Distinct Clinical Features and Novel Mutations in Taiwanese Patients With X-Linked Agammaglobulinemia

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Background: X-linked agammaglobulinemia (XLA) is caused by a mutation of the Bruton’s tyrosine kinase (BTK) gene and is the most common genetic mutation in patients with congenital agammaglobulinemia. The aim of this study was to analyze the clinical features, genetic defects, and/or BTK expression in patients suspected of having XLA who were referred from the Taiwan Foundation of Rare Disorders (TFRD).

Methods: Patients with recurrent bacterial infections in the first 2 years of life, serum IgG/A/M below 2 standard deviations of the normal range, and ≤2% CD19+B cells were enrolled during the period of 2004–2019. The frequency of infections, pathogens, B-lymphocyte subsets, and family pedigree were recorded. Peripheral blood samples were sent to our institute for BTK expression and genetic analysis.

Results: Nineteen (from 16 families) out of 29 patients had BTK mutations, including 7 missense mutations, 7 splicing mutations, 1 nonsense mutation, 2 huge deletions, and 2 nucleotide deletions. Six novel mutations were detected: c.504G>T [p.K168N], c.895-2A>G [p.Del K290 fs 23*], c.910T>G [p.F304V], c.1132T>C [p.T334H], c.1562A>T [p.D521V], and c.1957delG [Del p.D653 fs plus 45 a.a.]. All patients with BTK mutations had obviously decreased BTK expressions. Pseudomonas sepsis developed in 14 patients and led to both Shanghai fever and recurrent hemophagocytic lymphohistiocytosis (HLH). Recurrent sinopulmonary infections and bronchiectasis occurred in 11 patients. One patient died of pseudomonas sepsis and another died of hepatocellular carcinoma before receiving optimal treatment. Two patients with contiguous gene deletion syndrome (CGS) encompassing the TIMMBA/DDP1 gene presented with early-onset progressive post-lingual sensorineural Deafness, gradual Dystonia, and Optic Neuronopathy syndrome (DDON) or Mohr-Tranebjaerg syndrome (MTS).

Conclusion: Pseudomonas sepsis was more common (74%) than recurrent sinopulmonary infections in Taiwanese XLA patients, and related to Shanghai fever and...
INTRODUCTION

X-linked agammaglobulinemia (XLA; OMIM 300300), first described in 1952 (1), represents the prototype of primary B cell deficiencies caused by mutations of the Bruton’s tyrosine kinase (BTK) gene, a member of the Tec family of kinases localized on Xq21.3–Xq22, in the majority of male patients presenting with agammaglobulinemia (2). The XLA phenotype is characterized by a reduction or lack of mature B lymphocytes (≤2% of total lymphocytes), which is caused by a differentiation-transition blockage of B cell progenitors to mature B lymphocytes (3). Affected individuals have profound hypogammaglobulinemia and thus show increased susceptibility to bacterial infections, including sinusitis, otitis media, pneumonia, cellulitis, meningitis, gastroenteritis, and conjunctivitis (4). Onset usually occurs between 6 and 12 months of age, after consuming maternal transplacental IgG. The prognosis for individuals with XLA has markedly improved in the last 25 years as a result of earlier diagnosis, aggressive antibiotic therapy, and mainly through the use of replacement gamma globulin (intravenous or subcutaneous, IVIG or SCIG) to achieve an optimal serum IgG level (5, 6).

A total of 1,806 different BTK gene mutations are currently recorded in the Leiden Open Variation Database (https://databases.lovd.nl/shared/genes/BTK, last update on June 8, 2020). In accordance with the Rare Disease Control and Orphan Drug Act in Taiwan, patients who are suspected of having XLA are referred to our Primary Immunodeficiency Care and Research (PICAR) Institute for a molecular/genetic diagnosis and therapeutic suggestion. The aim of this study was to assess whether Taiwanese patients with XLA have unique manifestations and novel mutations.

