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22q and two: 22q11.2 deletion syndrome and coexisting conditions

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Abstract

22q11.2 deletion syndrome (DS) is the most frequent copy number variant (CNV) affecting ~1/1,000 fetuses and ~1/2,000–4,000 children, resulting in recognizable but variable findings across multiple organ systems. Patients with atypical features should prompt consideration of coexisting diagnoses due to additional genome-wide mutations, CNVs, or mutations/CNVs on the other allele, unmasking autosomal recessive conditions. Importantly, a dual diagnosis compounds symptoms and impacts management. We previously reported seven patients with 22q11.2DS and: SCID, Trisomy 8 mosaicism, Bernard-Soulier, and CEDNIK syndromes. Here we present six additional unreported patients with 22q11.2DS and concurrent diagnoses. Records on 1,422 patients with 22q11.2DS, identified via FISH, microarray, or MLPA, followed in our 22q and You Center at the Children's Hospital of Philadelphia (CHOP) were reviewed to identify a dual diagnosis. In addition to our seven previously reported cases, we identified an additional six with 22q11.2DS and another coexisting condition identified via: molecular/cytogenetic studies, newborn screening, coagulation factor studies, or enzyme testing; these include CHARGE syndrome (*CHD7* mutation), cystic fibrosis, a maternally inherited 17q12 deletion, G6PD deficiency, von Willebrand disease, and 1q21.1 deletion, resulting in an incidence of dual diagnoses at our center of 0.9%. The range of dual diagnoses identified in our cohort is notable, medically actionable, and may alter long-term outcome and recurrence risk counseling. Thus, our findings may support testing patients with 22q11.2DS using a combination of microarray, mutational analysis of the other allele/WES, to ensure appropriate personalized care, as formulating medical management decisions hinges on establishing the correct diagnoses in their entirety.

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CONFLICT OF INTEREST

None.

Keywords

CHARGE syndrome; cystic fibrosis; DiGeorge syndrome; dual diagnosis; G6PD deficiency; velocardiofacial syndrome; von Willebrand disease; 1q21.1 deletion; 22q11.2 deletion syndrome; 17q12 deletion syndrome

1 | INTRODUCTION

22q11.2 deletion syndrome (DS) is the most frequent chromosomal microdeletion syndrome (Botto et al., 2003; Devriendt, Fryns, Mortier, van Thienen, & Keymolen, 1998; Goodship, Cross, LiLing, & Wren, 1998; Oskarsdottir, Vujic, & Fasth, 2004; Tezenas Du Montcel, Mendizabai, Ayme, Levy, & Philip, 1996). The prevalence of 22q11.2DS has been reported to be 1 in ~1,000 unselected fetuses, those without congenital heart disease or cleft palate on prenatal ultrasound, (Grati et al., 2015) and anywhere from 1 per 3,000 to 1 per 6,000 livebirths (Botto et al., 2003; Devriendt et al., 1998; Goodship et al., 1998; Oskarsdottir et al., 2004; Tezenas Du Montcel et al., 1996). While this condition results in a heterogeneous clinical presentation, it leads to frequent associated features in multiple organ systems, including cardiac anomalies such as interrupted aortic arch type B, truncus arteriosus, tetralogy of Fallot and ventricular septal defects (Goldmuntz et al., 1993; Peyvandi et al., 2013); palatal abnormalities (velopharyngeal insufficiency [VPI], cleft lip, and/or palate); immunodeficiency and autoimmune disease, endocrinopathies (hypoparathyroidism with resultant hypocalcemia, thyroid dysfunction, growth hormone deficiency), genitourinary and gastrointestinal differences, and neurodevelopmental abnormalities (cognitive delays and neuropsychiatric involvement) (McDonald-McGinn et al., 2015). Patients also present with atypical features requiring the provider to consider the possibility of a concurrent diagnosis —such as a genetic condition related to/caused by the 22q11.2 microdeletion, or one that is unrelated/familial/coincidental. As the 22q11.2DS can affect multiple organ systems, it is crucial to consider that a secondary diagnosis may potentiate the symptoms of any single organ system.

Less common features reported in patients with 22q11.2DS and overlapping with other well-described conditions include: coloboma, sensorineural and conductive hearing loss, choanal atresia, tracheoesophageal fistula, imperforate anus, IUGR, vertebral anomalies, clubfoot, polydactyly, scoliosis, thrombocytopenia, microcephaly, idiopathic seizures, and hypotonia (McDonald-McGinn et al., 2015). These features, while known to be possibly associated with 22q11.2DS, are atypical enough that if they are noted, especially in conjunction with other atypical features, should raise a clinician’s suspicion to seek a secondary diagnosis.

Additionally, with the advent of newborn screening, certain highly prevalent genetic disorders are uncovered within a patient’s first weeks of life. If one of these patients additionally presents with findings reminiscent of 22q11.2DS, the clinician must still pursue the appropriate testing for this diagnosis. While statistically there is a lower likelihood of carrying two unrelated genetic diagnoses, this series demonstrates instances of these independently common dual diagnoses including cystic fibrosis, glucose 6 phosphate dehydrogenase (G6PD) deficiency, and severe combined immunodeficiency (SCID).

As has been previously reported, a deletion in one 22q11.2 allele can unmask an autosomal recessive disorder should the patient have a deletion/mutation in an important gene on the other allele (McDonald-McGinn et al., 2015). It is therefore crucial to give special consideration to these known conditions including a coagulopathy known as Bernard-Soulier syndrome type B due to a mutation in *GP1BB* (OMIM #231200), CEDNIK syndrome (Cerebral dysgenesis, neuropathy, ichthyosis and keratoderma) due to a mutation in *SNAP29* (OMIM #609528), and a condition with severe contractural arachnodactyly and skeletal anomalies known as van den Ende-Gupta syndrome (VDEGS) due to a *SCARF2* mutation (OMIM #600920), as well as other genes that may explain atypical features. For example, we have previously reported a patient with 22q11.2DS and Bernard-Soulier syndrome (Budarf et al., 1995) and four patients with 22q11.2DS and CEDNIK (McDonald-McGinn et al., 2013). Other groups have reported additional patients with Bernard-Soulier or VDEGS (Bedeschi et al., 2010; Kenny, Morateck, Gill, & Montgomery, 1999; Nakagawa, Okuno, Okamoto, Fujino, & Kato, 2001). Likewise, patients with 22q11.2DS and a second genetic diagnosis that is not a direct result of the copy number variant causing this deletion syndrome have been previously reported including: one patient from our center with SCID (Heimall et al., 2012), a patient with a sporadic FGFR3 mutation (Reardon et al., 1997), and a patient from our cohort with Trisomy 8 mosaicism (McDonald-McGinn et al., 2005). Similarly, here we present six unreported patients with coexisting conditions impacting medical management.

