The Journal of Veterinary Medical Science

NOTE

Internal Medicine

Investigation of the mutations in the genes involved in Janus kinase/signal transducer and activator of transcription pathway in canine large cell gastrointestinal lymphoma

Takumi TSURUTA[1)#], Naoki MATSUMURA[1)#], Keijiro MIZUKAMI[2)], Yuko GOTO-KOSHINO[3)], Tomomi AOI[2)], Ryoko YAMADA[2)], Itsuma NAGAO[1)], Megumi SAKAMOTO[3)], Taisuke NAKAGAWA[3)], Ray FUKUOKA[3)], Aki OHMI[3)], James K CHAMBERS[4)], Kazuyuki UCHIDA[4)], Yukihide MOMOZAWA[2)], Hirotaka TOMIYASU[1)*]

[1)]Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[2)]Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan
[3)]Veterinary Medical Center, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[4)]Laboratory of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

J Vet Med Sci
86(10): 1052–1055, 2024
doi: 10.1292/jvms.24-0096

Received: 5 March 2024
Accepted: 3 August 2024
Advanced Epub:
13 August 2024

ABSTRACT. Canine gastrointestinal lymphoma is known to be of T-cell origin in most cases, but the molecular biological aberrations have not been clarified. In human intestinal T-cell lymphoma, the mutations in the genes associated with Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway have been frequently observed. In this study, the gene mutations were investigated in 31 dogs with large cell gastrointestinal lymphoma (LCGIL) by focusing on the genes involved in JAK-STAT pathway. Next-generation sequencing analysis to examine the mutations in *STAT3*, *STAT5B*, and *JAK1* genes throughout the exon regions revealed the mutations in *STAT3* gene in two dogs and *JAK1* gene in one dog. In conclusion, this study could not indicate the associations of gene mutations in JAK-STAT pathway with LCGIL in most canine cases.

KEYWORDS: alimentary, *JAK1*, *STAT3*, *STAT5B*, T-cell lymphoma

Gastrointestinal lymphoma is the most common extranodal lymphoma in dogs, accounting for 5–7% of all lymphomas [5]. Canine gastrointestinal lymphoma is commonly of T-cell origin and generally affects small intestine [6]. Dogs with gastrointestinal lymphoma exhibit digestive symptoms such as weight loss, diarrhea, vomiting, and anorexia. Canine gastrointestinal lymphoma is further classified into large cell and small cell types based on the size of tumor cells, and cases with large cell gastrointestinal lymphoma (LCGIL) are usually treated with multi-drug combination chemotherapy including CHOP regimen or lomustine, resulting in a short median survival of 62–72 days [16, 17, 21].

In humans, enteropathy-associated T-cell lymphoma (EATL) and monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) are known as the common types of intestinal T-cell lymphoma, and refractory celiac disease type II (RCD2) is regarded as a precursor lesion to EATL [3, 23]. In human T-cell lymphoma, characteristic gene mutations associated with Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway have been identified; the somatic gain-of-function mutations in the *JAK1* and *STAT3* genes, which are suggested to be the main drivers in the malignant transformation, are observed in 90% of EATL patients and 80% of RCD2 patients [4, 8]. Also, the studies on MEITL have reported the frequent gain-of-function mutations in *JAK3* and *STAT5B* genes [18, 19].

Recently, $STAT5B^{N642H}$ mutation, the most frequent *STAT5B* mutation in human MEITL, was also found in 7 out of 42 (17%)

*Correspondence to: Tomiyasu H: atomi@g.ecc.u-tokyo.ac.jp, Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
#These authors contributed equally to this work.
(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

cases of feline gastrointestinal T-cell lymphomas, and activation of *STAT5B* was confirmed by immunohistochemical staining [9, 12]. Several studies have comprehensively searched for gene mutations also in canine lymphoma [7, 10], but no study investigated the gene mutations focusing on canine LCGIL.

Based on these backgrounds, we hypothesized that gene mutations associated with JAK-STAT pathway might be also associated with the pathogenesis of canine LCGIL. In this study, the mutations in the genes involved in JAK-STAT pathway were investigated in canine LCGIL cases using next-generation sequencing (NGS) analysis.

This study included 31 dogs referred to Veterinary Medical Center of the University of Tokyo and diagnosed with LCGIL. The diagnosis of LCGIL was made based on histopathological examinations using endoscopically or surgically obtained gastrointestinal lesions or cytological examinations using samples obtained by fine-needle aspiration for gastrointestinal lesions. PCR for antigen receptor gene rearrangements (PARR) was conducted using a part of DNA extracted from the tumor specimens as previously described to evaluate the clonal rearrangements of the *TCRγ* gene and the *immunoglobulin heavy chain* (*IgH*) gene in all cases [11]. gDNAs derived from peripheral blood were also available in 17 of the 31 cases and analyzed as normal controls for each case. The signalment and other information of each case are shown in Supplementary Table 1.

