10%程度にすぎない．CVIDでは最低10以上の未知遺伝子が背景としてあるのではと推測されている．マウスモデルや分子機能から推測された共刺激分子については遺伝子変異が見つかっていない．具体的にはBAFF-RのリガンドであるBAFF, APRIL, BCMAは少なくともmajorなCVID遺伝子ではない[2,30,31,34,35]．重症複合型免疫不全症あるいは複合型免疫不全症の責任遺伝子である*ADA*, *RAG*, *LIG4*, *Artemis*などの遺伝子異常症の軽症型を見逃している可能性は十分にある．しかしおそらくは主たる責任遺伝子ではない．SC-AのKRECs, TRECs正常群においては，家族例の解析や体系的あるいは網羅的遺伝子解析に機能解析を加えて遺伝子探索が行われるべきである．

私たちは今までに3つのアプローチを用いて探索をおこなっている．1つは家系例からSNP arrayにおいてhomozygosity mappingを行い，その領域にmapされる遺伝子をFLX454にてlong read sequenceする方法である．実際の検討では候補遺伝子が4000前後程度残り，塩基配列決定においての省力化にはつながらなかった．2つめの方法は，免疫に関連した分子群を抽出して，濃縮チップを作成し，全エクソン領域を解析する方法である．私たちはRAPID（Resource of Asian Primary Immunodeficiency Diseases）: URL http://rapid.rcai.riken.jp/RAPIDをベースに，既知遺伝子，候補遺伝子を抽出し，さらにRAPIDでの候補遺伝子抽出にも用いられているMGI（Mouse genome informatics）URL: http://www.informatics.jax.org/, RefDIC（Reference database of immune cells）URL: http://refdic.rcai.riken.jp/welcome.cgi, NetPath URL: http://www.netpath.org/などと共同研究者が独自に収集したB細胞亜群に特徴的に発現する分子群の情報などから，約2,500遺伝子を抽出し，その全エクソン解析を行っている．さらに3番目の方法として，すでに定法となった全エクソン解析も開始した．いずれの場合にも，SNP databaseが重要であり，現時点ではdbSNP135を元に日本人SNP情報を収集しつつ，標的を絞っているところである．いずれにせよできるだけ均一な集団での解析が重要であり，かつ家族歴があるものが優先して解析されることにより，新たな責任遺伝子同定も遠くないものと予想している．

VI. 再び臨床症状[1~5,14]

1. 感染症

多くは細菌感染症であり上下気道炎が多い．特に呼吸器感染症による気管支拡張症は重要で30-50%程度の患者で認められると共に，生命予後に大きく関与する．慢性感染症よりも重症感染症がその成立に関与しているとされている．多く，一方ニューモシスチス肺炎やMAC感染症ではT細胞性免疫不全症を疑う．

2. 消化管症状

消化管症状を呈する症例は多く，全国調査でも約1/3で認められた．多くは下痢・消化管感染症であり，この場合キャンピロバクターなどに加えて，サルモネラなどの細胞内寄生菌，CMV腸炎なども報告されている．また結節性リンパ様増殖（nodular lymphoid hyperplasia : NLH），萎縮性胃炎，炎症性腸疾患も有名である．NLHは約8%の患者で認められる．

3. 自己免疫疾患

自己免疫性溶血性貧血（Autoimmune hemolytic anemia : AIHA）や特発性血小板減少性紫斑病（Idiopathic thrombocytopenic purpura : ITP）が最も多い．また乾癬，悪性貧血，関節リウマチ，SLE，シェーグレン症候群なども認められる．

4. 悪性腫瘍

胃がんの発症危険度は7-16倍，悪性リンパ腫の発症危険度は12-18倍とされている．悪性リンパ腫ではB細胞由来が多く，EBVは陰性が多いとされている．

VII. 治　　療

1) γグロブリン補充

IgGは（500-）700 mg/dL以上を目標に補充を行うが，個人により至適IgGレベルが異なることに注意が必要である．1つには抗体の質（特異抗体の有無や親和性）の問題があるからであろう．またIgGは最低レベルを（500-）700 mg/dLとし，かつ発見時のIgGレベル＋300-500 mg/dL程度にするべきとの意見もある．いずれにせよ1,000 mg/dL程度に保ってはじめて感染症の頻度が減少する症例も多く経験し，それ以上とせざるを得ない場合もあ

る．

2. 合併する自己免疫疾患

AIHA や ITP に対してはステロイドやγグロブリン大量療法が試みられる．リツキサンを用いた報告もある．炎症性腸疾患に対しては 5-Aminosalicylic acid やステロイドを用い，ステロイドや TNF アンタゴニストが用いられている．合併したリウマチ性疾患に対する治療も，CVID の背景に関わらず標準治療が行われる．

3. 根治療法

いわゆる表 4 の SC-C, SC-D では造血細胞移植を考慮される場合がある．SC-A に対する造血細胞移植はまだ本格的には行われていないが，たとえば自己免疫疾患の管理や血液リンパ系腫瘍の治療に難渋する症例では考慮しても良いと思われる．

VIII. おわりに

CVID の病像と研究の現況について記載した．真の CVID とは何かという問題が残されているが，基本的には CVID は抗体産生不全型免疫不全症であり，特異抗体産生は不良で，かついわゆる T 細胞免疫不全症に合併するような感染症は稀と考えるべきである．成人領域で診療の機会も多く，また除外する疾患も多いため，もし診断に苦慮する場合も多い．筆者まで遠慮なくご相談いただければと思っている．

謝　辞：この研究は厚生労働科学研究・難治性疾患克服事業・症例研究分野「成人型分類不能型免疫不全症の実態把握，亜群特定に基づく診断基準策定及び病態解明に関する研究」班において行われたものである．KRECs, TRECs のアイデアによる分類及び SNP array からの責任遺伝子同定は防衛医科大学野々山恵章，現東京医科歯科大学今井耕輔先生のアイデアによる．また研究班のメンバーである筑波大学松本功先生，大阪大学田中敏郎先生，理化学研究所免疫アレルギー総合科学研究センター竹森利忠先生，かずさ DNA 研究所満生紀子先生，小原収先生に深謝したい．またこの研究は全国の CVID 患者さん，患者さんを紹介してくれた先生方によってはじめて可能になったものである．この場を借りて深謝申し上げたい．

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In conclusion, the associations among asthma, biofilm-forming bacteria, and revision ESS are strong and robust after adjusting for other factors in patients with CRS from a tertiary medical center. Despite its limitations, this study may improve our understanding of refractory CRS pathogenesis, possibly leading to more effective treatment strategies, such as incorporating the treatments of asthma and biofilm infection into conventional CRS therapies. Prospective cohort studies in diverse populations are needed to assess the causality of these associations.

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Quantification of κ-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects

To the Editor:

X-linked agammaglobulinemia (XLA) is a primary immunodeficiency caused by severely decreased numbers of mature peripheral B lymphocytes as a result of a mutation in the *BTK* gene. Non-XLA is characterized by hypogammaglobulinemia with decreased B-cell counts (less than 2% of mature B cells) in the absence of the *BTK* gene mutation. Both XLA and non-XLA are caused by an early B-cell maturation defect.[1] In patients with XLA and non-XLA, recurrent infections appear between 3 and 18 months of age, whereas the mean age at diagnosis is 3 years.[2] This delayed diagnosis results in frequent hospitalization because of pneumonia, sepsis, meningitis, and other bacterial infections, which frequently require intravenous administration of antibiotics and can be fatal. Frequent pneumonia results in a high incidence of chronic lung diseases.[3] Thus, early diagnosis and early treatment, including periodical intravenous immunoglobulin replacement therapy, is essential to improve the prognosis and the quality of life of patients with XLA and non-XLA.

In the process of B-cell maturation, immunoglobulin κ-deleting recombination excision circles (KRECs) are produced during κ-deleting recombination allelic exclusion and isotypic exclusion of the λ chain.[4] Coding joint (cj) KRECs reside within the chromosome, whereas signal joint (sj) KRECs are excised from genomic DNA. cjKREC levels remain the same after B-cell division, whereas sjKREC levels decrease, because sjKRECs are not replicated during cell division.[5] Because the B-cell maturation defects in XLA and non-XLA occur before κ-deleting recombination, KRECs are not supposed to be produced. Therefore, measurements of KRECs have the potential to be applied to the identification of these types of B-cell deficiencies in patients, which consist of around 20% of all B-cell defects.[6] In addition, some types of combined immunodeficiencies show an arrest in B-cell maturation and can also be identified by this method. The success of newborn screening for T-cell deficiencies by measuring T-cell–receptor excision circles[7] prompted us to develop a newborn screening method for XLA and non-XLA by measuring KRECs derived from neonatal Guthrie cards.

The study protocol was approved by the National Defense Medical College institutional review board, and written informed consent was obtained from the parents of normal controls, the affected children, and adult patients, in accordance with the Declaration of Helsinki.

