ORIGINAL ARTICLE

Investigation of 22q11.2 Deletion Syndrome in the first Moroccan Pediatric Patients series

Investigation du syndrome de délétion 22q11.2 dans la première série pédiatrique de patients marocains

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Abstract

Introduction: The 22q11.2 deletion syndrome (22q11DS) is an autosomal dominant genetic syndrome, frequently due to a microdeletion located on chromosome 22, presenting a wide variety of clinical manifestations. Cytogenetic methods, such as fluorescence in situ hybridization (FISH), and molecular biology techniques, such as multiplex ligation-dependent probe amplification (MLPA), are used to identify chromosomal deletions specific to the 22q11.2 region.

Aim: This study aimed to describe the first series of pediatric patients in Morocco, selected for their strong suspicion of DiGeorge syndrome.

Methods: As part of a collaboration between the University Hospital Center Hassan II in Fez and the University Hospital Center Abderrahim El Harouchi Ibn Rochd in Casablanca, Morocco, a prospective study was carried out from January 2021 to January 2024 on 30 patients screened for DiGeorge syndrome (DGS). The children included had at least two major signs of DGS. Diagnostic confirmation of 22q11DS was obtained by FISH analysis for all patients. In addition, MLPA analysis was performed on five patients among those confirmed by FISH. The MLPA process included DNA extraction, PCR amplification and capillary electrophoresis, with results analyzed using GeneMapper and Coffalyser software.

Results: Of the 30 patients selected, 22 were confirmed as having a 22q11DS. Among these, 19 had congenital heart disease and 17 had hypocalcemia, which was often associated with hypoparathyroidism. Facial dysmorphia was almost constant, and thymic abnormalities were observed in half the patients. Recurrent infections, hematological disorders and immune abnormalities were also common, underlining the clinical complexity of the syndrome.

Conclusion: Advances in molecular cytogenetics have enabled precise detection of microdeletions associated with 22q11DS, highlighting its global importance, but also revealing regional diagnostic challenges. Larger cohort studies are needed to strengthen the validity of results and improve clinical management approaches.

Key words: 22q11.2 deletion syndrome; DiGeorge syndrome; Fluorescence in situ hybridization (FISH); Multiplex ligation-dependent probe amplification (MLPA); Hypocalcemia; Congenital heart disease.

Résumé

Introduction: Le syndrome de délétion 22q11.2 (22q11DS) est un syndrome génétique autosomique dominant, fréquemment dû à une microdélétion située sur le chromosome 22, caractérisé par une grande variabilité clinique. Les méthodes cytogénétiques, telles que l'hybridation in situ en fluorescence (FISH), et les techniques de biologie moléculaire, telles que l'amplification multiplex de sondes dépendant d'une ligation (MLPA), sont utilisées pour identifier les délétions chromosomiques spécifiques de la région 22q11.2.

Objectif: Cette étude visait à décrire la première série de patients pédiatriques au Maroc, sélectionnés pour leur forte suspicion de syndrome de DiGeorge.

Méthodes: Dans le cadre d'une collaboration entre le Centre hospitalier universitaire Hassan II de Fès et le Centre hospitalier universitaire Abderrahim El Harouchi Ibn Rochd de Casablanca, au Maroc, une étude prospective a été menée de janvier 2021 à janvier 2024 sur 30 patients suspects d'être atteints du syndrome de DiGeorge. Les enfants inclus présentaient au moins deux signes majeurs de DGS. La confirmation diagnostique du 22q11DS a été obtenue par analyse FISH pour tous les patients. En outre, une analyse MLPA a été réalisée pour cinq patients parmi ceux dont le diagnostic a été confirmé par FISH. Le processus de MLPA comprenait l'extraction de l'ADN, l'amplification par PCR et l'électrophorèse capillaire, les résultats étant analysés à l'aide des logiciels GeneMapper et Coffalyser.