2 | METHODS

Patients with a laboratory confirmed chromosome 22q11.2 deletion via comparative genomic hybridization (CGH), multiplex ligation-dependent probe amplification (MLPA), fluorescent *in situ* hybridization (FISH), or SNP microarray, and a dual diagnosis were retrospectively identified from our cohort of 1,422 patients (including the seven previously reported cases with coexisting conditions) followed in the 22q and You Center at the Children's Hospital of Philadelphia (CHOP) under an IRB approved protocol. Of our 1,422 patients, 134 were relatives of the proband (69 mothers, 34 fathers, and 31 siblings), who were affected and presented to medical attention following a proband being diagnosed with 22q11.2 microdeletion syndrome.

3 | RESULTS

We identified six novel patients with a 22q11.2 deletion and a coexisting condition, resulting in an incidence of dual diagnoses at our center of 0.9%. Clinically available mutational analysis was performed to confirm a suspected diagnosis of cystic fibrosis (*CFTR*: F508del and c.1329_1330insAGAT, p.Ile444fs*3), and CHARGE syndrome (*CHD7*: c.3205C > T, p.Arg1069*). Microarray analysis was used to diagnose the 17q12 deletion syndrome and 1q21.1 deletion. Enzyme testing was used to confirm a newborn screen diagnosis of G6PD deficiency and coagulation assays were used to diagnose von Willebrand disease.

Six previously unpublished dual diagnosis patients are summarized in Table 1, with clinical features by organ system highlighted and attributed to one of the two diagnoses for each patient in Table 2. Using previous authors' delineation of distinct vs. overlapping phenotypes

(Boycott & Innes, 2017; Posey et al., 2017), our six patients fall into the following categories: patients 1, 3, and 6 each present with significantly overlapping phenotypes between their dual diagnoses, while patients 2, 4, and 5 demonstrate a mostly distinct secondary phenotype.

3.1 | CHARGE syndrome due to a *CHD7* mutation—Patient 1

Patient 1 is a now 26-year-old female with 22q11.2DS and CHARGE syndrome (OMIM #214800) who presented for a routine genetics evaluation soon after birth with a history of esophageal atresia. Perinatal history was complicated by severe polyhydramnios, absence of a stomach bubble, and a heart shifted to the left against the chest wall on fetal ultrasound. An amniocentesis during the pregnancy revealed a normal karyotype (46, XX). The persistent absence of gastric bubble raised the concern for a swallowing abnormality secondary to possible trachea-esophageal fistula. No choanal atresia or heart murmur was noted. A tube could not be passed into the stomach and abdominal X-ray was notable for a lack of gas. Workup as a neonate included X-rays to evaluate for VATER syndrome but vertebrae and other associated features were unremarkable. Ophthalmology exam discovered a unilateral chorioretinal coloboma involving the optic nerve. At 19 years of age the patient re-presented to the genetics department with an additional history of short stature and amenorrhea. At the time of this evaluation, her height, weight, and head circumference were all below the 5th percentile. She was noted to have a high arched palate, thick hair, narrow face, hypotelorism, mild osseous syndactyly of toes 2 and 3, distinctive ears, convex nasal ridge, a short philtrum, thick vermillion of the upper lip, and thinner vermillion of the lower lip. In light of the additional previous history of slight shortening and underdevelopment of the labia in the neonatal period, peripheral pulmonic stenosis, and anosmia, coupled with amenorrhea in adulthood and a coloboma involving the optic nerve, CHARGE syndrome was suspected. Mutational analysis for *CHD7* and a microarray were sent concurrently. The microarray revealed a nested 22q11.2 LCR22B-LCR22D deletion. This deletion does not include the important cardiac developmental gene *TBX1* but does include at least 15 other developmentally important genes including *SERPIND1, CRKL, LZTR1, SCARF2, PI4KA,* and *SNAP29* associated with features overlapping that of patients with a standard LCR22A-LCR22D 22q11.2 deletion. The patient was found to be heterozygous for a point mutation in the *CHD7* gene (c.3205C > T). This mutation changes a codon for arginine to a premature stop codon and has been reported previously in patients with CHARGE syndrome.

3.2 | Cystic fibrosis—Patient 2

Patient 2 has both 22q11.2DS and cystic fibrosis (CF) with a history of failure to thrive, sacral dimple, gastroesophageal reflux, pancreatic insufficiency, chronic otitis media, nasal congestion but no evidence of polypoid disease, right perforated tympanic membrane with bilateral myringotomy tubes, velopharyngeal insufficiency, anemia, vitamin D deficiency, and kidney stones. His state newborn screening study raised concerns for a diagnosis of cystic fibrosis (found to have a delta F508 mutation in *CFTR*); the diagnosis was confirmed with fecal elastase and a sweat chloride test. The patient's mother and father underwent *CFTR* sequencing. Mother was found to have a delta F508 mutation, and father was heterozygous for a c.1329_1330insAGAT mutation (previously described in association with CF). The insertion is a duplication and creates a stop codon caused by changing the reading

frame. A heart murmur was noted on day of life 1 and led to the patient remaining hospitalized for 5 days. A ventricular septal defect (VSD) (with concern for aortic arch hypoplasia) was discovered at 1 week of life requiring repair at 2 months of age. The patient received antibiotics in the newborn period for meconium aspiration concern. Noting the patient's long slender fingers and microcephaly, in conjunction with his VSD, the cardiologist sent testing for 22q11.2DS, which was positive. Parental testing for 22q11.2 deletion via FISH testing was normal.

