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SHORT REPORT

Novel *BTK* mutation presenting with vaccine-associated paralytic poliomyelitis

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Abstract Oral polio vaccine (OPV) has been used safely and efficiently for more than 40 years in preventive medicine. Vaccine-associated paralytic poliomyelitis (VAPP) is a rare adverse event of OPV due to reversion of the vaccine strain virus to a neurovirulent strain. VAPP can occur in healthy recipients or their close contacts. However, persons with primary humoral immunodeficiencies are at a much higher risk. X-linked agammaglobulinemia (XLA) is a prototypic humoral deficiency caused by mutations in the Bruton's tyrosine kinase (*BTK*) gene. In addition to susceptibility to bacterial infections, patients with XLA are especially prone to enteroviruses. Here, we describe the occurrence of VAPP in a 15-month old Iranian boy. The child had received four doses of OPV, administered at birth, 2, 4, and 6 months of age. The patient's infectious history was unremarkable. Laboratory evaluation revealed low levels of immunoglobulin G and $CD19^+$ B cells of less than 1% of the lymphocyte population. A novel insertion (c.685_686insTTAC) in the SH3 domain of the *BTK* gene was detected as the underlying cause. Immunodeficient recipients of OPV can excrete poliovirus vaccine strains for a long period and are at risk of developing flaccid paralysis. They could also serve as a source of reverted virulent poliovirus to be reintroduced into the general population. This patient presented for the first time with VAPP, without any history of other major infections in 15 months. This suggests that a negative history for recurrent infections does not exclude the presence of a primary defect in the immune system.

Keywords Bruton's tyrosine kinase (*BTK*) · Vaccine-associated paralytic poliomyelitis (VAPP) · X-linked agammaglobulinemia (XLA)

Abbreviations

AFP	acute flaccid paralysis
BTK	Bruton's tyrosine kinase
IPV	inactivated poliovirus vaccine
iVDPV	immunodeficiency-associated vaccine-derived polioviruses
OPV	oral polio vaccine
PBMCs	peripheral blood mononuclear cells
TLR-8	toll-like receptor 8
VAPP	vaccine-associated paralytic poliomyelitis

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VP1 viral capsid protein 1
XLA X-linked agammaglobulinemia

Oral polio vaccine (OPV) is a safe vaccine product which is rarely associated with adverse events [4]. It is known that, upon replication in the human gut, attenuated viruses may revert to a neurovirulent strain, especially in immunodeficient individuals [3, 13]. Vaccine-associated paralytic poliomyelitis (VAPP) is a rare complication occurring in OPV recipients or their close contacts [4]. The overall rate of VAPP is approximately one case per 6.2 million distributed doses of OPV [2]. We present a case of VAPP in a male recipient of OPV that was caused by a Sabin type 3–type 1 recombinant.

This patient was later found to be affected by a novel mutation of the Bruton's tyrosine kinase (*BTK*) gene.

Case report

Patient A.K., a 15-month-old boy, presented with fever and weakness of the lower limbs in active movements for 2 weeks before presentation. Prior to the presenting illness, he had had only minor upper respiratory infections. The patient had received four doses of OPV, administered at birth, and at ages 2, 4, and 6 months. Both his parents and his sister were in healthy condition, and there was no family history indicating increased susceptibility to infections.

On admission, the right leg was completely flaccid and the left was paretic. Deep tendon reflexes were 1+ in the upper extremities but unobtainable in the lower extremities. Plantar reflexes were absent. Sensory testing revealed hyperesthesia in the lower extremities. Bladder dysfunction was noted as recurrent episodes of urinary retention. Four days after admission, he experienced sudden cardiac arrest and was subjected to resuscitation.

Cerebrospinal fluid analysis showed 216 lymphocytes/μl and a protein level of 45 mg/dl.

