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ORIGINAL ARTICLE

Identification of 22q11.2 Deletion Syndrome via Newborn Screening for Severe Combined Immunodeficiency

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Abstract
Purpose Chromosome 22q11.2 deletion syndrome (22q11.2DS), the most common cause of DiGeorge syndrome, is quite variable. Neonatal diagnosis traditionally relies on recognition of classic features and cytogenetic testing, but many patients come to attention only following identification of later onset conditions, such as hypernasal speech due to palatal insufficiency and developmental and behavioral differences including speech delay, autism, and learning disabilities that would benefit from early interventions. Newborn screening (NBS) for severe combined immunodeficiency (SCID) is now identifying infants with 22q11.2DS due to T cell lymphopenia. Here, we report findings in such neonates, underscoring the efficacy of early diagnosis.
Methods A retrospective chart review of 1350 patients with 22q11.2DS evaluated at the Children's Hospital of Philadelphia identified 11 newborns with a positive NBS for SCID.
Results Five out of 11 would have been diagnosed with 22q11.2DS without NBS, whereas early identification of 22q11.2DS in 6/11 led to the diagnosis of significant associated features including hypocalcemia, congenital heart disease (CHD), and gastroesophageal reflux disease that may have gone unrecognized and therefore untreated.
Conclusions Our findings support rapidly screening infants with a positive NBS for SCID, but without SCID, for 22q11.2DS even when typically associated features such as CHD are absent, particularly when B cells and NK cells are normal. Moreover, direct NBS for 22q11.2DS using multiplex qPCR would be equally, if not more, beneficial, as early identification of 22q11.2DS will obviate a protracted diagnostic odyssey while providing an opportunity for timely assessment and interventions as needed, even in the absence of T cell lymphopenia.

Keywords 22q11.2 deletion · DiGeorge syndrome · newborn screening · SCID · TREC

Introduction

Severe combined immunodeficiency (SCID) is a genetic condition characterized by T cell lymphopenia, the absence or very low production and function of T cells [1]. The condition has historically presented with frequent and severe bacterial, fungal, and viral infections around 3 to 6 months of age, as well as persistent diarrhea and failure to thrive, typically leading to mortality within the first 2 years of life if untreated [1, 2]. The incidence of SCID is estimated to be 1 in 58,000 live births, with >20 genes known to be causally associated with both SCID and SCID-like disorders [1–6]. SCID is initially treated with immunoglobulin replacement, anti-microbial prophylaxis, nutritional supplementation for poor weight gain (e.g. nasogastric tube feeding or parenteral nutrition), and avoidance of live vaccines and non-irradiated blood products [1–3]. However, the definitive treatment for SCID is hematopoietic stem cell transplantation (HSCT), or in certain types,

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enzyme replacement therapy. The success rate has been demonstrated to be far superior if HSCT is performed within the first 3.5 months of life and/or prior to infection [4].

Newborn screening (NBS) for SCID was first piloted in the USA in 2008 and was subsequently added to the US Department of Health and Human Services' Recommended Uniform Screening Panel (RUSP) in 2010 [7]. As of April 2017, all 50 states in the USA plus the District of Columbia, Puerto Rico, and the Navajo Nation in Arizona are currently performing NBS for SCID or are in the pilot/planning phase of adding SCID to their respective NBS panels [8]. Concurrently, there is an emerging goal to promote global newborn screening for SCID, as the condition is initially asymptomatic but carries a high burden of morbidity/mortality if left untreated. Effective treatment options are currently available [2, 9–12].

NBS for SCID utilizes real-time quantitative polymerase chain reaction (RT-qPCR) to measure T cell receptor excision circles (TRECs) and extra-chromosomal DNA fragments that are formed by thymocytes during normal T cell maturation [13]. The exact TRECs cut-off levels that indicate a presumptive positive screen vary from state-to-state, but typically 0.1–0.2% of all NBS results will indicate a positive screen for SCID [14–17]. Following an abnormal NBS for SCID, testing via flow cytometry, is completed to enumerate lymphocyte counts and confirm a diagnosis of SCID or other primary immunodeficiency. As such, NBS for SCID can detect other conditions related to T cell lymphopenia including 22q11.2 deletion syndrome (22q11.2DS), the most common cause of DiGeorge syndrome, trisomy 21, ataxia telangiectasia, and CHARGE syndrome, in addition to secondary causes of T cell lymphopenia including congenital heart disease and prematurity [2, 5, 6]. To date, there have been a number of patients with 22q11.2DS identified through TREC NBS for SCID due to T cell lymphopenia [5, 13–16, 18, 19]. Kwan et al. (2014) reviewed the results of 3 million NBS panels including SCID and identified 411 infants with non-SCID T cell lymphopenia, including 78 neonates (19%) with 22q11.2DS.