Résultats: Sur les 30 patients sélectionnés, 22 ont été confirmés comme ayant un 22q11DS. Parmi eux, 19 présentaient une cardiopathie congénitale et 17 avaient une hypocalcémie, souvent associée à une hypoparathyroïdie. La dysmorphie faciale était presque constante et des anomalies thymiques ont été observées chez la moitié des patients. Les infections récurrentes, les troubles hématologiques et les anomalies immunitaires étaient également fréquents, soulignant la complexité clinique du syndrome.

Conclusion: Les progrès de la cytogénétique moléculaire ont permis une détection précise des microdélétions associées au 22q11DS, soulignant son importance mondiale, mais révélant également les défis diagnostiques régionaux. Des études de cohortes plus importantes sont nécessaires pour renforcer la validité des résultats et améliorer les approches de prise en charge.

Mots clés: Syndrome de délétion 22q11.2 ; syndrome de DiGeorge ; hybridation fluorescente in situ (FISH) ; amplification multiplex de sondes dépendant d'une ligation (MLPA) ; hypocalcémie ; cardiopathie congénitale.

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Introduction

DiGeorge syndrome (DGS), also known as 22q11.2 deletion syndrome (22q11DS) initially characterized by Angelo DiGeorge, is a clinical triad marked by exclusive occurrences of T-cell immune deficiency, hypoparathyroidism, and congenital heart disease (1). According to the expert committee of the International Union of Immunological Societies, DiGeorge syndrome represents an immune deficiency coupled with an autosomal dominant syndrome, categorized as a thymic anomaly associated with additional congenital anomalies (2). Globally, the prevalence of DiGeorge syndrome is estimated at 1/4000 - 1/5000 births (3). Though in Morocco, underestimations may occur due to the diverse clinical presentations, which can cause diagnostic delays. The etiology of DGS primarily stems from a microdeletion, predominantly found on chromosome 22 (4) and less frequently on chromosome 10p. Microdeletion on chromosome 22 often presents as de novo deletion spanning 1.5 to 3 million base pairs (Mbp) in most individuals. However, approximately 10% of cases exhibit an inherited pattern (5,6). The deletion at 22q11DS manifests with a broad spectrum of phenotypes, encompassing descriptions of over 180 clinical manifestations (7). Chromosome 22, which may harbor a 22q11.2 microdeletion, is 50 Mpb long. The microdeletion can range in length from 0.7 to 3 Mbp, representing 1.4 to 6% of the chromosome. Numerous low copy repeat (LCR) sequences that facilitate non-allelic homologous recombination and can result in copy number abnormalities are found in the 22q11.2 chromosome region (8) . There are eight sets of LCRs, referred to as LCR A through H. These LCRs share high homology with each other (9). However, during recombination events, they can become misaligned and result in deletion (10) .
Advances in molecular cytogenetics have facilitated the identification of tiny genomic deletions and duplications that are not systematically discernible by karyotyping. These techniques include fluorescence in situ hybridization (FISH) (11), multiplex ligation-dependent probe amplification (MLPA) (12), array comparative genomic hybridization (arrayCGH) (13) and whole genome sequencing (WGS) (14). Together, these methods can detect genetic alterations at a finer resolution than conventional karyotype analysis.
This study aimed to describe the first series of pediatric patients in Morocco diagnosed with 22q11DS, focusing on their clinical features and diagnostic confirmation methods.