MATERIALS AND METHODS

Patients

Patients with an initial diagnosis of XLA according to the European Society for Immunodeficiencies (ESID) criteria were enrolled from 2004 to 2019 (6), including (1) male patients with recurrent bacterial infections in the first 2 years of life, (2) serum IgG/A/M below 2 standard deviations of the normal range for age, and (3) ≤2% CD19+B cells. After excluding secondary etiologies of proteinuria, protein losing enteropathy, malnutrition, and severe burns, a definitive diagnosis of XLA was then made if a BTK mutation was identified and/or there was an obvious decrease in BTK expression in monocytes (7).

The patients and the healthy controls provided written consent for data collection and publication of this study. All human samples were obtained under protocols approved by the Institutional Review Board at Chang Gung Memorial Hospital (protocol 201601893A3, and 104-9578A3) and met the Institutional Review Board standards for ethical conduct of research with human subjects in accordance with the Declaration of Helsinki.

Molecular Analysis of the BTK Gene

Total RNA was isolated from peripheral blood mononuclear cells with TRizol (Invitrogen, Carlsbad, CA). Reverse transcription of messenger RNA followed by polymerase chain reaction (PCR) were performed as previously described (8, 9). Two pairs of oligonucleotide primers were designed to cover the entire coding region of the BTK gene. BTK1: CAG TGT CTT CTT CGA TCG AG; BTKC1: CAG TGG AAG GTG CAT TCT TG (1,277 b.p.); BTK5: TCA TTT TCA GAG ACT CCA GC; BTKC2: TGT CTC AGA AGC CAC TAT CC (1,253 b.p.). If a specific mutation was identified, genomic DNA responsible for the candidate exon was amplified and confirmed again.

Detection of BTK Gene Expressions

The expression of BTK in monocytes was evaluated by immunostaining from whole blood as previously described (7, 8). In brief, the red blood cells were removed using Lyse/Fix Buffer (BD Pharmingen, San Diego, CA), then incubated with CD14–PE (clone M5, BD Pharmingen) for 20 min, permeabilized using Perm Buffer II (BD Pharmingen), and then stained with AlexaFluor647-conjugated anti-BTK Ab (clone 53/BTK, BD Pharmingen) or isotype IgG2a (clone MOPC-173, BD Pharmingen) to identify epitopes between 2 and 172 amino acids of the Pleckstrin homology (PH) and Tec homology (TH) domains within the gating monocytes. The threshold of FSC was set as 5,000 and the data was analyzed by FlowJo 7.6 (Treestar, USA).

RESULTS

Demographic and Clinical Features

Of the 29 male patients (from 26 unrelated families) included in this study, 19 (from 16 unrelated families) had BTK mutations (Table 1). The median age at onset of XLA in these 19 patients was 1.2 (range 0–16; mean 2.5 ± 3.6) years. The median delay between the first significant infection and IVIG infusion was 2.7 (range 0–16; mean 3.7 ± 4.1) years. The median age at diagnosis and in April 2020 were 5.0 (range 0.5–27; mean 6.2 ± 6.6) years and 16.0 (range 0.5–28; mean 15.0 ± 11.1) years, respectively. All of the patients received regular IVIG supplements for their hypogammaglobulinemia to decrease susceptibility to infections.
### TABLE 1 | Clinical features of the XLA patients with BTK genetic mutations by the referred year.