First, we determined the sensitivity of detection levels of cjKRECs and sjKRECs in Guthrie cards using real-time quantitative PCR.[5] Normal B cells from a healthy adult were isolated from peripheral blood (PB; mean purity, 88.5%). PB was also obtained from 1 patient with XLA (P20) whose B-cell number was 0.09 in 1 μL whole blood and who was negative for sjKRECs ($<1.0 \times 10^2$ copies/μg DNA). Various numbers of normal B cells were serially added to 1 mL whole PB obtained from this patient with XLA. The B-cell–added XLA whole blood was then applied to filter papers, and 3 punches (3 mm in diameter) of dried blood spots were used for DNA extraction. At least 3 DNA samples containing the same B-cell concentrations (0.09-400 B cells/μL) were used for the real-time quantitative PCR of cjKRECs and sjKRECs. The percentages of the positive samples ($>1.0 \times 10^2$ copies/μg DNA) of cjKRECs and sjKRECs increased constantly

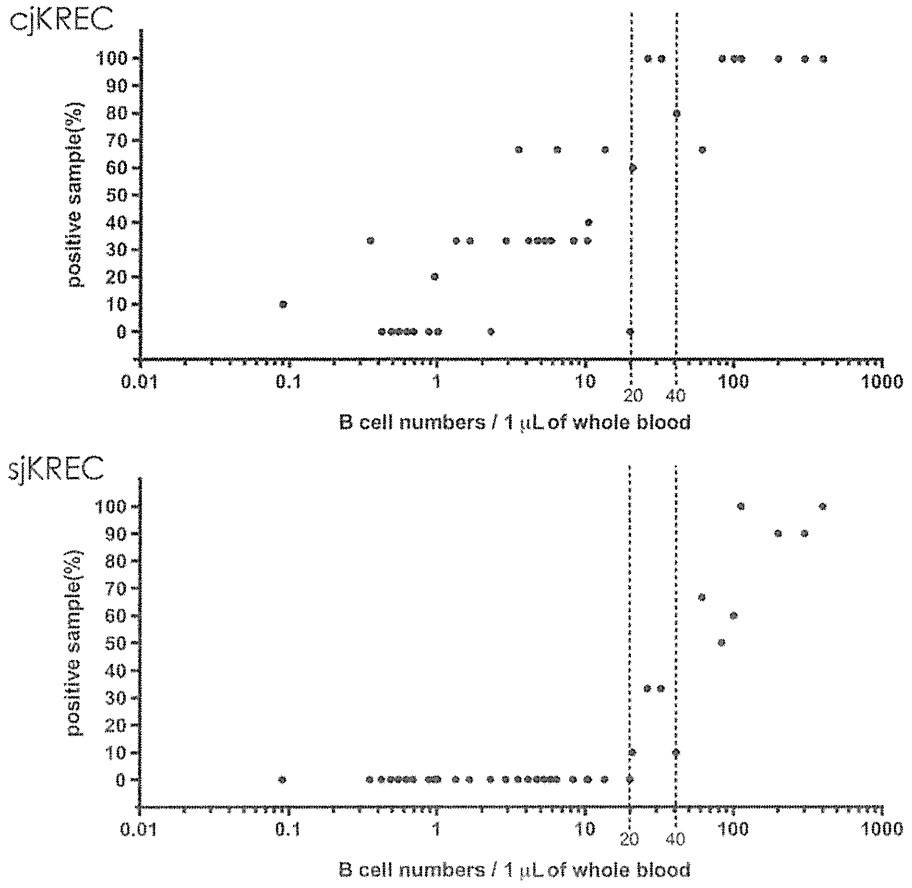

FIG 1. Sensitivity levels of cjKRECs and sjKRECs. Various numbers of purified normal B cells were serially added to whole PB from a patient with XLA (P20) to obtain B-cell–added XLA whole blood. cjKRECs and sjKRECs were measured in 3 to 10 samples of each concentration in triplicate. In all analyses, RNaseP (internal control) was positive ($2.3 \pm 0.2 \times 10^5$ copies/μg DNA). *X-axis,* B-cell numbers in 1 μL whole blood from a patient with XLA. *Y-axis,* Percentages of the KREC-positive results in the tests.

as the B-cell concentrations increased (Fig 1). None of the samples were positive for sjKRECs when the B-cell numbers were less than 20/μL, but cjKRECs were often positive. It has been reported that 90% of patients with XLA have less than 0.2% B cells in the PB at diagnosis.[1] Because peripheral lymphocyte numbers in neonates range from 1200 to 9800/μL,[8] the absolute B-cell numbers of 90% of patients with XLA are estimated to be 2.4 to 19.6/μL at the time of blood collection for Guthrie cards, although exact B-cell numbers of XLA in neonatal periods are not known at this moment. Because neonates are known to have fewer B cells than infants,[9] and we observed that B-cell numbers are constantly low in patients with XLA throughout infancy (Nakagawa, unpublished data, June 2010), which is consistent with the fact that BTK plays an essential role in B-cell maturation. It is likely that neonates with XLA also have severely decreased B cells. On the other hand, all samples obtained from 400 B cells/μL were positive for both cjKRECs and sjKRECs. We also observed that all healthy infants (1-11 months old; n = 15) were sjKREC-positive (Nakagawa, unpublished data, June 2010) and might have at least 600 B cells/μL whole blood.[9] From these data, it is assumed that at least 90% of patients with XLA are sjKREC-negative, and healthy neonates are positive for sjKRECs on neonatal Guthrie cards.

Next, we measured cjKRECs and sjKRECs in dried blood spots in filter papers or Guthrie cards from 30 patients with XLA and 5 patients with non-XLA and from 133 neonates born at the National Defense Medical College Hospital during this study period (August 2008 to October 2009) and 138 healthy subjects of various ages (1 month to 35 years old) to investigate the validity of this method. The levels of B cells of the patients ranged from 0.0% to 1.1% of total lymphocytes and 0.0 to 35.78/μL. IgG levels were 10 to 462 mg/dL (see this article's Tables E1 and E2 in the Online Repository at www.jacionline.org). Patients with leaky phenotypes[1,10] were included; 1 patient (P30) had more than 1% B cells and 34.22/μL total B cells, and 4 patients had more than 300 mg/dL serum IgG (P12, P30, P31, P33). All of the normal neonatal Guthrie cards were positive for both cjKRECs and sjKRECs ($7.2 \pm 0.7 \times 10^3$ and $4.8 \pm 0.6 \times 10^3$ copies/μg DNA, respectively). All healthy subjects of various ages were also positive for both cjKRECs and sjKRECs (Nakagawa, unpublished data, June 2010). In contrast, specimens from all 35 B-cell–deficient patients were sjKREC-negative ($<1.0 \times 10^2$ copies/μg DNA; Fig 2). All 5 patients with leaky phenotypes were also sjKREC-negative, which might be explained by the hypothesis that leaky B cells of patients with XLA are long-lived B cells that divided several times and have fewer sjKRECs than naive B cells.

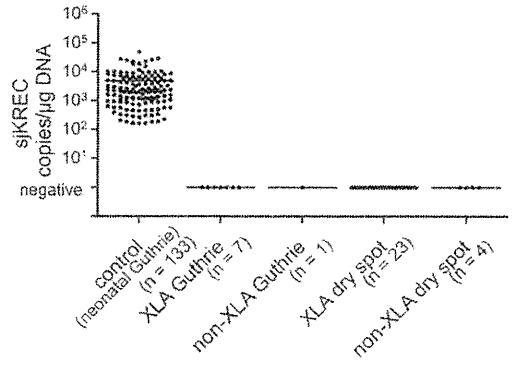

FIG 2. Copy numbers of sjKRECs measured in neonatal Guthrie cards or dried blood spots obtained from B-cell–deficient patients. On all samples from control, neonatal Guthrie cards (n = 133) were sjKREC-positive (4.8 ± 0.6 × 10^3 copies/μg DNA). B-cell–deficient patients were negative for sjKRECs in neonatal Guthrie cards (XLA, n = 7; non-XLA, n = 1) and dried blood spots (XLA, n = 23; non-XLA, n = 4).

One patient (P27) was positive for cjKRECs, but other patients were negative for it. *RPPH1* (internal control) was detectable at the same level as in normal controls in all samples.

These results indicate that sjKRECs are undetectable in XLA and non-XLA and suggest that measurement of sjKRECs in neonatal Guthrie cards has the potential for the use of newborn mass screening to identify neonates with early B-cell maturation defects. Greater numbers of neonatal Guthrie cards should be examined to confirm this potential, and the data obtained from dried blood spots on filter papers must be examined to prove that they truly reflect the data obtained from neonatal Guthrie cards. We should also examine whether screening can reduce the cost of treatment of the bacterial infections and chronic lung diseases in patients with XLA and non-XLA and increase the benefits for these patients. An anticipated pilot study using a large cohort of newborns must address these problems. We also found that T-cell–receptor excision circles and sjKRECs can be measured simultaneously on the same plate. Thus, a pilot study of neonatal screening for both T-cell and B-cell deficiencies could be performed simultaneously.