Methods

This prospective collaborative study was conducted between the Pediatric and Neonatology Department at the University Hospital Center Abderrahim El Harouchi Ibn Rochd in Casablanca and the Medical Genetics and Pathology Department at the University Hospital Center Hassan II in Fez, Morocco. The study adhered to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Ibn Rochd University Hospital (Approval Date: 2021/ File No 26/20). Written informed consent was obtained from all parents or legal guardians for participation and the disclosure of personal and clinical information. The study was conducted from January 2021 to January 2024.
Most of the patients were seen in consultation at the Pediatrics and Neonatology Department of the Abderrahim El Harouchi Ibn Rochd University Hospital in Casablanca, with only those presenting serious manifestations or complications being admitted to the hospital. Diagnostic tests were performed at the Medical Genetics and Pathology Department of the Hassan II University Hospital in Fez. The children included in this study exhibited at least two major signs of DiGeorge syndrome: facial dysmorphia, congenital heart disease, thymic hypoplasia or aplasia, hypocalcemia, and palatal anomalies.
A rigorous methodology was used to assess the clinical manifestations and complications associated with DiGeorge syndrome (22q11DS). Facial dysmorphia was analyzed by detailed clinical assessment, including standardized photographs and specialist consultations to identify specific facial features. Cardiac abnormalities were assessed by Doppler echocardiography using high-resolution ultrasound equipment. Hematological disorders were detected by complete blood counts, using samples taken in EDTA tubes. Specialist dental consultations included examinations to assess oral pathologies. Skeletal deformities were diagnosed by X-rays of the spine and lower limbs. Urological abnormalities were assessed by renal ultrasound and clinical examinations, using appropriate imaging techniques. Gastrointestinal disorders were examined by endoscopic and radiological examinations. Finally, psychological assessments were carried out to identify developmental delays, anxiety disorders and cases of autism, using standardized tests and specialist consultations. To assess the immunological characteristics of the patients, we measured the markers CD3, CD4, CD19, and CD8 as well as the immunoglobulins IgA and IgG. Blood samples were taken using tubes containing EDTA for cellular analyses and tubes without anticoagulant for serum. Blood samples were processed immediately, while serum was separated by centrifugation and stored at -20°C. Immunological markers were analyzed by flow cytometry using monoclonal antibodies conjugated with specific fluorochromes: CD3-FITC, CD4-PE, CD19-APC, and CD8-PerCP. Cells were incubated with these antibodies, washed and analyzed using a flow cytometer. Data were interpreted using FlowJo software. For IgA and IgG immunoglobulin assays, we used ELISA kits (Thermo Fisher Scientific) adapted for these analyses. The sera were diluted, incubated with the specific antibodies, and the complexes formed were revealed using a TMB substrate, followed by a reading at 450 nm with a microplate reader (BioTek Instruments). IgA and IgG concentrations were determined by comparison with the standardization curves provided.
To confirm 22q11DS, all patients in our series were diagnosed by FISH, performed on chromosome preparations. Metaphase chromosome spreads were

generated from lymphocyte cultures stimulated with PHA using conventional techniques prior to FISH analysis. The probes used were Vysis DiGeorge Region Probe - LSI TUPLE 1 SpectrumOrange (22q11.2) / LSI ARSA SpectrumGreen (22q13.3).

For five patients, MLPA was performed concurrently with FISH during a period when both techniques were used simultaneously at the Medical Genetics and Pathology Department at the University Hospital Center Hassan II in Fez. The process of MLPA involved extracting genomic DNA from peripheral blood using the PureLink Genomic DNA Mini Kit (Invitrogene), following the manufacturer's protocol. MLPA analysis utilized the SALSA® MLPA® Probemix P250 DiGeorge (MRC Holland, Amsterdam, Netherlands) encompassing 48 MLPA probes, including 29 probes for the 22q11.2 region, 1 probe for the 22q11.1 region, 17 probes for the 4q, 8p, 9q, 10p, 17p regions and 2 probes for the 22q13 region. Subsequent steps included PCR amplification and capillary electrophoresis conducted with an ABI 3500 Dx sequencer (Applied Biosystems). Raw data obtained from these procedures were analyzed using GeneMapper (Applied Biosystems) and Coffalyser software. The obtained DNA sample features were compared against a set of reference samples (negative controls) to deduce a probe ratio indicative of the gene's copy number. The deletion threshold was established at 0.75, while the duplication threshold was set at 1.30. Samples showing deletions and/or duplications underwent re-analysis to confirm the findings. As MLPA and FISH were performed concurrently, no additional re-analysis was required to confirm deletions detected by FISH. Karyotyping was not systematically performed for all patients. FISH analysis of the parents has been proposed. Genetic counselling was offered to couples wishing to plan a new pregnancy. In this context, the test has currently been performed in only one couple.

