FADS2 and *ELOVL6* mutation frequencies in Japanese Crohn's disease patients

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Summary Crohn's disease (CD) development is thought to involve genetic factors related to immune response as well as environmental factors, such as intestinal bacteria and diet, though no clear cause has yet been identified. In our previous study, we found that the concentrations of linoleic acid, stearic acid, and metabolites in erythrocytes differed between CD patients and healthy subjects. These factors related to lipid metabolism are controlled by $\Delta 6$ desaturase (fatty acid desaturase 2, FADS2) and elongase 6 (ELOVL6), respectively. In the present study, we analyzed the gene sequences of *FADS2* and *ELOVL6* in 52 Japanese CD patients, and then compared mutation frequencies with findings in healthy individuals. Nineteen *FADS2* mutations and 33 *ELOVL6* mutations were found. Furthermore, a new variant in the promoter region was shown in both genes, though no mutation in the coding region was found in either. For the *FADS2* intron, the allele frequency of rs227784 (0.3365; 95% CI = 0.0337-0.01460) was higher than that in healthy subjects (0.0190). Furthermore, allele rs227784 had a greater association with CD (odds ratio = 4.4; 95% CI = 2.1-9.3). As compared with healthy Japanese healthy individuals, no mutations were found with a largely deviated allele frequency in the present CD group. However, the number of patients examined was small, thus the reliability of our results is limited. The present findings regarding genetic effects on CD onset and lipid metabolism may be weak.

*Keywords:* FADS2, ELOVL6, Crohn's disease, Japanese

1. Introduction

Crohn's disease (CD) patients have discontinuous inflammation in the digestive tract extending from the mouth to anus. Currently, there are more than 40,000 such patients in Japan, twice the number of 20 years ago (*1*). CD occurrence is thought to be caused by genetic factors related to immune response as well as environmental factors, such as intestinal bacteria and diet, though no clear causes have been clarified (*2-*

5). One such factor is considered to be lipotoxicity due to excessive intake of lipids. Linoleic acid (LA), a vegetable oil-derived n-6 polyunsaturated fatty acid (n-6 PUFA), is metabolized to pro-inflammatory eicosanoids, such as prostaglandin, leukotriene, and thromboxane, *via* dihomo-γ-linoleic acid (DGLA) and arachidonic acid (AA) (Figure 1). The metabolic rate-limiting enzyme from LA to AA is $\Delta 6$ desaturase (fatty acid desaturase 2, FADS2), which is involved in desaturation of LA, with the *FADS2* gene located on chromosome 11 and mainly expressed in the liver, heart, and brain (*6*). Enzyme activities show individual differences, and it is thought that the onset of CD is caused by an imbalance between LA and its metabolites, as previous studies have reported that the ratio of LA metabolites to LA in erythrocyte

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membranes and plasma was higher in CD patients as compared to healthy individuals (*7,8*). Additionally, those studies found that the ratio of palmitic acid (PA) to stearic acid (SA) was different between CD patient and healthy groups. Elongation from PA to SA involves elongase 6 (elongation of the very long chain fatty acids protein 6, ELOVL6). The *ELOVL6* gene is encoded on chromosome 4 and lipid-induced inflammation has been found to be reduced in *ELOVL6* knockout mice (*9*). Therefore, we speculated that individual differences in *ELOVL6* activity can also have effects on intestinal inflammation in CD patients.
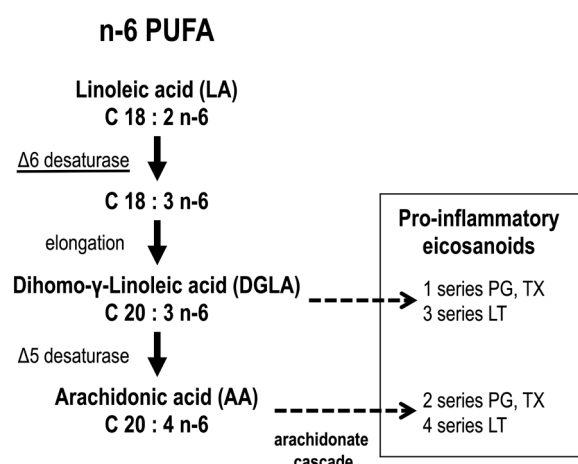
Figure 1. Metabolism of n-6 polyunsaturated fatty acids. LA is metabolized to DGLA and AA by Δ6 desaturase and elongation. The metabolites are converted to pro-inflammatory eicosanoids, prostaglandin, thromboxane, and leukotriene.