| Referred year | Onset/Dx/current [death] | CD19% | BTK expression % (>80%) | Mutation/Exon (E)/Domain* | Igs G/A/M/E mg/dL | Clinical manifestations (recurrent sinopulmonary infections, RSI; Bronchiectasis, B; Sepsis; Chronic diarrhea, CD), identified pathogens and eventful infections with family maternal carrier | New/References |
|---------------|--------------------------|-------|--------------------------|---------------------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| 2004 P1-1     | 1.5Y/3.3Y/19Y Y          | 0.2%  | 2.5%                     | c. 1562A>T; p.D521V; E15/Kinase | 320/18/32/4 <7 | CD, Pseudomonas aeruginosa sepsis, Pseudomonas oryzihabitans sepsis, meningitis, toxic megacolon, face lip gangrene | Novel |
|               |                          |       |                          |                           |                   |                                                                           |                 |
| P1-2          | 16Y/2Y/HCC               | 0.1%  | 1.8%                     |                           | 248/18/23/4 <7 | RSI, B, Strepococcus pneumonia hip arthritis, Staphylococcus aureus cutaneous abscess, appendicitis, chronic hepatitis B and C, hepatomegaly, hepatocellular carcinoma; mortality of hepatocellular carcinoma | Novel |
| 2004 P2       | 5.3Y/8Y/25Y Y           | 1.1%  | 0.5%                     | c. 1132T>C; p.T334H; E12/S2H | 117/-6/27.3/250 | RSI, B, Pseudomonas aeruginosa sepsis, Streptococcus pneumonia sepsis, Staphylococcus aureus cutaneous abscess | Novel |
| 2004 P3-1     | 1.2Y/3Y/23Y Y           | 0.1%  | 0.4%                     | Int 14 (-2) A>G/Kinase     | 106/-6/27.3/4 <7 | RSI, B, Streptococcus pneumonia sepsis, arthropath | (10) |
| P3-2          | 3Y/6Y/22Y Y             | 0.3%  | 1.5%                     |                           | 146/-6/27.3/25 | RSI, B, Pseudomonas aeruginosa sepsis, Campylobacter colitis | Novel |
| 2004 P4       | 4M/6M [Sepsis]          | 1.3%  | 1.1%                     | Int 10 (-2)A>G/SH3 c.895-2A>G Del p.A23 fs 5* | <150/-23/-31/-4 <7 | Pseudomonas aeruginosa sepsis and mortality | Novel |
| 2007 P5       | 9M/6Y/15Y Y             | 0.5%  | 2.2%                     | c.1921C>T; p.R641C/E19/Kinase | 142/-23/-18 <7 | Pseudomonas aeruginosa, sepsis, facial palsy | Novel |
| 2008 P6       | 3Y/5Y/15Y Y             | 0.2%  | 0.2%                     | c.910T>G; p.F304V/E11/S2H | 150/-23/-18 <7 | RSI, B, Pseudomonas Aeruginosa sepsis | (1) |
| 2009 P7       | 2.1Y/8Y/19Y Y           | 0.8%  | 1.5%                     | c. 504 G>T p. K168N/E6/TH | 201/-23/19 <7 | RSI, Streptococcus pneumonia sepsis | Novel |
| 2010 P8       | 8M/7Y/25Y Y             | 0.0%  | 2.1%                     | Int5(2)A>G/PH c.392-2A>G Del p.A174 fs 17a.a. | <136/-24/-17 <7 | RSI, B, empyema, failure to thrive | (12, 13) |
| 2015 P9       | 1.2Y/4Y/8Y Y            | 0.2%  | 1.1%                     | Int 17(-1) G>A/Kinase     | 113/-6/-27 <7 | RSI, B, Pseudomonas aeruginosa sepsis, osteomyelitis, ecthyma gangrenosum, failure to thrive, HLH-like | (11, 14, 15) |
| 2015 P10      | 4Y/20Y/28Y Y            | 0.6%  | 0.1%                     | Int8 (+2) T>C/TH2 c.776+2 T>C Del p.A197 fs 7* | 346/-23/22 <7 | RSI, B, Pneumococcus pericarditis, septic hip arthritis and sepsis, Pseudomonas Aeruginosa sepsis | (16) |
| 2016 P11-1    | 6M/1Y/3Y Y              | 0.0%  | 0.2%                     | Del c.1857G; Del D653 fs plus 45 a.a./E19/Kinase | 271/-23/14 <7 | RSI, B, Pseudomonas Aeruginosa sepsis | Novel |
| 2016 P11-2    | 0/1Y                    | 0.2%  | 0.3%                     |                           | 212/-23/-4 <7 | Prenatal diagnosis | Novel |
| 2017 P12      | 5M/6Y/16Y Y             | 1.0%  | 0.2%                     | c. 232 C>T; p.G78/E3/PH | 202/-24/6 <19 | RSI, B, Pseudomonas aeruginosa sepsis, Ecthyma gangrenosum, Campylobacter enterocolitis and right foot cellulitis with abscess | (2, 17) |
| 2018 P13      | 3.1Y/5Y/28Y Y           | 0.0%  | 2.1%                     | c.1385 G>A; p.G462D; E15/Kinase | 22.3/-5.9/-17 <7 | RSI, B, Streptococcus pneumonia sepsis, Pseudomonas aeruginosa sepsis, empyema, failure to thrive | (18) |
| 2018 P14      | 2.2Y/3Y/5Y Y            | 0.0%  | 0.4%                     | Del E19 DDP1              | 213/-7/12 <7 | RSI, otitis media, perianal cellulitis, Pseudomonas Aeruginosa sepsis, dysostosis | (19, 20) |
| 2018 P15      | 1.2Y/1.5Y/3Y Y          | 0.1%  | 1.1%                     | Del E6-19 DDP1            | 209/-7/-18 <7 | Rotavirus enteritis, Pseudomonas aeruginosa sepsis, ataxia, dystonia, hearing impairment | (19, 20) |
| 2019 P16      | 6M/1Y/2Y Y              | 1.2%  | 1.5%                     | Int14 (+1) G>A/Kinase c.1349 +1G>A Del p.W935 fs 32* | 186/-23/22 <7 | Pseudomonas aeruginosa sepsis (two times), Salmonella colitis | (21) |