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TABLE E1. Characteristics of patients with XLA

Patient no.	Unique patient no.	Age (y)	Sex	Serum Ig (mg/dL)			$CD19^+$		*BTK* mutation			Source	
				IgG	IgA	IgM	% Lymph	/μL	Genomic DNA	cDNA	Amino acid	Guthrie	Dry spot
P1	670	0	M	87	<6	10	0.21	12.99	29269G>T	1178-1G>T	Splice acceptor defect	x	
P2	718	0	M	215	<10	<10	0.07	7.04	11593_11594 insA	144_145insA	Arg49 frameshift	x	
P3	722	0	M	80	<1	1	<1.00	NA	25644C>T	763C>T	Arg255X	x	
P4	727	8	M	295	59	57	0.11	3.52	29269G>T	1178-1G>T	Splice acceptor defect		x
P5	732	34	M	1140*	<6	8	0.02	0.24	11631T>A	182T>A	Ile61Asn		x
P6	811	24	M	458*	0	13	0.50	5.32	23570T>G	426T>G	Tyr142X		x
P7	813	18	M	628*	109	6	0.60	6.87	23570T>G	426T>G	Tyr142X		x
P8	814	19	M	260	0	NA	0.20	3.01	16180C>T	344C>T	Ser115Phe		x
P9	815	13	M	600*	<10	<5	0.08	1.72	11590G>T	142-1G>T	Splice acceptor defect		x
P10	816	11	M	12	0	5	0.00	0.00	150kb deletion of *BTK, TIMM8A, TAF7L, DRP2*				x
P11	817	10	M	10	2	24	0.80	35.78	36288C>T	1928C>T	Thr643Ile		x
P12	824	13	M	462	6	27	0.41	14.49	27518C>A	895-11C>A	Splice acceptor defect		x
P13	834	5	M	<237	<37	43	0.00	0.00	25715_26210del	776+57_839+73del	Exon 9 deletion		x
P14	838	21	M	<50	<5	7	0.00	0.00	31596G>C	1631+1G>C	Splice donor defect		x
P15	839	16	M	604*	<1	<2	0.04	0.66	31596G>C	1631+1G>C	Splice donor defect		x
P16	847	11	M	698*	26	11	0.08	1.86	25536delG	655delG	Val219 frameshift		x
P17	877	14	M	20	19	8	0.21	NA	32357T>C	1750+2T>C	Splice donor defect		x
P18	880	5	M	233	39	41	0.06	NA	10941-?_14592+?del	1-?_240+?del	Exon 1-3 deletion		x
P19	888	8	M	<212	<37	150	0.15	6.60	11023G>A	83G>A	Arg28His		x
P20	891	21	M	195	<6	37	0.02	0.09	32243C>G	1638C>G	Cys502Trp		x
P21	958	0	M	<50	<10	9	0.80	27.14	31544_31547 delGTTT	1580_1583del GTTT	Cys527 frameshift		x
P22	701	2	M	115	<2	4	0.09	1.99	16172C>A	336C>A	Tyr112X		x
P23	911	0	M	<10	<6	<4	0.00	0.00	29955A>C	1350-2A>C	Splice acceptor defect	x	
P24	937	0	M	60	<2	58	0.00	0.00	11022C>T	82C>T	Arg28Cys	x	
P25	938	0	M	<20	<4	<6	0.00	0.00	36269-?_36778+?del	1909-?_2418+?del	Exon 19 deletion	x	
P26	939	0	M	60	<2	22	0.00	0.00	11022C>T	82C>T	Arg28Cys	x	
P27	890	12	M	<237	<37	<20	0.03	NA	36261G>A	1909-8G>A	Splice acceptor defect		x
P28	944	6	M	12	<1	1	0.02	NA	36281C>T	1921C>T	Arg641Cys		x
P29	948	5	M	<237	<37	<20	0.01	0.70	36261G>A	1909-8G>A	Splice acceptor defect		x
P30	1053	5	M	386	5	113	1.10	34.22	32259A>C	1654A>C	Thr552Pro		x

Age, Age at analysis of KRECs; *$CD19^+$ % Lymph,* CD19-positive cell percentage in lymphocytes; *$CD19^+$ /μL,* CD19-positive cell number in 1 μL whole peripheral blood; *M,* male; *NA,* not available; *Serum Ig,* serum levels of immunoglobulins at diagnosis.

BTK mutation's reference sequences are NCBI NC_000023.9, NM_000061.2, and NP_000052.1.

*Trough level during intravenous immunoglobulin therapy.

TABLE E2. Characteristics of patients with non-XLA

Patient no.	Unique patient no.	Age (y)	Sex	Serum Ig (mg/dL)			CD19$^+$		*BTK* mutation	Source	
				IgG	IgA	IgM	% Lymph	/μL		Guthrie	Dry spot
P31	596	4	F	386	<6	6	0.42	21.27	Normal		x
P32	719	0	F	<50	<5	<5	0.00	0.00	Normal	x	
P33	835	8	M	311	323	20	0.09	1.88	Normal		x
P34	915	0	M	<212	<37	<20	0.00	0.00	Normal		x
P35	947	0	M	<21	<37	<39	0.00	0.00	Normal		x

Age, Age at analysis of KRECs; *CD19$^+$ % Lymph*, CD19-positive cell percentage in lymphocytes; *CD19$^+$ /μL*, CD19-positive cell number in 1 μL whole peripheral blood; *F*, female; *M*, male; *Serum Ig*, serum levels of immunoglobulins at diagnosis.

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ORIGINAL ARTICLE

Genetic analysis of contiguous X-chromosome deletion syndrome encompassing the *BTK* and *TIMM8A* genes

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Patients with X-linked agammaglobulinemia (XLA) can present with sensorineural deafness. This can result from a gross deletion that not only involved the Bruton's tyrosine kinase (*BTK*) gene, but also *TIMM8A*, mutations in which underlie the Mohr-Tranebjærg syndrome (MTS). We analyzed the genomic break points observed in three XLA–MTS patients and compared these with deletions break points from XLA patients. Patient 1 had a 63-kb deletion with break points in intron 15 of *BTK* and 4 kb upstream of *TAF7L*. Patients 2 and 3 had 149.7 and 196 kb deletions comprising *BTK*, *TIMM8A*, *TAF7L* and *DRP2*. The break points in patients 1 and 3 were located in *Alu* and endogenous retrovirus (*ERV*) repeats, whereas the break points in patient 2 did not show involvement of transposable elements. Comparison of gross deletion sizes and involvement of transposable elements in XLA and XLA–MTS patients from the literature showed preferential involvement of *Alu* elements in smaller deletions (< 10 kb). These results show further insights into the molecular mechanisms underlying gross deletions in patients with primary immunodeficiency.
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Keywords: Alu; BTK; Mohr-Tranebjærg syndrome; TIMM8A; X-linked agammaglobulinemia

INTRODUCTION

X-linked agammaglobulinemia (XLA) is an inherited primary immunodeficiency characterized by early onset of recurrent bacterial infections, profound hypogammaglobulinemia and markedly reduced circulating B cells.[1] The gene responsible for XLA was identified in 1993,[2,3] and named Bruton's tyrosine kinase (*BTK*). The *BTK* gene is mapped to the Xq21.3–Xq22 region, encompasses 37.5 kb of genomic DNA and contains 19 exons (the initiation codon is in exon 2). Only 770 bp centromerically of the *BTK* gene is the gene *TIMM8A* (formerly *DDP1*) located; it consists of two exons and produces a 97 amino acid polypeptide.[4] Mutations in *TIMM8A* cause the rare X-linked neurodegenerative Mohr-Tranebjærg syndrome (MTS), which is clinically characterized by a progressive neurological deficits, including early onset of sensorineural deafness.[5–7]

A large number of *BTK* mutations, scattered over the entire gene, have been reported and deposited in an international mutation database (http://bioinf.uta.fi/BTKbase/). The most commonly found mutations are missense (34%), followed by nonsense mutations (20%). Mutations affecting splice sites were reported in 18% and small insertions and deletions in another 18% of XLA families. Disruption of the *BTK* gene by gross deletions occurs in about 3.5% of XLA families.[1] As *BTK* and *TIMM8A* are positioned in close genomic proximity, gross gene deletions can result in disruption of both genes causing a contiguous deletion syndrome of XLA and MTS, which has been observed previously in nine families.[4,8–10]

In this study, we describe three patients with gross deletions including the *BTK* and *TIMM8A* genes. We characterized the deletions and identified the genomic break points by combining comparative genomic hybridization (CGH) array, DNA fluorescence *in situ* hybridization (FISH) and long accurate (LA)-PCR analyses. The results of these investigations provide new insight into the genetic mechanisms causing the XLA–MTS deletion syndrome and allow accurate genetic analysis of patients and carriers.

MATERIALS AND METHODS

Patients

Patient 1 is a 15-year-old Japanese boy without a family history of immunodeficiency or neurological deficits. He was diagnosed with XLA at 7 years of age when he developed recurrent bacterial infections. Deafness was noticed 1 year earlier and gradually worsened.

Patient 2 is a 10-year-old Japanese boy, who has a history of recurrent otitis media and sinusitis since 12 months of age. He had been diagnosed with deafness and autism at 18 months of age. Agammaglobulinemia and lack of circulating B cells were recognized at 8 years of age, and was diagnosed with XLA.