In the present study, we focused on the *FADS2* and *ELOVL6* genes, known to be involved in lipid metabolism, and performed gene sequence analysis with CD patients. The obtained genetic information was compared with that of healthy subjects to detect mutations specific to CD.

2. Materials and Methods

2.1. *Subjects and ethical considerations*

Fifty-two CD patients receiving treatment at Jikei University Kashiwa Hospital in Japan were enrolled and DNA samples extracted from obtained blood were analyzed. This study was approved by the ethics committee of Jikei University {26-363(7869)}, as well as the ethics committee of Niigata University of Pharmacy and Applied Life Sciences (H27-005). All participants received an explanation regarding the purpose of the study and methods involved prior to enrollment, and each provided individual consent.

2.2. *PCR conditions*

We designed specific primers for amplification of the promoter, translated, and untranslated regions (5'UTR, 3'UTR) of human *FADS2* and *ELOVL6* (Figure 2, Tables S1 and S2, *http://www.ddtjournal.com/action/getSupplementalData.php?ID=52*). Oligo DNA synthesis was performed by Hokkaido System Science Co., Ltd. (Hokkaido, Japan). The composition of the reaction solution and PCR conditions are shown in
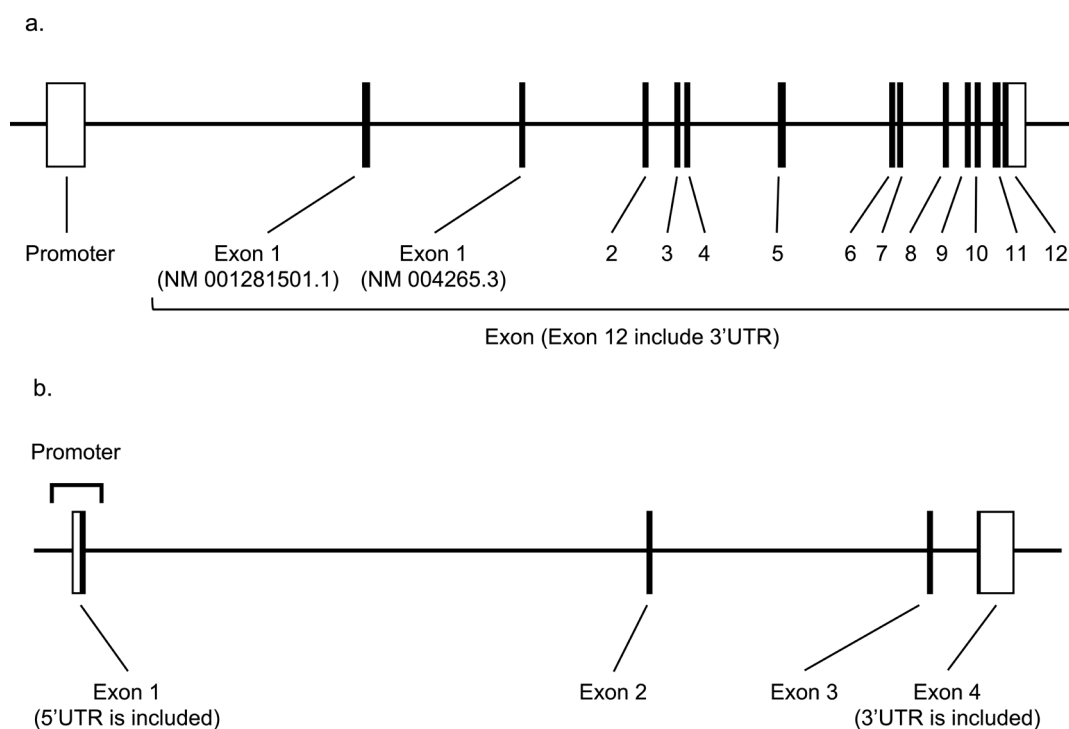
Figure 2. Amplified region of each gene. (a) *FADS2* gene region. **(b)** *ELOVL6* gene region.