Results

Inclusion criteria allowed us to select 30 patients with at least two major signs of 22q11DS for our study. The main reasons for consultation or referral were the suspicion of DiGeorge syndrome, particularly due to facial dysmorphia, congenital heart defects, or recurrent infections. The primary reasons for hospitalization were cyanosis, recurrent infections, and apyretic crises, as well as significant hypocalcemia, thymic hypoplasia, and other severe complications.

FISH analysis with specific probes was carried out in 26 cases. Unfortunately, three cases could not be analyzed due to death, and one patient was currently unavailable for follow-up. We confirmed the presence of a 22q11DS in 22 cases by FISH. An additional MLPA test was performed for five patients, which confirmed the deletion. The results of the parental FISH analysis were normal, showing no deletion of 22q11.2.

The 22q11DS was detected in 22 out of the 26 patients tested. These patients ranged in age from newborns to 5 years old: 12 were aged 0-1 year, 7 were aged 1-3 years, and 3 were aged 3-5 years. The cohort included 14 males and 8 females, with half coming from consanguineous marriages (11 out of 22). Almost all patients had facial dysmorphia (21 out of 22), described as a bulbous nose, hypertelorism, flattened philtrum, and other features detailed in Table 1. Hypocalcemia was present in 17 out of 22 patients, and among these, 8 out of 17 had hypoparathyroidism. Thymic hypoplasia was observed in 11 of 22 patients, including a single case of thymic aplasia. Additionally, palatal anomalies were observed in 5 of the 22 patients, consisting of one case of cleft lip and four cases of cleft palate.

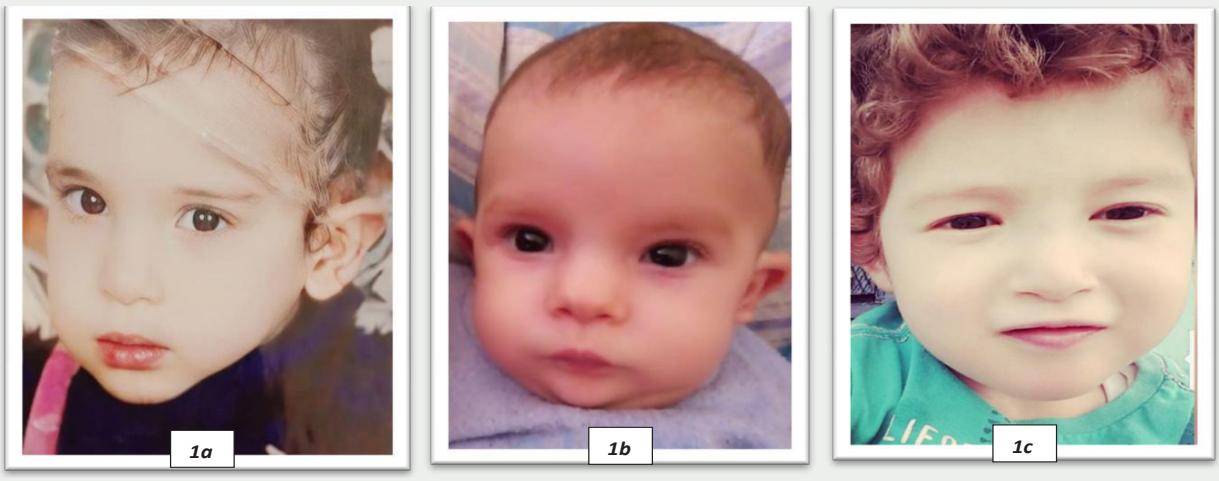

Figure 1. Facial dysmorphia in three patients with 22q11.2 deletion syndrome from our series: note the elongated face, hypertelorism, bulbous nose, flattened philtrum, and microstomia.

