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Defects of B Cell Terminal Differentiation in Patients with Type-1 Kabuki Syndrome

Andrew W. Lindsley, MD PhD[1,2,*], **Howard M. Saal, MD**[2,3], **Thomas A. Burrow, MD**[2,3], **Robert J. Hopkin, MD**[2,3], **Oleg Shchelochkov, MD**[4], **Pooja Khandelwal, MD**[2,5], **Changchun Xie, PhD**[6], **Jack Bleesing, MD PhD**[2,5], **Lisa Filipovich, MD**[2,5], **Kimberly Risma, MD PhD**[1,2], **Amal H. Assa'ad, MD**[1,2], **Phillip A. Roehrs, MD**[7], and **Jonathan A. Bernstein, MD**[8]

[1]Division of Allergy & Immunology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

[2]Department of Pediatrics, Univeristy of Cincinnati, Cincinnati, OH

[3]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

[4]Division of Genetics, Stead Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, Iowa

[5]Division of Bone Marrow Transplantation, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

[6]Division of Biostatistics and Bioinformatics, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio

[7]Division of Pediatric Hematology/Oncology, University of North Carolina, Chapel Hill, NC

[8]Division of Immunology, Allergy and Rheumatology, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH

Abstract

Background—Kabuki syndrome (KS) is a complex multi-system developmental disorder associated with mutation of genes encoding histone-modifying proteins. In addition to craniofacial, intellectual, and cardiac defects, KS is also characterized by humoral immune deficiency and autoimmune disease, yet no detailed molecular characterization of the KS-associated immune phenotype has previously been reported.

Objective—To characterize the humoral immune defects found in KS patients with KMT2D mutations.

Methods—We comprehensively characterize B cell function in a cohort (N = 13) of patients with KS (ages 4 months to 27 years).

*Corresponding Author: Andrew W. Lindsley, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH 45229, andrew.lindsley@cchmc.org, (513)-636-2016 –office, (513)-636-3310 –fax.

Results—Three-quarters (77%) of the cohort had a detectable heterozygous *KMT2D* mutations (50% nonsense, 20% splice site, 30% missense), and 70% of the reported mutations are novel. Amongst the patients with *KMT2D* mutations ($KMT2D^{Mut/+}$), hypogammaglobulinemia was detected in all but one individual, with IgA deficiency affecting 90% of patients and a deficiency in at least one other isoform seen in 40% of patients. Total memory ($CD27^{+}$) and class-switched memory B cells (IgM−) were significantly reduced in $KMT2D^{Mut/+}$ patients compared to controls (p-values < 0.001). $KMT2D^{Mut/+}$ patients also had significantly reduced rates of somatic hypermutation in IgG (p value = 0.003), but not IgA or IgM heavy chain sequences. Impaired terminal differentiation was noted in $KMT2D^{Mut/+}$ primary B cells. Autoimmune pathology was observed in patients with missense mutations affecting the SET domain and its adjacent domains.

Conclusions—In patients with KS, autosomal dominant KMT2D mutations are associated with the dysregulation of terminal B cell differentiation leading to humoral immune deficiency and in some cases autoimmunity. All patients with KS should undergo serial clinical immune evaluations.

Clinical Implications—$KMT2D^{Mut/+}$ Kabuki syndrome causes IgA deficiency in nearly all patients, although additional humoral defects (memory B cell deficiency, IgG hypogammaglobulinemia) have variable expressivity. Missense mutations in terminal domains may increase autoimmunity risk.

Keywords

Kabuki syndrome; *KMT2D*; *KDM6A*; hypogammaglobulinemia; memory B cells; $CD21^{lo}$ B cells; class-switch recombination; somatic hypermutation; *PTIP*; *AICDA*; *AID*; polymerase eta

Introduction

Kabuki syndrome (KS) or Niikawa-Kuroki syndrome is a congenital syndrome characterized by a stereotypical set of facial features, skeletal anomalies, dermatoglyphic abnormalities, intellectual disability, and post-natal growth deficiency (1, 2). Classic KS-associated facies include arched eyebrows with central notching; elongated palpebral fissures with eversion of the lateral lower eyelid; and large, prominent, cupped ears. Though consensus clinical diagnostic criteria have not been established for this rare disease, other frequently noted structural defects include congenital heart disease, cleft palate, gastrointestinal malformations, and ophthalmologic defects (3–5). Functional defects are also observed including immune dysfunction (recurrent infections and autoimmune disorders), seizures, hearing loss, and endocrinopathies (6–9).

Autosomal-dominant mutations in two epigenetic regulatory genes, lysine methyltransferase 2D (*KMT2D*/*MLL2*/*ALR*/*MLL4*; KS-1, OMIM 147920) and lysine demethylase 6A (*KDM6A*/*UTX*; KS-2, OMIM 300867), were recently found in large cohorts of patients with KS, suggesting that defects in histone-mediated regulation drive the diverse pathology of this syndrome (10, 11). KMT2D modifies gene expression via methylation of lysine 4 on histone 3 (H3K4), and mutations of this gene are the most common cause of KS (~60–70% of cases) (12, 13). *KMT2D* encodes a large, multi-domain protein that forms the core of one of the six mammalian complex of proteins associated with SET1 (COMPASS) protein

complexes (14). KDM6A is a cofactor physically associated with the KMT2D COMPASS complex and exhibits complementary demethylase activity at lysine 27 on histone 3 (H3K27) (15). Together, the components of the KMT2D COMPASS complex remove inhibitory epigenetic marks and add activating marks, specifically H3K4 mono-, di-, or trimethylation (H3K4me1,2,or 3). KMT2D has been implicated in the regulation of hundreds of genes via regulation of enhancer and promoter elements, providing a rationale for how defects in this single gene and its binding partners can lead to a complex and heterogeneous congenital phenotype (12, 16, 17).