the Tables S3-6 (*http://www.ddtjournal.com/action/getSupplementalData.php?ID=52*). The promoter region and 3'UTR of each gene were arbitrarily divided and amplified. Amplification of PCR products was detected by ultraviolet irradiation after agarose electrophoresis.

2.3. Direct sequencing

For pretreatment prior to direct sequencing, 1 μL of alkaline phosphatase and 1 μL of exonuclease I were added to 5 μL of the PCR product, then heated at 37°C for 15 minutes and 80°C for 15 minutes. DNA sequencing was performed by Eurofins Genomics K.K. (Tokyo, Japan). Direct sequencing was done using the obtained PCR products as templates. The sequences of Homo sapiens chromosome 11, GRCh 37.p5 primary assembly (NC_000011.10) for *FADS2*, and sequences of Homo sapiens chromosome 4 and GRCh 38.p12 primary assembly (NC_000004.12) for *ELOVL6* were used as controls to confirm the gene sequences.

2.4. Statistical analysis

Allele frequencies were calculated based on genotype frequencies. Hardy-Weinberg equilibrium was determined by comparing genotype frequency with the expected value using a χ^2 test. A *p*-value less than 0.05 was considered to indicate statistical significance. The 95% confidence interval (95% CI) of the allele frequencies was calculated on the basis of binomial distribution.

We used data in a genome database of healthy Japanese individuals as a control group to verify the association between mutations and CD. Fisher's exact test was utilized for statistical differences, with the level of rejection of a null hypothesis denoted as α = 0.05 for two tails. The odds ratio (OR) and its 95%CI were calculated for strength of relevance. All statistical processing was performed used the BellCurve® software package for Excel (Social Survey Research Information Co., Ltd.).

3. Results and Discussion

Sequence analyses of *FADS2* and *ELOVL6* were performed with 52 Japanese CD patients. In *FADS2*, we found 2, 12, and 5 mutations in the promoter, intron, and 3'UTR, respectively, while 14 mutations were detected in the promoter, 1 in the intron, and 18 in 3'UTR of *ELOVL6*. One novel mutation was confirmed in the promoter regions of both genes, whereas no mutation was detected in the coding region of either. The genotype and allele frequencies, and 95% CI of the mutations were calculated (Tables 1 and 2, *http://www.ddtjournal.com/action/getSupplementalData.php?ID=52*). Furthermore, we compared the 95%

CI values with those in database records containing human genome information (Tables 1 and 2, *http://www.ddtjournal.com/action/getSupplementalData.php?ID=52*). The Japanese Multi Omics Reference Panel (jMorp) (*10*) was used for comparisons with healthy Japanese and The Genome Aggregation Database (gnomAD) (*11*) for comparisons with other species.

In the comparison of *FADS2*, the allele frequency of rs2277284 (0.0769; 95% CI = 0.0337-0.1460) was higher than that in jMorp (0.0190) and the odds ratio was 4.4 (95% CI = 2.1-9.3). However, the genotype frequency of rs2277284 deviated from Hardy Weinberg's equilibrium and no heterozygotes were detected, in contrast to detection of 4 homozygotes. In addition, the rs2277284 allele frequency for East Asians (EAS) in gnomAD (0.0611) was nearly the same as the present result.