*TK, The tyrosine kinase domain; PH, Pleckstrin homology domain; SH2, Src homology 2 domain; SH3, Src homology 3 domain; TH, Tec homology domain; Del, deletion; fs, frameshift; “ meant “stop” codon. The white and gray columns represented 16 unrelated families.
Sepsis was the most common clinical feature (16/19, 84.2%) followed by recurrent sinopulmonary infections of sinusitis, otitis, and pneumonia (13/19, 68.4%), leading to bronchiectasis (in 11 patients). Nineteen sepsis episodes occurred in 16 patients (\textit{Pseudomonas} in 14 and \textit{Pneumococcus} sepsis in 5). Skin cellulitis and abscesses developed in 5 patients. Two patients had \textit{Campylobacter} colitis, 1 had \textit{Salmonella} colitis, 1 had rotavirus enteritis, and 1 had arthropathy, but none had \textit{Giardia lamblia}. Patient P9 suffered from recurrent hemophagocytic lymphohistiocytosis (HLH) before regular IVIG infusion. One patient died of \textit{Pseudomonas} sepsis at 6 months of age (P4), and one patient (P1-2) died due to hepatocellular carcinoma at 27 years before receiving IVIG infusion.

One pair of nephews (P1-1, P1-2) and one pair of siblings (P3-1, P3-2) were diagnosed in 2004. Three of these patients (P1-2, P3-1, and P3-2) developed bronchiectasis, and one uncle (P1-2) died of hepatocellular carcinoma when his nephew (P1-1) suffered from \textit{Pseudomonas} sepsis. The third pair of siblings (P11-1, P11-2) were diagnosed in 2016 and 2019, respectively. The index case (P11-1) developed \textit{Pseudomonas} sepsis, and the younger (P11-2) was identified by prenatal genetic analysis and was free of significant infections under regular IVIG infusion.

**Genetic Analysis**

The whole coding region was sequenced using two pairs of primers for PCR-amplification cDNA that was reverse transcribed from RNA. If a variant was detected in cDNA PCR-amplification, the responsible exons with the flanking intronic regions of the BTK gene were again confirmed. Ten patients with a low CD19 cell count but normal BTK expression were, as expected, compatible with the wild type of the BTK gene (Supplemental Table 1). The BTK expression was almost absent in the 19 included patients (Figure 1), of whom 7 had missense (6 unrelated families) mutations, 7 had splicing (6 unrelated families) mutations, 4 had deletions (3 unrelated families, including 2 huge deletions), and 1 had a nonsense mutation (Table 1). The involved domains from the N-end to the C-end of the amino acid were the Pleckstrin homology domain (PH) in 2, the Tec homology domain (TH) in 1, Src homology 3 domain (SH3) in 1, Src homology 2 domain (SH2) in 2, tyrosine kinase domain (TK) in 11, and an additional 2 huge deletions affecting the last exon 19. The genetic defects were distributed through the whole coding region, and over half (11/19) were clustered in exons 14, 15, and 19. All of the mothers were carriers. Six unique mutations (in 8 patients) were identified (2, 10–21), including c.1562A>T, c.1132T>C, c.895-2A>G, c.504G>T, c.910T>G, and c.1957delG.