In human medicine, the mutation in *STAT5B* gene (c.1924A >C, p.N642H) was observed in 43% of human MEITL cases, and two mutations in *STAT3* gene (c.1919A >T, p.Y640F and c.1981G >T, p.D661Y) were found in 7% of human EATL/MEITL cases. [12, 14, 18–20]. $STAT5B^{N642H}$ mutation was also found in 7 out of 42 (17%) cases of feline gastrointestinal T-cell lymphomas, and activation of *STAT5B* was confirmed by immunohistochemical staining [9, 12]. Therefore, we initially investigated these three mutations in the tumor samples of all 31 dogs as mutational hotspots. Primer sequences used for the targeted NGS to investigate these mutations are shown in Supplementary Table 2. The sequencing libraries for targeted NGS were prepared with gDNAs from each sample by two-step multiplex PCR; 1st PCR to amplify the target regions using Platinum™ Multiplex PCR Master Mix (Applied Biosystem, Carlsbad, CA, USA) and 2nd PCR to add index sequences, which were used to identify individuals and sequences that were used to bind the flow cell using KOD One PCR Master Mix (TOYOBO, Osaka, Japan). The library was purified using AMPure XP beads (Beckman Coulter, Blair, CA, USA). After assessing the quantity and quality using Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and KAPA Library Quantification Kit (Nippon Genetics, Tokyo, Japan), the library was sequenced on the MiSeq instrument (Illumina, San Diego, CA, USA). FASTQ files were trimmed with Cutadapt (v2.10). The processed reads were mapped to CanFam 3.1 using BWA (v0.7.17) and sorted using Samtools (v1.10). Variants calling in each sample were performed using Mutect2 tool in GATK (v4.2.6.1). The variants were extracted as mutations if the variant allele frequencies were more than 0.1.

Targeted NGS analysis was also performed using the primer pairs to cover the whole exon regions of *STAT3*, *STAT5B*, and *JAK1* genes as described above, except for that ROS_Cfam_1.0 was used as the reference genome. The mutations in *STAT3* gene could be investigated in all 31 cases, but those in *JAK1* and *STAT5B* could not in six dogs (Dog18, 19, 28–31) due to insufficient amounts of gDNAs. Primer sequences used for targeted NGS are shown in Supplementary Table 3. The variants were extracted as mutations according to the following criteria: 1) variants with the total read depth of more than 100 and variant allele frequency of more than 0.1 in the tumor sample where the variants were identified, 2) variants with total read depth of more than 100 and variant allele frequency of less than 0.1 in all peripheral blood samples examined. We also predicted the potential effect of the mutations on protein function using PolyPhen-2 [1].

In the examinations of two mutational hotspots in *STAT3* (c.1919A >T, p.Y640F and c.1981G >T, p.D661Y) and one in *STAT5B* (c.1924A >C, p.N642H) genes, a mutation in *STAT3* (c.1919A >T, p.Y640F) was identified in Dog 2 with variant allele frequency 0.122, while those in the other samples including the peripheral blood DNA from this dog were less than 0.01. A mutation in *STAT3* (c.1981G >T, p.D661Y) and *STAT5B* (c.1924A >C, p.N642H) were not found in any dogs.

Since the *STAT3* and *STAT5B* mutations in hot spots that are known in human and feline gastrointestinal lymphoma were not found in most canine LCGIL cases, we decided to investigate the mutations in *STAT3* and *STAT5B* genes throughout the exon regions of these genes. In addition, the mutations in *JAK1*, which is the upstream regulator of STAT3, were also investigated throughout the exon regions, because the common mutation in *STAT3* gene was found in a canine LCGIL case. After extraction based on the criteria as described above, point mutations in *STAT3* gene were found in two dogs; a missense mutation (c.1222T >C) in Dog 17 and a missense mutation (c.1847A >G) in Dog 13 (Table 1). A missense mutation (c.3125G >T) was also detected in *JAK1* gene in Dog 18 (Table 1). The analysis with polyphen-2 predicted probably damaging change for two missense mutations in *STAT3* (c.1222T >C and c.1847A >G) with a score of 0.926 and 0.920, respectively, and possibly damaging change for a missense mutation in *JAK1* (c.3125G >T) with a score of 0.832. However, no mutation in *STAT5B* gene was found in any cases. Although the criteria described above was not met, a mutation spot in *STAT3* in Dog 2 (c.1919A >T) was identified with variant allele frequency 0.098, and that in *JAK1* in Dog 3 (3073A >G) was identified with variant allele frequency 0.095, while those in the other samples including the peripheral blood DNA from this dog were less than 0.02 (Table 2). The analysis with polyphen-2 predicted possibly damaging change for a missense mutation in *STAT3* (c.1919A >T) with a score of 0.816, and probably damaging change for a missense mutation in *JAK1* (c.3073A >G) with a score of 0.999.