Both patients are doing well on immunoglobulin replacement therapy. They have no dystonia, but their hearing losses are severe and progressive.

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Patient 3 corresponds to patient 6 who was described in a previous report.[9] He was diagnosed with XLA at 8 months of age, and was found to have sensorineural hearing loss at 3 years of age. Genetic analysis resulted in the identification of a gross deletion involved the entire coding regions of the *BTK*, *TIMM8A*, *TAF7L* and *DRP2* genes.

Gene analysis of *BTK*

Informed consent for genetic analysis was obtained from the patients and their parents under a protocol approved by the Institutional Review Board of University of Toyama. *BTK* mutation analysis was performed by direct sequencing of complementary DNA and all 19 exons and exon–intron boundaries using genomic DNA as described previously.[11,12]

Fluorescence *in situ* hybridization

Peripheral blood mononuclear cells from patients and controls were stimulated in culture with phytohemagglutinin for 72 h, followed by treatment with a 0.075 M. KCl solution and fixation with Carnoy's solution (3:1 methanol and acetic acid) for metaphase preparation. A *BTK–TIMM8A*-specific genomic probe (5231 bp long) was prepared using the LA-PCR Kit (Takara, Kyoto, Japan) with primer pairs reflecting exon 19 of *BTK* and exon 2 of *TIMM8A* (5′-AGCATTCTGGCATGAATGTTCCCTGAAC-3′ and 5′-ATCTCTCCGGGT TGCAGATAATAACTG C-3′, respectively). In addition, probes were designed to detect the *TAF7L* and *DRP2* genes, which are located centromerically of TMM8A. The *TAF7L*- and *DRP2*-specific genomic probes (5662 and 5975 bp) were prepared similarly by LA-PCR Kit (Takara) with primers derived from exons 2 and 5 of *TAF7L* (5′-GCTTAGGTAGCCACCAACGTGTTGTTGA-3′ and 5′-GACGTCCCTGTTTCACAAGGAATTAGGA-3′) and from exons 7 and 12 of *DRP2* (5′-CGTGACTGTATTAAAGGGCTCAACCATG-3′ and 5′-GCAG TACTTCCTGTCACAAAGCAGTTGC-3′), respectively. For FISH analysis, denatured metaphase spreads were hybridized with probes labeled with digoxigenin-11-deoxyuridine triphosphate using a nick translation kit (Roche Diagnostics KK, Tokyo, Japan). The Spectrum Green-labeled X-chromosome probe (DXZ1, Abbott Japan, Tokyo, Japan) was used as a control. The *BTK–TIMM8A*-, *TAF7L*- and *DRP2*-specific probes were detected with anti-digoxigenin rhodamine (Roche Diagnostics KK) providing a red signal, whereas DXZ1 was detected by a green signal. Metaphases were counterstained with 4′, 6-diamino-2-phenylindole dihydrochloride, and the images of the hybridization captured by fluorescence microscopy (Carl Zeiss Co., Tokyo, Japan). At least 20 metaphases were observed in each setting.

Array CGH analysis

Array CGH analysis was performed using the Agilent kit (Agilent Technologies, Palo Alto, CA, USA) as described.[13] A total of 1 μg DNA from patients and male controls were double-digested with *Rsa*I and *Alu*I for 4 h at 37 °C. After column purification, each digested sample was labeled by random priming for 2 h using Cy3-deoxyuridine triphosphate for the patient DNA and Cy5-deoxyuridine triphosphate for the control DNA. Labeled products were purified by Microcon YM-30 filter units (Millipore, Billerica, MA, USA). After probe determination and pre-annealing with Cot-1 DNA (Invitrogen, Carlsbad, CA, USA), hybridization was performed at 65 °C with rotation for 40 h. The hybridized array was analyzed with the Agilent scanner and the Feature Extraction software (v9.5.3.1; Agilent). A graphical overview was obtained using the CGH analytics software (v3.5.14; Agilent). The UCSC Genome Browser was used to retrieve the reference genome sequence (http://www.genome.ucsc.edu).

LA-PCR and sequencing

LA-PCR reaction was performed with the appropriate forward and reverse primers that were used to map the gross deletion boundaries as described previously.[14] PCR products were excised from the gel, purified with the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA), and sequenced on ABI Prism 3130XL sequence detection system (Applied Biosystems, Foster City, CA, USA).

RESULTS

BTK mutation analysis

PCR analysis of genomic DNA resulted in the amplification of *BTK* exons 1–15, but not of exons 16–19 in patient 1, and amplifications of exons 1–5, but not of exons 6–19 in patient 2.

FISH analysis

To confirm a large deletion in the *BTK* gene and to investigate a possible deletion of the *TIMM8A* gene, we performed FISH analysis with a *BTK–TIMM8A*-specific probe. Cells from both patients 1 and 2 lacked signals of this probe (Figures 1a and b), indicating a contiguous

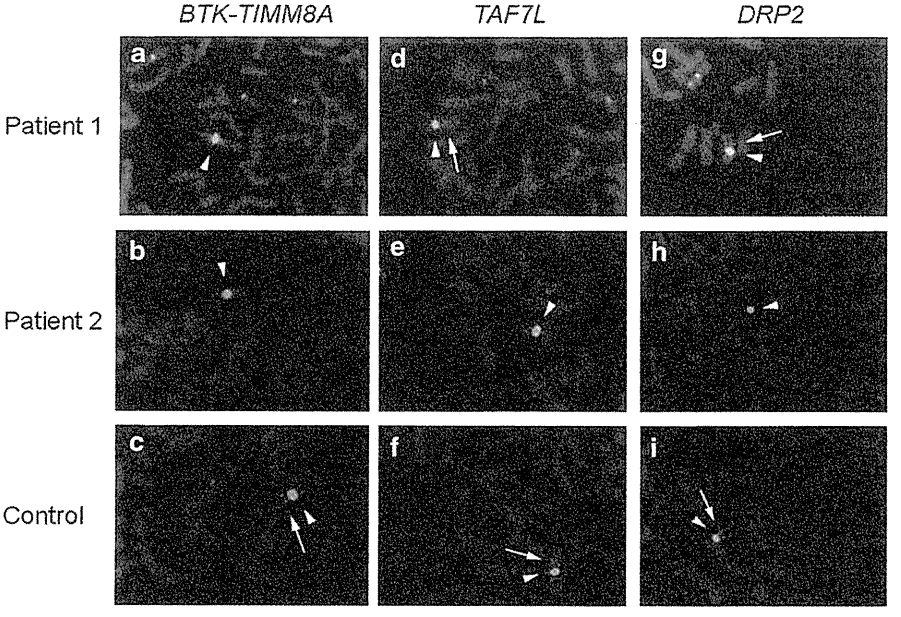

Figure 1 Identification of multigene detections by fluorescence *in situ* hybridization analysis with Bruton's tyrosine kinase (*BTK*)–*TIMM8A*- (**a–c**), *TAF7L*- (**d–f**) and *DRP2*-specific probes (**g–i**) and the X-chromosome-specific DXZ1 probe in patient 1 (**a**, **d** and **g**), patient 2 (**b**, **e** and **h**) and healthy male control (**c**, **f** and **i**). The DZX1 probe is shown in green (arrow heads), whereas the *BTK–TIMM8A*, *TAF7L* and *DRP2* probes are shown in red (arrows).

gene deletion syndrome of XLA and MTS. To study the extent of the deletion further, we performed additional FISH experiments using *TAF7L*- and *DRP2*-specific probes. Lymphocytes from patient 1 showed normal signals for both probes (Figures 1d and g), but cells from patient 2 failed to give signals for either *TAF7L* or for *DRP2* (Figures 1e and h). We conclude that the genomic deletion in patient 1 is restricted to the *BTK* and *TIMM8A* genes, whereas the deletion in patient 2 is considerably larger involving *TAF7L* and *DRP2*.

Array CGH analysis

To study the extent of the deletions in patients 1 and 2, we performed array CGH analysis of genomic DNA. In patient 1, we found an interstitial loss in copy number in the Xq22 region, involving the *BTK* and *TMM8A* genes, spanning a minimum of 63 kb (Figure 2). In patient 2, we observed a deletion of at least 138 kb including the *BTK*, *TIMM8A*, *TAF7L* and *DRP2* genes (Figure 2). These results confirmed the FISH analysis of patient 1 having a deletion of *BTK* and *TIMM8A*, and of patient 2 having a deletion involving the *BTK*, *TIMM8A*, *TAF7L* and *DRP2* genes, similar to what was found for patient 3.[9]

Analysis of the break point junctions

On the basis of FISH and array CGH results, PCR primers were designed to span the putative break points. Sequence analysis of the PCR products from patient 1 revealed that recombination had occurred between a site in intron 15 of *BTK* and a site 4.4 kb upstream of *TAL7L* (Figure 3). It is an unequal crossover, which shares a 22-bp stretch of 100% homology and resulted in the deletion of 63 kb. To determine whether the break points were located in transposable elements, we analyzed the ± 1000 bp genomic sequences flanking the break point regions against reference collection repeats.[15] The results revealed that both the distal and proximal deletion break points of patient 1 are located within short interspersed element of the *Alu* subclass (Figure 4).