3.3 | 17q12 Deletion syndrome—Patient 3

Patient 3 was evaluated by clinical genetics at 22 months of age for a history of polyhydramnios (and maternal uterus didelphys), failure to thrive, anemia, thrombocytopenia, unilateral multicystic dysplastic kidney, chronic constipation, developmental delay, and short stature. Physical exam revealed a nondysmorphic child at the 3rd percentile for height, 49th percentile for weight, and 40th percentile for head circumference. A sacral dimple and pilonidal cyst were found and a tethered cord was diagnosed by follow-up MRI. Fragile X testing was normal. On genome wide microarray, the child was found to have a 1.49 Mb maternally inherited pathogenic recurrent deletion of 17q12 and a *de novo* 395 kb nested deletion of chromosome 22q11.2 (LCR22-C to LCR22-D). His 17q12 deletion contains more than 28 RefSeq genes including *PIGW, ACACA,* and *HNF1B*. Haploinsufficiency of the *HNF1B* gene is associated with renal cysts and diabetes syndrome (RCAD) also known as MODY5 with clinical features including nondiabetic renal disease resulting from abnormal renal development, and diabetes. The severity among the patients can be variable (Mefford et al., 2007). Additional features associated with the 17q12 deletion syndrome (OMIM #614527) include pancreatic abnormalities, facial dysmorphism, genitourinary differences (including uterus didelphys and cryptorchidism), as well as cognitive and developmental differences (Loirat et al., 2010; Moreno-De-Luca et al., 2010; Palumbo et al., 2014).

3.4 | Glucose 6 phosphate dehydrogenase deficiency—Patient 4

Patient 4 is a 13-year-old African American male with a nested LCR22-B to LCR22-D 22q11.2 deletion, which was detected by MLPA testing ordered by his pediatrician; the test was performed due to a history of autism spectrum disorder, hypernasal speech (possible VPI), a high T4 count, overfolded auricles, intermittent episodes of vomiting, asthma, headaches, bilateral pes planus, and a learning disability. Fragile X testing was also performed and was normal. The patient is mildly dysmorphic with slightly overfolded helices, bilateral crumpled ears, long narrow nose with narrow nasal bridge and nasal tip, a high arched palate, malar flattening, and hyperpigmentation on his phallus. Glucose-6-phosphate dehydrogenase deficiency is included in the newborn screen in some states and was confirmed in this child at 9 months of age, when his Glucose-6-phosphate dehydrogenase studies revealed a low level—1.3 U/g Hb (normal range: 4.6–13.5 U/g Hb), with normal hematocrit, hemoglobin, and mean corpuscular volume. The G6PD level was verified by repeat analysis according to the lab report. He has never had a blood transfusion.

3.5 | Von Willebrand disease—Patient 5

Patient 5 was diagnosed with 22q11.2DS at 5 years 9 months of age when his cardiologist noted, in addition to his previously diagnosed VSD and interrupted aortic arch, that the child was experiencing speech difficulties due to palatal insufficiency. He too was found to have a nested LCR22A-LCR22B 22q11.2 deletion initially detected by FISH analysis and later sized by MLPA. The pregnancy was complicated by polyhydramnios and maternal gallstones. The child had both feeding difficulties and failure to thrive in infancy. He had a protruding left ear, VPI, mild epicanthus on the left, bulbous nose, high palate, heart murmur, a broad left thumb, normal left kidney and cystic right kidney on renal ultrasound (small simple cyst), and a sacral dimple with the diagnosis of spina bifida occulta. He was evaluated at 7 years of age for excessive bruising and a prolonged partial thromboplastin time of 42 s (normal 26.5 to 36.5 s), although he tolerated multiple surgical procedures without bleeding or clotting concerns. Given the fact that the patient's platelet counts were not low enough to explain his bleeding/bruising concerns, and he was not found to have Bernard-Soulier syndrome, the hematology team investigated for other diagnoses. His prothrombin time was 13.0 s (INR 1.14). His repeat aPTT was very nearly normal. His peripheral blood smear revealed generally large platelets, and a very abnormal result on the platelet function analysis showing closure time >255 s with collagen/epinephrine (normal <184 s), and closure time >300 s with collagen/ADT (normal <110 s). Factor VIII activity level was 67% (normal 60% to 180%), von Willebrand factor antigen 58% (normal 50% to 160%), a low level of ristocetin cofactor activity 40% (normal 46% to 150%), and normal von Willebrand factor multimer analysis. These findings are consistent with the diagnosis of "low vWF levels" or mild type 1 von Willebrand disease.

3.6 | 1q21.1 deletion syndrome—Patient 6

Patient 6 was diagnosed at 2 years of age via microarray with both a standard LCR22A to LCR22D 22q11.2 deletion and a 1.207 Mb deletion on chromosome 1q21.1 due to his history of dysphagia, mild developmental delay, and dysmorphic features that were not clearly consistent with a known diagnosis. The pregnancy was complicated by a spinal abnormality noted on ultrasound. While the phenotype of 1q21.1 deletion syndrome (OMIM #612474) can be variable, patient 6 presented with findings consistent with this diagnosis including microcephaly, and skeletal anomalies including scoliosis (Bernier et al., 2016; Gamba et al., 2016). The patient also presented with features consistent with 22q11.2DS including feeding difficulties, constipation, short stature, laryngeal cleft, developmental delay, low IgM and moderate CD8 T cell lymphopenia, anterior arch C1 hypoplasia, velopharyngeal insufficiency and hypernasal speech, underdeveloped alae nasi, and long, slender, tapered fingers. Interestingly, the patient does not present with any cardiac anomaly, which can be associated with either of his chromosome deletion diagnoses. The 1q21.1 deletion has been previously reported to be inherited with incomplete penetrance (Brunetti-Pierri et al., 2008); parental studies are pending at the time of this publication.