KMT2D binds multiple co-factors, including the paired box (PAX) transactivation domain–interacting protein (*PTIP*), which plays a key role in B cell terminal differentiation. PTIP has dual activities, binding regions of DNA double-strand breaks, such as those that occur during class-switch recombination (CSR), and interacting with PAX transcription factors. PTIP directly binds the B cell master transcription factor PAX5, targeting KMT2D-mediated H3K4me3 methylation to switch regions within the immunoglobulin (Ig) heavy chain locus (IGH) during CSR (18–20). B cell–specific knockout of *Ptip* in primary murine B cells dramatically impairs CSR (20). Studies of PTIP have demonstrated the important role of the KMT2D COMPASS complex in regulating the IGH locus during B cell differentiation, thus linking the molecular functions of the two KS-associated genes with a central mechanism of humoral immunity.

Immune dysfunction is a common feature of KS. Described as having a common variable immune deficiency-like (CVID-like) immune profile in early studies (7), this initial observation was later correlated with clinical findings in three larger cohorts of patients with heterozygous *KMT2D* mutations ($KMT2D^{Mut/+}$). Approximately half of the patients in these studies had clinical features of immune deficiency, including recurrent otitis media [47% (21)] and frequent/repeat infections [41.9% (22)/64% (23)]. A more recent study found recurrent otitis media to be a nearly universal clinical finding (85%) in $KMT2D^{Mut/+}$ KS patients (24). In addition, autoimmune diseases, especially immune thrombocytopenia (ITP), hemolytic anemia, and vitiligo, have also been reported in patients with KS; however, all of these early cases were in clinically diagnosed patients without a defined genetic etiology. Two new case reports of ITP in genetically defined $KMT2D^{Mut/+}$ patients, however, have strengthen the link between KS and autoimmunity (25, 26). Together, these foundational studies provide a general outline of the KS immune phenotype, but reveal little about the underlying pathogenesis of the observed immune dysfunction.

Despite a growing clinical interest in KS, the cellular characteristics and molecular mechanisms of KS-associated immune dysfunction remain poorly understood. A variety of recent studies, however, have now shown that histone-modifications regulate key events in terminal B cell differentiation. Specifically, CSR and the related phenomenon, somatic hypermutation (SHM), are two critical B cell processes subject to epigenetic regulation (27–29), but despite their importance to humoral immunity, these processes have never previously been evaluated in patients with KS. To address these knowledge gaps, we characterize herein the specific cellular and molecular B cell defects found in a cohort of 13 patients with KS (10 pediatric, 4 adult cases). Our findings provide mechanistic insight into

the humoral immune deficiency and autoimmunity found in this understudied patient population.

Methods

Patient cohort

Patients with a clinical diagnosis of KS by a board-certified clinical geneticist were recruited to participate. All participants and/or their parents/guardians provided written consent/assent for enrollment in this study (including explicit authorization to publish images of patients). The study protocol (#2012–4636) was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center (CCHMC). Phenotypic data were collected from medical record review and interviews with patients, guardians and physicians.

KMT2D/KDM6A sequencing analysis and multiplex ligation-dependent probe amplification (MLPA)

All 13 patients underwent *KMT2D* sequencing (reference: NM_003482.3). KMT2D domains were mapped according to UniProt Database (O14686). Per the step-wise approach to genetic diagnosis of KS in clinical practice, the 3 patients (A, B, C) in whom no *KMT2D* mutation was identified underwent multiplex ligation-dependent probe amplification (MLPA) analysis of 23 *KMT2D* exons, sequencing of the *KDM6A* coding region, and MLPA of 26 *KDM6A* exons. (See Online Methods for MLPA exon numbers).

Splice site analysis

To confirm the presence of mis-spliced mutant variant transcripts, putative splice site variants were analyzed using NNSPLICE software (30). cDNA from patients 3 and 7, who have mutations in *KMT2D* splice sites, was generated from peripheral blood mononuclear cells (PBMCs). Primers flanking the predicted splice site mutation amplified regions of interest. Bands were purified, cloned, and sequenced.

Clinical laboratory evaluations

Immunoglobulin (Ig) levels, post-vaccination serology, lymphocyte subpopulation data, and mitogen studies were derived from physician review of medical records. All clinical evaluations were performed at Clinical Laboratory Improvement Amendments (CLIA)–certified clinical laboratories and reported with the age-matched normal ranges. B cell flow cytometry was performed in the Diagnostic Immune Laboratory, CCHMC.

Somatic hypermutation analysis

V(D)J libraries were generated from PBMC-derived cDNA, cloned, and sequenced. Briefly, we combined the BIOMED-2 FR1 pool of variable gene primers (31) with individual antisense, constant region–specific (μ, γ, α) primers to amplify pools of isotype-specific Ig-derived clones (32, 33). After gel purification, these libraries were cloned (TOPO TA cloning, Life Technologies), screened for inserts, and 20–30 clones per library were sequenced. Ig sequences were aligned with the human IGH locus using the IgBLAST

software and the IMTG database (34). Non-productive clones were excluded from analysis. Mutation analysis performed with SHMTool software (35).

In vitro terminal B cell differentiation

B cell differentiation was induced using a modified version of the protocol described by Ettinger *et al*. (36). (See Online Methods for culture and staining details). Cells were analyzed after 3 days or 7 days of culture by flow cytometry (LSR-II flow cytometer, BD Biosciences).

Statistical Analysis

Patient-derived B cell data was compared to age-matched controls using a linear regression model in which the p-value was calculated on the basis of the F-test (GLM procedure, SAS ver9.4 software). All other statistical analyses were performed using an unpaired Student *t* test. $p < 0.05$ was considered statistically significant.

Results

Cohort characteristics

Each patient's KS phenotype was evaluated for the presence of 4 of the 5 cardinal manifestations of KS (Table 1) (dermatoglyphic analysis not done) (37) Images of each patient's face and right ear are provided (Supplemental Figure E1). Additional KS-associated structural and functional anomalies were also evaluated. The frequency of selected traits in our cohort were calculated and compared with the meta-analysis of Bogershausen and Willnik (4 studies, total n = 287 patients) (9). All but two categories of KS-associated traits showed similar frequency rates between our cohort and the meta-analysis; congenital heart disease and genitourinary anomalies were more common in our cohort than in the meta-analysis cohorts. Adult KS patients also exhibit a variety of CVID-associated comorbidities, especially splenomegly and pulmonary disease (Supplemental Table E2, A).