As for *ELOVL6*, the allele frequency of rs150566425 (0.0192; 95% CI = 0.0023-0.0677) deviated from the findings in jMorp (0.0004). On the other hand, the frequencies of allele rs755746 (0.0288; 95% CI = 0.0060-0.0820) and that in jMorp (0.0315) were lower than the frequency of EAS in gnomAD (0.1137).

In the present CD patients, no mutation was detected in the coding region of either *FADS2* or *ELOVL6*. At present, none of the genes that cause amino acid mutations have been reported to have a frequency greater than 1%. Therefore, it can be concluded that there are no contradictions with these analysis results.

For *FADS2*, the allelic frequencies of rs174538 and rs174578 in our results and in jMorp were approximately 20% lower than those of EAS in gnomAD. The frequency of these mutations is considered to be low in Japanese as compared to other East Asians. Allele rs2277284 was more frequent in the present CD patients than in healthy Japanese, suggesting that this mutation might be related to disease onset. However, there was no divergence of the allele frequency between our results and that of EAS in gnomAD, as well as only homozygotes with no heterozygosity, thus it will be necessary to examine for verification for relevance to the disease. The allele mutations rs174589, rs526126, and rs2072114 have been reported to have influence on lipid metabolism (*12-16*). However, there was no divergence of frequency in this study, and no association with CD onset was found.

Regarding *ELOVL6*, the mutation frequency of allele rs755746 in our results and in jMorp was about 10% lower than that of EAS in gnomAD. Among East Asian races, Japanese are thought have a low frequency of this mutation. Allele rs150566425 is suggested to be related to onset and mutation, because it was found more frequently in CD patients than healthy individuals. The allele frequencies of rs5860996 and rs200600528 obtained in the present study were slightly lower than

Table 1. Allele frequencies and odds ratio of *FADS2*.

Items	rsID	Position[†]	Mutation	Genotype (%, N = 52)			Allele frequency (95%CI)		Odds ratio (95%CI)		p value	jMorp (5)	gnomAD (6)				
				A/A	A/a	a/a							EAS	AMR	ASJ	NFE	AFR
Promoter	rs174538	61,792,609	g > a	36.5	42.3	21.2	0.4231	(0.3267~0.5239)	1.2170	(0.8224~1.8011)	0.3590	0.3770	0.5722	0.5955	0.2690	0.3060	0.0685
	New	61,792,811	a > c	98.1	1.9	0.0	0.0096	(0.0002~0.0524)		NA		NA	NA	NA	NA	NA	NA
Intron	rs2072114	61,837,743	a > g	48.1	36.5	15.0	0.3365	(0.2468~0.4358)	1.2689	(0.8420~1.9121)	0.2743	0.2872	0.4310	0.3085	0.1379	0.1523	0.1293
	rs174578	61,838,027	t > a	36.5	40.4	23.1	0.4327	(0.3358~0.5335)	1.2391	(0.8380~1.8322)	0.3091	0.3803	0.5788	0.6312	0.3414	0.3495	0.3738
	rs174589	61,848,331	c > g	78.9	21.2	0.0	0.1058	(0.0539~0.1814)	1.1140	(0.5933~2.0916)	0.7364	0.0990	0.1477	0.3042	0.1621	0.1887	0.0432
	rs144659223	61,848,406	c > a	92.3	7.7	0.0	0.0385	(0.0105~0.0956)	2.5193	(0.9110~6.9665)	0.0843	0.0158	0	0.0035	0	0.0005	0
	rs374558244	61,857,127	c > t	98.1	1.9	0.0	0.0096	(0.0002~0.0524)		NA		NA	0.0006	0	0	0.0001	0.0005
	rs526126	61,857,413	g > c	1.9	21.2	76.9	0.8750	(0.7957~0.9317)	0.6837	(0.3801~1.2297)	0.2222	0.9131	0.8329	0.8491	0.7862	0.8235	0.3297
	rs553613434	61,857,613	g > a	98.1	1.9	0.0	0.0096	(0.0002~0.0524)		NA		0.0017	0.0026	0	0	0	0
	rs2277284	61,857,643	g > t	92.3	0.0	7.7	0.0769	(0.0337~0.1460)	4.4218	(2.1055~9.2863)	0.0009	0.0190	0.0611	0.0378	0.0103	0.0055	0.2950
	rs141055240	61,863,162	t > c	86.5	13.5	0.0	0.0673	(0.0274~0.1338)	2.1457	(0.9849~4.6746)	0.0856	0.0334	0.0783	0.0024	0.0069	0.0004	0.0358
	rs145771202	61,863,592	c > t	98.1	1.9	0.0	0.0096	(0.0002~0.0524)	0.9600	(0.1320~6.9817)	1.0000	0.0104	0	0	0	0	0
	rs1162570716	61,863,638	c > a	98.1	1.9	0.0	0.0096	(0.0002~0.0524)		NA		NA	NA	NA	NA	NA	NA
	rs520298	61,863,810	c > a	98.1	1.9	0.0	0.0096	(0.0002~0.0524)		NA		0.0003	0	0	0	0	0
3'UTR	rs139145063	61,865,702	c > t	86.5	13.5	0.0	0.0673	(0.0274~0.1338)	2.1579	(0.9908~4.7001)	0.0849	0.0334	0.0770	0.0012	0.0069	0.0002	0.0053
	rs11539527	61,866,116	a > g	86.5	13.5	0.0	0.0673	(0.0274~0.1338)	2.0804	(0.9555~4.5296)	0.0901	0.0336	0.0770	0.0024	0.0069	0.0005	0.0547
	rs142344045	61,866,134	c > t	98.1	1.9	0.0	0.0096	(0.0002~0.0524)		NA		0.0086	0	0	0	0	0
	rs17156516	61,866,232	g > a	86.5	13.5	0.0	0.0673	(0.0274~0.1338)	2.1178	(0.9726~4.6115)	0.0874	0.0335	0.0764	0.0024	0.0069	0.0006	0.0547
	rs1359586116	61,866,402	c > t	98.1	1.9	0.0	0.0096	(0.0002~0.0524)		NA		NA	NA	NA	NA	NA	NA