The results of the Doppler echocardiography are as follows: three cases had no cardiac malformations, while a significant majority, 19/22, had cardiac malformations detailed in Table 2.

Specific biological examinations were conducted for each patient, revealing a range of associated anomalies. Twelve patients had recurrent infections since birth, including one case with recurrent lung infections and elevated levels of immunoglobulins (IgA, IgG). Nine patients had hematological disorders, including anemia in seven cases and lymphopenia in two cases.

Table 1. Distribution of patients with 22q11.2 deletion syndrome based on facial characteristics

Different facial characteristics	Number of patients
Bulbous nose	16/21*
Hypertelorism	14/21
Flattened philtrum	9/21
Elongated face	6/21
Microtia	5/21
cleft lip and palate	5/21
Prominent forehead	4/21
microstomia	4/21
Retrognathism	2/21

*: Facial dysmorphism was present in 21 out of 22 patients with confirmed 22q11.2 deletion syndrome

Table 2. Distribution of patients with 22q11.2 deletion syndrome based on the type of congenital heart disease.

Heart disease	Number of patients
TOF	8/22
VSD	4/22
PA/VSD	4/22
ASD	1/22
TA	1/22
VSD + ASD	1/22

TOF: Tetralogy of Fallot; VSD: Ventricular septal defect; PA/VSD: pulmonary atresia with ventricular septal defect; ASD: Atrial septal defect; TA: Truncus arteriosus.

Six patients exhibited oral disorders, including four cases of dental caries and two cases of oral candidiasis, which led to additional dental and feeding issues. Three patients had scoliosis, three had clubfoot, and one had pes planus. Urological anomalies included renal agenesis in two patients and bilateral cryptorchidism in one patient, which required surgical intervention. Gastrointestinal anomalies included gastroesophageal reflux in 10 cases, and anorectal malformation in one case.

Furthermore, eleven patients exhibited developmental delays, including both motor and language development issues. The age at which these patients began walking ranged between 19 and 24 months. Additionally, ten patients displayed fearfulness or anxiety, and one patient was diagnosed with autism. One patient also presented with an acute fever of unknown origin.

An essential aspect of our study was to analyze the immune profiles of our patients. Table 3 shows the distribution of immune parameters: CD3, CD4, CD8, IgA and IgG.

Table 3. Overview of Immune Profile in patients with 22q11.2 deletion syndrome from our series with thymic conditions

Immune Profile	Number of patients	Thymic condition
↘ CD3	1	thymic hypoplasia
↘ CD3, CD4, CD19	1	thymic hypoplasia
↘ CD3, CD4, CD8	1	thymic hypoplasia
↘IgA	1	thymic hypoplasia
↗ IgA, IgG	1	thymic hypoplasia
↘ CD3, CD4	3	2 thymic hypoplasia 1 thymic aplasia
Normal	14	3 thymic hypoplasia

Discussion

Our study highlighted the diversity of clinical manifestations of 22q11.2 deletion syndrome in a sample (22q11DS) of the Moroccan population. Among the 22 patients diagnosed, the FISH technique was primarily used for initial diagnosis, while the MLPA method was concurrently applied to 5 cases to confirm the detected deletions. Our results revealed a significant male predominance and a wide age distribution. Furthermore, congenital heart disease was present in 19 out of 22 cases, consistent with worldwide data, with tetralogy of Fallot being the most common cardiac anomaly. Endocrine and immunological anomalies, such as hypocalcemia and thymic hypoplasia, were also frequent.

The confirmed cases showed a significant male predominance, with a distribution of 14 boys and 8 girls. Statistical analysis using a proportion test revealed a significantly higher proportion of boys than would be expected in an equal gender distribution. The same predominance has been observed in different studies (15,16). It should be noted that other studies have reported slight female predominance (17), underlining the diversity of results.

Our patients diagnosed with this syndrome present a wide age spectrum, from newborns to 5-year-olds. This observation underlines the importance of considering various age groups when studying and understanding the clinical manifestations of the syndrome in the Moroccan population. The inclusion of such a wide age range not only enhance the comprehensiveness of our results, but also highlights potential differences in symptomatology and disease progression across different developmental stages.

