypic and genotypic profile of an Egyptian sample of children with a group of neurodevelopmental disorders (NDD).*** ***Methodology:*** ***A cross sectional descriptive study was implemented on fifty Egyptian children with NDD ; 43 of them were males (86%) and 7 were females (14%) and their ages ranged from 2 to 12 yrs with a mean age of 7.92± 1.95 yrs. Full clinical history taking, thorough clinical examination, ECG recording, plain X-ray of the chest and heart, echocardiography, and pelvi-abdominal sonographic examination were carried out for all enrolled children in addition to CBC, serum calcium, Pediatric Symptom Checklist (PSC) score, and IQ assessment. DSM 5 diagnostic criteria were used to confirm the type of NDD. On the other hand, chromosomal analysis using HRB and FISH searching for microdeletion 22q11.2 was done for 16 out of 50 studied cases (32%) when such deletion was clinically highly suspected.*** ***Results:*** ***Craniofacial dysmorphism, congenital heart diseases (CHD), intellectual disability (ID), psychosocial impairment (PSI), hypocalcaemia, abnormal CBC, abnormal ECG, and abnormal echocardiographic findings were reported in 32%, 10%, 80%, 70%, 30%, 28%, 6%, and 10% of enrolled cases respectively. Different degrees of ID, Autism Spectrum Disorder (ASD) with different degrees of ID, and specific learning disabilities (LD) with normal or borderline IQ were diagnosed in 40%, 40%, and 20% of examined cases respectively. HRB and FISH revealed microdeletion 22q11.2 in 2 out of the16 examined cases (12.5%).*** ***In conclusion,*** ***Pediatricians' high index of suspicion of DiGeorge/velocardiofacial syndrome especially among children with different types of NDD is crucial to pick up such cases as early as possible. When confirmed, providing their families with efficient genetic counseling service and implementation of individualized management plan with early physical, behavioral, and or educational interventions for these cases are mandatory to improve their overall prognosis and minimize recurrence risk whenever possible.***

Keywords: Autism Spectrum Disorder (ASD), Learning Disabilities (LD), Neurodevelopmental Disorders (NDD), DiGeorge/velocardiofacial syndrome, microdeletion 22q11.2, Fluorecence In Situ Hybridization (FISH) technique, High Resolution Banding (HRB)

1.Introduction

Almost all cases of DiGeorge syndrome (DS), velocardiofacial syndrome (VCFS), and conotruncal anomaly face syndrome result from a common deletion of chromosome 22q11.2. These syndromes are usually referred to as the 22q11.2 deletion syndrome (22q11.2 DS), which has a wide phenotypic spectrum [1] and an estimated incidence of one in 4000 births [2,3].This phenotypic spectrum includes a wide variety of malformations and abnormalities occurring in different combinations and with widely differing severity. The typical clinical picture includes congenital heart defects, recurrent infections, velopharyngeal insufficiency, learning difficulties, behavioral abnormalities, and characteristic facial features [1, 4].

The reported neuropsychiatric and behavioral disorders associated with 22q11.2 DS include elevated rates of shyness, disinhibition, autism spectrum disorders, psychosis, severe attentional difficulties, executive dysfunction, behavioral phenotype reflective of non-verbal learning disabilities, concomitant language deficits, and socio-emotional concerns [5,6].

Most people with 22q11.2 deletion syndrome are missing a sequence of about 3 million DNA building blocks (base pairs) on one copy of chromosome 22 in each cell. This region contains 30 to 40 genes, many of which have not been well characterized. A small percentage of affected individuals have shorter deletions in the same region. This condition is described as a contiguous gene deletion syndrome because it results from the loss of many genes that are close together. Researchers are working to identify

all of the genes that contribute to the features of 22q11.2 deletion syndrome. They have determined that the loss of a particular gene on chromosome 22, TBX1, is probably responsible for many of the syndrome's characteristic signs (such as heart defects, a cleft palate, distinctive facial features, hearing loss, and low calcium levels). Some studies suggest that a deletion of this gene may contribute to behavioral problems as well. The loss of another gene, COMT, in the same region of chromosome 22 may also help to explain the increased risk of behavioral problems and mental illness. The loss of additional genes in the deleted region likely contributes to the varied features of 22q11.2 deletion syndrome [7].

2.Problem Definition

Nowadays, most children with DiGeorge/velocardiofacial syndrome will survive into adulthood, particularly if heart problems are detected and treated early. Less than 5% of these babies die before their first birthday. As the child with DiGeorge/velocardiofacial syndrome gets older, physical features such as heart and speech problems tend to become less of an issue, but behavioral, learning, and mental health problems may significantly impair his daily activities all through his life [1,4].