3'UTR, 3' untranslated region; 95%CI, 95% Confidence interval; AFR, African; AMR, American (Latino); ASJ, Ashkenazi Jewish; EAS, East Asian; NFE, Non-Finnish European; NA, Not applicable. [†]NC_000011.10.

Table 2. Allele frequencies and odds ratio of Elovl 6.

Items	rsID	Position†	Mutation	Genotype (%, N = 52)			Allele frequency (95%CI)	Odds ratio (95% CI)		p value	jMorp (5)	gnomAD (6)				
				A/A	A/a	a/a						EAS	AMR	ASJ	NFE	AFR
Promoter	rs6824447	110,199,557	g > a	46.2	38.5	15.4	0.3462 (0.2555~0.4458)	0.9354	(0.6228~1.4051)	0.8372	0.3615	0.3404	0.3955	0.5931	0.5032	0.7302
	rs3813830	110,199,112	g > a	94.2	5.8	0.0	0.0288 (0.0060~0.0820)	0.9501	(0.2980~3.0294)	1.0000	0.0314	0.0508	0.0498	0	0.0025	0.0002
	rs3813829	110,199,010	a > g	61.5	32.7	5.8	0.2212 (0.1457~0.3131)	0.8716	(0.5467~1.3894)	0.6463	0.2466	0.2487	0.2783	0.4688	0.3431	0.3761
	rs3813828	110,198,992	a > g	76.9	21.2	1.9	0.1250 (0.0683~0.2043)	1.2040	(0.6699~2.1637)	0.5206	0.1066	0.0721	0.0330	0.0345	0.0496	0.0362
	rs1402223270	110,198,740	g > t	96.2	3.8	0.0	0.0192 (0.0023~0.0677)	NA	NA		NA	0	0	0	0	0.0001
	rs1054469231	110,198,568	g > a	98.1	1.9	0.0	0.0096 (0.0002~0.0524)	NA	NA		0.0014	0	NA	NA	0	NA
	rs755746	110,198,070	a > t	94.2	5.8	0.0	0.0288 (0.0060~0.0820)	0.8608	(0.2708~2.7364)	1.0000	0.0315	0.1137	0.0979	NA	0.0046	0.0320
	rs755747	110,197,906	g > a	76.9	21.2	1.9	0.1250 (0.0683~0.2043)	1.2312	(0.6851~2.2125)	0.5159	0.1064	0.0718	0.0330	0.0345	0.0497	0.0360
	rs990095271	110,196,887	ggaggag > ggag	98.1	1.9	0.0	0.0096 (0.0002~0.0524)		NA		0.0001	NA	NA	NA	NA	NA
	rs968600	110,196,633	g > a	5.8	23.1	71.2	0.8269 (0.7403~0.8941)	0.9731	(0.5833~1.6236)	0.8953	0.8311	0.8245	0.8534	0.8138	0.8284	0.8595
	rs575275791	110,196,465	g > a	98.1	1.9	0.0	0.0096 (0.0002~0.0524)	NA	NA		0.0045	0.0006	NA	0	0	NA
	New	110,196,388	g > a	98.1	1.9	0.0	0.0096 (0.0002~0.0524)	NA	NA		NA	NA	NA	NA	NA	NA
	rs569200155	110,196,189	g > c	98.1	1.9	0.0	0.0096 (0.0002~0.0524)	NA	NA		0.0023	0.0090	NA	0	0	0
	rs11098070	110,196,091	a > g	13.5	44.2	42.3	0.6442 (0.5440~0.7361)	1.4213	(0.9487~2.1291)	0.0912	0.5602	0.5613	0.5260	0.4552	0.5206	0.3405
Intron	rs3733623	110,105,364	t > c	5.8	36.5	57.7	0.7596 (0.6659~0.8380)		NA		0.7242	0.7089	0.3389	0.2743	0.2344	0.4178
3'UTR	rs537457824	110,050,844	atata > atatatata	98.1	1.9	0.0	0.0096 (0.0002~0.0524)	NA	NA		0.0160	0.0167	0	0	0.0001	0.0001