Furthermore, our study highlights the crucial importance of early diagnosis. Detecting the syndrome at an early stage is essential for effective management, and helps to mitigate the syndrome's potential long-term impact on the health and well-being of those affected, including intellectual and psychiatric problems that may arise (18,19).

It's worth mentioning that, although outside the scope of our study, prenatal diagnosis is a crucial aspect that deserves attention. Despite its absence in our research, the importance of prenatal diagnosis cannot be understated (20,21). This diagnostic approach is likely to provide valuable information on the manifestations of the syndrome before birth, enabling parents and healthcare professionals to make informed decisions. Future studies should explore the nuances of prenatal diagnosis in the context of this syndrome to improve our understanding and contribute to comprehensive healthcare strategies.

In our study, we observed a consanguinity rate of 50% among patients with 22q11.2 deletion syndrome, a figure that far exceeds the consanguinity rates reported at the national level, which vary between 19.64% and 38.9%, depending on the region of Morocco (22). This high rate could be attributed to several factors, including the relatively small size of our sample as well as the social and cultural factors specific to certain Moroccan communities, where consanguineous marriages are common. Finally,

limited access to genetic counselling and health services in these regions could contribute to the persistence of these practices, thereby increasing the prevalence of genetic diseases. These observations underline the importance of a larger study to better understand the impact of consanguinity on 22q11DS in Morocco. This high rate raises questions about the complexity of the syndrome's mechanisms, even though it is not generally associated with consanguinity (5). The syndrome results from a de novo hemizygous deletion at 22q11.2 initiated by meiotic non-allelic homologous recombination events between low-copy repeats (LCRs), commonly referred to as segmental duplications, with a statistically significant predominance of maternal origin (23). This paves the way for a better understanding of its prevalence and modes of transmission in diverse populations.

Facial dysmorphia, although usually present (in 21 out of 22 patients in our series), often represents a diagnostic challenge in newborns due to its subtlety. This characteristic facies is manifested by a bulbous nose, hypertelorism, flattening of the philtrum and an elongated face (24). It may be accompanied by microtia, a prominent forehead and retrognathism (24,25).

Anomalies affecting the otorhinolaryngeal tract, such as cleft palate, are often considered major diagnostic criteria, despite their relatively low incidence in our series (5 out of 22 patients) (26,27). Congenital heart disease is diagnosed in most patients with a 22q11.2 microdeletion and is the main reason for referral.

The prevalent subset of cardiac anomalies within this group includes conotruncal defects, such as tetralogy of Fallot, pulmonary atresia with ventricular septal defect, truncus arteriosus, interrupted aortic arch type B, conoventricular and/or atrial septal defects, and aortic arch anomalies. Tetralogy of Fallot is the most common cyanotic congenital heart disease in children beyond the neonatal period (26,28,29), accounting for 8 out of 22 cases in our study. Overall, heart disease was present in 19 out of 22 cases, which aligns with the literature, where tetralogy of Fallot is estimated to occur in 3-5 cases per 10,000 live births, representing 7%-10% of all congenital heart defects. The incidence of tetralogy of Fallot in patients with 22q11.2 deletion syndrome (22q11DS) ranges from 20% to 45%, which is consistent with the findings in our series. A ventricular septal defect (VSD) is also among the most prevalent congenital heart diseases and is frequently observed as a cardiovascular anomaly in individuals with chromosome 22q11.2 deletion syndrome. A larger study suggests the need to systematically test for chromosome 22q11.2 deletion in patients with certain types of VSD associated with aortic arch or pulmonary artery anomalies (30). Pulmonary atresia with ventricular septal defect (PA/VSD) was observed in 4 out of 22 patients, representing a congenital heart defect present at birth. Approximately 10-25% of individuals with a Digeorge Syndrome have PA/VSD. This congenital heart defect is characterized by malformation of the pulmonary valve and the presence of a hole in the ventricular septum. The association between 22q11DS and PA/VSD is significant, necessitating regular medical follow-up and timely interventions. The Truncus Arteriosus (TA) cardiac anomaly observed in our series, is reported in 5-10% of individuals with 22q11DS (31). This heart condition results from abnormal development of the heart during gestation, where a single major artery exits the heart instead of the two separate arteries normally present (32). Although the precise cause remains unknown, TA is often linked with genetic mutations in the 22q11.2 deletion (33). While most patients showed improvement in cardiac issues following surgical treatment, within the first year post-diagnosis of 22q11DS, cardiovascular complications remained the primary cause of death (26).