In patient 2, the deletion extended from a site 4.4 kb upstream of *DRP2* to a site in intron 5 of *BTK*, encompassing 149.7 kb (Figure 3). Break point junction analysis revealed 3 bp microhomology between the two break point regions. Neither of the break points was located in DNA sequences derived from transposable elements (Figure 4).

The gross deletion in patient 3 encompassed 196 kb, and the junction showed microhomology between the 5′ and 3′ break points (Figure 3). The 5′ break point was located upstream of the *DRP2* gene in a repeat derived from an endogenous retrovirus 2 (ERV2), whereas the 3′ break point in intron 1 of *BTK* was not located in a transposable element, but close to an *Alu* element (Figure 4).

Thus, two of the three break point regions of the XLA–MTS patients we studied showed involvement of transposable elements. Although six out of eight break points of *BTK* deletions were found to be located in an *Alu* element in previous studies,[14,16,17] we observed *Alu* element involvement only in patient 1.

Transposable element involvement in XLA and XLA–MTS

It was previously shown that *BTK* gross deletion break points were frequently located in *Alu* elements. To study whether gross deletions resulting in XLA or XLA–MTS are derived from similar deletion mechanisms, we analyzed all reported gross deletions in XLA and XLA–MTS patients (Figure 5).[9,14,16,17] Including our patients, we collected data from five XLA and six XLA–MTS deletions. The deletion size clearly differed: XLA–MTS patients, as expected, had much larger disease-causing deletions. As *BTK* and *TIMM8A* are in close genomic proximity, a deletion < 10 kb can already disrupt both genes.

Interestingly, all deletions < 10 kb involved *Alu* elements, whereas only two out of six deletions > 10 kb had break points located in *Alu* elements (Figure 5). These results imply that *Alu* elements are more frequently associated with smaller deletions, whereas other elements, such as long interspersed elements and ERV elements, seem to be more frequently associated with larger deletions. As XLA–MTS deletions are on average larger than XLA deletions, they show less frequently involvement of *Alu* elements.

DISCUSSION

The contiguous gene deletion syndrome involving *BTK* and *TIMM8A* has previously been described in 12 patients from nine unrelated families with deletion sizes ranging from 20–196 kb.[8–10] Of these, only three break point junctions have been cloned.[14,16] In this study, we describe three patients with 63, 150 and 196 kb large deletions at the Xq22 region, which included *BTK* and *TIMM8A*.

Short interspersed elements of the *Alu* subclass are the most frequently occurring interspersed repeat elements in the human genome: the 280-bp sequence occurs approximately every 4 kb in the human genome.[18] Mispairing between such repeats has been shown to be frequent causes of deletions and duplications. *Alu/Alu*-mediated genomic rearrangements are classical homologous recombination that result in a loss or gain in the number of nucleotide bases,

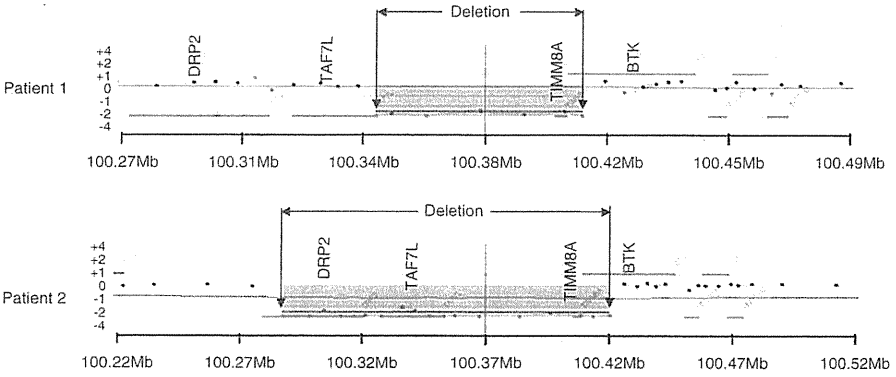

Figure 2 Array comparative genomic hybridization analysis of the X-chromosomes of patient 1 (upper panel) and patient 2 (lower panel). Grey belts indicate regions of continuously reduced copy number around Xq22. Upper panel discloses a deletion of at least 65.8 kb including the Bruton's tyrosine kinase (*BTK*) and *TIMM8A* genes in patient 1, and lower panel discloses a deletion of at least 138 kb including the *BTK*, *TIMM8A*, *TAF7L* and *DRP2* genes in patient 2. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

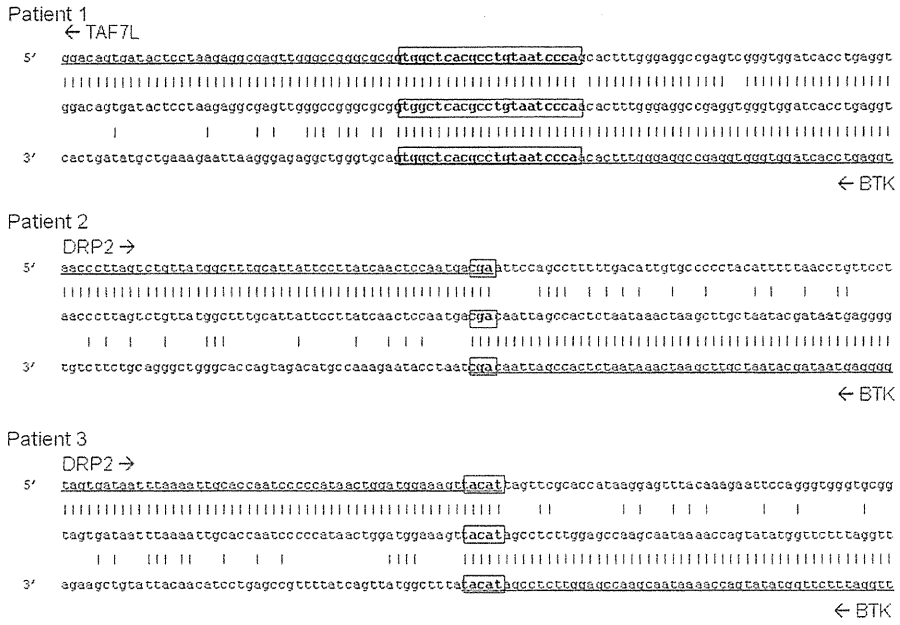

Figure 3 Sequences of the gross deletion break point junctions of three patients compared with control sequences. The upper, middle and lower panels indicate patient 1, patient 2 and patient 3. Microhomology regions at the junctions are boxed. BTK, Bruton's tyrosine kinase.

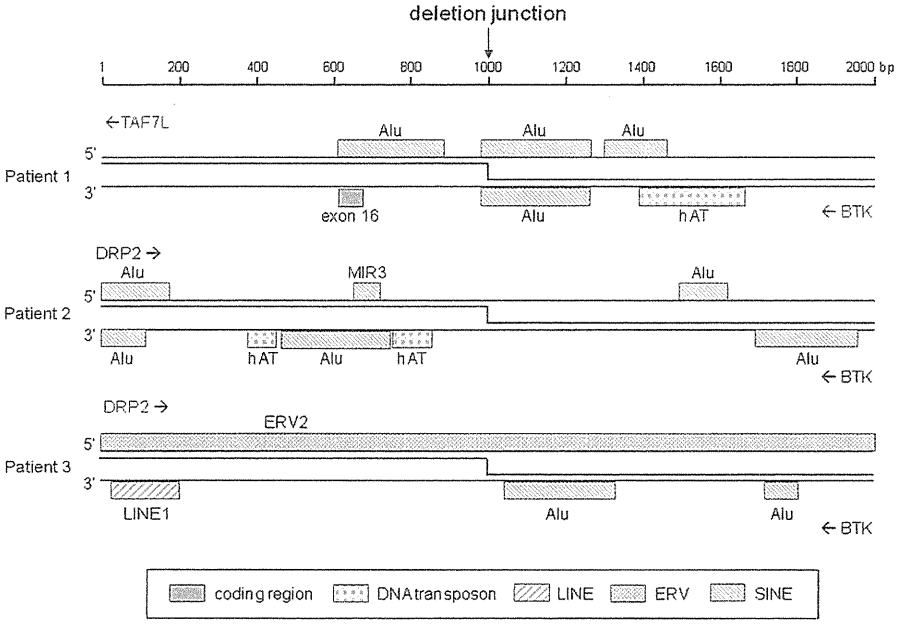

Figure 4 A ±1000 bp flanking the gross deletion break points of patient 1 (upper panel), patient 2 (middle panel) and patient 3 (lower panel). Besides coding elements, four types of transposable elements are indicated: DNA transposons, endogenous retrovirus (ERV), long interspersed element (LINE) and short interspersed element (SINE). BTK, Bruton's tyrosine kinase.

causing approximately 0.3% of human genetic diseases.[19,20] Recently, the break point junctions of eight XLA patients with a *BTK* gross deletion were analyzed for the presence of transposon-derived repetitive elements. Most of the break points were located in *Alu* elements.[14] These observations have suggested a general role for *Alu* sequences in promoting recombination in the *BTK* gene. Besides deletions, *Alu*-mediated recombination may also promote genomic duplications in *BTK*, depending on how the break points are joined.[16] The 5′ and 3′ break points in patient 1 were located in *Alu* elements, similar to most XLA patients with gross deletions affecting the *BTK* gene.[14,16,17] The break points of patients 2 and 3, however, were not located in *Alu* elements, and, intriguingly, the homology regions were very small repeat fragments of only 3 or 5 bp nucleotides, implying that the deletion was not due to an unequal homologous recombination.