Molecular genetic evaluation

All of the patients underwent *KMT2D* gene sequencing, and 10 out of 13 (77%) were found to have mutations predicted to be pathogenic (Supplemental Figure E2), a rate consistent with prior reports (9, 10). Seven of these *KMT2D* mutations were novel. Half of the 10 identified $KMT2D^{Mut/+}$ patients harbored nonsense/frameshift mutations predicted to generate a truncated protein or induce nonsense-mediated decay (NMD) of the mutated transcript (38).

Two patients, 3 and 7, had splice site mutations identified (Supplemental Figure E2). Bioinformatic analysis of patient 3's mutation predicted a deleterious effect (loss of intron 26 acceptor site). This prediction was confirmed by PCR amplification of a mutant transcript fragment (Mut) from patient-derived PBMC cDNA (Supplemental Figure E3,A). Cloning and sequencing of this aberrant band revealed retained intronic sequence encoding a premature stop codon. Patient 7 was also found to have a splice site mutation (adjacent to the donor site of intron 29), and bioinformatic analysis predicted reduced confidence in the

functionality of the site. Similar PCR analysis of PBMC-derived cDNA from patient 7 failed to detect a mutant band (Supplemental Figure E3, B).

Three patients, 5, 6, and 8, had missense mutations in the 3' end of the *KMT2D* locus (exons 48, 51) within highly conserved functional domains (Supplemental Figure E2). The previously reported missense mutation identified in patient 8 (c.16294C>T) was recently shown to disrupt the Kabuki interaction surface (KIS), which forms a secondary active site in the COMPASS complex (39).

The three patients (A, B, C) who were clinically diagnosed with KS but in whom no *KMT2D* mutation were detected (clinical KS, cKS) also underwent *KMT2D* exon copy number variant testing (MLPA) and *KDM6A* sequencing and copy number testing (MLPA). In all three cases, these additional studies failed to detect a pathogenic mutation, insertion, or deletion in these two previously described KS-associated genes.

Autoimmune disease clinical histories

Autoimmune disease is commonly associated with humoral immune deficiency and is observed in ~20% of patients with CVID (40). Three of our patients have a history of marked autoimmune disease (patients 5, 6, and 8; 23% of total cohort; detailed clinical histories provided in Supplemental Figure E4). All 3 patients experienced ITP, and in 2 cases, ITP developed within several months of contracting Epstein-Barr virus (EBV) (patient 5 and 8). Patient 6 also developed vitiligo, which required phototherapy. Patient 8 has had recurrent ITP, autoimmune neutropenia, autoimmune hepatitis, and insulin-dependent diabetes mellitus. Interestingly, these patients all harbor similar missense mutations in the 3'-end of the *KMT2D* gene near the SET domain region (Supplemental Figure E2).

Immune evaluations

Lymphocyte Evaluations—Peripheral lymphocyte subpopulations were evaluated for 12 out of the 13 patients, and the majority of patients had normal or minor deviations from age-matched control values(41) (Supplemental Table E1). Similarly, mitogen studies were performed in 11 out of 13 subjects, with >80% having essentially normal results. Two patients (6,8) had diminished, but not absent, mitogen responses (Supplemental Table E1). With regard to lymphocyte subpopulations, four out of 12 patients (33%), were found to have a reduced percentage of $CD19^+$ B cells (vs. age-matched controls). Four patients (2, 5, 6, 8) had lymphopenia and abnormal lymphocyte subpopulations at the time of enrollment. Patient 2 has a large, retroperitoneal lymphatic malformation, resulting in significant chylous ascites and loss of peripheral lymphocytes, especially T cells; patient 2's absolute B cell count was low (556 cells/mcl vs. age-adjusted normal range 700–2500 cells/mcl), despite an elevated B cell percentage because of preferential loss of T cells. Patient 5 underwent thymectomy at 3 days of life, with intermittent lymphopenia noted since that time. Patient 6 was noted to have lymphocyte subpopulation levels within normal, age-matched ranges at initial evaluation (5.5 years of age) but subsequently developed lymphopenia with onset of autoimmune disease (Supplemental Figure E4). Similarly, patient

8 had a normal absolute lymphocyte count at 13 years of age prior to contracting EBV, but since that time has had chronic borderline lymphopenia (Supplemental figure E4).

Immunoglobulin levels—We found that 90% of our *KMT2D*$^{Mut/+}$ patients had IgA deficiency, whereas our 3 cKS patients (A–C) had normal or above-normal serum IgA levels (Figure 1A). In patients greater than 5 years of age, the IgA levels were significantly decreased in *KMT2D*$^{Mut/+}$ patients compared with cKS patients (A–C) (p value = 0.003) (Figure 1A, right panel). Low serum IgM was noted in 40% of *KMT2D*$^{Mut/+}$ patients (4, 6, 8, and 9) but in none of the cKS patients (Figure 1B). Low IgG was also noted in 40% of patients with *KMT2D* mutations (patients 6, 8, and 9) and in 1 cKS patient (Figure 1C). Three KMT2D$^{Mut/+}$ patients (6, 8, 9) had pan-hypogammaglobulinema.

Specific antibody titers against tetanus were also evaluated for all patients greater than 2 years of age except for patients 4 and 5. All patients had protective tetanus titers except for patient 6. One month after revaccination, patient 6 mounted a 10-fold increase in her anti-tetanus titers (still below protective level), but this level returned to her previously low baseline by 1 year later. An alternative measure of vaccine responsiveness was available for patient 5, who mounted protective responses to hepatitis A and B vaccinations at 6 years of age. Of note, 5 adult *KMT2D*$^{Mut/+}$ patients (patients 6–10) underwent pre- and post-vaccination pneumococcal titer testing, and 3 out of 5 (60%) failed to mount a normal response (defined as 2-fold increase in 70% of tested titers)(Supplemental Table E2) (42).