22q11.2DS is the most frequent chromosomal microdeletion syndrome with an estimated prevalence of 1 in 3000 to 6000 live births and 1 in 1000 unselected fetuses [20, 21]. The submicroscopic deletion has been identified as the most frequent cause of DiGeorge syndrome, velocardiofacial syndrome, and conotruncal anomaly face syndrome, and in a subset of patients with Opitz G/BBB syndrome and Cayler Cardiofacial syndrome [20]. The clinical manifestations of 22q11.2DS involve multiple organ systems, most notably cardiac and palatal abnormalities, immune differences, endocrine and gastrointestinal problems, and variable cognitive and psychiatric deficits [22–24]. Most individuals (~85%) with 22q11.2DS have a typical 3-Mb deletion that occurs as the result of non-allelic homologous recombination between chromosome 22 specific low copy repeats (LCR) LCR22A to LCR22D. Smaller, atypical nested deletions (LCR22A-LCR22B, LCR22A-LCR22C, LCR22C-LCR22D, etc.) are identified in a subset of patients (Fig. 1), often with the same phenotypic features identified in individuals with the standard LCR22A-LCR22D deletion [24].

As the 22q11.2 deletion affects neural crest cell migration, immunodeficiency is observed in approximately 75% of individuals with 22q11.2DS due to thymic aplasia/hypoplasia with resultant impaired T cell production and function [22–25]. Mild to moderate T cell lymphopenia is the most common presentation in these patients, though other immunodeficiency phenotypes can be expected including humoral dysfunction (delayed immunoglobulin production, impaired responses to vaccines) as well as autoimmune and allergic conditions [22–26]. T cell counts in patients with 22q11.2DS are typically low in infancy but may be normal later in life [22, 23, 26]. Immune therapy is not required in the majority of patients with 22q11.2DS with mild to moderate T cell lymphopenia [22, 25]. However, individuals with more severe compromise may require protective isolation, immunoglobulin replacement, avoidance of live viral vaccines, and a therapeutic thymus or matched sibling donor T cell transplant, similar to the management of SCID patients [25].

There is currently enthusiasm within the clinical community to initiate NBS for 22q11.2DS. Here, we examine existing NBS data to understand the frequency of positive NBS for SCID among patients with 22q11.2DS, and the impact early diagnosis has had in these instances. We emphasize the importance and value of early screening and diagnosis for patients with 22q11.2DS, who may otherwise be missed due to a lack of significant clinical findings early in life.

Methods

This study was carried out within the 22q and You Center, a multidisciplinary clinic for patients with a laboratory confirmed chromosome 22q11.2 deletion, at The Children's Hospital of Philadelphia (CHOP) under IRB Protocol 07-005352. The Center, housed within the Division of Human Genetics in the Department of Pediatrics, includes coordinated examination by subspecialists as needed from across the Medical and Surgical Departments at CHOP including but not limited to genetics, allergy, immunology, cardiology, endocrinology, otolaryngology, speech, audiology, plastic surgery, gastroenterology, general pediatrics, general surgery, orthopedics, urology, hematology, neurology, developmental pediatrics, neuropsychology, psychiatry, and social work. The CHOP 22q database contains information for 1350 patients, with a confirmed 22q11.2 microdeletion using standard laboratory methodologies including fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe

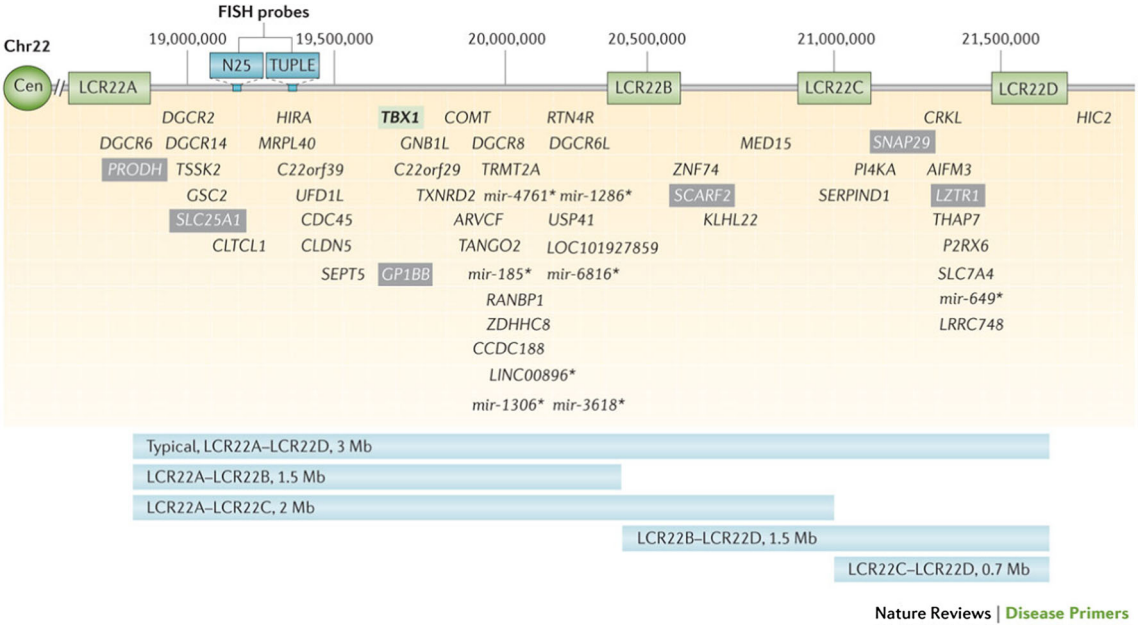

Fig. 1 Low copy repeats and genes within the 22q11.2 deletion. Schematic representation of the 3-Mb 22q11.2 region that is commonly deleted in 22q11.2 deletion syndrome, including the four low copy repeats (LCR22s) that span this region (LCR22A, LCR22B, LCR22C, and LCR22D). Common commercial probes for fluorescence in situ hybridization (FISH) are indicated (N25 and TUPLE). The protein-coding and selected non-coding (*) genes are indicated with respect to their relative position along chromosome 22 (Chr22). T-box 1 (*TBX1*; *green box*) is highlighted as the most widely studied gene within the 22q11.2 region. Mutations in this gene have resulted in conotruncal cardiac anomalies in animal models and humans. Known human disease-causing genes that map to the region are indicated in *gray boxes*. These include proline dehydrogenase 1 (*PRODH*; associated with type I hyperprolinaemia), solute carrier family 25 member 1 (*SLC25A1*; encoding the tricarboxylate transport protein and is associated with combined D-2- and L-2-hydroxyglutaric aciduria), platelet glycoprotein Ib β-polypeptide (*GP1BB*; associated with Bernard–Soulier syndrome), scavenger receptor class F member 2 (*SCARF2*; associated with Van den Ende–Gupta syndrome), synaptosomal-associated protein 29 kDa (*SNAP29*; associated with cerebral dysgenesis, neuropathy, ichthyosis and palmoplantar keratoderma (CEDNIK) syndrome), and leucine-zipper-like transcription regulator 1 (*LZTR1*; associated with schwannomatosis 2). Further details on the location of non-coding RNAs and pseudogenes in the 22q11.2 region may be found in Guna et al.[89]. Common 22q11.2 deletions are shown, with the typical 3-Mb deletion flanked by LCR22A and LCR22D (LCR22A–LCR22D) on *top* and the nested deletions, with their respective deletion sizes indicated *below*. Each of the deletions portrayed is flanked by a particular LCR22. Those rare deletions not mediated by LCRs are not shown. *AIF3M* apoptosis-inducing factor mitochondrion-associated 3, *ARVCF* armadillo repeat gene deleted in velocardiofacial syndrome, *CDC45* cell division cycle 45, Cen centromere, *CLDN5* claudin 5, *CLTCL1* clathrin heavy chain-like 1, *COMT* catechol-*O*-methyltransferase, *CRKL* v-crk avian sarcoma virus CT10 oncogene homolog-like, *DGCR* DiGeorge syndrome critical region, *GNB1L* guanine nucleotide-binding protein (G protein), β-polypeptide 1-like, *GSC2* goosecoid homeobox 2, *HIC2* hypermethylated in cancer 2, *HIRA* histone cell cycle regulator, *KLHL22* kelch-like family member 22, *LINC00896* long intergenic non-protein-coding RNA 896, *LOC101927859* serine/arginine repetitive matrix protein 2-like, *CCDC188* coiled-coil domain-containing 188, *LRRC74B* leucine-rich repeat-containing 74B, *MED15* mediator complex subunit 15, *mir* microRNA, *MRPL40* mitochondrial ribosomal protein L40, *P2RX6* purinergic receptor P2X ligand-gated ion channel 6, *PI4KA* phosphatidylinositol 4-kinase catalytic-α, *RANBP1* Ran-binding protein 1, *RTN4R* reticulon 4 receptor, *SEPT7* septin 7, *SERPIND1* serpin peptidase inhibitor clade D (heparin cofactor) member 1, *TANGO2* transport and golgi organization 2 homolog, *THAP7* THAP domain-containing 7, *TRMT2A* tRNA methyltransferase 2 homolog A, *TSSK2* testis-specific serine kinase 2, *TXNRD2* thioredoxin reductase 2, *UFD1L* ubiquitin fusion degradation 1-like, *USP41* ubiquitin-specific peptidase 41, *ZDHHC8* zinc-finger DHHC-type-containing 8, *ZNF74* zinc-finger protein 74. Reprinted with permission from McDonald-McGinn et al. (2015) [24] (color figure online)