4 | DISCUSSION

With the advent of whole exome sequencing (WES), the phenomenon of multiple genetic diagnoses in a single patient is becoming more widely recognized (Boycott & Innes, 2017).

Recent studies have found a multiple genetic diagnosis rate in patients found to have at least one molecular diagnosis on WES of 4.9% (Posey et al., 2017), 3.2% (Retterer et al., 2016), and 6.5% (Yang et al., 2013). Of the 2,076 patients in Posey et al.'s article who were found to have at least one molecular diagnosis, 12 patients (0.6%) were found to have a causal copy-number variant as one of their multiple diagnoses (Posey et al., 2017). Similarly, from our cohort of 1,422 patients who each harbor at least one causal copy-number variant, specifically the 22q11.2 microdeletion, 0.9% of these patients were found to have a second genetic diagnosis. We have reason to believe that this 0.9% dual diagnosis rate is an underestimation, since clinicians may stop searching for a cause after confirming an early diagnosis of 22q11.2DS and may not consider alternate explanations for an atypical phenotype, especially due to this microdeletion's known heterogeneous pathogenicity. Conversely, patients referred for WES will inevitably have all of their genetic diagnoses uncovered simultaneously, preventing the bias of prematurely ending a diagnostic odyssey when one reasonable explanation is found.

This case series highlights the clinical importance of attending closely to patients who present with features that are inconsistent with a diagnosis of 22q11.2 deletion or exceed the severity generally associated with this diagnosis. Previous reports of clinicians investigating for a secondary diagnosis often occur when a disease with a well-known phenotype presents with atypical severity (Jehee et al., 2017). For example, while the individual features of esophageal atresia, optic nerve coloboma, and sensorineural hearing loss, can each be observed in 22q11.2DS, the combination of all three in a single patient triggered consideration of an alternative unifying diagnosis, in this case CHARGE syndrome.

4.1 | Previous reports of 22q11.2DS and a second diagnosis

Previously published cases of 22q11.2DS with unrelated concomitant secondary genetic disorders include trisomy 8 mosaicism, Artemis deficiency (a rare form of severe combined immunodeficiency), and a sporadic case of *FGFR3* mutation (Table 3). Autosomal recessive disorders unmasked by a 22q11.2 microdeletion, which have also been previously reported, include CEDNIK, Bernard-Soulier syndrome, and van den Ende-Gupta syndrome (Table 3) (Budarf et al., 1995; McDonald-McGinn et al., 2013).

For the seven cases previously reported by our center, six patients carried the diagnosis of 22q11.2DS prior to the determination of their secondary diagnosis. The *SNAP29* mutations (four patients) were all discovered on research testing of the opposite allele; three of the patients presented with structural brain anomalies and one presented with a cleft lip. The patient with Bernard-Soulier syndrome was uncovered a few months following the 22q11.2DS diagnosis, after a significant episode of epistaxis, moderate thrombocytopenia, and large platelets amid a history of significant bleeding. The patient with an unusual form of SCID was noted to have severe immunodeficiency beyond the typical presentation of a 22q microdeletion. The trisomy 8 mosaicism was uncovered as the first diagnosis on traditional cytogenetic studies in the setting of a congenital diaphragmatic hernia. The 22q11.2DS diagnosis was made in the setting of specific testing conducted to explain the patient's truncus arteriosus and polydactyly.

For the previously reported cases in the literature, Bernard-Soulier syndrome was often the initial or simultaneous diagnosis made (Kenny et al., 1999; Nakagawa et al., 2001). The VDEGS diagnosis was made secondarily upon sequencing *SCARF2* in a patient who phenotypically presented with VDEGS and was initially found to have a heterozygous deletion of 22q11.2 (Bedeschi et al., 2010). The *FGFR3* mutation was uncovered after the 22q11.2 deletion, as an explanation for the patient's craniosynostosis and broad thumbs (Reardon et al., 1997).

4.2 | Clues to pursuing a second diagnosis

It is worth noting that most of the six newly reported dual diagnosis cases presented in this series harbor nested deletions. This may have been the impetus in certain cases to search for an alternative diagnosis, when the severity seemed out of proportion especially for a patient harboring a nested deletion. In regards to patient 1, the gene *CHD7* has been shown to operate in the same developmental pathway as *TBX1* (Corsten-Janssen & Scambler, 2017); the latter is a known developmental gene located near the FISH probes N25 and TUPLE with the 22q11.2 LCR22A-LCR22B region. Interestingly, the patient presented in this series with a *CHD7* mutation had a B-D 22q11.2 deletion, which does not include the *TBX1* gene. While this patient's nested deletion is often associated with a milder phenotype, her course complicated by a concomitant diagnosis of CHARGE syndrome was more involved. Mouse model studies have demonstrated increased incidence of cardiac anomalies and reduced viability in double *Chd7* and *Tbx1* heterozygote knockouts (Randall et al., 2009). Therefore, it seems unlikely that a patient with a full A-D deletion would present with a secondary diagnosis as severe as CHARGE syndrome, since these concurrent mutations would reduce the likelihood of viability. Patient 3 who harbors an LCR22-C to LCR22-D nested deletion exhibits the many phenotypic features of his maternally inherited 17q12 deletion, including severe developmental delay and cryptorchidism, coupled with gastrointestinal problems (reflux and constipation) known to be more often associated with 22q11.2DS. The cases of patients 1 and 3 demonstrate that those with nested deletions who have a somewhat severe phenotype are a subset in which to pursue a secondary cause.

Family history can help direct diagnosis as well. The proband in case 3 has an extensive family history of renal disease, some of it fatal in the newborn period. Mother of proband, who is also affected with 17q12 deletion, has a learning disability (she completed high school), and uterus didelphys (two uteri and two cervices). The proband had a maternal half-brother who died at several hours of life due to multi-cystic kidneys and an unspecified spinal cord/neural tube defect. The patient had two maternal aunts, one with a cardiac anomaly and another with kidney problems. The maternal grandfather had kidney issues, two great aunts had uterus and cervix anomalies, and a maternal great uncle died after birth with kidney disease.

