Abstract. Background/Aim: The underlying etiology of Crohn’s disease remains unknown. The aim of this study was to identify genomic alterations associated with the development of Crohn’s disease in one Japanese family with a family history of Crohn’s disease. Materials and Methods: We performed whole-exome sequence and pedigree analysis of a Japanese family in which both sisters developed Crohn’s disease. Whole-exome sequencing was performed using the Ion Torrent Proton™ system. Data from the Proton runs were initially processed using the Ion Torrent platform-specific pipeline software Ion Reporter. An autosomal dominant mode of inheritance was assumed, and stringent selection criteria were applied. Results: A substitution in the NR4A1 gene at codon 293 resulting in an amino acid change from arginine to serine was identified only in the affected sisters. Conclusion: The impaired DNA-binding capacity of the NR4A1 protein due to an NR4A1 germline mutation may be a possible cause of Crohn’s disease.

Crohn’s disease (CD) (OMIM 266600) is an inflammatory bowel disease (IBD) that forms discontinuous erosions and ulcers throughout the digestive tract. Various genetic and environmental factors (1) and several risk loci associated with susceptibility to CD have been reported (2, 3). However, the definitive etiology remains elusive.

In this study, we used whole-exome sequencing and pedigree analysis of a Japanese family with CD to evaluate which germline alterations affected the onset of CD.

Mutations in the NR4A1 gene were identified by whole-exome sequencing. The NR4A1 gene encodes nuclear receptor subfamily 4, group A, member 1 (NR4A1), which is a member of the steroid orphan receptor family. The NR4A1 gene has been implicated in several diseases, including Crohn’s disease (CD) and inflammatory bowel disease (IBD). In this study, we identified a novel NR4A1 Arg293Ser mutation in two affected sisters with CD. This mutation results in a change from arginine to serine at codon 293 of the NR4A1 protein.

In conclusion, the impaired DNA-binding capacity of the NR4A1 protein due to an NR4A1 germline mutation may be a possible cause of Crohn’s disease.
sequences, and filter to remove poor signal-profile reads using the optimized parameters provided by the manufacturer of the AmpliSeq exome. The other filter employed was visual examination of the mutations using CLC Genomics Workbench version 9.0.1 (Qiagen, Hilden, Germany) in addition to the removal of possible strand-specific errors (i.e., a mutation detected only in either the “plus” or “minus” strand but not in both strands). A limitation of this analysis is that structural variants such as large deletions or exon skipping variants could not be detected.

**Results**

Whole-exome sequencing was performed with DNA samples from patients III-6 and III-7, their unaffected father (II-3), and their unaffected mother (II-4) (Figure 1A). A mutation at codon 293 resulting in an amino acid substitution from arginine to serine, Arg293Ser, was identified in NR4A1 in patient III-6, patient III-7, and their unaffected father (II-3) (Figure 1B). An autosomal dominant mode of inheritance, which is speculative, was assumed, and stringent selection criteria were applied (Tables I and II). The variant did not exist in the data of 10 non-consanguineous, unrelated persons whose library was created using AmpliSeq Exome Kit and sequenced by Ion Torrent System as in the present study.

**Discussion**

NR4A1 was initially known as a nerve growth factor (4), and was also identified as a member of the “orphan” nuclear steroid receptor superfamily (5). NR4A1 is transcriptionally active in many types of cells (6, 7) under different culture conditions. Furthermore, the suppression of NR4A1 can inhibit apoptosis (8). Moreover, NR4A1 plays a significant role in the functioning of macrophages (9). The loss of NR4A1 in myeloid cells leads to the acceleration of leukocyte infiltration to the central nervous system (10). The loss of NR4A1 also exacerbates organ fibrosis in the experimental mouse model by dysregulating transforming growth factor-β pathway (11). Thus, NR4A1 is involved in the regulation of fundamental cellular functions, including inflammation and cell survival (12, 13).

The NR4A1 gene contains seven exons. Exon2 encodes an N-terminal transactivation domain, exon3 and 4 encode a DNA-binding domain, and dimerization and ligand-binding domains are encoded by exons 5 to 7 (14). The DNA-binding domain is a highly conserved amino acid sequence also found in other DNA-binding proteins such as the retinoid X receptor (RXR), estrogen receptor 2, steroid receptor, and androgen receptor (2135-2140 (2021)).