A comparison of the deletion size and the presence of transposable elements resulting in gross deletions causing XLA and XLA–MTS strongly suggests that *Alu* elements are mainly involved in <10 kb

	Patient	Disease	Schematic representation of genomic deletion (DRP2, TAF7L, TIMM8A, BTK)	Deletion size (kb)	Transposable element	reference
1	ID113	XLA		2.6	Alu	14
2	0850	XLA		2.8	Alu	16
3	P4	XLA		6.1	Alu	17
4	2430	XLA-MTS		7.5	Alu	16
5	ID434	XLA		8.2	Alu	14
6	ID440	XLA		11.5	–	14
7	2433	XLA-MTS		11.9	Alu	16
8	0703	XLA-MTS		38.2	LINE1	16
9	Patient 1	XLA-MTS		63	Alu	This study
10	Patient 2	XLA-MTS		149.7	–	This study
11	Patient 3	XLA-MTS		196	ERV2	9

Figure 5 Schematic representation of all molecularly characterized gross deletions in patients with XLA and XLA–MTS. The patients are listed according to the size of the gross deletion and for each patient, the disorder (XLA or XLA–MTS) is given as well as the involvement of transposable elements. BTK, Bruton's tyrosine kinase; ERV, endogenous retrovirus; LINE, long interspersed element; MTS, Mohr-Tranebjærg syndrome; XLA, X-linked agammaglobulinemia.

deletions. As most deletions underlying XLA–MTS are >10 kb, the involvement of *Alu* elements in deletions causing XLA–MTS is less frequent than in those associated with XLA. Thus, it is likely that the majority of the deletions underlying XLA–MTS arise from mechanisms that are different from those underlying XLA.

We used a combined approach of DNA FISH, array CGH and LA-PCR to characterize the break point regions in our patients and clone the break point regions. In doing so, we were able to establish a genetic basis of the disease. This is important for patient care with respect to genetic counseling and carriership analysis in family members. Wide use of DNA FISH and array CGH analysis to establish genetic abnormalities will support higher rates of gross lesions. This will not contribute to patient care, but to general understanding of complexity and frequency of these types of genetic lesions.

In patients 2 and 3, the *TAF7L* and *DRP2* genes were deleted in addition to *BTK* and *TIMM8A*. *TAF7L* encodes an RNA polymerase II TATA-box-binding protein-associated factor II protein, which is expressed only in male spermatogonia and may have a role in pre-meiotic stages of mammalian spermatogenesi.[21] The *DRP2* gene encodes the dystrophin-related protein 2, which is a member of the dystrophin family of proteins performing a critical role in the maintenance of membrane-associated complexes at points of intercellular contact in vertebrate cells.[22] It is expressed principally in the brain and spinal cord. Disruption of the DRP2-dystroglycan complex is followed by hypermyelination and destabilization of the Schwann cell-axon unit in *Prx* (−/−) mice.[23] However, no human pathology has been reported due to any mutation in these genes. Both patients presented with recurrent infections and hearing loss as typical symptoms of contiguous gene deletion syndrome of XLA and MTS, and showed no pathological findings which might be associated with the deletion of *TAF7L* and *DRP2* genes. Therefore, these genes might not have a critical function in humans or other proteins might compensate for their loss.

In conclusion, sensorineural hearing loss in XLA should raise concerns about a possible contiguous gene deletion syndrome. Array CGH analysis combined with FISH analysis can provide a more accurate diagnosis of large deletions. Not only transposable elements and microhomology, but also other mechanisms may contribute to gross rearrangements involving the *BTK* gene.

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Letter to the Editor

Atypical case of X-linked agammaglobulinemia diagnosed at 45 years of age

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X-linked agammaglobulinemia (XLA) is a humoral inherited immunodeficiency, and mutations in *Bruton's tyrosine kinase* (*BTK*) gene have been identified to be responsible for XLA. We describe an atypical Japanese case of XLA diagnosed at 45 years of age. We think that it is the oldest case in Japan so far.

A 45-year-old man was referred to our hospital because of recurrent bacterial infections. His family history demonstrated no

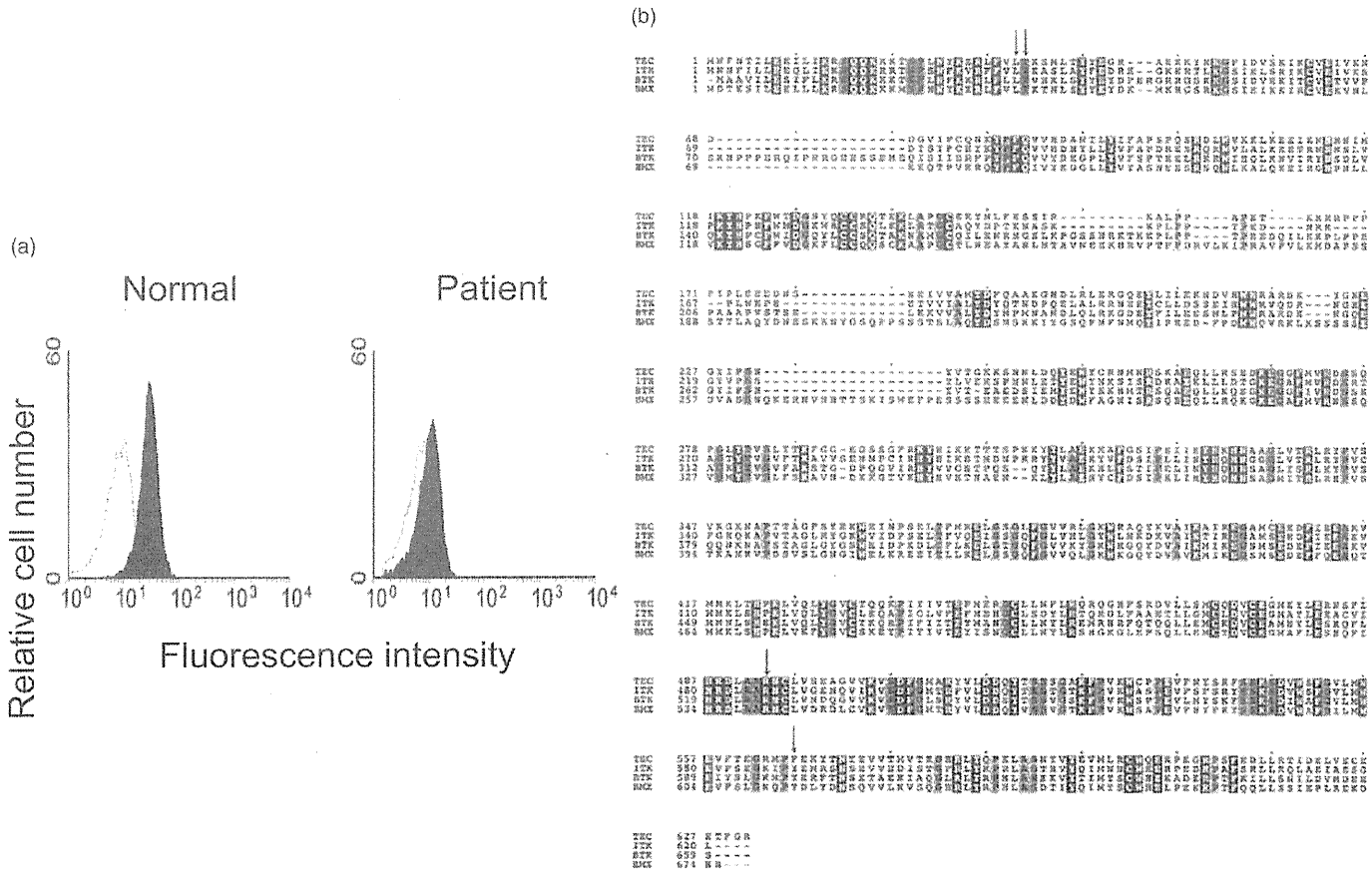

Fig. 1 (a) Flow cytometric detection of BTK in monocytes from a normal donor and the patient. The closed and dashed lines indicate staining with anti-BTK (48-2H) and control immunoglobulin (Ig) G1 monoclonal antibodies in CD14-gated monocytes, respectively. (b) The alignment of the BTK family of tyrosine kinases (human ITK, TEC, BTK and BMX). Amino acids conserved in all four proteins are shown in colored boxes. Arrows indicate L32, T33, R525 and Y598, which are mutated in the adult-onset X-linked agammaglobulinemia patient.