4.3 | Implications to clinical management

Accurate diagnostics allows for more personalized and appropriate medical management. The gastrointestinal complications that can be seen in 22q11.2DS include esophagitis, aspiration, choking and recurrent pneumonia, failure to thrive, malnutrition, and feeding refusal (Dyce et al., 2002; Eicher et al., 2000). Older children and adults often experience

recurrent and chronic sinus infections as a sign of nasopharyngeal reflux as this results in repeated contamination of the nasal cavity (Bassett et al., 2011; Dyce et al., 2002; McDonald-McGinn et al., 1997; McDonald-McGinn et al., 1999; McDonald-McGinn et al., 2001; Ruotolo et al., 2006; Solot et al., 2001; Stransky et al., 2015; Widdershoven et al., 2013). Patients with cystic fibrosis often first present with failure to thrive followed by recurrent pneumonia. The sinopulmonary infections in 22q11.2DS are often exacerbated by immune deficiency, susceptible anatomy, and the increased risk of allergies (Staple, Andrews, McDonald-McGinn, Zackai, & Sullivan, 2005). When a patient with 22q11.2DS also has cystic fibrosis, this dual diagnosis can exacerbate the sinus and pulmonary infections resulting from both underlying conditions. Early and aggressive management of symptoms can impact long-term outcome and quality of life.

The recommendations for vaccines and prophylactic treatment must be carefully examined in patients with 22q11.2DS and cystic fibrosis. 22q11.2DS patients with very low T cell counts should not be administered live viral vaccines. Patients with no CD45RA T cells should receive prophylaxis against pneumocystis (Butcher et al., 2012; Hamilton, Husein, & Dworschak-Stokan, 2008; Hofstetter et al., 2014; Moylett, Wasan, Noroski, & Shearer, 2004; Perez, Bokszczanin, McDonald-McGinn, Zackai, & Sullivan, 2003). According to 2016 *Clinical Practice Guidelines From the Cystic Fibrosis Foundation for Preschoolers With Cystic Fibrosis* (Lahiri et al., 2016), children should receive age-appropriate immunizations and annual seasonal influenza vaccination along with family members and caregivers. Furthermore, it is recommended that 22q11.2DS patients with autoimmune diseases should be managed with minimal immunosuppression treatment if possible (Maggadottir & Sullivan, 2013). Patients with CF may require a lung transplant at a certain stage of their life and disease, which would be accompanied by subsequent necessary immunosuppression. These are the types of medical decisions that need to be carefully weighed and considered in treating patients with dual diagnoses.

With a diagnosis of CF comes a risk of cystic fibrosis related diabetes (CFRD). Diabetes and adult-onset obesity has been studied in 22q11.2DS. The age of onset for type 2 diabetes was noted to be similar to the age of onset in adults with Prader-Willi syndrome; the median age of diagnosis for type 2 diabetes in 22q11.2DS (10 patients in a cohort of 207) was 39.5 years compared to all types of diabetes for adults in the general U.S. population which is 54.2 years (Sinnema et al., 2011; Voll et al., 2017). Voll et al.'s study suggests that psychotic illness (and therefore psychotropic medications) may be a contributing factor to the obesity observed in adults with 22q11.2DS, but not the sole cause. It is important that clinicians are aware of this risk when considering prescription of psychotropic medication, especially if coupled with an even greater risk for diabetes due to a secondary diagnosis. Similar to patient 2, with a dual diagnosis of CF and 22q11.2DS, the risk of diabetes in patient 3 also comes from two separate diagnoses. The gene included in patient 3's 17q12 deletion, which is associated with renal anomalies and increased risk of diabetes, is *HNF1B* (Palumbo et al., 2014).

Patient 5 demonstrates that despite a phenotypic feature being consistent with an underlying diagnosis, it is important to investigate further when a symptom seems more severe than expected for one particular diagnosis. 22q11.2DS is sometimes associated with platelet

abnormalities, such as Bernard-Soulier syndrome or storage pool deficiency. These patients will have bleeding symptoms. Many more patients with the deletion have mildly low platelet counts with elevated mean platelet volume (Lambert et al., 2017). Most of these patients do not have abnormal bleeding. Patient 5 had additional evidence of mild von Willebrand disease. This information was important to identify since the intervention in the case of minor bleeding could be treatment with aminocaproic acid (antifibrinolytic agent) or nasal DDAVP, which transiently increases circulating levels of von Willebrand factor.

Finally, anticipatory guidance and counseling is entirely dependent on accurate diagnosis. For example, there is reduced reproductive fitness of men with 22q11.2DS (Costain, Chow, Silversides, & Bassett, 2011); this coupled with the infertility seen in patients with cystic fibrosis is an important counseling concern when discussing reproductive health with patients.

5 | CONCLUSION

It is imperative that clinicians diagnose and remain cognizant of the phenotypic overlap within the dual diagnoses of their patients. Overlapping features often make a secondary diagnosis more difficult to discover, or lead to a delay in secondary diagnosis, since medical teams often conclude a diagnostic odyssey when a fitting explanation for a symptom is found. The phenotypic overlap between 22q11.2DS and CHARGE syndrome may both make the diagnosis more difficult and may also lead to more severe organ involvement. Retinal coloboma, choanal atresia, and butterfly vertebrae (McDonald-McGinn et al., 2015) are features common to both 22q11.2DS and CHARGE syndrome. Feeding difficulties and failure to thrive or short stature are nonspecific features that underlie 22q11.2DS as well as many of the secondary diagnoses presented in this series. The immunodeficiency seen in 22q11.2DS is often secondary to thymic hypoplasia and impaired T-cell production (Jyonouchi, McDonald-McGinn, Bale, Zackai, & Sullivan, 2009; McDonald-McGinn & Sullivan, 2011; Piliero, Sanford, McDonald-McGinn, Zackai, & Sullivan, 2004; Verhagen et al., 2012). T-cell lymphocytopenia is the finding that is detected on the newborn screen for SCID (McDonald-McGinn et al., 2015). An overlapping ophthalmologic feature between 22q11.2DS and van den Ende-Gupta syndrome is sclerocornea.

