# 学位論文内容の要旨
## （Summary of Dissertation）

博士の専攻分野の名称 博士（医　学）　　氏 名　シャイマ セイド モハメド アリ アブドラブ
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学 位 論 文 題 名（Dissertation Title）

Diagnosis and Characterization of Endoscopic Findings in XIAP Deficiency
(XIAP 欠損症の診断と内視鏡所見の特徴に関する研究)

**Background and Objectives:** Inflammatory bowel disease (IBD) is a diverse group of disorders with multifactorial etiology. Recent studies have reported increasing numbers of monogenic IBD caused by mutations in various genes including XIAP, IL10R, FOXP3, CTLA4, LRBA, CYBB, WASP, IKBKG, especially in very early-onset IBD (VEO-IBD) and refractory IBD. For the diagnosis of monogenic IBD, whole exome sequence analysis (WES) and panel sequence analysis of various genes responsible for IBD are widely available. However, the proportion of monogenic IBD in VEO-IBD varies between centers and cohorts and ranges from 5-31%. Furthermore, deep intronic mutations and mutations only affecting untranslated regions (UTR) are difficult to identify with DNA sequencing analysis, which could be associated with undiagnosed monogenic IBD. X-linked inhibitor of apoptosis protein (XIAP) deficiency is one of inborn errors of immunity characterized by recurrent hemophagocytic lymphohistiocytosis (HLH) and IBD. This disorder is especially one of the monogenic causes of refractory IBD mimicking Crohn's disease (CD). Hematopoietic stem cell transplantation (HSCT) is the only curative therapy of XIAP deficiency and is especially required in patients complicated with refractory IBD or HLH. Although variants in XIAP were demonstrated in about 4% of male patients with pediatric-onset CD, there could be still underdiagnosed patients. IBD phenotype in XIAP deficiency is hypothesized to be caused by impaired NOD2 signaling resulting in granulomatous colitis and perianal disease mimicking Crohn's disease. However, specific clinical characteristics of IBD in XIAP deficiency, including endoscopic findings are poorly understood. Therefore, we tried to establish simple and accurate methods for the diagnosis of XIAP deficiency based on genetic and protein expression studies and to investigate endoscopic findings shared by patients with this disease.

**Materials and Methods:** Four unrelated Japanese male patients with IBD and histories of HLH were studied for the diagnosis of XIAP deficiency. XIAP expression was studied in isolated peripheral blood mononuclear cells (PBMC) and in phytohemagglutinin (PHA)-stimulated T cell blasts from patients and controls as follows: fresh peripheral heparinized blood samples were drawn from the patients and controls, PBMC were isolated and PHA-stimulated T cell blasts were obtained. Then, SDS-PAGE, and Western blot analysis of XIAP expression were performed with cytoplasmic extract. Anti-XIAP antibody and anti-actin antibody were used as primary antibodies. The rest of PBMC was used for RNA isolation, cDNA synthesis, and subsequent TOPO-TA cloning. DNA was extracted from the patients and controls' granulocytes. PCR and RT-PCR primers sets were designed to cover full-length XIAP gene coding, non-coding exons and to determine the deletion breakpoints in 5'UTR of Patient 4. Parallelly, we retrospectively evaluated endoscopic findings of four patients with XIAP deficiency and those of 127 pediatric-onset conventional CD patients.
**Results:** These four patients were diagnosed with XIAP deficiency based on the absent XIAP expression in cultured T-cell blasts. Sequence analysis of the responsible gene, XIAP, demonstrated two distinct novel nonsense mutations of p.Gln114X and p.Glu25X, and a previously reported nonsense mutation of p.Arg381X. Although no mutations in the coding region were detected in the fourth patient, further studies demonstrated a novel 2199 bp deletion encompassing non-coding exon 1, presumably affecting transcription/stability but also translation of XIAP mRNA possibly due to the lack of internal ribosomal entry sites sequences. Patient 4's mother was shown to be an

asymptomatic heterozygous carrier of the deletion while his brother did not have this deletion. These four patients shared endoscopic findings of wide and longitudinal ulcers with straight and non-raised edge with "scooped-out appearance" in the colon, which was not observed in other 127 pediatric-onset conventional CD patients, indicating this finding could be specific to XIAP deficiency. All of the patients eventually underwent hematopoietic stem cell transplantation, leading to complete or partial remission of IBD.

**Discussion:** One of the concerns to identify genetic causes accurately is whether some disease-causing mutations located deep within introns or UTR were overlooked in some cases, since detection and validation of these mutations are challenging. The deletion mutation encompassing non-coding exon 1 (5'UTR) of XIAP observed in Patient 4 is one of the cases unidentified by panel sequence analysis or WES focusing only on coding exons and exon-intron boundaries. In Patient 4, absent XIAP expression in Western blot analysis of T-cell blasts led to the diagnosis of XIAP deficiency. Therefore, this analysis should be considered as one of the first screening methods for the diagnosis of this disease especially in shipped patients' samples with increased apoptotic conditions and in limited samples from neonates or infants, since T-cell blasts can be easily obtained from small amount regardless of the conditions of blood and patients.

To the best of our knowledge, there have been no reports of a deletion mutation affecting only non-coding exon 1 (5'UTR) of XIAP, while gross deletion mutations encompassing coding exons of 1-2, 1-4, 1-6, and 4-6 have been reported.
Electrophoresis of RT-PCR products in this patient showed a faint but detectable band in contrast with complete absence of XIAP expression at protein levels. Our additional studies with a French group indicated an active promoter region in 5'UTR determined by gene reporter expression assays in HEK 293 cell lines (under minor revision). And about translation, there are some previous findings that XIAP mRNA is controlled at the translational level, specifically through an internal ribosome-entry site (IRES) residing at 5'UTR. Therefore, complete absence of XIAP expression at protein levels in Patient 4 could be attributable to abrogated translation in addition to impaired transcription or stability of XIAP mRNA due to the deletion of 5'UTR.

One of the challenges is to differentiate XIAP deficiency from conventional CD, which would facilitate early HSCT to avoid life-threatening events like HLH and the occurrence of refractory IBD. Therefore, we retrospectively evaluated endoscopic findings of the four patients with XIAP deficiency and those of 127 patients with pediatric-onset conventional CD. And we detected an endoscopic finding exclusively observed in XIAP deficiency: multiple wide and longitudinal ulcers with straight and non-raised edge. We named "scooped-out appearance", since the ulcer bed looked like a track scooped out by a spoon. Although multiple longitudinal ulcers are also observed in conventional CD, their ulcers are mostly narrow and sharp, and their ulcer edges were irregular shaped and slightly raised. This endoscopic finding has not been reported from elsewhere, but needs to be reproduced with more patients. All patients underwent allogeneic HSCT, which led to a complete remission of IBD in P1, P2, and P3 while P4 showed partial remission of IBD due to mixed chimeric state.

**Conclusion:** Four patients were diagnosed with XIAP deficiency who had presented for 3-10 years with refractory IBD mimicking CD. We report three distinct novel mutations in XIAP gene including one in XIAP 5'UTR. Collectively, panel sequence analysis of sequence analysis including XIAP and XIAP expression in T-cell blasts should be performed for the early and accurate diagnosis of XIAP deficiency especially in patients with refractory IBD with HLH, and splenomegaly. Endoscopic findings of multiple wide and longitudinal ulcers with scooped-out appearance could also a clue to the diagnosis of XIAP deficiency. Further studies including more patients with XIAP deficiency are necessary to determine whether this endoscopic finding is specific to XIAP deficiency.