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Table 1 Serum Ig levels and mutations of *BTK*

Patient No.	Age at diagnosis	Serum Ig levels (mg/dL)			*BTK* mutations		
		IgG	IgA	IgM	Nucleotide change	Consequence	Severity
P1 [P65 in [3]]	24	NA	0	1	1942-1943delAG	Frameshift	S
P2 [[4]]	27	635	<5	11	605-606delAG	Frameshift	S
P3 [P40 in [3]]	27	346	16	8	1705C>T	R525X	S
P4 [[5]]	27	132	7	17	230C>T	T33I	S
P5 [P23-2 in [3]]	28	454	95	38	1924T>G	Y598D	LS
P6 [P67-2 in [3]]	31	527	8	30	2008-2040del (33 nt)	Inframe	S
P7 [P14 in [3]]	32	702	185	<25	1706G>A	R525Q	S
P8 [P2 in [3]]	32	462	<8	<7	227T>C	L32S	S
P9 [Present case]	45	679	570	17	637G>T	E169X	S

Ig, immunoglobulin; LS, less severe; NA, not applicable; S, severe.

episodes of recurrent infections. He had become febrile once a month in his childhood. He was hospitalized at 8 years of age for about 1 year to investigate, however a clear diagnosis was not determined. The frequency of fever decreased to once a year after he turned 15 years old.

At the age of 42 years, he was admitted because of fever and headache, and was diagnosed as having bacterial meningitis. *Streptococcus anginosus* was detected by blood and cerebrospinal fluid culture examination. After he was discharged he suffered from bacterial meningitis, coxitis and spondylitis. Because of recurrent serious bacterial infections, immunological studies were done at the age of 45 years. Laboratory tests were as follows: white blood cells, 4800/μL (neutrophils 62.6%, eosinophils 3.3%, basophils 0.6%, monocytes 12.4%, and lymphocytes 21.1%); hemoglobulin, 14.7 g/dL; platelets, 239×10^3/μL; serum immunoglobulin (Ig) G, IgA and IgM levels, 679 (normal range: 870–1700), 570 (110–410), and 17 (35–220) mg/dL, respectively. Serum IgE and allergen-specific IgE for cedar pollen and alternaria was detected. The percentage of T cells and B cells in peripheral blood were 85% and 1%, respectively. Flow cytometric analysis of the peripheral monocytes using the anti-BTK antibody showed BTK deficiency (Fig. 1a). The patient's *BTK* gene was sequenced and disclosed a nonsense mutation (637G>T, E169X) in exon 6, which has not been reported in the BTK database (http://bioinf.uta.fi/BTKbase/).

There have been several reports of atypical cases of XLA that were diagnosed in adulthood.[1–3] In those cases, Ig levels were moderately low and the patients did not suffer from any severe infection during their childhood. Kanegane *et al.*[1] reported that higher concentrations (>300 mg/dL) of serum IgG were evident in the cases diagnosed among adults. Genotype–phenotype correlations in XLA have been studied,[4,5] but have not been established clearly. López-Grandos *et al.*[4] classified the mutations of Spanish XLA patients according to their severity on the basis of the proposal by Conley and Howard[5] to analyze a genotype–phenotype correlation. The severe categories include: (i) amino acid substitutions at sites that are conserved in other members of the BTK family of tyrosine kinases; (ii) frameshift mutations; (iii) splice-site alterations that occur at the invariant two base pairs at the beginning and end of an intron; (iv) premature stop codons; and (v) in-frame deletions. They reported that less severe mutations or minimal detection of protein by means of flow cytometry were associated with less severity in clinical data and mild hypogammaglobulinemia, although there were some exceptions. We reviewed nine Japanese patients with adult-onset XLA, including our case (P9) (Table 1).[1–3] We classified these patients into the severe and the less severe group. The alignment of BTK families discloses that L32, T33 and R525 are highly conserved, but Y598 is not conserved (Fig. 1b). Therefore, only the patient with Y598D mutation belongs to the less severe group. In spite of severity, serum IgG levels were relatively high except P4. Those results may suggest that severity in mutations is not associated with severity in clinical data in Japanese cases. However, the number of patients was too small, so further studies are required.

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Nationwide Survey of Patients with Primary Immunodeficiency Diseases in Japan

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Abstract To determine the prevalence and clinical characteristics of patients with in Japan, we conducted a nationwide survey of primary immunodeficiency disease (PID) patients for the first time in 30 years. Questionnaires were sent to 1,224 pediatric departments and 1,670 internal medicine departments of Japanese hospitals. A total of 1,240 patients were registered. The estimated number of patients with PID was 2,900 with a prevalence of 2.3 per 100,000 people and homogenous regional distribution in Japan. The male-to-female ratio was 2.3:1 with a median age of 12.8 years. Adolescents or adults constituted 42.8% of the patients. A number of 25 (2.7%) and 78 (8.5%) patients developed malignant disorders and immune-related diseases, respectively, as complications of primary immunodeficiency disease. Close monitoring and appropriate management for these complications in addition to prevention of infectious diseases is important for improving the quality of life of PID patients.

Keywords Primary immunodeficiency disease · epidemiology · nationwide survey · Japan

Abbreviations

APECED	Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy
BTK	Bruton's tyrosine kinase
CGD	Chronic granulomatous disease
CID	Combined T and B cell immunodeficiency
CVID	Common variable immunodeficiency disease
FMF	Familial Mediterranean fever
IPEX	Immune dysregulation polyendocrinopathy enteropathy X-linked
NEMO	Nuclear factor kappa B essential modulator
PID	Primary immunodeficiency disease
SIgAD	Selective IgA deficiency
SLE	Systemic lupus erythematosus

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TRAPS	Tumor necrosis factor receptor-associated periodic syndrome
WAS	Wiskott–Aldrich syndrome
WHIM	Warts hypogammaglobulinemia, infections, and myelokathexis

Introduction

Patients with primary immunodeficiency disease (PID) show susceptibility to infections due to congenital immune system defects. These patients are also associated with noninfectious complications including autoimmune diseases and malignant disorders. Recent studies have revealed the causes of many PIDs to be mutations in various genes encoding molecules involved in the host defense mechanisms [1]. In addition, various new PIDs including defects in innate immunity and autoinflammatory disorders were identified under the recent progress in immunology and molecular genetics [2]. PID classification has been revised according to the identification of new PIDs and on the basis of new findings in PID pathophysiology. For a more precise clinical analysis, data should be obtained in accordance with the latest PID classifications.

The first nationwide survey of patients with PID in Japan was conducted between 1974 and 1979, which included 497 registered cases [3]. By 2007, a total of 1,297 patients were cataloged by a small number of PID specialists into a registration system [4]. The approximate prevalence of PID patients in Japan in the first nationwide survey was 1.0 in 100,000 people, which was much lower than that in other countries [5–7]. This difference in PID prevalence between Japan and other countries suggested that some PID patients in Japan remained unregistered. To determine the prevalence and clinical characteristics of patients with PID in Japan on the basis of the recent international classification system for PID, we conducted a nationwide survey of PID for the first time in 30 years.

Methods

This study was performed according to the nationwide epidemiological survey manual of patients with intractable diseases (2nd edition 2006, Ministry of Health, Labour, and Welfare of Japan) as described previously [8]. PID classification was based on the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee in 2007 [2]. Patients with chronic benign neutropenia and syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis were excluded because these were considered to be acquired diseases. The survey was conducted on PID patients who were alive on December 1, 2008 and those who were newly diagnosed and dead between December 1, 2007 and November 30, 2008 in Japan. Among the 2,291 pediatric departments and 8,026 internal medicine departments in Japan, hospitals participating in the survey were randomly selected after setting the selection ratio according to the number of beds (overall selection rate: 53.4% for pediatric departments, 20.8% for internal medicine departments; Table I). University hospitals and pediatric training hospitals, where many PID patients were considered to be treated, were stratified separately (Table I). Primary questionnaires regarding the number of patients and disease names based on PID classification were sent to the selected hospitals. Secondary questionnaires regarding age, gender, clinical manifestations, and complications of individual PID patients were sent to respondents who answered that they observed at least one PID patient with characteristics listed in the primary questionnaires.

Results

Questionnaires were distributed to 1,224 pediatric departments and 1,670 internal medicine departments of hospitals in Japan, and the response rate was 55.0% and 20.1%, respectively (Table I). A total of 1,240 patients (1,146 patients from pediatric departments and 94 patients from internal medicine departments) were registered (Table I). The estimated number of patients with PIDs in Japan was 2,900 (95% confidence interval: 2,300–3,500), and the prevalence was 2.3 per 100,000 inhabitants. We also determined the regional distribution on the basis of the patients' addresses. The estimated regional prevalence ranged from 1.7 to 4.0 per 100,000 inhabitants, and no significant differences were observed between different regions in Japan (Fig. 1). The most common form of PID was predominantly antibody deficiencies (40%), followed by congenital defects of phagocyte number, function, or both (19%) and other well-defined immunodeficiency syndromes (16%; Table II). Autoinflammatory disorders were observed in 108 cases (9%). The most common PID was Bruton's tyrosine kinase (BTK) deficiency (182 cases, 14.7%), followed by chronic granulomatous disease (CGD; 147 cases, 11.9%). However, common variable immunodeficiency disease (CVID) and selective IgA deficiency (SIgAD) were observed only in 136 (11.0%) and 49 cases (4.0%), respectively. Among patients registered from internal medicine departments, antibody deficiencies were the most common disorder (71%).