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Figure 1. Pedigree plotting and sequencing results. A). Pedigree of the study family and the disease status of each member. Squares denote male family members, circles denote female members, shaded symbols denote affected members, and slashes denote deceased members. We performed whole-exome sequencing using DNA from peripheral blood samples from the following four family members: II-1, II-3, III-1, and III-2. B). Sequence chromatogram and direct sequence chart of the mutant genome. The NR4A1 germline mutation c.877C>T, a missense substitution of cysteine for arginine at position 293 (encoding p.Arg293Cys; position chr12:52449814 T/C), was detected with Ion Torrent technology.
There have been two reports on experimental colitis using the NR4A1⁻/⁻ mouse model. In the first report, Hamers et al. defined the role of NR4A1 in IBD in induced mouse IBD models. Inflammation was increased in NR4A1⁻/⁻ mice and the mediated invasion of T-cells and monocytes into the colon was more pronounced. The expression of MCP-1, TNFα and IL-6 was increased and the transcription of Foxp3 was decreased. Moreover, the over-expression of NR4A1 in activated RAW macrophages contributed to the upregulation of IL-10 and the down-regulation of TNFα, MIF-1, and MCP-1 transcription via NFκB repression. The authors concluded that NR4A1 has a protective function in the induced colitis model (17).

In the second report, Wu et al. found genetic variants of NR4A1 associated with susceptibility to both ulcerative colitis (UC) and CD in the most recent GWASs on UC and CD. They confirmed that NR4A1 expression was suppressed in the colon tissues of UC or CD patients. They also revealed that deficiency...
of NR4A1 increased vulnerability to colitis, whereas treatment with an NR4A1 agonist significantly reduced the induced colitis in the NR4A1–/– mouse model. The authors concluded that NR4A1 was a key regulator of the TRAF6/TLR/IL-1R-initiated inflammatory pathway in IBD (18).

In an in vitro model, Nr4a1 deletion in B2 cells increased atherosclerosis and was associated with increased T follicular helper cell-Germlinal Center response and NR4A1 expression in Marginal Zone B cells regulating PD-L1 expression, limiting the T follicular helper cell-Germlinal Center response and protecting from atherosclerosis (19). The NR4A1 expression was elevated in human osteoarthritis cartilage and in an in vitro osteoarthritis model, which could be blocked by the NF-xB signal inhibitor JSH23 (20).

Recently, Klepsch et al. identified that the loss of NR2F6 induced spontaneous late-onset colitis in the NR2F6–/– mouse model (21). They suggested that agonists of NR2F6 might be a potential treatment strategy of human IBD. Onuki et al. reported that the activation of RXR/peroxisome proliferator-activated receptor δ and NR4A1/RXR heterodimers diminished the monocyte-mediated inflammatory response in the gut (22). Liu et al. also reported that NR4A1 is an important regulator of T cell function and a potential target for tumor immunotherapy (23). These observations also support our findings.

In conclusion, we speculate that the onset of CD in this family may be due to the impaired DNA-binding capacity of the NR4A1 protein caused by the NR4A1 Arg293Ser germline mutation. As treatment with an agonist for NR4A1 has been reported to attenuate excessive inflammation in an experimental mouse colitis model (18), NR4A1 protein impairment may be a possible cause of IBD. NR4A1 agonists may be future targets for preventing and treating IBD, and screening for NR4A1 germline mutations will provide important information that can help elucidate the etiology of IBD in the future.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors’ Contributions

KM: project development, manuscript writing, study conceptualization and design, data collection and abstraction, and statistical analyses; SF: study conceptualization and design, and manuscript preparation and revision; All Authors read and approved the final version of the manuscript.