The fecal specimens were processed at the Iranian National Polio Laboratory (NPL), School of Public Health and Institute for Public Health Research, University of Tehran, Iran. Recombinant Sabine type 3–type 1 viruses were isolated. The isolates showed a VP1 nucleotide variation of 2% from the Sabin type 3 vaccine strain. His close contacts were not shown to excrete poliovirus, as examined on several occasions.

The baseline immunologic workup was remarkable for hypogammaglobulinemia and diminished $CD19^+$ B lymphocytes (Table 1).

He was commenced on intravenous immunoglobulin (500 mg/kg every 4 weeks) and physical therapy. Follow up fecal cultures showed disappearance of the virus from 20 months of age. Currently, at 24 months, he is completely bedridden and breathes through a tracheotomy tube.

Table 1 Laboratory data

Test	Patient	Normal
WBC (cells/μl)	6,500	6,400–12,000
Hb (mg/dl)	12.8	10–14
Platelets (cells/μl)	538,000	>150,000
ALC (cells/μl)	3,375	3,400–9,000
CD3 (cells/μl)	2,700	1,900–5,900
CD4 (cells/μl)	1,404	1,400–4,300
CD8 (cells/μl)	1,290	500–1,700
CD19 (cells/μl)	35	610–2,600
CD16 (cells/μl)	640	150–950
IgG (mg/dl)	556	650–1,410
IgA (mg/dl)	<10	83–255
IgM (mg/dl)	<10	55–210
DTH to PPD antigen	10 mm	

Sequence analysis of the *BTK* gene

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ) centrifugation. Total RNA was isolated from PBMCs with TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Reverse transcription of mRNA followed by polymerase chain reaction (RT-PCR) was performed as previously reported [6]. Human *BTK* cDNA sequence in the Genbank database (accession no. NM 000061) was chosen as the reference. The following oligonucleotide primers were used: Btk exons 1 to 13 (sense: CAG TGT CTG CTG CGA TCG AG; antisense: CAG TGG AAG GTG CAT TCT TG) and Btk exons 11 to 19 (sense: TCA TTG TCA GAG ACT CCA GC; antisense: TTG CTC AGA AGC CAC TAT CC).

Genomic DNA was obtained from whole blood by the conventional salting out method. The mutation identified from cDNA was confirmed by sequence analysis of the genomic DNA for exon 8 using primers GGG AGA GAA GAG AAG AGT GC (sense), and AGG AAG GGC TGG TGT GGA C (antisense). PCR products were supplied for direct sequencing with an automated ABI PRISM 310 Gene Analyzer (PE Applied Biosystems, USA).

Result

The sequence analysis of *BTK* cDNA showed a frame shift insertion (c.685_686insTTAC) in exon 8, leading to a

premature stop codon in the SH3 domain. The analysis of genomic DNA confirmed the mutation corresponding to the SH3 domain. Neither of the two internet databases that update the mutations of *BTK*, BTKbase (http://bioinf.uta.fi/BTKbase/) and HGMD (Human Gene Mutation Database; http://www.hgmd.cf.ac.uk), has yet documented a similar altered *BTK* sequence. The mutation analysis revealed the carrier status of the mother; however, the daughter of the family was not affected (Fig. 1).

Discussion

X-linked agammaglobulinemia (XLA) is a primary immunodeficiency caused by mutations in the Bruton's tyrosine kinase (*BTK*) gene [9, 10]. Affected patients have a selective defect in antibody production and usually present after 6 months of birth with recurrent bacterial infections. Cellular immunity is normal and, consequently, they recover normally from most viral infections. Enteroviruses require specific antibodies to be eliminated from an established infection, as shown by the unequivocal predisposition of patients with XLA to polio and other enteroviral infections. Moreover, the impaired Toll-like receptor 8 (TLR-8)-mediated recognition of viral ssRNA by *BTK*-deficient dendritic cells has been anticipated to be responsible for the susceptibility of XLA patients to enteroviral infections [8].

Although OPV is free of adverse effects in immunocompetent children, reverting base substitutions occurs during virus replication in the human intestine. In rare circumstances, these revertants cause VAPP [4]. In 95% of cases, VAPP occurs before the first year of age and usually after the first dose of vaccine [11].