**Genotype–Phenotype Correlation**

In contrast to other reports that patients with missense mutations had maintained some residual capability to therefore present with milder phenotypes than those with other types of mutation (7, 12, 13), all of our patients with missense mutations developed similar severity as sepsis and bronchiectasis to those with other mutation types. However, early initiation of IVIG seemed to attenuate the exacerbation of bronchiectasis and prevent sepsis episodes, because the patients who received regular IVIG before 2 years of age did not develop bronchiectasis.

Of note, in the 2 huge deletions, the designed PCR primers amplified the coding region of cDNA covering the segment of exons 1–13 in P14, but none were detected in P15 (Figure 2A). Furthermore, each exon from 1 to 19 was amplified, and the absence of exon 19 and the absence of exons 6–19 was in patients P14 and P15 (Figure 2B), who both gradually developed dystonia and ataxia. The TIMM8A/DDP1 (Translocase of Inner Mitochondrial Membrane 8A/Defaelessness-Dystonia Peptide 1) gene is 770 b.p. downstream from the 3’end of the BTK gene in the X chromosome, and it encodes DDP1 which imports metabolite transporters from the cytoplasm to mitochondria and mainly orchestrates neural development and muscle coordination. Contiguous gene deletion syndrome (CGS) affected the BTK and TIMM8A genes supported by the evidence of undetectable two exons of the TIMM8A gene (Figure 2C). Compared to a normal female with two X alleles, two carrier-mothers of P14 and P15 had only half the relative concentration of exon 2 in the TIMM8A gene (Figure 3) and exon 19 of the BTK gene (data not shown) that was equal to a healthy male with one X allele.

**DISCUSSION**

Referred from the Taiwan Foundation of Rare Disorders (TFRD), 19 of 29 male patients (from 26 unrelated families) with hypogammaglobulinemia, low B cell populations, and infections were diagnosed with XLA. The prevalence of XLA based on approximately 3,200,000 live births during the 16-year study period (https://www.ncbi.nlm.nih.gov/books/NBK448170/) is estimated around 1 case per 170,000 live births in Taiwan, close to that of Norway (1/100,000–1/285,000) (22) and Switzerland (1/200,000) (23), but higher than Italy (1/250,000) (24) and USA (1/379,000) (4). As well as geno-geographic diversity for higher prevalence, our national health insurance (NHI) and medicine strategy covering such rare disorders encourage affected patients to urge effective management for life-quality improvement.

As expected, obviously decreased intracellular BTK staining in monocytes prompts analysis of genetic defects in those with lower B cell percentage. Eight patients (8/19, 42%) had novel mutations, and two (2/19, 10.5%) had huge deletions involving the neighboring TIMM8A gene encoding mitochondrial import inner membrane translocase subunit TIMM8A that is expressed at much higher levels in Purkinje cells of the cerebellum (19). Thus, patients with CGS develop deafness-dystonia-optic neuronopathy syndrome (DDON) [or Mohr-Tranebjaerg syndrome (MTS)] characterized by progressive dystonia, ataxia, hearing impairment, cortical blindness, and early dementia. However, XLA patients present with hearing impairment which is more commonly ascribed to complications of recurrent sinopulmonary infections, and those with unstable gait are often suspected of having chronic encephalitis caused by enterovirus or prion (John Cunningham) infections. Both neurological dysfunctions of hearing impairment and unstable gait are often
thought to be due to B cell deficiencies rather than neuropathy in auditory brain stem responses caused by the loss of TIMM8A in mitochondrial dysfunction. Thus, it is important for clinicians to consider the possibility of a deletion of the last exon 19 in the BTK gene in patients suspected of having DDON syndrome, and to further test for the existence of the neighboring TIMM8A gene to allow for an effective therapeutic strategy. Applying to Italian, Chinese, American, and African cohort studies (4, 12, 24, 25), those with huge deletions of the last exon 19 in the BTK gene should have, but not yet, further evaluated the existence of TIMM8A gene for the DDON/MTS phenotype.