3.Study Objectives

The current study was conducted to explore the phenotypic and genotypic profile of an Egyptian sample of children with a group of neurodevelopmental disorders (NDD) and to screen for Microdeletion 22q11.2 Syndrome whenever clinically suspected in this high risk group.

4.Study Design & Research Methodology

The current cross sectional descriptive study was carried out in accordance to the code of ethics of the ***World Medical Association (Declaration of Helsinki, 1989)*** [8] for experiments involving humans. Written informed consent of legal caregivers of enrolled infants and children was taken after explanation of the study objectives and its benefits for their children and other children who suffer from similar disorders. The study protocol was approved by Ain Shams Faculty of Medicine Ethical Committee.

Participants:

Fifty Egyptian children with different types of NDD were enrolled in the current study. They aged between 2 to 12 yrs with a mean age of 7.92± 1.95 yrs; 43 of them were males (86%) and 7 were females (14%). Thirty six cases were consecutively recruited from children regularly attending the Child Psychiatry Clinic, Children's Hospital, Ain Shams University (72%), 10 cases were referred from a Specialized Center located in Alexandria governorate, Egypt (20%), and 4 cases were referred from a Specialized Center located in Shobra El-Khema, Elkaliobia governorate, Egypt (8%).

Procedure

- Full history taking laying stress on parental consanguinity, antenatal and perinatal events, detailed developmental history, and family history of similar conditions [9,10,11].
- Thorough clinical general and all body systems' examination [10,11] with special emphasis on craniofacial dysmorphism and any other concomitant congenital malformations [12].
- ENT examination laying stress on palatal examination and hearing assessment using audiometry [13] and or brain stem auditory evoked potential [14].
- Family pedigree construction to elicit consanguinity and its degree if any as well as family history of similar conditions [15].
- Photo- recording of any detected craniofacial dysmorphism and or any other phenotypic malformations
- Laboratory Investigations:

1. CBC
2. Serum calcium determination: Total serum calcium (normal levels range from 9-10.5 mg/dl [16].

- Cardiac evaluation using ECG, plain X -ray of the chest and heart, and echocardiography [17, 18].
- Pelvi-abdominal sonographic examination searching for any associated pelvi-abdominal malformations [19, 20].
- **Psychometric assessment using a group of questionnaires and instruments:**

a. Psychosocial function assessment using an Arabic validated version of Pediatric Symptom Checklist (PSC) [21]. The PSC is a psychosocial screen designed to facilitate the recognition of pediatric cognitive, emotional, and behavioral problems so that appropriate interventions can be initiated as early as possible. It consists of 35 items that are rated as "Never," "Sometimes," or "Often" present and scored as 0, 1, and 2, respectively. The total score was calculated by adding the score for each of the assessed 35 items. Psychosocial impairment (PSI) is considered for children aged <6 years at a cutoff score of 24 points or higher and for those aged 6 -16 years, at a cutoff score of 28 points or higher. If four or more items were left blank, the questionnaire was considered invalid. A positive total score on the PSC suggests the need for further evaluation by a qualified health or mental health professional as both false positives and false negatives occur [22,23].

b. Vineland Adaptive Behavioral Scales (VABS); an Arabic validated version of which was used in assessment of enrolled infants and children as it forms an aid in diagnosing and classifying intellectual disability and other disorders such as autism, Asperger syndrome, and developmental delays [24]. It is a diagnostic tool that helps measuring the capabilities of both children and adults in dealing with everyday life. Its content and scales are organized within a 3 domains structure: communication, daily living, and socialization. It offers also, a motor skills

domain and an optional maladaptive behavior index. VABS composite-standard scores have a mean of 100 and a SD of 15 and its sub domains' have means of 15 with SD of 3[25, 26]. The degree of ID was subsequently categorized into profound (IQ < 20 - 25), severe (IQ: 20-25 to 35-40), moderate (IQ 35-40 to 50-55), mild (IQ level 50-55 to approximately 70). Meanwhile, Borderline Intellectual Functioning or below average intelligence was considered at IQ 71 - 84

c. An Arabic validated version of Childhood Autism Rating Scale (CARS) was used for both identification and rating of autism [27]. This test can be used to determine the severity of autistic symptomatology and can thus be useful in its periodic monitoring. CARS test consists of 15 items, each rated on a 4-point scale (may be extended to 7 points by insertion of intermediate points). The child may be rated between two descriptions by using rating of 1.5, 2.5 or 3.5. The items of CARS include relation to people, imitation, emotional response, body use, object use, adaptation to change, visual response, listening response, taste, smell and touch response and use, fear or nervousness, verbal communication, non verbal communication, activity level, level and consistency of intellectual response, and general impressions. The total score of the test can range from 15 to 60 points according to severity of autism. The score can be categorized into: non-autistic (15-29 points) as grade 0, mild to moderately autistic (30-36 points) as grade 1 and severely autistic (37-60 points) as grade 2 [28, 29].