The virulent strain isolated from our patient revealed 2% drift from the original Sabin type 3 vaccine strain. As the poliovirus accumulates mutations at a rate of 1% per year [1], it is most likely that our case acquired the vaccine strain virus at his first dose of OPV at birth. He might be protected by the transplacental transfer of maternal antibody [12] during the first few months of life. An IgG level of 556 mg/dL at 15 months of age and an absence of early recurrent infections in his medical history substantiates this notion.

Vaccination with four doses of OPV is a routine practice in Iran. The last case of indigenous wild poliovirus was identified in 1997; however, Iran shares borders to two endemic countries (Pakistan and Afghanistan) and the risk of imported wild polio cases is high.

The Iranian office of national OPV immunization and acute flaccid paralysis (AFP) surveillance were established in 1994. Covering 100% of infants younger than 1 year of age by OPV, viral-induced poliomyelitis is close to eradication in Iran. Reports of VAPP cases in immunodeficient patients bring about concern regarding the endgame strategies of polio eradication in Iran [7]. As OPV is administered at birth when most primary immunodeficien-

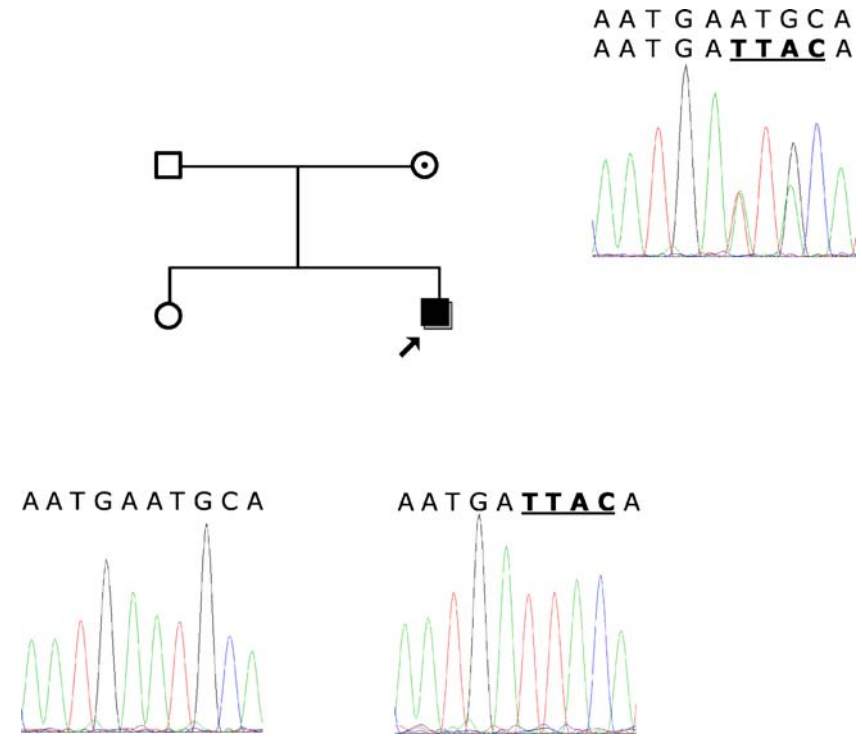

Fig. 1 *BTK* sequence of the patient showing TTAC insertion at c.685. The carrier status of his mother and the normal sequence of his sister are also depicted

cies are hardly identifiable, almost all immunodeficient subjects have indeed been vaccinated with OPV.

Immunodeficient recipients of OPV are potentially predisposed to become long-term excretors of poliovirus vaccine strains (immunodeficiency-associated vaccine-derived polioviruses [iVDPV]), with the possible risk of both developing paralysis and reintroducing reverted virulent poliovirus into the population after polio vaccination is stopped [5].

Switching to an all-inactivated poliovirus vaccine (IPV) strategy or sequential schedules of IPV/OPV will reduce the risk of VAPP in the future.

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