A mutation in mutational hot spot which is known in *STAT3* gene was found in only one dog, and targeted NGS for *STAT3* and *JAK1* genes throughout the exon regions revealed other mutations, including the possible ones, in three and two dogs, respectively. Furthermore, analysis with polyphen-2 predicted probably or possibly damaging change for these mutations. These results indicated that aberrations in JAK1-STAT3 pathway might be associated with the pathogenesis of LCGIL in these cases but not in many other cases.

JAK–STAT pathway mainly regulates cell division, apoptosis, chromatin regulatory processes or cell fate decisions, and it involves many of the genes that define cancer pathways [22, 24]. There are several members of the JAK family and the TYK protein that are

Table 1. The gene mutations identified by targeted next-generation sequencing analysis throughout the exon regions of *STAT3* and *JAK1* genes

Gene	Chromosome	Mutation Position (ROS_Cfam_1.0)	Mutation Position (CamFam3.1)	Reference sequence	Altered sequence	Annotation	Variant allele frequency	Dogs that harbored the mutations
STAT3	9	21321114	20601542	T	C	Missense	0.111	Dog 17
	9	21327310	20607589	A	G	Missense	0.173	Dog 13
JAK1	5	45587580	45404151	G	T	Missense	0.11	Dog 18

Table 2. The variants whose variant allele frequencies were near 0.1 in targeted next-generation sequencing analysis throughout the exon regions of *STAT3* and *JAK1* genes

Gene	Chromosome	Mutation Position (ROS_Cfam_1.0)	Mutation Position (CamFam3.1)	Reference sequence	Altered sequence	Annotation	Variant allele frequency	Dogs that harbored the mutations
STAT3	9	21327882	20608161	A	T	Missense	0.098	Dog 2
JAK1	5	45587528	45404099	A	G	Missense	0.095	Dog 3

involved in the JAK/STAT pathway [2]. *STAT3* facilitates tumor progression in various human cancers, particularly leukemia and lymphomas, and is mainly activated by *JAK1* [2, 24]. Actually, the mutations in *JAK1* and/or *STAT3* have been observed in human EATL [4, 8], but similar results were not observed in the present study.

In feline T-cell gastrointestinal lymphoma, $STAT5B^{N642H}$ mutation was found in 7 of 42 (17%) cases [12]. On the other hand, the mutations in *STAT5B* gene were not found in any canine LCGIL cases in the present study. It should be noted that most feline cases with the mutation were diagnosed as EATL type II, which corresponds to small cell gastrointestinal lymphoma, in the previous study. In fact, they have different clinical aggressiveness; median survival time of canine LCGIL cases is 62–72 days, but feline small cell gastrointestinal lymphoma cases have better prognosis with median survival of 513–704 days [13, 15–17, 21]. The results of the present study suggested that the molecular pathogenesis could be also different between canine LCGIL and feline small cell gastrointestinal lymphoma.

The limitation of the present study include that we could not confirm the immunophenotype of tumor cells by immunohistochemistry, although the results of PARR suggested that the tumor cells of most cases were derived from T-cell as previously reported [6]. The lack of histopathological evaluation is also limitation in the present study. Furthermore, mucosal samples collected by endoscopy should contain non-neoplastic cells such as epithelial cells and immune cells, and these contaminations might result in low sensitivity to detect the mutations in the samples. Future studies that use tumor cells isolated from the tumor samples are needed to investigate the mutations with higher sensitivity.

In conclusion, the gene mutations in *JAK1*, *STAT3*, and *STAT5B* were not found in most canine gastrointestinal lymphoma cases. Future studies to investigate the comprehensive gene mutations may be helpful for the better understanding of the molecular pathogenesis of canine LCGIL.

CONFLICTS OF INTEREST. The authors declare no conflict of interest.

ACKNOWLEDGMENTS. This study was supported by the Japan Society for the Promotion of Science, KAKENHI [grant number 22K15007]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We wish to thank the members of Laboratory of Veterinary Internal Medicine, the University of Tokyo and Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences for advice.