d. Alziat (2007) Learning Disabilities Diagnostic Rating Scale Battery (LDDRS): an Arabic validated psychoeducational skills's assessment battery that was used as a test of academic achievement. It enables examiners to jointly evaluate domain-specific achievement skills and the cognitive abilities related to those skills [30].

e. DSM 5 (2013) diagnostic criteria for NDD were used to settle the diagnosis of the different encountered types of these disorders [31,32].

- **Cytogenetic evaluation** carried out using the following techniques:

1. Routine conventional karyotyping using G-banding [33].
2. High resolution karyotyping (high resolution banding study; HRB) by synchronization using MTX, FUDR and thymidine release [34].
3. Molecular cytogenetic evaluation using Fluorescence In Situ Hybridization (FISH) analysis for diagnosis of 22q11.2 deletion was performed on metaphase spreads from standard lymphocyte cultures using the appropriate probe. The requirement for proven 22q11.2 deletion was the demonstration of one signal in 11 metaphase spreads with fair quality. Accordingly, the presence of two signals in 11 metaphases was taken as a proof of a normal 22q11.2 region [35].

Data Analysis:

Analysis of the obtained data was done by IBM computer using ***SPSS (statistical program for social science version 16)*** [36] as follows: description of quantitative variables as means, SDs, and ranges and description of studied categorical variables as numbers and percentages (frequencies). Chi-square test was used to compare qualitative variables while unpaired t-test was used to compare quantitative variables between groups whenever possible. Results were considered statistically insignificant at $p>0.05$, significant at $p<0.05$, and highly significant at $p<0.01$.

5.Results

Analysis of the collected data of the enrolled fifty Egyptian children with different types of NDD showed that 86% of them were males and 14% were females with ages ranged from 2 to 12 yrs with a mean age of 7.92± 1.95 yrs. The overall consanguinity rate recorded among them was 46% (23 cases) but with no similar conditions among their family members; **Table (1).**

Phenotypically, craniofacial dysmorphism, congenital heart diseases (CHD), intellectual disability (ID), psychosocial impairment, hypocalcaemia, abnormal CBC, abnormal ECG, and abnormal echocardiographic findings were reported in 32%, 10%, 80%, 70%, 30%, 28%, 6%, and 10% of enrolled cases respectively; **Table (1)**. Congenital heart diseases (CHD) that were diagnosed in 5 cases (10%) were Fallot tetralogy (one case), atrial septal defect (ASD) in one case, and ventricular septal defects (VSD) in 3 cases.

Different degrees of ID (Group I), Autism Spectrum Disorder (ASD) with different degrees of ID (Group II), and specific learning disabilities (LD) with normal or borderline IQ (Group III) were diagnosed in 40%, 40%, and 20% of examined cases respectively; **Fig (1)**. Enrolled cases in group I (ID) showed statistically insignificant differences compared to group II (ASD) concerning mean values of age and total scores of PSC and VABS scores while cases included in group III (LD) were significantly older and had lesser mean value of PSC score and higher mean values of VABS score; i.e. showed better psychosocial functioning and higher IQ compared to those enrolled in both group I and II; **Table (2)**.

On the other hand, mild, moderate, and severe ID were reported in 8 (40%), 7 (35%), and 5 (25%) of studied cases of group I (ID) and in 6 (30%), 10 (50%), and 4 (20%) of studied autistic children (group II) respectively; with significantly more prevalent moderate degree of ID in group II (ASD); $p = 0.0319$. Meanwhile, according to CARS' scores, 60% of studied autistic children had mild to moderate autism (12 cases) and 40% (8 cases) had severe autism. On the other hand, out of studied 10 cases with LD, 70% (7 cases) had combined dyslexia and dyscalculia while 30% (3 cases) had dyscalculia and impairment in written expression; **Table (3)**.