In our study, hematological abnormalities, including anemia and lymphopenia, were observed in 9 out of 22 patients with 22q11DS. These findings are consistent with recent research highlighting the prevalence of hematological problems in this condition. A study by Rosa et al. (34), revealed that hematological abnormalities are an integral part of the clinical spectrum of the syndrome. While their study focused primarily on platelet problems, our results highlight the broader hematological impact of 22q11DS, including anemia and lymphopenia. These observations reinforce the need for comprehensive hematological assessment in patients with 22q11DS and support the growing recognition of these abnormalities as key features of the syndrome.

In our study, skeletal abnormalities were documented in seven patients, with scoliosis and feet abnormalities noted. This aligns with literature indicating that skeletal anomalies are widespread (35). Additionally, there is a significantly increased risk of clubfoot and other extremity abnormalities, such as polydactyly, which can aid in the diagnosis of 22q11DS (36). This emphasizes the importance of comprehensive skeletal evaluations in patients with 22q11DS to effectively address these potential complications.

In our study, urological anomalies were observed, including renal agenesis in two patients and bilateral cryptorchidism in one patient requiring surgery. These results are consistent with the literature, which reports that abnormalities of the genitourinary system affect around a third of patients with 22q11DS. The urological problems observed in our series are well documented. The literature also highlights that genital anomalies, such as cryptorchidism, are more common in males with 22q11DS (26,27,37). In addition, our study revealed gastrointestinal anomalies, including gastro-esophageal reflux in 10 cases and anorectal malformation in one case. These findings are consistent with the literature, which indicates that up to 66% of patients with 22q11DS have a variety of gastrointestinal problems, ranging from common functional disorders to rare congenital anomalies (38). This high prevalence highlights the importance of systematic screening and management of gastrointestinal conditions in 22q11DS patients for effective management of complications.

Endocrine abnormalities are widespread, with hypocalcemia being a frequently occurring lifelong symptom of 22q11DS, mostly linked to either relative or absolute hypoparathyroidism. Hypocalcemia is recognized as a characteristic trait of 22q11.2 deletion syndrome. In our study, we found that 17

out of 22 patients had hypocalcemia, and 8 out of 22 were diagnosed with hypoparathyroidism. These findings reinforce the significant association between hypocalcemia and hypoparathyroidism, consistent with existing data (39). Thymic hypoplasia was observed in 11 out of 22 patients, including one case of thymic aplasia. In patients with 22q11.2 deletion syndrome, thymic abnormalities such as hypoplasia and aplasia significantly impact the immune system (1). Thymic hypoplasia often results in low T-cell counts, which can vary in severity. Some infants may initially show improvement in T-cell counts, but these levels may decline as they grow (1,40). Thymic aplasia can lead to significant immune deficiencies, but patients generally maintain intact antibody production and function. Although most individuals with 22q11DS exhibit normal antibody function and avidity, some may show functional antibody defects (41). We observed notable variations in immune markers, including reductions in CD3, CD4, CD8, and CD19, confirming the correlation between the absence of functional thymic tissue and reduced T-cell numbers. One patient had increased levels of IgA and IgG. While elevated IgG and IgA levels are not commonly reported, they have been observed in other studies, including a large cohort of 1023 patients with 22q11DS, where elevated immunoglobulin levels were seen even in cases of severe combined immunodeficiency (42).