amplification (MLPA), array comparative genomic hybridization (CGH), or SNP microarray analysis, who have been evaluated at CHOP since 1992. The database was retrospectively reviewed to identify patients born after 2008 who had positive NBS results for SCID. Deletion size (e.g. LCR22A to LCR22D; LCR22A to LCR22C) was also noted. Concurrently, we reviewed medical records from outside hospital(s), as well as the immunology consult and outpatient notes and laboratory study results from within CHOP.

Results

Eleven patients (six male, five female) had an abnormal TREC NBS result for SCID due to T cell lymphopenia. A diagnosis of SCID was ruled out via flow cytometry testing in all 11 patients (Table 1), which demonstrated T cell deficits, but not the profound lymphocytopenia expected to be seen with SCID (<2000 cells/μL) [27]. All but one patient had a standard typical LCR22A-LCR22D 22q11.2 deletion, with the exception being a patient with an LCR22A to LCR22C

Table 1 Flow cytometry data in patients with 22q11.2DS with abnormal NBS for SCID

Patient	Age at evaluation	CD3 (cells/μL)	CD4 (cells/μL)	CD8 (cells/μL)	CD19 (cells/μL)	NK (cells/μL)	CD4/CD45 RA (cells/μL)	CD4/ CD45 RO (cells/μL)	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)
1	3 weeks	1874*	1505*	362*	976	387	No data	No data	No data	No data	No data
2	13 days	812*	549*	262*	408*	312	No data	No data	No data	No data	No data
3	9 days	1314*	924*	390*	241*	545	701	226	282	19	22
4	7 days	384*	266*	117*	520*	216*	197	75*	408*	58	38
5	13 days	1806*	1460*	333*	1283	1030	886	367	No data	No data	No data
6	1 month	1168*	770*	388*	803	326	No data	No data	No data	No data	No data
7	1 month	2701	1861	660	1501	1681	1741	240*	682	74	46*
8	10 days	1260*	1032*	218*	478*	842	884	114*	739	<6	34
9	8 days	977*	626*	321*	208*	58*	333	313	No data	No data	No data
10	1 month	828*	557*	253*	1574	563	445	108*	No data	No data	No data
11	2 weeks	1400*	995*	398*	448*	1219	885	215	No data	No data	No data

All data are absolute values

*Values that were below the reference range

deletion. Most patients were Caucasian (n = 7; Black, n = 3; Other, n = 1) and had NBS completed in New Jersey (n = 4), Pennsylvania (n = 3), New York (n = 2), Texas (n = 1), and Connecticut (n = 1).

Five of 11 patients (45%) (Group 1) had significant congenital heart disease (CHD)/other major congenital anomalies (MCAs), resulting in a diagnosis of 22q11.2DS prenatally or postnatally following a clinical genetics consultation. Timing of TREC NBS results in this group was either following or concurrent with confirmatory cytogenomic results for 22q11.2DS, made via fluorescent in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), or SNP-microarray. Importantly, the remaining six patients (55%) (Group 2) only came to attention via an abnormal TREC NBS result followed by 22q11.2 deletion studies (MLPA, CGH-microarray, or SNP-microarray) performed by an astute immunologist or pediatrician when a diagnosis of SCID was excluded.