References

1 Xavier R and Podolsky D: Unravelling the pathogenesis of inflammatory bowel disease. Nature 448(7152): 427-434, 2020. PMID: 17653185. DOI: 10.1038/nature06005

2 Momozawa Y, Dmitrieva J, Théâtre E, Deffontaine V, Rahmouni S, Charletoua B, Crins F, Docampo E, Elansary M, Gori AS, Lecut C, Mariman R, Mni M, Oury C, Altkhov I, Alexeev D, Aulchenko Y, Amininejad L, Bouma G, Hoentjen F, Löwenberg M, Oldenburg B, Pierik MJ, Vander Meulen-de Jong AE, Janneke van der Woude C, Visschedijk MC, International IBD Genetics Consortium., Lathrop M, Hugot JP, Weersma RK, De Vos M, Franchimont D, Vermeire S, Kubo M, Louis E and Georges M: IBD risk loci are enriched in multigenic regulatory modules encompassing putative causative genes. Nat Commun 9(1): 2427, 2018. PMID: 29930244. DOI: 10.1038/s41467-018-04365-8

3 Alonso A, Domènech E, Juliá A, Panés J, García-sánchez V, Mateu P, Gutiérrez A, Gomollón F, Mendoza J, Garcia-planella E, Barreiro-de acosta M, Muñoz F, Vera M, Saro C, Esteve M, Andreu M, Chaparro M, Manyé J, Cabrè E, López-lasanta M, Tortosa R, Gelpi J, García-montero A, Bertranpetit J, Absher D, Myers R, Marsal S and Gisbert J: Identification of risk loci for Crohn’s disease phenotypes using a genome-wide association study. Gastroenterology 148(4): 794-805, 2020. PMID: 25557950. DOI: 10.1053/j.gastro.2014.12.030

4 Milbrandt J: Nerve growth factor induces a gene homologous to the glucocorticoid receptor gene. Neuron 1(3): 183-188, 2020. DOI: 10.1016/0896-6273(88)90138-9

5 Safe S, Jin U, Morpurgo B, Abdalayeh A, Singh M and Tjalkens R: Nuclear receptor 4A (NR4A) family – orphans no more. J Steroid Biochem Mol Biol 157: 48-60, 2020. PMID: 25917081. DOI: 10.1016/j.jsbmb.2015.04.016

6 Davis I, Hazelt J, Chen R, Blenis J and Lau L: Functional domains and phosphorylation of the orphan receptor Nur77. Mol Endocrinol 7(8): 953-964, 2013. PMID: 8232315. DOI: 10.1210/mend.7.8.8232315

7 Paulsen RE, Weaver CA, Fahrner TJ and Milbrandt J: Domains regulating transcriptional activity of the inducible orphan receptor NGFI-B. J Biol Chem 267(23): 16491-16496, 1992. PMID: 1644831.

8 Woronicz J, Calnan B, Ngo V and Winoto A: Requirement for the orphan steroid receptor Nur77 in apoptosis of T-cell hybridomas. Nature 367(6460): 277-281, 2020. PMID: 8121493. DOI: 10.1038/367277a0

9 Pei L, Castrillo A and Tontonoz P: Regulation of macrophage inflammatory gene expression by the orphan nuclear receptor Nur77. Molecular Endocrinology 20(4): 786-794, 2020. PMID: 16339277. DOI: 10.1210/me.2005-0331

10 Shaked I, Hanna R, Shaked H, Chodaczek G, Nowyehed H, Tweet G, Tacke R, Basat A, Mikulski Z, Togher S, Miller J, Blatchley A, Salek-ardakani S, Darvas M, Kaikonen M, Thomas G, Lai-wing-sun S, Rezk A, Bar-or A, Glass C, Bandukwala H and Hedrick C: Transcription factor Nr4a1 couples sympathetic and inflammatory cues in CNS-recruited macrophages to limit neuroinflammation. Nat Immunol 16(12): 1228-1234, 2020. PMID: 26523867. DOI: 10.1038/s41551-018-03321

11 Palumbo-zerr K, Zerr P, Distler A, Fiehr J, Mancuso R, Huang J, Mielenz D, Tomcik M, Fürnrohr B, Scholtysek C, Dees C, Blatchley A, Salek-ardakani S, Darvas M, Kaikonen M, Thomas G, Lai-wing-sun S, Rezk A, Bar-or A, Glass C, Bandukwala H and Hedrick C: Transcription factor Nr4a1 couples sympathetic and inflammatory cues in CNS-recruited macrophages to limit neuroinflammation. Nat Immunol 16(12): 1228-1234, 2020. PMID: 26523867. DOI: 10.1038/s41551-018-03321