Conversely, exon 1 of the TIMM8A gene follows exon 19 of the BTK gene. If patients with the DDON phenotype are identified to have a deletion of exon 1 of the TIMM8A gene, the diminished BTK expression in flow cytometry and/or a B cell percentage <2% are considered to be clues to speculate whether the deletion expands to the BTK gene, thereby allowing for timely IVIG treatment. Overall, of 19 patients identified with CGS in a Medline search (19, 20, 26–32) including our patients P14 and P15, only one of five with a deletion of the last exon 19 in the BTK gene and the whole TIMM8A gene succumbed to pneumonitis and respiratory failure because of rapid progressive severe spasticity at 6 years of age. In contrast, the other four who had larger deletions expanding from exon 6 in the BTK gene seemed to have gradual psychomotor retardation, speech impairment, and sensorineural hearing loss.

FIGURE 1 | After PBMC purified by centrifugation, we utilized FSC and SSC to locate the monocyte region and gated them by CD14+ in a representative patient (P15) with the BTK mutations showed an almost complete absence of BTK expression (1.1%) and a bimodal pattern in his carrier mother (38.9%) compared to the normal healthy control (85.7%).
FIGURE 2 | RT-PCR amplification of cDNA included two designed two pairs: BTK1-BTKC1 for the coding region from exon 1 to exon 13 (product 1,277 b.p.); and BTK5-BTKC2 (1,253 b.p.) for the coding region from exon 11 to exon 19. Compared to the healthy control and mother of P15, only cDNA amplification by RT-PCR of the product from BTK1-BTKC1 was detectable in P14, and the others were all undetectable. PMNs should express BTK, but much lower than PBMCs.

(Continued)
FIGURE 2 | We evaluated BTK expression in two cell lines of PBMCs and PMNs in P15. The two leukocyte components did not express any BTK after PCR-amplification in P15 (A). Therefore, each exon was amplified from genetic DNA. Exon 19 in the BTK gene in P14 and exons 6–19 in P15 were missing (B). The contiguous gene TIMM8A with two exons was amplified, but it revealed only non-specific products in exon 1 (GGA GTT GGA CGC CTG CCT GG; CTT GAA TCC TGT CAT GAT GAA for exon 1, product 1,593 b.p.) and undetectable in exon 2 (GAA CCT GGC GGA GGT TAC AGT; CCT TGG AAT CAG CCC ATG CT, product 1,742 b.p.). In the normal control, two white-arrows pointed at the correct locations (C). Two duplications were performed each.

Their phenotypic severity did not correlate to the extent of the deletion (20).

Under NHI coverage and support by social welfare, Taiwanese infants are obligated to receive regular vaccination and evaluation of neurodevelopment. If they have cough and yellow rhinorrhea over 5 days in suspicion of sinusitis or otitis media, primary physicians often give empiric antibiotics (Augmentin or cefaclor). Our vaccine schedules of Haemophilus influenzae type B (Hib) and pneumococcus 13-valent conjugate vaccine are both at 2 and 4 months old (plus the third dose of pneumococcus 13-valent conjugate vaccine at 6 months old) and therefore decrease Hib and pneumococcus infections in all children, including those with BTK mutations. Our previous sepsis study in PIDs patients showed that the most identified pathogen was pseudomonas followed by strep. pneumococcus (33, 34). Until now, there have been no available pseudomonas vaccines to prevent pseudomonas infections. These aspects may explain how pseudomonas sepsis has become the most common presentation in patients with BTK mutations. Thus, our patient P1-1 had a [D521V] BTK missense mutation and presented with the Shanghai fever phenotype consisting of severe bloody diarrhea, neutropenia, eczema gangrenosum, and pseudomonas sepsis (35). Because the 10 warning signs proposed by the Jeffery Model Foundation were not widely known in Taiwan in 2004, patient P4 died of pseudomonas sepsis when he was 5 months old despite receiving the first IVIG infusion at...
that time. Patient P1-2, the maternal uncle of P1-1, died of hepatocellular carcinoma with chronic hepatitis B and C at 29 years of age. Recently, our thalassemia patient (manuscript preparation) received three doses of anti-CD20 deletion therapy (rituximab 375 mg/m² per week for 3 doses) for post-transplant autoimmune pancytopenia. Rituximab continuously inhibited B cell generation until now, unexpectedly over 6 years, and led to persistent hypogammaglobulinemia. The precise mechanism of malignant transformation remains elusive despite higher virus load of hepatitis B virus in hypogammaglobulinemia patients than those without hypogammaglobulinemia. Hepatitis B vaccine can enhance memory B cells to make high-affinity antibody to effectively resist against hepatitis B virus and therefore may prevent hepatocellular carcinoma (36). However, hypogammaglobulinemia patients (with BTK mutations or CD20 deletion by biologics) are presumed not to produce enough high-affinity antibodies to the hepatitis B virus. Whether such insufficiency antibodies for neutralization and opsonization to hepatitis B virus in hypogammaglobulinemia patients relate to malignancy transformation should be further investigated.