HRB and FISH revealed microdeletion 22q11.2 in 2 out of the16 examined cases (12.5%); **Fig (2). Table (4)** shows the clinical and investigational findings of these 2 reported cases with 22q11.2 DS and **Fig (3)** shows the craniofacial dysmorphic features of one of them. On the other hand, **Fig (4)** shows the FISH findings of one of the two reported cases with microdeletion 22q11.2. Lastly, **Fig (5)** reveals the normal FISH findings of one of the studied cases with NDD (LD in the form of dyslexia and dyscalculia) and atrial septal defect.

Table 1: Frequency distribution of all studied categorical variables of enrolled cases with NDD:

Variable	No	%
Sex distribution Males	43	86
Females	7	14
Parental Consanguinity	23	46
Family history of similar conditions	0	0
Craniofacial dysmorphism	16	32
Normal ENT examination & hearing assessment	50	100
Abnormal CBC	14	28
Decreased Serum Ca (Hypocalcaemia)	15	30
Cardiac evaluation Abnormal ECG	3	6
Abnormal echocardiograhic findings	5	10
Normal pelvi-abdominal sonographic evaluation	50	100
Intellectual Disabilities (ID)	40	80
Psychosocial Impairment (PSI)	35	70
+ CARS (Autism Spectrum Disorder)	20	40
Specific Learning Disabilities	10	20
microdeletion 22q11.2 proved by HRB and FISH	2/16	12.5
Total number of enrolled cases	**50**	**100**

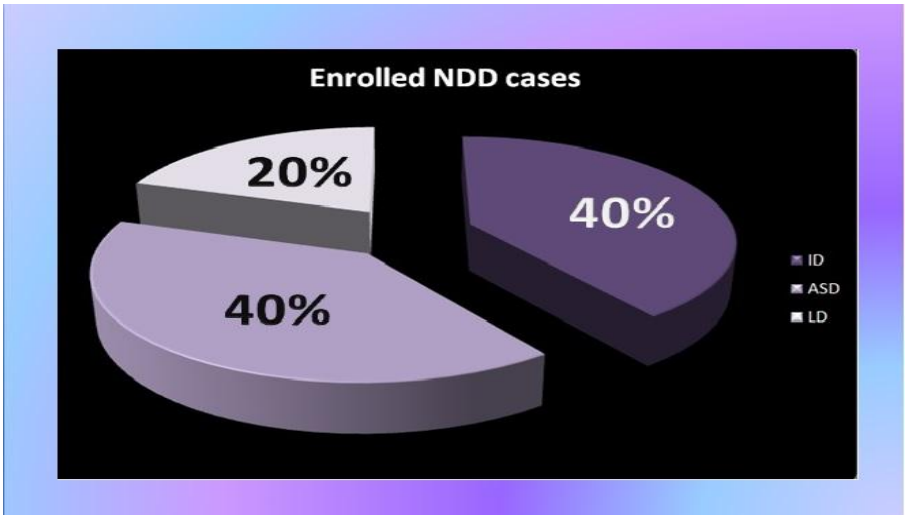

Figure 1: Diagnostic classification of studied cases with NDD according to DSM 5 diagnostic criteria and the results of performed psychometric assessment into Intellectual Disabilities (ID), Autism Spectrum Disorder (ASD), and Specific Learning Disabilities (LD)

Table 2: Mean values of age and total PSC, VABS, and CARS scores of studied cases with different types of NDD:

Studied quantitative variables	Group I ID Cases No=20		Group II ASD Cases No=20		Group III LD Cases No=10		I VS II	I VS III	II VS III
	Mean	±SD	Mean	±SD	Mean	±SD			
Age in years	6.99	2.21	7.55	1.99	9.33	1.31	0.41	0.001**	0.0071**
Total PSC score	33.25	3.11	31.91	1.89	22.00	2.45	0.11	<0.0001**	<0.0001**
Total VABS score	46.51	14.86	53.88	15.37	85.22	14.11	0.13	<0.0001**	<0.0001**
Total CARS score			33.74	1.74					

Pediatric Symptom Checklist (PSC), Vineland Adaptive Behavioral Scales (VABS), Childhood Autism Rating Scale (CARS)
t = unpaired t-test was used for statistical comparison, P>0.05 = statistically insignificant, p<0.01**=statistically highly significant

Table 3: Frequency distribution of different ID degrees, autistic features' rating, and type of specific LD of enrolled cases:

Studied categorical variables	Group I ID Cases No=20		Group II ASD Cases No=20		Group III LD Cases No=10		I VS II	
	No	%	No	%	No	%	X^2	P
Degree of ID according to VABS: Mild	8	40	6	30			2.1978	0.1382
Moderate	7	35	10	50			4.6036	0.0319*
Severe	5	25	4	20			0.7168	0.3972
Rating of autistic features according to CARS: Mild-Moderate			12	60				
Severe			8	40				
Type of specfic LD according to LDDRS: Dyslexia + Dyscalculia					7	70		
Dyscalculia + Impairment in written expression					3	30		

Vineland adaptive behavioral scales (VABS), Childhood Autism Rating Scale (CARS), Learning Disabilities Diagnostic Rating Scale Battery (LDDRS),
X^2 = Chi square test was used for statistical comparison, P>0.05 = statistically insignificant, P<0.05* = statistically significant

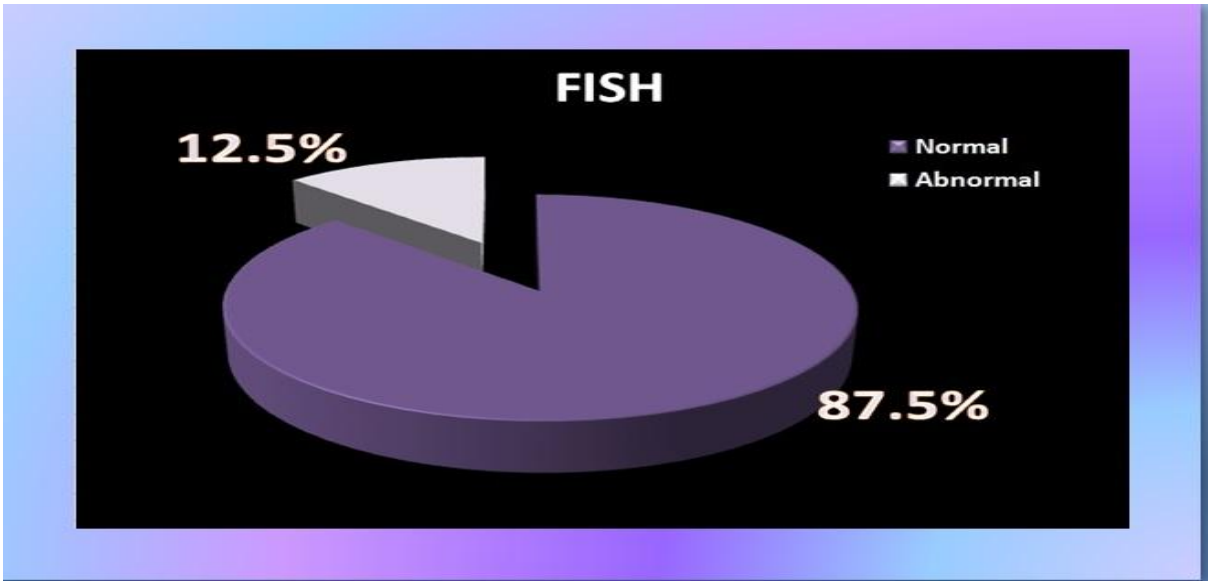

Figure 2: Prevalence of microdeletion 22q11.2 among FISH studied cases (2 out of 16; 12.5%)

Table 4: Clinical and investigational findings of the 2 reported cases with 22q11.2 DS:

Variable	Case 1	Case 2
Age	11 yrs	9 yrs
Sex	male	Female
FH of similar conditions	No	No
Consanguinity	No	No
Hazardous perinatal events	No	No
Growth	Short stature (4 SD < mean for age)	Fair
Recurrent infections	No	Chest infections
Craniofacial dysmorphism	Low anterior hair line, mongoloid slant of narrow palpebral fissures, bulbous nose, low set ears, long philtrum, thin lips, and prominent upper central incisors	No
Visual assessment	Free	Free
ENT examination & hearing assessment	Free	Free
ECG	Right bundle branch block	Normal
Echocardiography	Fallot tetralogy	VSD
CBC	Microcytic hypochromic anemia	Normal
Serum Ca	Hypocalcaemia	Hypocalcaemia
IQ	60 (mild ID)	71 (BLIQ)
PSC score	18 (Fair psychosocial function)	19 (Fair psychosocial function)
NDD subtype	Mild ID	Specific LD (dyslexia, dyscalculia)
Conventional karyotype	No abnormality detected	No abnormality detected
HRB	No abnormality detected	No abnormality detected
FISH	del 22q11.2	del 22q11.2