In another study, 43% of patients with 22q11DS exhibited signs of antibody deficiencies, such as IgA deficiency, IgM deficiency, IgG subclass deficiency, or specific antibody deficiency. Additionally, a significant correlation was found between recurrent infections and abnormalities in humoral factors ($P < 0.01$) (43). The variability in immune profiles, including both decreased and increased immunoglobulin levels, highlights the importance of a comprehensive approach to understanding immune dysfunction in 22q11DS. A detailed examination of these immune alterations is essential for elucidating the mechanisms driving immune variability in this syndrome. Dental anomalies are prevalent in patients with 22q11DS, with a prevalence reported at 76% and 78% in different studies (44,45). These include abnormalities in tooth shape, eruption, and number, as well as enamel alterations such as hypomineralization and hypoplasia, with a high prevalence of dental caries (46). In our study, we identified only six cases of oral anomalies, including four cases of dental caries and two cases of oral candidiasis. The observed differences in prevalence may be attributed to the age range of our patient population. Notably, dental caries typically begin to emerge after six months of age, whereas some of the children in our series were younger than six months old (47). This age-related factor could explain the discrepancies when compared to the existing literature.

Developmental delays, including psychomotor delays, were observed in 11 patients, with one patient also diagnosed with autism. In addition to developmental issues, psychiatric symptoms such as fearfulness or anxiety were observed in 10 of our patients. These findings highlight the significant prevalence of psychiatric disorders, particularly anxiety, in individuals with 22q11DS. A study shows that anxiety disorders have an estimated average incidence of 39% among individuals with developmental delays (19), which aligns with our findings and underscores the need for heightened attention to anxiety management in these patients. Although autism is less common, with 20-50% of patients with 22q11DS meeting the diagnostic criteria for autism spectrum disorders, it still requires targeted assessment and support. The combination of developmental delays and psychiatric symptoms, such as anxiety and autism, underscores the importance of comprehensive screening and early intervention. These observations suggest the need for personalized diagnostic and treatment approaches that address both developmental delays and psychiatric symptoms to improve patient outcomes and quality of life. A recent study involving mothers of children with 22q11DS highlighted the significant challenges these children face in school, including the impact of psychiatric symptoms like anxiety on learning, communication, and social behavior (48). Given the complex neurodevelopmental profile of children with 22q11DS, multidisciplinary care including psychological, educational, and therapeutic support is crucial for managing both cognitive and psychosocial challenges.

Clinical variability in 22q11DS is influenced by a combination of genetic, epigenetic, and environmental factors. Cillo et al. (49) point out that this variability can result from diverse mutations within the same gene, the modulatory effect of other genes, and interactions between genes and the environment, such as epigenetic modifications and prenatal and postnatal environmental conditions. Our study illustrates this variability by showing abnormalities in some cases and different symptoms in others. Our results highlight a high rate of consanguinity in the Moroccan cohort studied, which may influence the manifestation and diagnosis of 22q11DS, underscoring the need for regionally adapted genetic screening and counselling. Further research is needed to explore the impact of inbreeding, identify additional homozygous variants, and assess implications for clinical management. A multidisciplinary approach is recommended for comprehensive and personalized management of 22q11DS, taking into account regional specificities and patient needs in Morocco.

Conclusion

This pioneering Moroccan study has provided several important insights into 22q11DS. Our findings confirm that 22q11DS presents a wide range of clinical features, including facial dysmorphia, hypocalcemia, thymic hypoplasia, and congenital heart disease. The use of both FISH and MLPA techniques proved effective in accurately confirming 22q11DS and identifying associated immune abnormalities, psychomotor developmental delays, and anxiety disorders. These results underscore the importance of increased awareness and genetic counselling tailored to the Moroccan context, especially considering the notably high consanguinity rate. Future research should delve deeper into the regional variations

observed, explore their implications for clinical practice, and aim to refine screening and intervention strategies to enhance patient outcomes and address the unique needs of the Moroccan population.

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