Overall, average age at time of 22q11.2DS diagnosis was 25.1 days. Patients in Group 1 came to diagnosis on average by 10.0 days ± 8.5 days, whereas patients in Group 2 came to attention by 37.6 days ± 21.7 days. This is in contrast to 3.89 years ± 7.08 years, which is the average age of 22q11.2DS diagnosis in 767 patients followed in our 22q and You Center at CHOP, where such data was available.

Table 2 summarizes the major clinical findings for the 11 patients. Within Group 1, testing for 22q11.2DS was prompted by observation of MCAs, especially severe cardiovascular anomalies. An exception was patient 1 who was diagnosed prenatally via amniocentesis following a high-risk maternal serum screening for trisomy 13/18. Prenatal FISH detected the 22q11.2DS in patient 1, and MCAs were subsequently identified on prenatal ultrasound following the diagnosis of 22q11.2DS.

Major congenital anomalies identified in Group 1 included CHD (n = 3) (interrupted aortic arch type B [IAA-B, n = 2], right aortic arch with a vascular ring [n = 1]), soft palate cleft (n = 1), esophageal atresia with tracheoesophageal fistula (EA/TEF) (n = 1), choanal stenosis (n = 1), and unilateral multicystic kidney (n = 2). Patient 4 expired following EA/TEF surgical repair. Anomalies in Group 2 included two patients with CHD including pulmonary stenosis (n = 1) and ventricular and aortic septal defects (n = 1), as well as one patient with unilateral renal agenesis. Patient 9 died of sudden infant death syndrome (SIDS).

Aside from initial T cell lymphopenia/T cell deficits, additional immunologic problems included frequent and prolonged illnesses (upper respiratory infections, sinusitis, bronchitis, otitis media) in 7/11 (64%) patients, eosinophilia (n = 1), dermatitis/eczema (n = 1), and asthma (n = 1). Other immunologic associations related to 22q11.2DS, such as humoral dysfunction, cannot be ruled out due to limited bloodwork and the young ages of these patients. Four patients with no listed immunologic problems were deceased or lost to follow-up. No immunologic interventions were reported among any of the 11 patients, though patients 1 and 7 received prophylaxis for renal complications.

Six of 11 patients (55%) had hypocalcemia and 5/11 (45%) had gastroesophageal reflux disease including 2/6 and 3/5 from Group 2, respectively. Additional common features found in both groups included otolaryngologic problems (e.g. chronic otitis media), palatal abnormalities (e.g. velopharyngeal insufficiency), genitourinary, and endocrinologic findings, in addition to hypocalcemia (Table 2). Despite a small sample size, the prevalence of most of the phenotypic

Table 2 Clinical findings in patients with 22q11.2DS with abnormal NBS for SCID

Patient	Cardiovascular	Endocrine	Palatal	Otolaryngologic	Genitourinary	Gastrointestinal	Hematologic	Facial dysmorphology
Group 1								
1		Hypocalcemia	*Soft palate cleft*	Chronic OM, adenoid hypertrophy	*Multicystic kidney (R)* [prenatally detected]			Eyes: hooded Ears: thick overfolded helices, attached lobes (bil) Nose: bulbous nasal tip Mouth: small mouth/chin Other: microcephalic
2	*IAA type B*, VSD, ASD	Hypocalcemia, Hypomagnesemia		Chronic OM	Hydronephrosis (L)	GERD, constipation		Eyes: hyperteloric, downslanting Ears: cupping (bil) Nose: bulbous nasal tip, hypoplastic nasal alae Mouth: wide spaced teeth, tented upper lip
3	*IAA type B*, aberrant R subclavian artery, VSD, pulmonary artery stenosis	Hypocalcemia		Laryngomalacia		GERD		Ears: thickened helix (R), thin wide rim (L) Nose: bulbous nasal tip and bridge Mouth: small mouth
4[a]	*Vascular ring*, RAA, VSD	Hypocalcemia	Submucosal cleft palate	*EA/TEF*			Thrombocytopenia	Eyes: small palpebral fissures Ears: posteriorly rotated (bil), overfolded helix (R) Nose: elevated nasal bridge Mouth: micrognathia
5		Abnormal thyroid levels		*Choanal stenosis*	*Multicystic dysplastic kidney (L)*			Eyes: hooding (bil) Ears: pointed with kinks and indented helices (bil) Nose: bulbous nasal tip, hypoplastic nasal alae Mouth: small mouth, high arched palate Other: asymmetric crying facies
Group 2								
6		Vitamin D deficiency	VPI	Chronic OM	Renal agenesis (L), hydronephrosis, hypospadias	Inguinal hernia (L)		Eyes: strabismus Ears: thick overfolded helices, lobes attached (bil) Nose: depressed nasal root, bulbous nasal tip, hypoplastic nasal alae Mouth: small mouth/chin
7	Peripheral pulmonic stenosis			Chronic OM, sinusitis	Hydronephrosis (bil), hypospadias, posterior urethral valves			Ears: thick helices (bil) Nose: bulbous nasal tip Mouth: small mouth, tented upper lip
8		Hypocalcemia	VPI	Sensorineural hearing loss (bil)	Redundant foreskin	GERD, constipation, inguinal and umbilical hernia		Ears: thick, crumpled, square helices (bil) Nose: depressed nasal root Mouth: small mouth
9[a]				Obstructive sleep apnea		GERD		