While in 22q11.2DS the mean IQ is ~70 compared with a reference IQ mean of 100 in the typically developing population, it is important to consider secondary insults, including cardiac arrest, hypocalcemia, or seizures (De Smedt, Swillen, Verschaffel, & Ghesquiere, 2009; Glaser et al., 2002; Wang, Woodin, Kreps-Falk, & Moss, 2000) versus a secondary diagnosis known to impact neurodevelopment. It is important to counsel families on the expectation of developmental outcomes with even greater caution when there is a possibility that a secondary diagnosis may be contributing to the patient’s phenotype.

Premature mortality occurs in 22q11.2DS, and early deaths in the infant age group are in part due to cardiac defects, hypocalcemia, and airway malacia (McDonald-McGinn et al., 2005; Repetto et al., 2014). The fact that overall mortality for those with 22q11.2DS is greater than the mortality rates observed in nonsyndromic individuals with similar cardiac defects (Repetto et al., 2014) is one piece of evidence pointing to the fact that syndromes

themselves can affect overall morbidity and mortality; it is not merely the exact sum of the parts of symptomatic organ involvement that can contribute to overall prognosis. It is unclear how two concurrent genetic syndromes in a single patient may impact that patient's overall clinical course. As cytogenetic and molecular diagnoses become more widespread, and there are more instances of dual diagnoses uncovered, we may be able to provide better prognostic evidence for this cohort of patients.

From a practical standpoint, it is important that one diagnosis not over-shadow the needs of the other; the impact that treatment of one condition may have on the other must be considered. Medications and interactions are one example of consideration when dealing with two potentially conflicting diagnoses. For instance, calcium supplementation, which is often necessary for 22q11.2DS treatment, can cause increased risk of renal calculi. In 17q12 deletion syndrome, one of the dual diagnoses presented in this series, cystic kidneys are a feature of the syndrome and renal calculi in the unaffected kidney may prove detrimental.

The methods for diagnostic testing of 22q11.2DS are an important discussion point in the context of potential dual diagnoses, since the use of FISH will not identify nested deletions outside of the LCR22A-LCR22B region nor CNVs on other chromosomes. Even multiplex ligation-dependent probe amplification (MLPA) will not detect CNVs, deletions and duplications, or gene mutations in genes not related to the 22q11.2 deletion site. This case series may provide an additional argument for analyzing a genome-wide microarray in lieu of MLPA or FISH, which will accomplish the task of diagnosing 22q11.2 microdeletion syndrome, while also drawing a clinician's attention to other potential CNVs that can cause a pathogenic phenotype, and/or regions of homozygosity that can direct a clinician to a hotspot for potential autosomal recessive genetic disorders. In our center, FISH is no longer used as a diagnostic method, as this would result in missed nested LCR22B-LCR22D and LCR22C-LCR22D deletions. While cost of genetic testing may be a concern in pursuing secondary diagnoses, there may also be health care costs saved by correctly finding a secondary diagnosis and employing the appropriate preventative health care.

In light of our findings, it is not necessarily prudent or cost-effective to consider whole exome sequencing in all of these patients but is instead wise to consider multiplex clinical tests on all 22q11.2DS patients, instead of only an MLPA. This can be accomplished by assessing for a CNV at 22q11.2 and a mutation in a presumed involved or important gene on the nondeleted allele, along with conducting a full microarray. Furthermore, WES can then be reserved for patients with atypical findings, which will prove a prudent economic decision based on current cost and resource utilization. With the high utilization of hospital visits (inpatient and outpatient) of patients with 22q11.2DS, it is especially important to investigate and document all secondary diagnoses, in order to provide the safest and highest quality care to this population.

New cases of dual diagnoses within the 22q and You Center's cohort are continually surfacing as our clinical knowledge regarding unmasked recessive diseases on the non-deleted allele and the clinical presentations associated with these genes becomes more well defined. Investigations into these mutations on the nondeleted allele are currently underway as part of our international 22q11.2 Brain and Behavior Consortium.

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TABLE 1

Laboratory results for six dual diagnosis patients

Patient case	22q11.2 deletion type	Diagnostic test	Confirmed secondary diagnosis	Laboratory testing results
1	B-D; 744 kb het deletion on 22q11.21 (contains 15 genes including *SERPIND1, SNAP29*; *TBX1* is not deleted)	Microarray	CHARGE syndrome	*CHD7*: c.3205C > T, p.Arg1069*
2	22q11.21 deletion	Microarray	Cystic Fibrosis	*CFTR*: F508del and c.1329_1330insAGAT, p.Ile444fs*3
3	C-D; 395 kb het deletion on chr22: 21069073–21463730 (contains 10 RefSeq genes; does not include *TBX1*)	Microarray	17q12 deletion syndrome	1.49 Mb pathogenic recurrent deletion on chromosome 17q12
4	B-D	MLPA	G6PD deficiency	Glucose-6-phosphate dehydrogenase level low: 1.3 U/g Hb
5	A-B	MLPA	von Willebrand disease	Low vWF levels, low level of ristocetin cofactor activity
6	A-D; 2.406 Mb deletion on 22q11.21	Microarray	1q21.1 deletion syndrome	1.207 Mb deletion on chromosome 1q21.1q21.2

Abbreviations (het = heterozygous; MLPA = multiplex ligation-dependent probe amplification).