B cell subpopulations—Given our finding of hypogammaglobulinemia in the majority of our *KMT2D*$^{Mut/+}$ patients, we next evaluated peripheral B cell subpopulations in 12 out of 13 patients in our cohort (patient 5- no data; patient 6- not all data available). Consistent with our lymphocyte subpopulation analysis, 4 (33%, 4 of 12) of our patients had a low percentage of total B cells (CD19^{+}/CD20^{+}, Figure 2A); all 4 were *KMT2D*$^{Mut/+}$ patients (44%, 4 of 9). We next evaluated total memory B cells (CD27^{+}) and found that 89% (8 of 9) of *KMT2D*$^{Mut/+}$ patients were deficient in this cell type, a feature shared with 67% (2 of 3) of cKS patients (Figure 2B). When compared to control subjects, we found that *KMT2D*$^{Mut/+}$ patients had a significant reduction in memory B cells after adjusting for age (p-value = 0.0005). Class-switched memory B cells (IgM^{-}, IgD^{-}, CD27^{+}) were also low in 5 *KMT2D*$^{Mut/+}$ patients (63%) and in 1 patient lacking a *KMT2D* mutation (Figure 2C). Compared to age-adjusted control subjects, we found that *KMT2D*$^{Mut/+}$ patients had a significant reduction in class-switched memory B cells (p-value = 0.0077). Finally, we evaluated CD19^{+}CD21^{lo} B cells, a cell population previously associated with CVID in adults (43) and found that 4 *KMT2D*$^{Mut/+}$ patients had an expanded population of these cells; no expansion of these cells was seen in cKS patients (Figure 2D). When compared to control subjects, *KMT2D*$^{Mut/+}$ patients had a significant expansion in CD19^{+}CD21^{lo} B cells after adjusting for age (p-value < 0.0001). Collectively, these findings indicate that many patients with KS exhibit abnormal terminal B cell differentiation.

Somatic hypermutation (SHM) rates—A critical step in terminal B cell differentiation is post-activation SHM of the IGH locus. We therefore quantified the rate of SHM in V(D)J transcripts cloned from *KMT2D*$^{Mut/+}$ B cells to investigate whether this process was disrupted by *KMT2D* genetic lesions. We focused our analysis on our 4 adult *KMT2D*$^{Mut/+}$

patients (7–10) because of the availability of age-matched controls. Using a set of diverse variable gene primers (BioMED-2 FR1) paired with a constant region primer (IgM, pan IgG, or IgA specific), we isolated and sequenced patient-specific V(D)J clones and quantified their divergence from germline sequence. SHM rates for IgM and IgA V(D)J clones from *KMT2D*$^{Mut/+}$ patients did not significantly differ from rates derived from control subject clones (Figure 3A, B) and were consistent with previously published rates of SMH in adults (44–47). Interestingly, the SHM rate for IgG V(D)J clones was significantly lower in 3 out of 4 *KMT2D*$^{Mut/+}$ patients when compared to controls clones (p-values <0.003)(Figure 3C). These findings show that a subset of adult patients with KS have diminished IgG affinity maturation in circulating memory B cells. IgG affinity maturation appears to be more susceptible to KMT2D insufficiency than IgA SHM, possibly the result of the divergent cytokine signaling mechanisms and cell trafficking that drive mucosal (IgA) vs systemic (IgG) humoral immunity(48).

To explore the mechanism driving this reduction in the SHM rate, we next performed mutation analysis on IgG clones derived from *KMT2D*$^{Mut/+}$ patients (Supplemental Table E3). SHM-associated mutations are targeted to the V(D)J and occur in non-random patterns with certain nucleotide motifs being preferentially targeted (“hotspot”) or ignored (“coldspot”). We observed no differences in the fraction of transition or transversion mutations detected in V(D)J sequences derived from *KMT2D*$^{Mut/+}$ patients versus from normal controls. Likewise, the percentage of total V(D)J mutations occurring at activation-induced cytidine deaminase (*AICDA*, also known as *AID*) “hotspot” and “coldspot” motifs also did not differ between *KMT2D*$^{Mut/+}$ patients and controls. In contrast, we did observe a trend toward reduced utilization of the preferred polymerase η (eta) motif in *KMT2D*$^{Mut/+}$ patients (KS average 9.01% vs. Control 15.96%, p = 0.058). This ~40% reduction was seen in all *KMT2D*$^{Mut/+}$ patients examined, including patient 10 who had a normal overall SHM rate.

In vitro terminal B cell differentiation—In order to clarify whether the humoral defects seen in many of our patients with KS were the result of intrinsic B cell defects (cell autonomous or nonautonomous), we induced *in vitro* class-switch recombination in B cells isolated from an adult *KMT2D*$^{Mut/+}$ patient with hypogammaglobulinemia, but no prior exposure to rituximab or other immune suppressive medication (patient 9). Total B cells were isolated from patient 9 and two age-matched control patients and incubated in media alone or media plus germinal center differentiation factors/stimuli (IL-21, anti-CD40 antibody, anti-IgM antibody). Cells were analyzed after 3 days and 7 days of incubation by flow cytometry. Control cells exposed to the differentiation factors efficiently downregulated IgD and induced CD38 (Figure 4), a marker of memory and plasma cell differentiation (36). B cells derived from patient 9 appeared to initiate normal differentiation at day 3 of culture by downregulating surface IgD but failed to upregulate CD38 on IgD^{-} cells by day 7 of culture (2.8% versus 36.3% IgD^{-}CD38^{+} in control cells). CD27 was also examined and followed the same pattern as CD38 (data not shown). These findings suggest a specific role for KMT2D signaling during germinal center–mediated terminal differentiation.