Table 2 (continued)

Patient	Cardiovascular	Endocrine	Palatal	Otolaryngologic	Genitourinary	Gastrointestinal	Hematologic	Facial dysmorphology
								Ears: pointed with thin helix (R), squared helix (L), prominent antitragus (bil)
10	VSD, ASD	Hypocalcemia		Laryngomalacia		GERD, failure to thrive		Ears: lowset, posteriorly rotated Mouth: small mouth, micrognathia
11		Hypoglycemia		Helical rim deformity			Thrombocytopenia	Ears: Overfolded and crumpled helices (bil) Nose: bulbous nasal tip with hypoplastic nasal alae

Italicized findings indicate the major congenital anomaly clinical finding(s) that prompted a genetic work-up

OM otitis media, *EA/TEF* esophageal atresia/tracheoesophageal fistula, *VPI* velopharyngeal insufficiency, *IAA* interrupted aortic arch, *VSD* ventricular septal defect, *ASD* atrial septal defect, *RAA* right aortic arch, *GERD* gastroesophageal reflux disease

[a] Patient died after edema following EA/TEF surgical repair (patient 4) or due to Sudden Infant Death Syndrome (SIDS) (patient 9)

features seen in our cohort (e.g. hypocalcemia, palatal abnormalities) is comparable to the frequency reported in the 22q11.2DS population [24–26].

A formal dysmorphology exam was completed by a clinical geneticist at CHOP for 10/11 patients. The age at evaluation ranged from 1 day to 3.5 years. Three of four patients who were initially evaluated at an outside hospital were noted to have grossly normal physical features as described by the outside physician, supporting previous reports that typically associated facial features may be mild, variable, and difficult to identify at early ages and by untrained clinicians.

Discussion

Eleven CHOP patients with 22q11.2DS were found to have an abnormal TREC NBS result suggestive of a diagnosis of SCID. In more than half of these cases (Group 2, n = 6/11 patients), the abnormal NBS result prompted evaluation by an immunologist or pediatrician leading to the diagnosis of 22q11.2DS at an average of 37.6 days ± 21.7 days of life. The remaining cases (Group 1, n = 5/11 patients) were found to have an abnormal NBS for SCID following or concurrent to their 22q11.2DS diagnosis, made by a geneticist due to a history of MCAs at an average age of 10.0 days ± 8.5 days. In comparison to our overall cohort (n = 767), the time to diagnosis for Group 2, who would otherwise not have come to attention due to a lack of major congenital findings, is significantly shorter (37.6 days vs. 3.8 years ± 7.08 years; $p < .001$). Importantly, several patients in this group had comorbid diagnoses, including hypocalcemia and GERD (2/6 and 3/6 respectively), that were identified only following the diagnosis of 22q11.2DS when screening for these associated abnormalities was initiated. Moreover, earlier diagnosis of 22q11.2DS, particularly among the patients identified in Group 2, allowed for earlier surveillance and treatment for identified problems, early interventions for associated developmental/behavioral differences, and appropriate condition-specific genetic counseling.