Recurrent hemophagocytic lymphohistiocytosis (HLH) occurred before regular IVIG infusions in our 4-year-old patient P9 who suffered from pseudomonas sepsis or/and recurrent sinopulmonary infections, which could trigger HLH. The mechanism of hypercytokine storm from HLH resembling macrophage activation syndrome infers that the absence of BTK can augment inflammation cytokines through Toll-like receptor signaling pathways (TLR4, 7, 8, and 9) (37–39), possibly driving to the HLH process as two brothers in a previous report (40). IVIG infusion serves as an induction medication in the TPOG-2004-HS protocol for HLH to suppress hypercytokinemia and therefore modulate the overactive innate immunity related to the BTK mutations.

This study should be interpreted in light of its limitations. First, our patients had hypogammaglobulinemia, lower B cell percentage, and typical antibody-deficiency phenotypes. However, additional two Taiwanese male siblings with the p.P116L BTK mutation had selective IgM deficiency and focal proliferative glomerulonephritis initially presenting with proteinuria and hematuria (41). Both <1% B cells reminded physicians of XLA despite a normal IgG level. Thus, male patients with a low B cell percentage and increased susceptibility to bacterial infections should be screened for the BTK mutation. Second, to those with the DDON phenotype accompanying recurrent otitis media, sinusitis, or/and pneumonia, we recommend assessing their BTK expression level and investigating whether they belong to CGS involving the BTK and TIMM8A genes. Third, although carrier detection can be indirectly predicted by half-dose of X-linked targeted genes compared to non-carrier females or a bimodal flowcytometric pattern of the BTK expression in CD14+ monocytes, the direct breakpoints at genomic DNA should be possibly located by designed “walking” primers with/without ligation-mediated PCR (28, 42) through ligation-adapter as well as whole genome sequencing (WGS) that could explore the un-amplified sequence alignment by bioinformatics software program. Fourth, genetic mutations of non-X linked agammaglobulinemia encoding for pre-BCR and/or BCR complex (such as IGHM, CD79a, CD79b, and IGLL1 genes) and for activating mTOR signaling (such as PI3KD and PIK3R1 genes). These candidate genes and others are investigated in referred patients with the wild BTK gene by whole exome sequencing (some patients in Supplemental Table 1), but negative findings (43).

In conclusion, regular IVIG infusions and adequate prophylactics prevented recurrent sinopulmonary infections in Taiwanese patients with BTK mutations except two who died due to hepatocellular carcinoma and pseudomonas sepsis before IgG infusion in 2004. Pseudomonas sepsis was the most common manifestation, and these patients also presented with severe diarrhea and eczema gangrenosum, relating to Shanghai fever and recurrent HLH. In addition to the higher rate of novel mutations of the BTK gene (42% in this study), approximately 10% of patients were CGS affecting the BTK and TIMM8A genes and presented with the DDON/MTS phenotype characterized by early-onset progressive post-lingual sensorineural deafness, gradual dystonia, and optic atrophy, which indeed required aggressive psychomotor re-education and physical therapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and all human samples were obtained under protocols approved by the Institutional Review Board at Chang Gung Memorial Hospital (protocol 201601893A3 and 104-9578A3) and met the Institutional Review Board standards for ethical conduct of research with human subjects in accordance with the Declaration of Helsinki. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Y-HY, M-YH, and W-IL carried out the molecular genetic studies, analyzed the sequence alignment, and drafted the manuscript. S-JL and C-YW performed the immunoassays. W-IL designed the study and the genetic analysis. L-CC, K-WY, T-CY, C-YW, L-SO, and J-LH participated in the study to care for critical patients. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.02001/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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