	rs149752874	110,050,418	c > g	94.2	5.8	0.0	0.0288 (0.0060~0.0820)	NA	NA		0.0235	NA	NA	NA	NA	NA
	rs4698806	110,050,333	a > g	21.2	51.9	26.9	0.5288 (0.4285~0.6275)	1.2414	(0.3897~3.9543)	0.7368	0.6056	0.5889	0.5849	0.4103	0.3346	0.8035
	rs9995789	110,049,874	g > t	30.8	55.8	13.5	0.4135 (0.3177~0.5142)	0.7308	(0.4958~1.0772)	0.1294	0.4720	0.4478	0.3631	0.2448	0.1740	0.6874
	rs150566425	110,049,550	t > c	96.2	3.8	0.0	0.0192 (0.0023~0.0677)	0.7887	(0.5323~1.1684)	0.2764	0.0004	NA	NA	NA	NA	NA
	rs371671518	110,049,258	a > g	98.1	1.9	0.0	0.0096 (0.0002~0.0524)	NA	NA		0.0034	0.0006	0	0	0	0
	rs17041272	110,048,926	c > g	73.1	25.0	1.9	0.1442 (0.0822~0.2277)	0.7527	(0.4341~1.3051)	0.3707	0.1834	0.1722	0.0932	0.0655	0.0524	0.1759
	rs5860996	110,048,716	aaaaaaaa > aaaaaaa	19.2	42.3	25.0	0.4615 (0.3633~0.5620)		NA		0.5345	0.5715	0.5095	0.4097	0.3779	0.8208
	rs200600528	110,048,706	tgtttgtttta > t	80.8	17.3	1.9	0.1058 (0.0540~0.1814)		NA		0.1251	0.1701	0.0221	0.0242	0.0351	0.0647
	rs6837303	110,048,429	c > t	23.1	50.0	26.9	0.5192 (0.4189~0.6186)	0.7028	(0.4770~1.0355)	0.0855	0.6068	0.5938	0.4941	0.4034	0.3407	0.8006
	rs11947713	110,048,416	c > t	76.9	21.2	1.9	0.1250 (0.0683~0.2043)	0.6370	(0.3552~1.1426)	0.1582	0.1834	0.1727	0.0934	0.0655	0.0525	0.1767
	rs6836309	110,048,057	a > c	80.8	17.3	1.9	0.1058 (0.0540~0.1814)	0.8485	(0.4524~1.5915)	0.7624	0.1223	0.1211	0.0165	0.0207	0.0306	0.0202
	rs78610984	110,047,988	t > c	80.8	17.3	1.9	0.1058 (0.0540~0.1814)	0.8586	(0.4577~1.6107)	0.7619	0.1220	0.1210	0.0154	0.0207	0.0306	0.0078
	rs77807740	110,047,676	g > a	73.1	23.1	3.8	0.1538 (0.0906~0.2378)	0.9018	(0.5273~1.5423)	0.7920	0.1722	0.1497	0.0165	0.0207	0.0305	0.0078
	rs3733624	110,047,354	c > g	42.3	53.8	3.8	0.3077 (0.2209~0.4058)	1.0726	(0.7050~1.6317)	0.7453	0.3397	0.3077	0.2043	0.0182	0.0339	0.0155
	rs3813826	110,046,632	t > g	73.1	25.0	1.9	0.1442 (0.0822~0.2277)	0.7475	(0.4311~1.2961)	0.3710	0.1852	0.1735	0.0884	0.0655	0.0520	0.1116
	rs3813825	110,046,513	t > a	28.8	55.8	15.4	0.4327 (0.3359~0.5335)	0.8557	(0.5789~1.2647)	0.4888	0.4718	0.4402	0.3113	0.1793	0.1982	0.0883
	rs9030	110,046,154	a > g	28.8	55.8	15.4	0.4327 (0.3359~0.5335)	0.8542	(0.5779~1.2625)	0.4888	0.4721	0.4406	0.4005	0.3379	0.2881	0.6462