Discussion

Based on our results and accumulating evidence from multiple model systems (27, 49, 50), we believe the hypogammaglobulinemia, reduced memory B cells, and impaired SHM observed in our *KMT2D*$^{Mut/+}$ patients are likely the result of disrupted epigenetic regulation of the IGH locus. During B cell terminal differentiation, the IGH locus undergoes a variety of dynamic, stepwise epigenetic modifications. In activated naive mature B cells, H3K4me3 modifications accumulate throughout the V(D)J region and IGH switch regions. These targeted H3K4me3 marks are associated with an open chromatin state, and initiate a cascade of secondary H3 histone modifications that drive AICDA/AID recruitment to the switch regions, and ultimately induce recombination of the IGH locus (51). The V(D)J sequence also is subject to similar epigenetic regulation during SHM, with initial H3K4me3 accumulation at the V(D)J coding sequence leading to secondary histone modifications, recruitment of AICDA/AID and the error-prone DNA polymerase-η (pol-η), and subsequent mutation-driven affinity maturation of the antibody (49). Impaired H3K4 trimethylation of the IGH locus reduces both CSR and SHM efficiency (27, 50), linking these independent, but simultaneous processes to epigenetic regulatory mechanisms. These findings are highly relevant to KS-associated immune dysfunction since KMT2D catalyzes H3K4 methylation at the IGH locus by binding it's COMPASS cofactor PTIP (20), therefore supporting a direct link between KMT2D function and CSR/SHM (Supplemental Figure E5).

The CVID-like phenotype observed in our KMT2D$^{Mut/+}$ patients implicates altered epigenetic signaling as an etiology for human B cell dysfunction. Multiple knockout murine models have shown that a variety of chromatin modifier genes (*Ezh2*, *Suv39h1, Spt6*) have important roles in terminal B cell differentiation, CSR, and SHM (27–29). Future investigations into the etiology of CVID may benefit from closer inspection of B cell epigenetic regulation. Other important aspects of B cell differentiation may also be affected in some *KMT2D*$^{Mut/+}$ patients, such as impaired deletion of autoreactive B cell clones. One intriguing observation is that all three of our *KMT2D*$^{Mut/+}$ patients with autoimmune disease were found to have missense point mutations in the terminal carboxyl end of *KMT2D* gene. Of the two other *KMT2D*$^{Mut/+}$ patients reported in the literature to have developed ITP, one patient exhibited a highly similar missense mutation in the same gene region (exon 52, c. 16384G>C, p.D5462H) (26). A recent enzymatic study of the KMT2D pre-SET domain region modeled five previously reported KS missense mutations and found that all clustered onto a solvent-exposed surface termed the KIS (39). These mutations interfered with KMT2D's formation of a second active site with critical protein cofactors rendering the protein only capable of performing histone monomethylation. Additional studies, perhaps using animal models that recapitulate human genetic lesions, are needed to clarify the *in vivo* effects of specific *KMT2D* point mutations on the development of autoreactive B cells.

KS is a complex, heterogeneous disease and patients are at increased risk for recurrent sinopulmonary infections and serious autoimmune sequela, as seen in our adult *KMT2D*$^{Mut/+}$ patients(Supplemental Table E2, A&B). Unfortunately, no disease-specific clinical treatments are currently approved for use in patients with KS, though this may change now that the underlying gene defects have been identified. Several lysine demethylase (KDM) inhibitors have recently been described and may prove useful in

directly targeting H3K4 methylation defects (52). B cells are interesting initial targets for therapeutic epigenetic treatment given their lifelong, ongoing differentiation from stem cells, providing a broad developmental window for aberrant epigenetic signaling to be normalized by pharmacologic intervention.

In summary, we show that greater than 80% of *KMT2D*$^{Mut/+}$ patients have hypogammaglobulinemia and diminished memory B cell populations. In addition, we present evidence associating *KMT2D* mutations with expansion of the CVID-associated CD21^{lo} B cell population, impaired SHM in IgG transcripts, and disrupted terminal B cell differentiation We also note a clustering of missense mutations in the terminal region of the *KMT2D* gene and that such mutations may increase the risk for autoimmune disease in *KMT2D*$^{Mut/+}$ patients. In summary, this report reveals that epigenetic dysregulation of the B cell compartment can lead to clinically relevant primary immune deficiency and autoimmunity in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

Disclosures

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Abbreviations used

AICDA	activation-induced cytidine deaminase
CCHMC	Cincinnati Children's Hospital Medical Center
cKS	Clinical Kabuki syndrome (no identifiable KMT2D/KDM6A mutations)
COMPASS	complex of proteins associated with SET1
CSR	class-switch recombination
H3K4	histone 3, lysine 4
Ig	immunoglobulin
IGH	immunoglobulin heavy chain locus
KDM6A	lysine demethylase 6A
KIS	Kabuki interaction surface
KMT2D	lysine methyltransferase 2D
***KMT2D*$^{Mut/+}$**	autosomal dominant mutation in *KMT2D*
KS	Kabuki syndrome
PTIP	paired box (PAX) transactivation domain–interacting protein