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TABLE 2

Clinical features of six dual diagnosis patients

Patient case number	Organ system involved	Clinical features outside of 22q11.2 deletion syndrome and consistent with secondary diagnosis	Clinical features consistent with 22q11.2 deletion syndrome
1	Neurology/development	***Cavum septum pellucidum***; DEVELOPMENT ***Delayed milestones:*** sat 12 mo; walked 24 mo; speech 24 mo; ***Asperger's syndrome;*** anxiety; 19 yo at 8th grade academic level; IEP; repeated one grade during schooling	***Cavum septum pellucidum;*** sagittal synostosis; DEVELOPMENT ***Delayed milestones:*** sat 12 mo; walked 24 mo; speech 24 mo; ***Asperger's syndrome;*** anxiety; 19 yo at 8th grade academic level; IEP; repeated one grade during schooling
	Otolaryngology	Anosmia (MRI: olfactory sulci hypoplastic; no visualization of well-formed olfactory bulbs); ***bilateral sensorineural hearing loss***	Three otitis media infections in 12 mo, recurrent, monthly sinus infections; small mandible, narrow nasal valves bilaterally; ***bilateral sensorineural hearing loss***
	Cardiology	Peripheral pulmonic stenosis	NR
	Ophthalmology	***Coloboma involving optic nerve***	***Coloboma involving optic nerve***
	Orthopedics	NR	Scoliosis
	Endocrinology	Amenorrhea, low follicle stimulating hormone (FSH)	Bone age at 19 yo was 13 yo (beyond 2SD)
	Gastrointestinal	***Esophageal atresia;*** colonic interposition with surgical repair and gastrostomy tube placement; hospitalized multiple times for GI concerns	***Esophageal atresia***
	Genitourinary	Slight shortening of labia (underdeveloped) in neonatal period; diminutive uterus	NR
2	Neurology/development	NR	Deep sacral dimple, normal spine MRI; DEVELOPMENT: delayed crawling; walked at typical age; speech delay; Bayley Scale of Infant Development: general cognitive abilities avg to low avg; Vineland Adaptive Behavior Scales: avg to low avg adaptive behavioral skills; No concerns on Child Behavior Checklist. 23 mo old: development quotient 92 (avg range); receptive language skills at 18 mo level and emerging to the 19 to 24 mo level; Expressive language skills 13 to 18 mo level. At 5.5 yo attended regular school for kindergarten but in a special classroom with IEP. Received PT briefly
	Otolaryngology	NR	Chronic otitis media infections and persistent nasal regurgitation with emesis; possible subglottic cyst on laryngoscopy and a bifid uvula; mild low-frequency conductive hearing loss in the right ear and abnormal results in the left ear due to excessive cerumen; right tympanic membrane perforation and left myringotomy; hypernasal speech with glottal stops
	Cardiology	NR	Ventricular septal defect (VSD) (with concern for aortic arch hypoplasia)
	Ophthalmology	NR	Bilateral esotropia without his glasses
	Orthopedics	NR	Toes: two overlapping three bilaterally, long, slender fingers with bilateral transitional palmar creases; extra rib and hyperextensibility
	Immunology	NR	Significant immune deficiency: inadequate responses to vaccines; 3 yo with normal B and T-cell subsets, normal immunoglobulin levels, and normal lymphocyte stimulation assay, except for low-normal candida response: cleared to receive live vaccines

Patient case number	Organ system involved	Clinical features outside of 22q11.2 deletion syndrome and consistent with secondary diagnosis	Clinical features consistent with 22q11.2 deletion syndrome
	Pulmonology	MRSA pneumonia; bronchomalacia, minimal membranous subglottic stenosis; increased airway secretions; chest CT with diffuse mosaicism of the lung without obvious bronchiectasis, recurrent pneumonia	NR
	Endocrinology	Vitamin D deficiency	Hypocalcemia following VSD repair
	Gastrointestinal	***Failure to thrive requiring overnight G-tube feeds; constipation*** without distal intestinal obstruction syndrome; elevated liver function studies at 5yo	***Failure to thrive requiring overnight G-tube feeds; constipation;*** gastroesophageal reflux disease
	Nephrology	NR	Renal calculi seen on ultrasound at 5yo
3	Neurology/development	DEVELOPMENT significant delays: sat 12 mo; walked 22–25 mo, severe mixed receptive and expressive language disorder (at 28 mo had 20 words; at 3 yo 4 mo total language score of 11 mo equivalent (expressive 1 yo 1 mo and comprehension 11 mo); Autism spectrum disorder; not toilet trained at 3 yo. At 4 yo 3 mo: fine motor 9–12 mo for pincer grasp, other skills at 6 mo level; OT noted impaired strength, muscle tone, coordination, cognition and acquisition of milestones; developmental pediatrician was concerned for ID at his 4 yo visit based on skills assessed at <1 yo	Sacral dimple, pilonidal cyst and a tethered cord (slightly low position of the conus terminating at upper L3 level); two regions of fatty infiltration in the filum terminale noted
	Otolaryngology	NR	Sensitivity to noise; normal audiogram at 25 mo old; one ear infection between 12 and 24 mo old
	Ophthalmology	NR	Esophoria and moderate hyperopia with no need for corrective lenses
	Immunology	NR	NR; T cells unaffected
	Pulmonology	NR	Mild intermittent asthma well controlled on albuterol
	Endocrinology	HbA1C normal; ***short stature; growth hormone response to stimulation: borderline deficient***	***Short stature; growth hormone response to stimulation: borderline deficient***
	Gastrointestinal	NR	Constipation; poor weight gain; frequent reflux leading to cough, treated with ranitidine
	Nephrology	***Multicystic dysplastic kidney (prenatal onset),*** and no obstruction on the left but with compensatory hypertrophy in the left kidney and normal kidney function; elevated spot urine oxalate:creatinine ratios, but urine hyperoxaluria panel was normal; normal creatinine and PTH levels	***Multicystic dysplastic kidney;*** 2 mo old: small nonobstructing stones in left kidney
	Urology	Cryptorchidism; 3 yo: moderate atrophy of the right testis and a normal left testis with compensatory hypertrophy	NR
	Hematology	NR	Thrombocytopenia in newborn period; anemia at 2 mo
4	Neurology/development	NR	At 9 yo, taking risperidone; difficulty expressing emotions and opening up to discuss his worries; intermittent headaches with vomiting; benign developmental venous anomaly in the left cerebellum with associated signal abnormality; no associated cavernoma. Benign developmental venous anomaly in the left frontal lobe. Probable synovial cyst with hemorrhage fluid level between the dens and left lateral mass of C1; DEVELOPMENT: rolled over 6 mo; sat/crawled 9 mo; pulled to stand 12 mo; ran/hopped/walked 2 yo; climbed stairs 3 yo; first words 12 mo; sentences 2 yo;