12 Pols T, Bonta P and De vries C: NR4A nuclear orphan receptors: protective in vascular disease? Curr Opin Lipidol 18(5): 515-520, 2021. PMID: 17885421. DOI: 10.1097/MOL.0b013e3282cf77d1
13 Maxwell M and Muscat G: The NR4A Subgroup: Immediate early response genes with pleiotropic physiological roles. Nucl Recept Signal 4(1): nrs.04002, 2020. PMID: 16604165. DOI: 10.1621/nrs.04002

14 Saucedo-cardenas O, Kardon R, Ediger T, Lydon J and Conneely O: Cloning and structural organization of the gene encoding the murine nuclear receptor transcription factor, NURR1. Gene 187(1): 135-139, 2020. PMID: 9073077. DOI: 10.1016/s0378-1119(96)00736-6

15 Ham J, Thomson A, Needham M, Webb P and Parker M: Characterization of response elements for androgens, glucocorticoids and progestins in mouse mammary tumour virus. Nucleic Acids Res 16(12): 5263-5276, 2017. PMID: 2838812. DOI: 10.1093/nar/16.12.5263

16 Sultan C, Lumbrasso S, Poujol N, Belon C, Boudon C and Lobaccaro J: Mutations of androgen receptor gene in androgen insensitivity syndromes. J Steroid Biochem Mol Biol 46(5): 519-530, 2019. PMID: 8240973. DOI: 10.1016/0960-0760(96)90178-y

17 Hamers A, Van dam L, Teixeira duarte J, Vos M, Marinkovic G, Van tiel C, Meijer S, Van stallbacka S, Hunevee A, De jonge W and De vries C: Deficiency of nuclear receptor Nur77 aggravates mouse experimental colitis by increased NFĸB activity in macrophages. PLOS ONE 10(8): e0135598, 2020. PMID: 26241646. DOI: 10.1371/journal.pone.0135598

18 Wu H, Li X, Wang J, Gan W, Jiang F, Liu Y, Zhang X, He X, Zhao Y, Lu X, Guo Y, Zhang X and Li J: NUR77 exerts a protective effect against inflammatory bowel disease by negatively regulating the TRAF6/TLR-IL-1R signalling axis. J Pathol 238(3): 457-469, 2019. PMID: 26564988. DOI: 10.1002/path.4670

19 Nus M, Basatemur G, Galan M, Cros-brunso L, Zhao T, Masters L., Harrison J, Figg N, Tsiantoulas D, Geissmann F, Binder C, Sage A and Mallat Z: NR4A1 Deletion in Marginal Zone B Cells Exacerbates Atherosclerosis in Mice—Brief Report. Arterioscler Thromb Vasc Biol 40(11): 2598-2604, 2020. PMID: 32907369. DOI: 10.1161/ATvbaha.120.314607

20 Xiong Y, Ran J, Xu L, Tong Z, Adel abdo M, Ma C, Xu K, He Y, Wu Z, Chen Z, Hu P, Jiang L, Bao J, Chen W and Wu L: Reactivation of NR4A1 Restrains Chondrocyte Inflammation and Ameliorates Osteoarthritis in Rats. Front Cell Dev Biol 8: 158, 2020. PMID: 32258036. DOI: 10.3389/fcell.2020.00158

21 Klepsch V, Gerner R, Klepsch S, Olson W, Tilg H, Moschen A, Baier G and Hermann-kleiter N: Nuclear orphan receptor NR2F6 as a safeguard against experimental murine colitis. Gut 67(8): 1434-1444, 2020. PMID: 28779026. DOI: 10.1136/gutjn1-2016-31466

22 Onuki M, Watanabe M, Ishihara N, Suzuki K, Takizawa K, Hirota M, Yamada T, Egawa A, Shibahara O, Nishii M, Fujihara M, Makishima M, Takahashi D, Furuhashi Y, Kakuta H and Hase K: A partial agonist for retinoid X receptor mitigates experimental colitis. Int Immunol 31(4): 251-262, 2019. PMID: 30590577. DOI: 10.1093/intimm/dxy089

23 Chen J, Lopez-moyado I, Seo H, Lio C, Hempleman L, Sekiya T, Yoshimura A, Scott-browne J and Rao A: NR4A transcription factors limit CAR T cell function in solid tumours. Nature 567(7749): 530-534, 2020. PMID: 30814732. DOI: 10.1038/s41586-019-0985-x

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