SHM	somatic hypermutation

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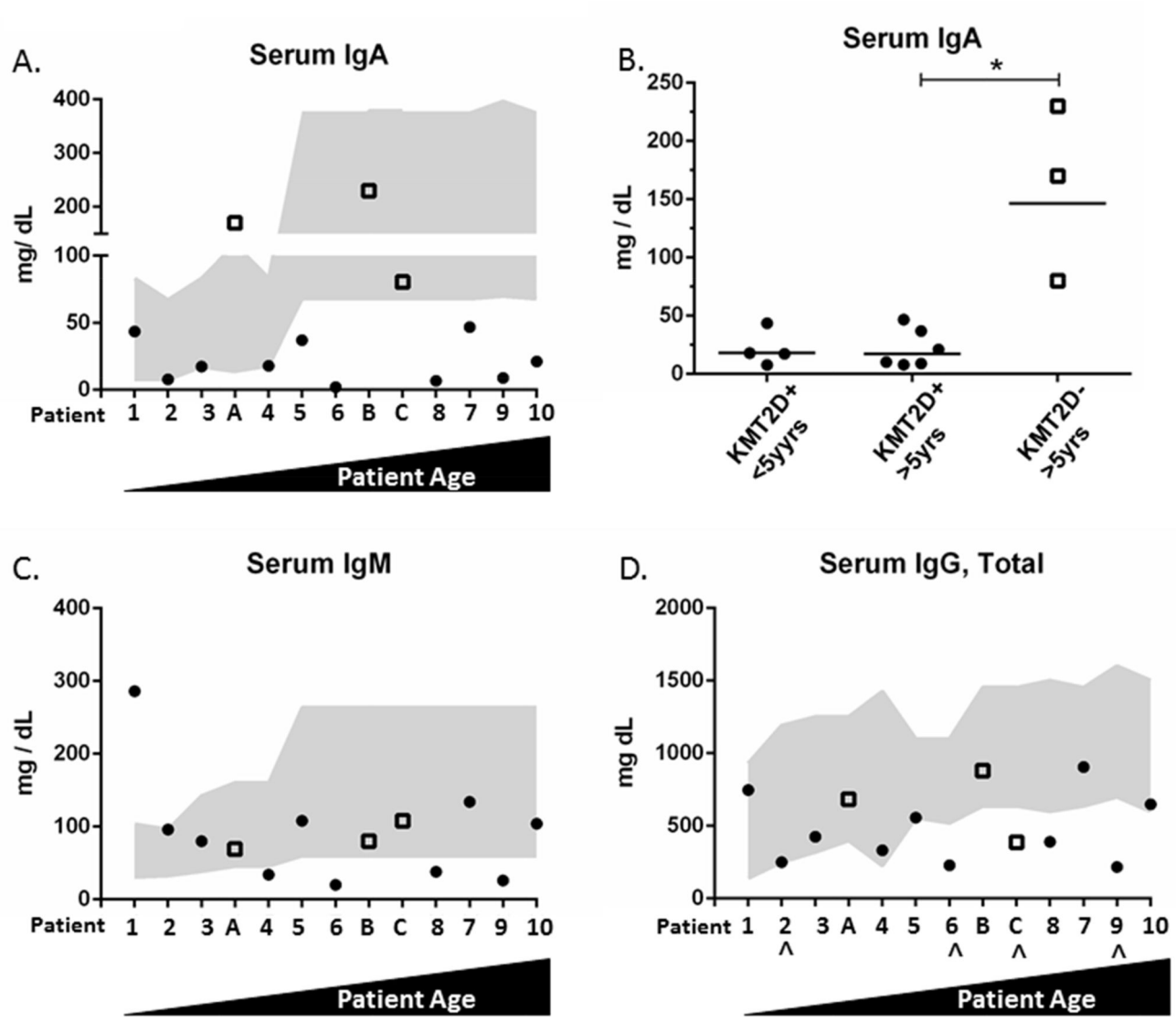

Figure 1. Serum Ig levels

Patients arranged by ascending age. Gray shading represents age-matched normal range (2.5th–97.5th percentile). (A) IgA levels in KMT2D$^{Mut/+}$ patients (#1–10, solid circles) and cKS patients (A–C, hollow squares). (B) IgA values (bar=mean) are significantly reduced in KMT2D$^{Mut/+}$ patients > 5 yrs old versus cKS patients. *$p < 0.05$. (C, D) IgM levels and IgG levels. IgG values for patients 2, 6, C, and 9 (^) are the last levels acquired before IVIG/ SCIG treatment.