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Patient case number	Organ system involved	Clinical features outside of 22q11.2 deletion syndrome and consistent with secondary diagnosis	Clinical features consistent with 22q11.2 deletion syndrome
			toilet trained 2 yo; Autism spectrum disorder; mixed receptive-expressive language disorder; 13 yo attends 7th grade at a public school in full time autism support placement, with no aide in school; reads at a 4th–5th grade level and does well with basic math; previously received PT/OT/speech
	Otolaryngology	NR	Normal hearing assessment
	Cardiology	NR	Normal echocardiogram with some question of an additional vessel; unlikely vascular ring
	Ophthalmology	NR	Corrective lenses
	Orthopedics	NR	Scoliosis; pectus excavatum; pes planus bilaterally with tight heel cords and hamstrings; head tilt; very long slender fingers, sandal gap of his toes
	Immunology	NR	Normal immunology testing
	Pulmonology	NR	Past history of asthma now resolved
	Endocrinology	NR	Slightly high T4 levels with normal thyroid stimulating hormone
	Gastrointestinal	NR	Weight low for height percentile at 13 yo; sporadic and intermittent emesis since age 10 yo thought to be abdominal migraines
	Hematology	Glucose-6-phosphate dehydrogenase deficiency	NR
5	Neurology/development	NR	Seizures from 18 mo to 5 yo 6 mo; anxiety, bipolar disorder, social anxiety, delusional thinking and psychosis. He had symptoms of hyperactivity and aggressive behaviors; headaches; insomnia, past history of night terrors; hospitalized once for an episode of mania with psychotic features; DEVELOPMENT: walked 18 mo; first words 2 yo 6 mo; hypernasal speech, IEP, PT/OT/speech therapies in childhood, ADHD, social anxiety; full scale IQ 78; 17 yo: completing high school and obtaining GED
	Otolaryngology	NR	Frequent otitis media and myringotomies, tympanostomy and adenoidectomy; velopharyngeal insufficiency
	Cardiology	NR	VSD and interrupted aortic arch; developed a subaortic web; patching of the supravalvular region to relieve supravalvular aortic stenosis at the site of the aortotomy from the original surgery; cardiac MRI showed mild residual subaortic obstruction as well as mild stenosis of the left subclavian artery
	Orthopedics	NR	Normal C spine films
	Immunology	NR	Low T-cell count at around 4 yo; history of chronic rhinitis and chronic sinus infections
	Pulmonology	NR	Obstructive sleep apnea due to narrow palate; asthma
	Endocrinology	NR	Hypocalcemia and hypothyroidism
	Gastrointestinal	NR	Constipation; chronic, recurrent, crampy abdominal pain since age 3 yo; feeding difficulties and failure to thrive in infancy
	Urology	NR	Inguinal hernia; hydrocele and phimosis
	Nephrology	NR	Cystic right kidney on renal ultrasound (small simple cyst)

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Patient case number	Organ system involved	Clinical features outside of 22q11.2 deletion syndrome and consistent with secondary diagnosis	Clinical features consistent with 22q11.2 deletion syndrome
	Hematology	Excessive bruising and a prolonged partial thromboplastin time of 42 s; low level of ristocetin cofactor activity 40% (normal 46% to 150%): consistent with the diagnosis of mild type 1 von Willebrand disease	NR
6	Neurology/development	***Microcephaly; DEVELOPMENT: rolled over 7mo, sat 9 mo, walked 20 mo, single words 2.5 yo, short sentences 3 yo, difficulty toilet training***	Low muscle tone; ***microcephaly, DEVELOPMENT: rolled over 7 mo, sat 9 mo, walked 20 mo, single words 2.5 yo, short sentences 3 yo, difficulty toilet training***
	Otolaryngology	NR	Otitis media, aspiration, laryngeal cleft type I, nasopharyngeal reflux, velopharyngeal insufficiency and hypernasal speech
	Orthopedics	***Congenital scoliosis and mild kyphosis; anterior arch C1 hypoplasia, increased atlantodental interval with flexion, lumbar butterfly vertebra, and vertebral fusion anomalies***	***Congenital scoliosis and mild kyphosis; anterior arch C1 hypoplasia, increased atlantodental interval with flexion, lumbar butterfly vertebra, and vertebral fusion anomalies***
	Immunology	NR	Frequent upper respiratory infections, low IgM, and nonprotective pneumococcal titers, moderate CD8 T-cell lymphopenia
	Endocrinology	NR	Short stature
	Gastrointestinal	NR	Dysphagia, feeding difficulties, reflux, vomiting, constipation, failure to thrive, gastrostomy tube dependent

Abbreviations (mo = months old; yo = years old; IEP = individualized education plan; NR = none reported; avg = average; PT = physical therapy; ID = intellectual disability; OT = occupational therapy; ADHD = attention deficit and hyperactivity disorder; ***italics*** = overlapping features.

TABLE 3

Previously reported cases of dual diagnoses

Confirmed secondary diagnosis	Classification of secondary diagnosis	Publication
CEDNIK syndrome	Unmasked AR	McDonald-McGinn et al., 2011
Bernard-Soulier syndrome	Unmasked AR	Budarf et al., 1995; Kenny et al., 1999; Nakagawa et al., 2001
Van den Ende-Gupta syndrome	Unmasked AR	Bedeschi et al., 2010
Trisomy 8 mosaicism	Mosaic aneuploidy	McDonald-McGinn et al., 1997
Severe combined immunodeficiency syndrome (rare form known as Artemis deficiency)	Coincidental AR	Heimall et al., 2012
FGFR3 mutation	Heterozygous for pro250arg mutation	Reardon et al., 1997

Abbreviations (AR = autosomal recessive).