Based on these findings and the technical availability of NBS using multiplex qPCR [24] developed specifically for 22q11.2DS, one major question arises as to whether general population NBS for 22q11.2DS should be implemented on a global (national/international) level. The World Health Organization's Wilson-Jungner policy guidelines originally defined suitability of a condition for NBS, and in 2006, the American College of Medical Genetics (ACMG) Newborn Screening Expert Group redefined evaluation criteria to be used to determine the inclusion of medical conditions in newborn screening programs [28, 29]. Based on the ACMG criteria (Table 3), conditions should be well-understood with acceptable treatments and/or interventions available and the screening test should be effective and cost-efficient [28–30].

Table 3 ACMG Newborn Screening Expert Group criteria applied to 22q11.2DS

Guidelines	Applicability in 22q11.2DS	
1. Clinical characteristics of the condition	1:3000–1:6000 births in the USA	✓
Incidence of the condition	Average age of diagnosis of CHOP cohort is 3.89 years	
Clinically identifiable signs and symptoms in the first 48 h	Comorbidity and mortality is significant if undiagnosed and untreated	
Burden of disease (natural history if not treated)		
2. Screening test availability and characteristics	Several PCR-assays have demonstrated high sensitivity and specificity, rapid turn-around-time, are cost efficient, and use dried blood spots	✓
Availability of a sensitive and specific test algorithm	PCR assays can be developed to detect other microdeletion syndromes simultaneously	
Ability to test on either neonatal blood spots or an alternative specimen type or by a simple, in-nursery procedure		
Test is based on a platform that offers high-throughput capability		
Cost of test is < $1 per infant screened		
Multiple analytes relevant to one condition can be detected in the same run		
Other conditions (secondary targets) can be identified by the same analytes		
Multiple conditions can be detected by the same test (multiplex platform)		
3. Diagnosis, follow-up, treatment, and management.	Basic interventions (e.g. management of hypocalcemia, GERD, speech-language, occupation, and physical therapies, etc.) are widely available	✓
Availability of treatment	Certain treatments (e.g. cardiac surgery and treatment of hypocalcemia) can be life saving	
Cost of treatment	Preventive management leads to decreased healthcare costs	
Potential efficacy of existing treatment	Early identification avoids “diagnostic odyssey” and decreases burden on family	
Individual benefits of early intervention		
Familial and societal benefits of early identification		
Prevention of mortality through early diagnosis and treatment		
Availability of diagnostic confirmation		
Acute management		
Simplicity of therapy		

22q11.2DS is quite common with an estimated prevalence of ~1 in 3000–6000 live births and ~1 in 1000 unselected fetuses [20, 21]. 22q11.2DS has wide phenotypic variability, but many features conferring significant morbidity and some mortality are quite common, e.g., CHD [24], as evidenced by the variability observed among the 11 patients in this study (Table 2). Importantly, early detection leads to early intervention and condition-specific management, which can improve or even prevent associated comorbidities/mortality. Screening for cardiac, immune, endocrine, gastrointestinal, renal, and other associated abnormalities may identify and prevent significant complications related to 22q11.2DS. These comorbidities may include cardiac crisis related to an undetected IAA-B (which may not be identified via newborn pulse oximetry monitoring), seizures secondary to hypocalcemia, or feeding refusal due to silent gastroesophageal reflux disease and dysmotility, which may go undiagnosed prior to the identification of 22q11.2DS. Likewise, early diagnosis provides prompt access to services and interventions that may improve long-term prognosis and forgo a prolonged diagnostic odyssey for both the family and the primary healthcare provider [24, 31–33]. For example, developmental evaluations and therapies such as speech therapy initiated prior to the onset of frustrations associated with delays in emergence of language and social support networks for families struggling with managing the care of a child with complex medical needs. The “diagnostic odyssey” that many families currently face is a period of time between when a family is concerned about their child’s health and development and when a diagnosis is reached. This can be an emotional and distressing time for families, and resolution of a diagnosis has been shown to provide parents with a sense of validation, improved prognosis through available therapies and targeted medical interventions, and access to support networks specific to them and their child [33]. Finally, the identification of 22q1.2DS will allow for appropriate genetic counseling, as ~10% of cases are

familial. Parental deletion studies are recommended when a child is diagnosed with this contiguous gene deletion syndrome, as the recurrence risk, if identified in a parent, is 50% for all subsequent pregnancies [24].