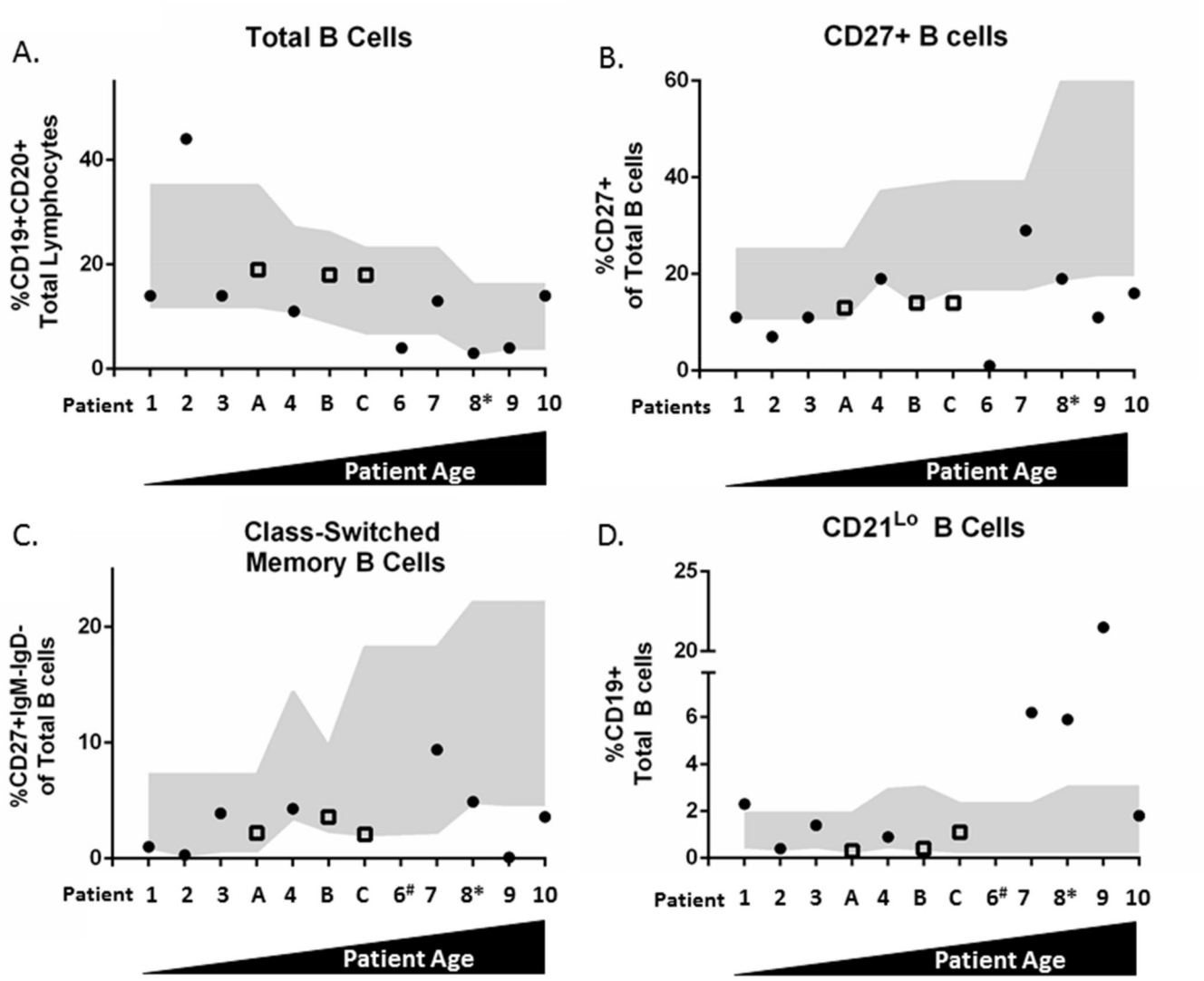

Figure 2. B cell subset analysis

Patients arranged by ascending age; gray shading represents age-matched normal range (5^{th}–95^{th} percentile). (A) Total B cells ($CD19^+CD20^+$) in $KMT2D^{Mut/+}$ (solid circles) and cKS patients (hollow squares). (B) Total memory B cells ($CD19^+$, $CD27^+$). (C) Class-switched memory B cells ($CD19^+CD27^+IgM^-IgD^-$). (D) $CD19^+$ $CD21^{lo}$ B cells. # = data were not available for patient 6 (n = 11). *Received anti-CD20 (rituximab) antibody therapy 3 yrs prior to analysis; had normal total B cell count pre-therapy. Lo, low.

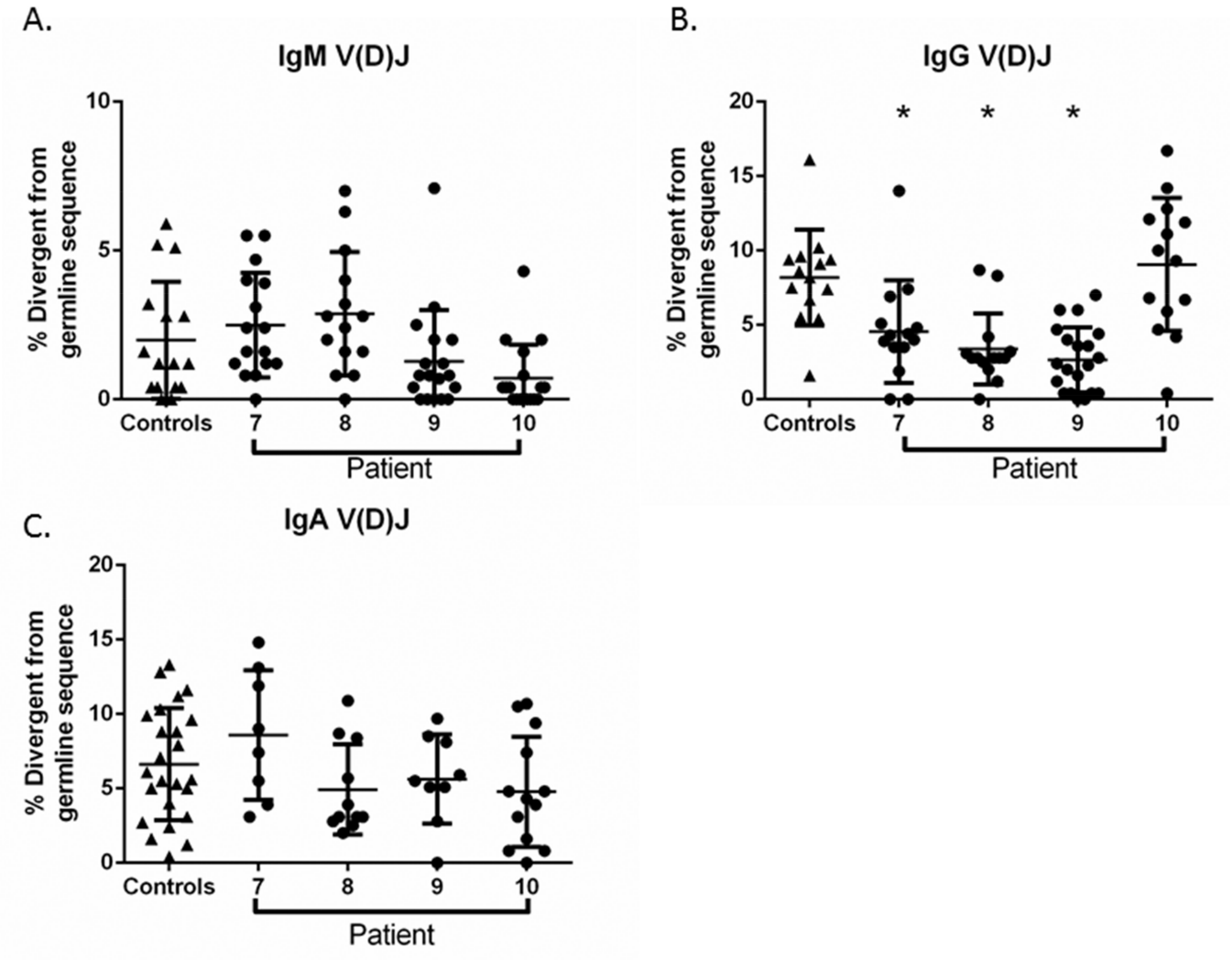

Figure 3. Somatic hypermutation (SHM) rate

Isoform-specific V(D)J clones analyzed in adult *KMT2D*$^{Mut/+}$ patients(circles) and age-matched adult controls (triangles). Presented as percent (%) divergent from germline IGH sequence. (A,B, C) IgM, IgG and IgA mutations rates in *KMT2D*$^{Mut/+}$ patients and controls. IgG mutation rates (B) were significantly lower in 3 of 4 *KMT2D*$^{Mut/+}$ patients than in controls (*$p < 0.05$). All data (A,B, C) are present as mean ± standard deviation of the mean.