ID = Intellectual Disability, LD = Specific Learning Disability, BLIQ = Borderline Intelligent Quotient, NDD = Neurodevelopmental Disorder, VSD = Ventricular Septal Defect, High Resolution Banding (HRB), Fluorecence In Situ Hybridization (FISH)

Figure 3: Craniofacial dysmorphic features of one of the two reported cases with 22q11.2 DS showing low anterior hair line, mongoloid slant of narrow palpebral fissures, bulbous nose, low set ears, long philtrum, thin lips, and prominent upper central incisors

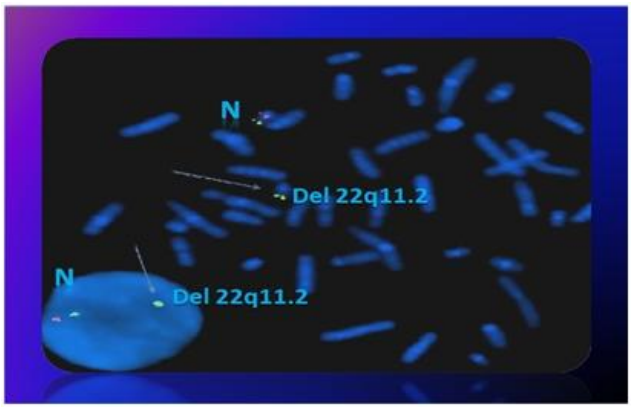

Figure 4: FISH findings of one of the 2 reported cases with microdeletion 22q11.2 as indicated by the absence of the red signal on the mutated chromosome 22 while N refers to normal chromosome 22 i.e. it has both green and red signals

Figure 5: Normal FISH findings of one of the studied cases with specific LD in the form of dyslexia and dyscalculia and atrial septal defect (each chromosome 22 has both green and red signals)

6.Discussion

Many children with 22q11.2 deletion syndrome have growth and developmental delays, including delayed speech and learning disabilities. Later in life, they are at an increased risk of developing mental illnesses such as schizophrenia, depression, anxiety, and bipolar disorder. Additionally, affected children are more likely than children without 22q11.2 deletion syndrome to have attention deficit hyperactivity disorder (ADHD) and developmental conditions such as autism spectrum disorders that affect communication and social interaction [5,6]

Botto et al., (2003) [37] has collected data revealing that even if under- ascertained, the prevalence of the 22Q11.2 deletion seems to be high, twice that of phenylketonuria. Extrapolating those data on a national scale, they have estimated that 700 children or more are born with the deletion every year in the United States alone. Many of these children will present with a wide spectrum of cardiac, neurodevelopmental, gastrointestinal, and other anomalies. Accordingly, along with their families, such cases will face complex clinical, surgical, and developmental challenges.

Deletion of one critical gene or several contiguous genes on the deleted portion of the long arm of chromosome 22 in 22q11.2 DS is thought to be the basis of this syndrome. Although several genes in this area have been mapped, which genes must be deleted to cause this syndrome remains unknown. The TBX1 gene may be one important gene in the deleted region. Several gene products from within the deleted region have been identified and are being further characterized. The result of this deletion is a developmental field defect involving the third and fourth pharyngeal pouches caused by defective migration of the neural crest cells during the fourth week of embryogenesis. Portions of the heart, head and neck, thymus, and parathyroids derive from these pouches [7].

The cardiac aspects of this deletion syndrome lead to the greatest morbidity and mortality. Cardiac defects are observed in 74-80% of 22q11.2 DS patients. Only a small fraction of patients experiences severe recurrent infections secondary to T cell immunodeficiency due to severe thymic hypoplasia. Failure to thrive may be observed during early infancy in those with cleft palates and swallowing difficulties [1, 4]. The 2 reported cases in the current study with 22q11.2 DS had conotruncal CHD i.e. 100% (one case had VSD and the other had Fallot tetralogy). The case with VSD had recurrent chest infections because of the lung plethora while that with Fallot tetralogy had short stature.

The different neuropsychological disorders associated with 22q11.2 deletion syndrome include elevated rates of psychosis, severe attentional difficulties, executive dysfunction, and behavioral phenotype reflective of non-verbal learning disabilities, concomitant language deficits, and social-emotional concerns [5]. Bearden et al., (2005) [38] claimed that behavioral manifestations in 22q11.2 DS could result from haploinsufficiency of the catechol-O-methyltransferase (COMT) gene located within the 22q11 region. Such claim created a unique opportunity for investigating the interface between genetics and brain - behavior.