3'UTR, 3' untranslated region; 95%CI, 95% Confidence interval; AFR, African; AMR, American (Latino); ASJ, Ashkenazi Jewish; EAS, East Asian; NFE, Non-Finnish European; NA, Not applicable. †NC_000004.12.

those in jMorp and EAS in gnomAD. These deletion mutations are very close to each other and overlap in registered defective bases, thus it is unclear whether they can be correctly distinguished in the existing databases. Although there is no report regarding *ELOVL6* in CD, an association with diabetes has been shown. *ELOVL6* activity was reported to have effects on development of diabetes and inflammation associated with onset of that disease (*9*). Also, Morcillo *et al.* reported that the risk of insulin resistance was decreased when the rs6824447 mutation was present, while the risk increased in individuals with the rs17041272 mutation (*17*). On the other hand, Liu *et al.* reported that there were no significant differences regarding those mutations between healthy and type 2 diabetic patients (*18*). We consider that the association with CD onset is low, because no divergence was found in the frequency of any of the mutations examined in this study.

In the present study, gene sequence analyses of *FADS2* and *ELOVL6* in CD patients were performed. In general, no mutations were found that largely deviated in allele frequency as compared with healthy Japanese individuals. However, the number of patients examined was few, which limits the reliability of our results. The present findings suggest that genetic effects may be weak between CD onset and lipid metabolism. Nevertheless, it is possible that some healthy individuals may carry mutations associated with CD development. In the future, we intend to analyze lipid distribution in plasma and erythrocyte membrane specimens, and investigate the causal relationship with genetic information for CD patients obtained in this study.

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