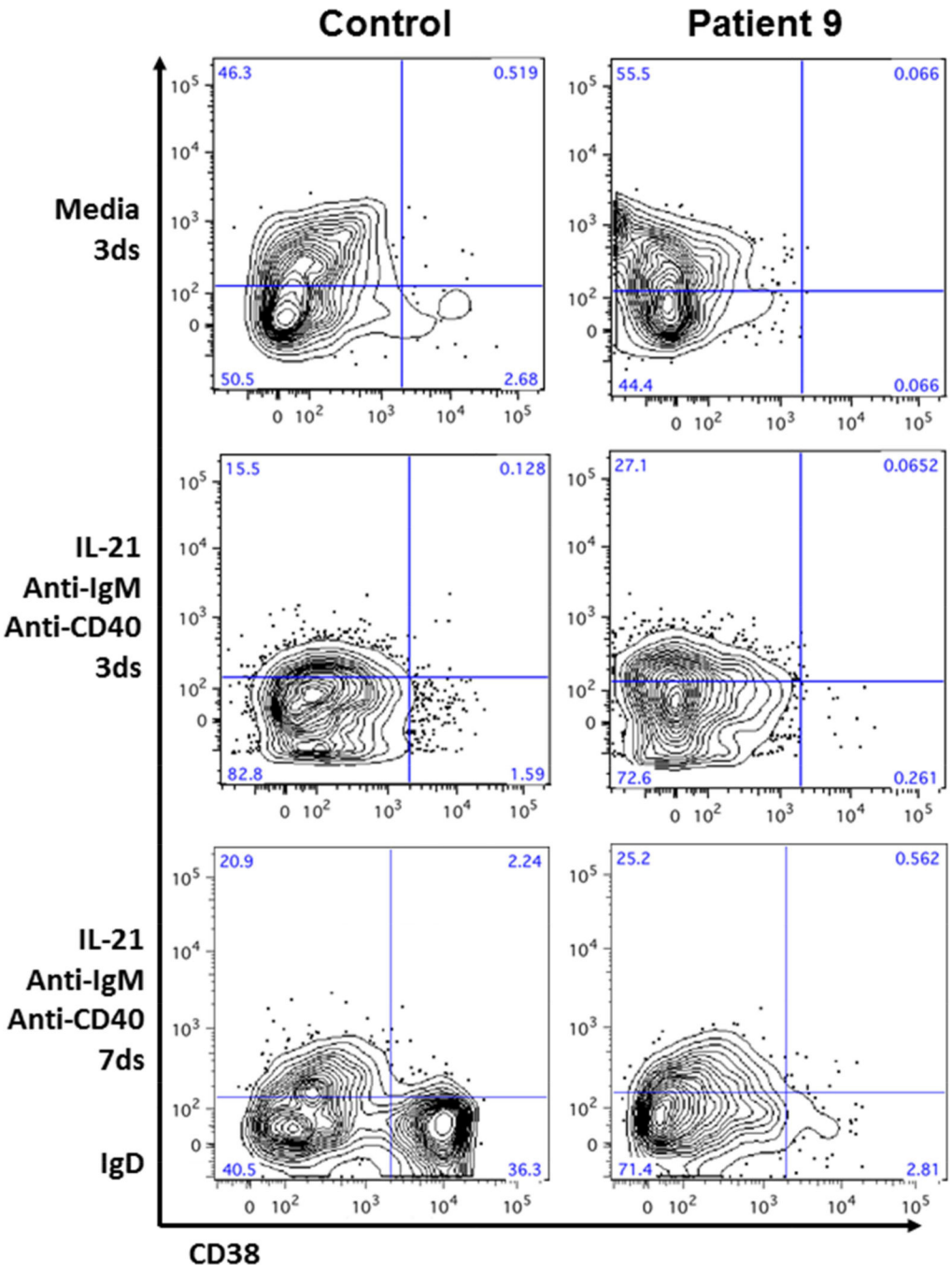

Figure 4. In vitro B cell differentiation

Isolated total B cells cultured in media alone or media plus differentiation factors. Flow cytometry performed on day 3 and day 7 of culture. Performed in duplicate with representative flow plots shown.

TABLE 1

KS Phenotype

	SUBJECT :	1	2	3	4	5	6	7	8	9	10	A	B	C		
	Age at Enrollment	0 yrs, 4 m	0 yrs, 5 m	0 yrs, 11 m	3 yrs, 8 m	6 yrs, 10 m	15 yrs, 5 m	15 yrs, 6 m	23 yrs, 0 m	24 yrs, 10 m	27 yrs, 2 m	2 yrs, 11 m	12 yrs, 0 m	13 yrs, 6 m	Present in this Cohort	Meta-analysis (Ref. 9)
CARDINAL FEATURES	Typical Facial Features	+	+	+	+	+	+	+	+	+	+	+	+	+	100%	87%
	Skeletal Anomalies	−	+	+	+	+	−	+	+	−	+	+	+	+	77%	ND
	Mild-Moderate Intellectual disability	NA	NA	NA	NA	+	+	+	+	+	+	+	+	+	100%*	ND
	Post-natal growth deficiency	+	−	+	+	−	+	+	+	+	+	−	+	+	77%	70%
ADDITIONAL FEATURES	Congenital Heart Defects	+	+	+	+	+	+	+	−	−	+	−	−	+	69%	46%
	Genitourinary anomalies	−	+	−	+	−	+	+	+	+	+	−	+	+	69%	44%
	Cleft lip and/or palate	+	−	+	+	+	−	+	−	+	−	−	−	−	46%	37%
	Gastrointestinal anomalies	−	−	−	−	−	−	+	−	+	−	−	−	−	15%	ND
	Ophthalmologic anomalies	+	+	−	−	−	+	+	+	−	−	−	+	+	54%	ND
	Autoimmune Complications	−	−	−	−	+	+	−	+	−	−	−	−	−	23%	ND
	Recurrent Infections OM/PNA/UTI	−/−/−	−/−/−	−/−/−	+/+/+	+/−/−	+/+/+	+/+/−	+/+/+	+/+/−	−/−/−	+/−/−	+/−/−	+/+/−	77%	61%

LEGEND: yrs, years; m, months; NA, not available; ND not done. OM, otitis media; PNA, pneumonia; UTI, urinary tract infection

*indicates percentage of subjects over 5 years of age.