More recently, there has been a lot of debate about the role of micro RNAs in the development of different psychiatric and behavioral disorders. Micro RNAs (miRNAs) are important regulators of post-transcriptional gene expression. They may up- or down-regulate the translation of messenger RNA (mRNA) or render it unstable. A single mRNA may be regulated by multiple miRNAs, and, conversely, some miRNAs have the potential to target hundreds of mRNAs. MiRNAs primarily act to negatively regulate gene expression i.e. miRNA over-expression should lead to down-regulation of their gene targets resulting in a negative correlation [39].

MiRNAs are approximately 22 nucleotide (nt) long small non coding RNAs. MiRNA biogenesis is initiated via transcription by RNA polymerase II, generating primary transcripts known as pri-miRs. Pri-miRs are cropped by ribonuclease III Drosha and its cofactor, DiGeorge syndrome critical region gene 8 (DGCR8), to generate approximately 65 nt long hairpin-shaped precursors known as pre-miRs. Drosha and DGCR8 form a protein complex

called a microprocessor, crucial for initial miR biogenesis [40].

Chromosome 8p, from which at least seven micro RNAs are transcribed, is an important area for neurodevelopmental disorders including autism and schizophrenia [41]. Patients with DiGeorge 22q11.2 deletion have shown a deficiency in DGCR8 (a key micro RNA processing gene) expression, resulting in decreased micro RNAs biosynthesis, imposing a 30-fold increased risk of schizophrenia [42]. The functional targets of these miRNAs include a number of genes that have been implicated in schizophrenia, such as BDNF, the dopamine receptor DRD1, the synaptic protein neuregulin 1 (NRG1) and early growth response gene 3 (EGR3). On the other hand, hypo-functional NMDA-receptor signaling in dorsolateral prefrontal cortex and superior temporal gyrus is consistent with cognitive and behavioral disturbances of schizophrenia, autism, and attention deficit hyperactivity disorder (ADHD) [43].

Reviewing the foregoing data was the stimulus for the current study that aimed at exploring the phenotypic and genotypic profile of an Egyptian sample of children with a group of neurodevelopmental disorders (NDD) and to screen for Microdeletion 22q11.2 Syndrome whenever clinically suspected in this high risk group. The clinical features that were used as guidelines in the clinical diagnostic process preceding genetic testing for 22q11.2 DS were those grouped by Oskarsdóttir et al., (2005) [1] into a core set of eight features: cardiac defects, non-visible/hypoplastic thymus or infection problems, hypocalcaemia, feeding difficulties, cleft palate/speech-language impairment, developmental delay/learning difficulties, characteristic dysmorphic features, and other malformations and deformities.

Phenotypically, enrolled cases in the current study had craniofacial dysmorphism, congenital heart diseases (CHD), intellectual disability (ID), psychosocial impairment, hypocalcaemia, abnormal CBC, abnormal ECG, and abnormal echocardiographic findings in 32%, 10%, 80%, 70%, 30%, 28%, 6%, and 10% of them respectively. Congenital heart diseases (CHD) that were diagnosed in 5 cases (10%) were Fallot tetralogy (one case), atrial septal defect (ASD) in one case, and ventricular septal defects (VSD) in 3 cases while different degrees of ID (Group I), Autism Spectrum Disorder (ASD) with different degrees of ID (Group II), and specific learning disabilities (LD) with normal or borderline IQ (Group III) were diagnosed in 40%, 40%, and 20% of examined cases respectively. One of the 2 reported cases with 22q11.2 DS had mild ID and the other had specific LD in the form of dyslexia, dyscalculia with borderline IQ (71).

Antshel et al., (2007) [44] studied a sample consisted of 41 children (20 were females) with VCFS, ranging in age from 6.5 years to 15.8 years. Seventeen out of those 41 children met the formal DSM-IV diagnostic criteria for an autistic spectrum disorder (i.e. VCFS + ASD). Generally, sixty percent of their studied children with VCFS proved to have a psychiatric disorder while 94% of the children with VCFS + ASD had a co- occurring psychiatric disorder. On the other hand, De Smedt et al (2007) [6] reported that long-term complications of 22q11.2 DS may include learning disabilities, mild mental retardation, and psychiatric disorders.