NBS for SCID is not a universally reliable form of detection for 22q11.2DS, as it will identify only a subset of patients with 22q11.2DS, specifically those with marked T cell lymphopenia. Based on population data using the 1 in 4000 estimated prevalence rate, published data from the states of California, Wisconsin, and New York would suggest that only 3–15% of the expected number of infants with 22q11.2DS would have an abnormal NBS study for SCID [14, 15, 34]. Additionally, the limited immunologic data from our 11 patients suggests that the subset of infants with 22q11.2DS identified via NBS for SCID does not necessarily correlate with severe immunologic compromise. Furthermore, the time to final diagnosis of 22q11.2DS is delayed among this subset of individuals, while the neonate undergoes further immune evaluation and the family awaits confirmatory cytogenomic testing, often SNP microarray, which may take several months for insurance approval/results. Testing for 22q11.2DS in a newborn with a positive NBS for SCID, but without evidence of SCID, requires evaluation by an astute and knowledgeable clinician. Significant comorbidities, such as major congenital anomalies, may be a clear indicator that further testing is required once a diagnosis of SCID is excluded. However, healthcare providers who are unfamiliar with the complex nature of 22q11.2DS and the variability of its presentation may overlook patients with a mild presentation without performing specific deletion studies. Even the distinct facies often associated with 22q11.2DS can be difficult to identify in all affected individuals, particularly in infants [35]. Indeed, four patients within our cohort evaluated by physicians at an outside hospital were thought to have grossly normal facies. Implementation of a NBS platform designed specifically to identify newborns with 22q11.2DS may well identify affected neonates with any clinical presentation and will avoid delayed diagnosis due to multi-step clinical and laboratory evaluations while concurrently reducing overall healthcare costs [36].

Multiple PCR methods have been shown to be effective in rapidly identifying a 22q11.2 deletion at low costs, with several assays utilizing the dried blood spot specimen currently used for newborn screening panels [17, 37–39]. When considering the use of these techniques in population-based screening for 22q11.2DS, it is critical that the assays target multiple loci along the typical 22q11.2 deletion region to ensure that atypical nested or distal deletions, occurring in 15% of patients, are not missed [24, 38, 39]. For example, according to P. North, PhD (written communication, December 2016), a team from Wisconsin has now developed a clinically validated multiplex assay using both *TBX1* within the LCR22A-B region and *CRKL* within the LCR22C-D region which would detect the vast majority (>99%) of patients with a standard or nested 22q11.2 deletion. Should this assay be considered cost-effective and adoptable for general population NBS, it would identify virtually all patients with 22q11.2DS, thus circumventing both the unfortunate sequelae related to late diagnosis, in particular those patients with undetected ductal-dependent congenital heart disease, hypocalcemia and dysphagia, and the diagnostic odyssey, with which so many families have struggled.

Conclusion

Our case series demonstrates the utility of NBS for SCID in identifying patients with non-SCID-related T cell lymphopenia diseases, in particular 22q11.2DS. Moreover, our findings provide support for inclusion of 22q11.2DS in general population NBS, as NBS for 22q11.2DS will result in earlier diagnosis of affected individuals, while concurrently reducing morbidity and likely mortality and overall healthcare costs. The addition of 22q11.2DS to NBS panels has been evaluated in the past [29, 30] but warrants further discussion, as our knowledge of technology, treatment, and disease phenotype continues to rapidly evolve. The criteria guidelines implemented by the ACMG Newborn Screening Expert Group, while a modernized version of Wilson and Jungner criteria, are becoming rapidly outdated in this advancing genomic era [28, 29].

Assays which screen for 22q11.2DS using dried blood spots have been developed and proven to be effective and efficient [17, 37–39]. Population-based studies should be completed to demonstrate the efficacy of these assays on a larger scale [16]. However, the clinical characteristics, diagnosis, management, and treatment of 22q11.2DS have been shown to meet the criteria for NBS programs and support the need for earlier diagnosis. Furthermore, healthcare costs of patients with CHDs that are not immediately identified (e.g. vascular ring, IAA-B not detected with postnatal pulse oximetry monitoring) have been demonstrated to be as high as 20 million dollars [36]. An early diagnosis of 22q11.2DS in these children would lead to targeted screening and interventions, streamlined care, and appropriate medical management, thus dramatically decreasing healthcare costs. Early diagnosis can lead to improved medical and developmental outcomes, access to appropriate services and supports, decreased emotional distress for families, and appropriate genetic counseling collectively making 22q11.2DS a reasonable candidate for newborn screening (using genomic methodologies [e.g. qPCR] on blood spots).

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Compliance with Ethical Standards

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Conflict of Interest Professor McDonald-McGinn has given lectures on 22q11.2DS for Natera. The remaining authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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