REFERENCES

1. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. 2010. A method and server for predicting damaging missense mutations. *Nat Methods* **7**: 248–249. [Medline] [CrossRef]
2. Agashe RP, Lippman SM, Kurzrock R. 2022. JAK: not just another kinase. *Mol Cancer Ther* **21**: 1757–1764. [Medline] [CrossRef]
3. Chander U, Leeman-Neill RJ, Bhagat G. 2018. Pathogenesis of enteropathy-associated T cell lymphoma. *Curr Hematol Malig Rep* **13**: 308–317. [Medline] [CrossRef]
4. Cording S, Lhermitte L, Malamut G, Berrabah S, Trinquand A, Guegan N, Villarese P, Kaltenbach S, Meresse B, Khater S, Dussiot M, Bras M, Cheminant M, Tesson B, Bole-Feysot C, Bruneau J, Molina TJ, Sibon D, Macintyre E, Hermine O, Cellier C, Asnafi V, Cerf-Bensussan N. CELAC network. 2022. Oncogenetic landscape of lymphomagenesis in coeliac disease. *Gut* **71**: 497–508. [Medline] [CrossRef]
5. Couto CG, Rutgers HC, Sherding RG, Rojko J. 1989. Gastrointestinal lymphoma in 20 dogs. A retrospective study. *J Vet Intern Med* **3**: 73–78. [Medline] [CrossRef]
6. Coyle KA, Steinberg H. 2004. Characterization of lymphocytes in canine gastrointestinal lymphoma. *Vet Pathol* **41**: 141–146. [Medline] [CrossRef]