In the secondary survey, 923 cases were registered. The male-to-female ratio was 2.3:1 (n=914, unanswered: 9 cases) with a median age of 12.8 years (range: 0 to 75 years; n= 897, unanswered: 26 cases). The number of adolescent or

Table 1 Stratification and selection of hospitals and the survey results

	Stratification	Departments in Japan	Departments selected	Selection rate (%)	Return[a]	Response	Response rate (%)	PID Patient	Patients per department	Patients estimated
Pediatrics	University hospital	118	118	100	0	80	67.8	661	8.3	975
	Training hospital	402	402	100	4	242	60.8	376	1.6	618
	≥500 beds	92	92	100	5	48	55.2	24	0.5	44
	400–499 beds	118	118	100	3	63	54.8	42	0.7	77
	300–399 beds	287	230	80.1	4	122	54.0	31	0.3	72
	200–299 beds	289	116	40.1	4	53	47.3	6	0.1	32
	100–199 beds	486	98	20.2	0	44	44.9	4	0.1	44
	<99 beds	499	50	10.0	1	10	20.4	2	0.2	100
	Subtotal	2,291	1,224	53.4	21	662	55.0	1,146	1.7	1,961
Internal medicine	University hospital	156	156	100	1	47	30.3	37	0.8	122
	≥500 beds	374	374	100	1	86	23.1	35	0.4	152
	400–499 beds	328	263	80	1	54	20.6	6	0.1	36
	300–399 beds	692	278	40.2	6	49	18.0	10	0.2	140
	200–299 beds	1,008	202	20.0	0	36	17.8	2	0.1	56
	100–199 beds	2,460	246	10.0	1	36	14.7	1	0.0	68
	<99 beds	3,008	151	5.0	6	24	16.6	3	0.1	375
	Subtotal	8,026	1,670	20.8	16	332	20.1	94	0.3	950
Total		10,317	2,894	28.1	37	994	34.8	1,240		2,911

[a] Due to the closure of departments

adult cases (≥15 years) was 384 (42.8%; Fig. 2a). The male-to-female ratio of the younger generation (<15 years) was 2.7:1, while that of the older generation (≥15 years) was 2.0:1. Combined T and B cell immunodeficiencies (CIDs) were predominantly observed in the younger generation, while antibody deficiencies were more common with

Region	Reported PID Patients	Population ($\times 10^5$)	Estimated prevalence per 10^5 (95% C.I.)
Hokkaido	73	55.4	4.0 (0.2-7.8)
Tohoku	81	94.3	2.1 (0.9-3.3)
Kanto	387	419.8	1.8 (1.2-2.5)
Chubu	236	236.9	2.3 (1.4-3.1)
Kinki	158	208.4	2.0 (1.2-2.8)
Chugoku/Shikoku	105	116.1	1.7 (1.0-2.3)
Kyushu/Okinawa	200	146.0	2.5 (1.7-3.3)
Total	1240	1276.9	2.3 (1.8-2.7)

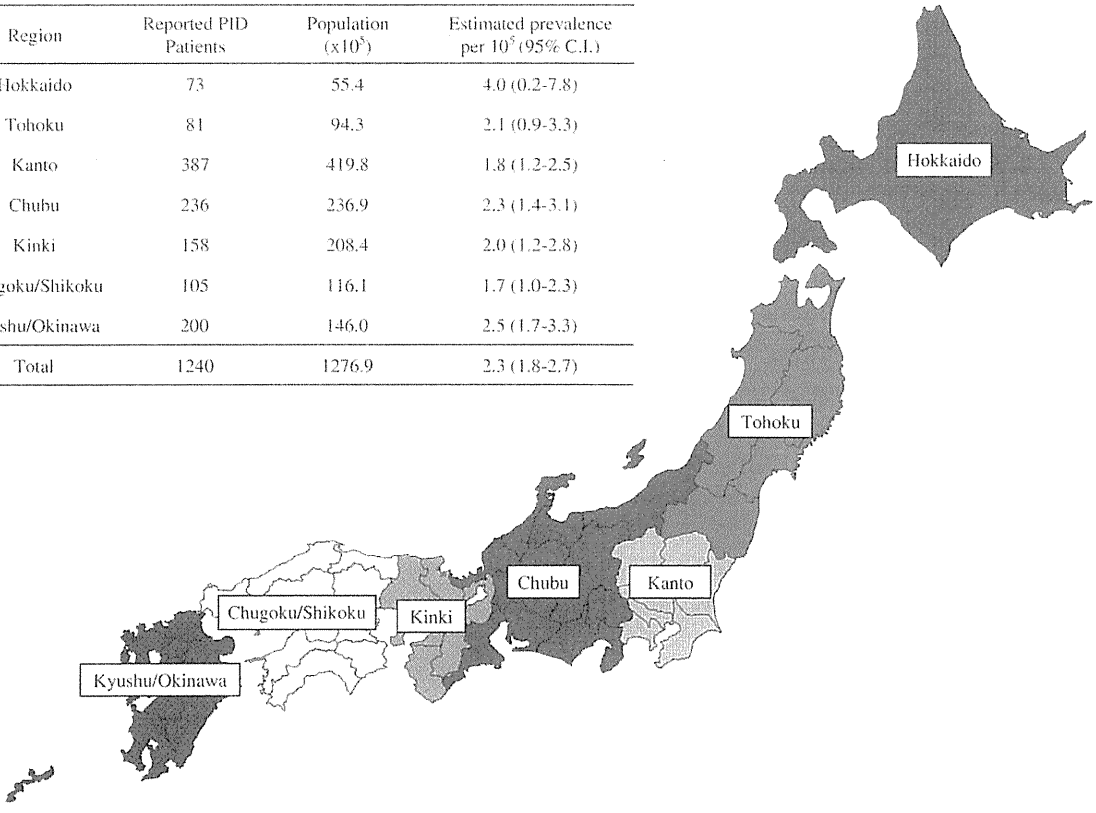

Fig. 1 Regional distribution of PID patients. *CI* Confidence interval

Table II Reported number of PID

Category	Total number	Pediatric department	Internal medicine department
I. Combined T and B cell immunodeficiencies	93 (7%)	93 (8%)	0 (0%)
γc deficiency	47	47	0
Adenosine deaminase deficiency	9	9	0
Omenn syndrome	4	4	0
Others	23	23	0
Untested or undetermined	10	10	0
II. Predominantly antibody deficiencies	501 (40%)	434 (38%)	67 (71%)
BTK deficiency	182	173	9
Common variable immunodeficiency disorders	136	107	29
Selective IgG subclass deficiency	66	58	8
Selective IgA deficiency	49	34	15
Hyper IgM syndrome	34	34	0
Transient hypogammaglobulinemia of infancy	7	7	0
Others	11	7	4
Untested or undetermined	16	14	2
III. Other well-defined immunodeficiency syndromes	194 (16%)	189 (17%)	5 (5%)
Wiskott–Aldrich syndrome	60	60	0
DNA repair defects (other than those in category I)	15	15	0
DiGeorge anomaly	38	38	0
Hyper-IgE syndrome	56	52	4
Chronic mucocutaneous candidiasis	17	16	1
Others	5	5	0
Untested or undetermined	3	3	0
IV. Diseases of immune dysregulation	49 (4%)	48 (4%)	1 (1%)
Chediak–Higashi syndrome	9	8	1
Familial hemophagocytic lymphohistiocytosis syndrome	5	5	0
X-linked lymphoproliferative syndrome	8	8	0
Autoimmune lymphoproliferative syndrome	8	8	0
APECED	4	4	0
IPEX syndrome	7	7	0
Others	2	2	0
Untested or undetermined	6	6	0
V. Congenital defects of phagocyte number, function, or both	230 (19%)	223 (19%)	7 (8%)
Severe congenital neutropenia	44	42	2
Cyclic neutropenia	19	17	2
Chronic granulomatous disease	147	144	3
Mendelian susceptibility to mycobacterial disease	5	5	0
Others	9	9	0
Untested or undetermined	6	6	0
VI. Defects in innate immunity	15 (1%)	15 (1%)	0
Anhidrotic ectodermal dysplasia with immunodeficiency	7	7	0
Interleukin-1 receptor-associated kinase 4 deficiency	2	2	0
Others	5	5	0
Untested or undetermined	1	1	0
VII. Autoinflammatory disorders	108 (9%)	101 (9%)	7 (8%)
Familial Mediterranean fever	44	40	4
TNF receptor-associated periodic syndrome	13	12	1
Hyper IgD syndrome	4	4	0
Cryopyrin-associated periodic syndrome	22	22	0