22q11.2 DS is a congenital condition, but age at diagnosis largely depends on the severity and the types of concomitant birth defects. Thus, those with more serious cardiac defects, hypocalcaemia, or both observed in classic DGS are diagnosed in the neonatal period. Recurrent infections usually present in patients older than 3-6 months. Some individuals without hypocalcaemia, with normal immune function, mild cardiac defects, and minimal facial anomalies may not be diagnosed until late childhood. Late diagnosis into adulthood continues to be reported, especially in those with isolated mild symptoms. Hence, diagnosis in fetuses with a congenital heart anomaly should be offered to the pregnant woman [1].

O´ skarsdo´ttir et al., (2004) [3] stated that the number of individuals diagnosed to have 22q11.2 DS depends on the experience and awareness of the syndrome among specialists who encounter these children and also on the severity of the phenotype as they found a high frequency of 22q11.2 DS in Gothenburg, Sweden. They described it as an example of increased awareness of the syndrome in their region where a confirmatory FISH test for the syndrome was requested by many different specialists from different hospitals and Outpatient Clinics. Nineteen patients of their described series (19/41) (46%) were referred by cardiologists, 11 (27%) by pediatric neurologists or child psychiatrists, and seven (17%) by speech pathologists. Two patients were referred by pediatric immunologists, one by an audiologist, and one patient was diagnosed antenatally.

Later, Oskarsdóttir et al., (2005) [1] investigated and described the presenting phenotype of 100 children with the 22q11.2 deletion syndrome. The median age at diagnosis in their study was 6.7 years. Of all patients, 26% were diagnosed in infancy and 92% had a congenital cardiac defect, whereas 54% of those diagnosed later, had a cardiac defect. A cleft palate was present in 25 cases and 44 had some other malformation or deformity. All presented with a combination of many of the core features of the syndrome. Of those diagnosed after 2 years of age, the majority presented with speech-language impairment, developmental delay or learning difficulties, and recurrent infections. Characteristic mild dysmorphic features were noticed in all their studied children.

In the current study, FISH analysis documented microdeletion 22q11.2 in 2 out of 16 FISH examined cases with neurodevelopmental disorders (12.5%); one was diagnosed at the age of 11 yrs and the other at the age of 9 yrs in spite of the concomitant CHD and hypocalcaemia in both cases; a finding that shows the importance of screening for this deletion syndrome in patients suffering from neurodevelopmental disorders with or without other concomitant suggestive clinical features. Without such screening, 22q11.2 DS detection would be missed or lately diagnosed.

Patients with 22q11 DS usually have characteristic facies, which become more pronounced as the children grow into the second decade. These are often recognized in white children and consist of a high and broad nasal bridge, long face, narrow palpebral fissures, and micrognathia. Microcephaly and asymmetric crying face may be present [45]. In the current study, craniofacial dysmorphism was recorded in 16 out of 50 enrolled cases with NDD (32%) while it was reported in one of the 2 cases diagnosed as 22q11.2 DS (50%) and it was in the form of low anterior hair line, mongoloid slant of narrow palpebral fissures, bulbous nose, low set ears, long philtrum, thin lips, and prominent upper central incisors.

It is worth considering that both reported cases were born to non consanguineous parents and had no family history of similar conditions. McDonald-McGinn et al., (2001) [46] reported the first unselected cohort of patients with the 22q11.2 deletion who were identified through an affected relative. Analysis of their series of 30 patients, many with very mild manifestations of the deletion, allowed them to examine the outcome in individuals who lacked specific features for this disorder. They emphasized the importance of broadening the index of suspicion in order to provide an appropriate recurrence risk counseling, cognitive remediation, and medical management. Further, they underscored the lack of familial concordance and the current lack of genotype-phenotype correlations in 22q11.2 DS and raised the possibility that the deletion is more common than previously reported.

7.Conclusion

Pediatricians' high index of suspicion of DiGeorge / velocardiofacial syndrome among children with different types of NDD is crucial to pick up such cases as early as possible. When confirmed, providing their families with efficient genetic counseling service and implementation of individualized management plan with early physical, behavioral, and or educational interventions for these cases are mandatory to improve their overall prognosis and minimize recurrence risk whenever possible.

8.Future Scope

The true incidence and prevalence of this syndrome in Egypt or any other country will only be available through population-based screening, but this would be too expensive and ethically questionable. So, screening of specific high risk populations would be more justified. Increasing the awareness and knowledge of general pediatricians and other specialists who are more likely to encounter 22q11.2 DS cases early in life, is crucial, in order to reduce the possibility of diagnostic delay or missing cases.

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Conflict of Interest

The authors declare no conflict of interest, no financial, and or personal relationships with other people or organizations that could inappropriately influence our study or theirs

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