7. Elvers I, Turner-Maier J, Swofford R, Koltookian M, Johnson J, Stewart C, Zhang CZ, Schumacher SE, Beroukhim R, Rosenberg M, Thomas R, Mauceli E, Getz G, Palma FD, Modiano JF, Breen M, Lindblad-Toh K, Alföldi J. 2015. Exome sequencing of lymphomas from three dog breeds reveals somatic mutation patterns reflecting genetic background. *Genome Res* **25**: 1634–1645. [Medline] [CrossRef]
8. Ettersperger J, Montcuquet N, Malamut G, Guegan N, Lopez-Lastra S, Gayraud S, Reimann C, Vidal E, Cagnard N, Villarese P, Andre-Schmutz I, Gomes Domingues R, Godinho-Silva C, Veiga-Fernandes H, Lhermitte L, Asnafi V, Macintyre E, Cellier C, Beldjord K, Di Santo JP, Cerf-Bensussan N, Meresse B. 2016. Interleukin-15-dependent T-cell-like innate intraepithelial lymphocytes develop in the intestine and transform into lymphomas in celiac disease. *Immunity* **45**: 610–625. [Medline] [CrossRef]
9. Freiche V, Cordonnier N, Paulin MV, Huet H, Turba ME, Macintyre E, Malamut G, Cerf-Bensussan N, Molina TJ, Hermine O, Bruneau J, Couronné L. 2021. Feline low-grade intestinal T cell lymphoma: a unique natural model of human indolent T cell lymphoproliferative disorder of the gastrointestinal tract. *Lab Invest* **101**: 794–804. [Medline] [CrossRef]
10. Giannuzzi D, Marconato L, Fanelli A, Licenziato L, De Maria R, Rinaldi A, Rotta L, Rouquet N, Birolo G, Fariselli P, Mensah AA, Bertoni F, Aresu L. 2022. The genomic landscape of canine diffuse large B-cell lymphoma identifies distinct subtypes with clinical and therapeutic implications. *Lab Anim (NY)* **51**: 191–202. [Medline] [CrossRef]
11. Goto-Koshino Y, Mochizuki H, Sato M, Nakashima K, Hiyoshi S, Fujiwara-Igarashi A, Maeda S, Nakamura K, Uchida K, Fujino Y, Ohno K, Tsujimoto H. 2015. Construction of a multicolor GeneScan analytical system to detect clonal rearrangements of immunoglobulin and T cell receptor genes in canine lymphoid tumors. *Vet Immunol Immunopathol* **165**: 81–87. [Medline] [CrossRef]
12. Kieslinger M, Swoboda A, Kramer N, Freund P, Pratscher B, Neubauer HA, Steinborn R, Wolfesberger B, Fuchs-Baumgartinger A, Moriggl R, Burgener IA. 2021. A Recurrent STAT5BN642H driver mutation in feline alimentary T cell lymphoma. *Cancers (Basel)* **13**: 5238. [Medline] [CrossRef]
13. Kiselow MA, Rassnick KM, McDonough SP, Goldstein RE, Simpson KW, Weinkle TK, Erb HN. 2008. Outcome of cats with low-grade lymphocytic lymphoma: 41 cases (1995-2005). *J Am Vet Med Assoc* **232**: 405–410. [Medline] [CrossRef]
14. Küçük C, Jiang B, Hu X, Zhang W, Chan JKC, Xiao W, Lack N, Alkan C, Williams JC, Avery KN, Kavak P, Scuto A, Sen E, Gaulard P, Staudt L, Iqbal J, Zhang W, Cornish A, Gong Q, Yang Q, Sun H, d'Amore F, Leppä S, Liu W, Fu K, de Leval L, McKeithan T, Chan WC. 2015. Activating mutations of STAT5B and STAT3 in lymphomas derived from γδ-T or NK cells. *Nat Commun* **6**: 6025. [Medline] [CrossRef]
15. Lingard AE, Briscoe K, Beatty JA, Moore AS, Crowley AM, Krockenberger M, Churcher RK, Canfield PJ, Barrs VR. 2009. Low-grade alimentary lymphoma: clinicopathological findings and response to treatment in 17 cases. *J Feline Med Surg* **11**: 692–700. [Medline] [CrossRef]
16. Maeda S, Ohno K, Fujiwara-Igarashi A, Uchida K, Tsujimoto H. 2016. Changes in Foxp3-positive regulatory T cell number in the intestine of dogs with idiopathic inflammatory bowel disease and intestinal lymphoma. *Vet Pathol* **53**: 102–112. [Medline] [CrossRef]
17. Maeda S, Tsuboi M, Sakai K, Ohno K, Fukushima K, Kanemoto H, Hiyoshi-Kanemoto S, Goto-Koshino Y, Chambers JK, Yonezawa T, Uchida K, Matsuki N. 2017. Endoscopic cytology for the diagnosis of chronic enteritis and intestinal lymphoma in dogs. *Vet Pathol* **54**: 595–604. [Medline] [CrossRef]
18. Nairismägi ML, Tan J, Lim JQ, Nagarajan S, Ng CCY, Rajasegaran V, Huang D, Lim WK, Laurensia Y, Wijaya GC, Li ZM, Cutcutache I, Pang WL, Thangaraju S, Ha J, Khoo LP, Chin ST, Dey S, Poore G, Tan LHC, Koh HKM, Sabai K, Rao HL, Chuah KL, Ho YH, Ng SB, Chuang SS, Zhang F, Liu YH, Pongpruttipan T, Ko YH, Cheah PL, Karim N, Chng WJ, Tang T, Tao M, Tay K, Farid M, Quek R, Rozen SG, Tan P, Teh BT, Lim ST, Tan SY, Ong CK. 2016. JAK-STAT and G-protein-coupled receptor signaling pathways are frequently altered in epitheliotropic intestinal T-cell lymphoma. *Leukemia* **30**: 1311–1319. [Medline] [CrossRef]
19. Nicolae A, Xi L, Pham TH, Pham TA, Navarro W, Meeker HG, Pittaluga S, Jaffe ES, Raffeld M. 2016. Mutations in the JAK/STAT and RAS signaling pathways are common in intestinal T-cell lymphomas. *Leukemia* **30**: 2245–2247. [CrossRef]
20. Roberti A, Dobay MP, Bisig B, Vallois D, Boéchat C, Lanitis E, Bouchindhomme B, Parrens MC, Bossard C, Quintanilla-Martinez L, Missiaglia E, Gaulard P, de Leval L. 2016. Type II enteropathy-associated T-cell lymphoma features a unique genomic profile with highly recurrent SETD2 alterations. *Nat Commun* **7**: 12602. [Medline] [CrossRef]
21. Sogame N, Risbon R, Burgess KE. 2018. Intestinal lymphoma in dogs: 84 cases (1997–2012). *J Am Vet Med Assoc* **252**: 440–447. [Medline] [CrossRef]
22. Stark GR, Darnell JE Jr. 2012. The JAK-STAT pathway at twenty. *Immunity* **36**: 503–514. [Medline] [CrossRef]
23. van Vliet C, Spagnolo DV. 2020. T- and NK-cell lymphoproliferative disorders of the gastrointestinal tract: review and update. *Pathology* **52**: 128–141. [Medline] [CrossRef]
24. Wingelhofer B, Neubauer HA, Valent P, Han X, Constantinescu SN, Gunning PT, Müller M, Moriggl R. 2018. Implications of STAT3 and STAT5 signaling on gene regulation and chromatin remodeling in hematopoietic cancer. *Leukemia* **32**: 1713–1726. [Medline] [CrossRef]