

Various Expression Patterns of $\alpha 1$ and $\alpha 2$ Genes in IgA Deficiency

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ABSTRACT

Background: IgA deficiency (IgAD) is the most common immunodeficiency, however the pathogenesis in most cases of IgAD is unknown. There are 2 subclasses of IgA, IgA1 and IgA2, and its heavy chains are encoded by 2 different genes, the $\alpha 1$ and $\alpha 2$ genes. To investigate the molecular pathogenesis of IgA deficiency, it is important to evaluate each of the expressions of IgA1 and IgA2 separately.

Methods: In this study, we report on the reverse transcriptase (RT)-PCR method in which $\alpha 1$ and $\alpha 2$ mRNAs can be separately evaluated. This method is based on electrophoretic separation using the difference of 39 bases between $\alpha 1$ and $\alpha 2$ mRNAs. Three selective, 5 partial and 2 secondary IgAD patients were examined.

Results: In the 3 selective IgAD patients, no $\alpha 1$ or $\alpha 2$ mRNA expression was detected. In the 5 partial IgAD patients, various $\alpha 1$ and $\alpha 2$ mRNA expression patterns were found. One of the partial IgAD patients showed only $\alpha 2$ gene expression, but not $\alpha 1$ gene expression, and was found to show an $\alpha 1$ gene deletion together with $\gamma 2$ and ϵ gene deletions. His plasma IgA2 level was within the normal range.

Conclusions: Patients with an $\alpha 1$ gene deletion can be considered as having partial IgAD. Using this method, we identified the second case of $\alpha 1$ gene deletion in Japan, and classified IgAD patients on the basis of $\alpha 1$ and $\alpha 2$ expression.

KEY WORDS

gene expression, IgA subclasses, partial IgA deficiency, selective IgA deficiency, $\alpha 1$ gene deletion

ABBREVIATIONS

IgA, immunoglobulin A; IgAD, immunoglobulin A deficiency; RT, reverse transcriptase; CVID, common variable immunodeficiency; TACI, transmembrane activator and calcium-modulator and cyclophilin ligand interactor; SD, standard deviation; PBMCs, peripheral blood mononuclear cells; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; BSA, bovine serum albumin; PBS, phosphate-buffered saline

INTRODUCTION

Human immunoglobulin A (IgA) is the most abundant immunoglobulin in secretions. IgA has 2 subclasses, IgA1 and IgA2. The ratios of IgA1 : IgA2 are approximately 9 : 1 in serum and 6 : 4 in saliva.¹ In mucosal tissue, IgA synthesis greatly exceeds that of other immunoglobulin classes. These 2 subclasses play important roles in the first line of defense, and the amount ratio of these molecules in secretions varies.

Selective IgA deficiency (IgAD) is the most common immunodeficiency, and has been found with a

frequency ranging from 0.03 to 0.3%. The prevalence differs according to ethnic groups, with a lower frequency in the Japanese population, namely, 1/18,000 people.^{2,3} Most IgAD patients remain healthy, but some suffer from a variety of infections, allergies, autoimmune disorders, gastrointestinal diseases, malignancies, endocrinopathies, neurological diseases, and genetic disorders.^{2,4} The pathogenesis of IgAD has not yet been completely clarified. IgAD has been found to be associated with IgG2, IgG4, and IgE deficiencies.⁵⁻⁸ The class switch disorder in IgA-producing B lymphocytes is one of the most important factors in IgAD patients.⁹ Asano *et al.* suggested

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Table 1 Immunological data of patients

Patient No.	Sex	Age	Serum level (mg/dl)			IgG subclass (mg/dl)			
			IgG	IgA	IgM	IgG1	IgG2	IgG3	IgG4
Selective IgA deficiency									
1	M	10 years	1363	< 5	146	619	255	57.3	33.9
2	F	11 years	1640	< 5	117	949	400	47.7	90.2
3	F	17 years	1261	< 5	137	630	625	35.1	18.4
Partial IgA deficiency									
4	M	4 years	1223	17	120	933	< 8.0	22.8	< 3.0
5	F	3 years	1644	15	150	878	47.9	17.3	32.1
6	F	3 years	869	27	106	306	69.3	27.6	3.8
7	M	4 years	887	45	101	295	97.0	40.0	4.4
8	M	4 years	1624	8	100	1090	120	74.7	< 3.0
Secondary IgA deficiency									
9	F	7 years	913	12	105	602	148	52.8	16.5
10	M	13 years	705	9	39	852	345	49.8	6.4

that decreased expression levels of $I\alpha$ germline transcripts before a class switch may be the cause of selective IgAD, and B-cell differentiation might be disturbed after a class switch in partial IgAD patients.¹⁰ Husain *et al.* reported that the increased destruction of a subset of B cells is the cause for the inability to produce IgA in IgAD patients.¹¹

The association between IgAD and common variable immunodeficiency (CVID) has been discussed,³ and it was reported recently that some CVID and IgAD patients have mutations in TNFRSF13B (encoding TACI; transmembrane activator and calcium-modulator and cyclophilin ligand interactor).^{12,13}

The molecular weights of the IgA1 and IgA2 heavy chains are both approximately 53 kD. The $\alpha 1$ and $\alpha 2$ genes show about 97% of its identity.¹⁴ This high homology between them makes it difficult to analyze the $\alpha 1$ and $\alpha 2$ genes separately. The α -chain constant region of both $\alpha 1$ and $\alpha 2$ genes is encoded by 3 exons. The hinge region of the human α chain is encoded at the beginning of the second exon. The hinge region of the $\alpha 2$ gene shows a deletion of 39 nucleotides (corresponding to 13 amino acids) when compared with that of the $\alpha 1$ gene.^{1,15} To clarify the pathogenesis and immunological reactions of IgAD clear, we analyzed $\alpha 1$ and $\alpha 2$ gene expression in IgAD patients. In this study, we devised a new method to determine the expression levels of the $\alpha 1$ and $\alpha 2$ genes, and analyzed selective, partial and secondary IgAD patients.

METHODS

SUBJECTS

As shown in Table 1, we analyzed 3 selective IgAD patients (patients number 1, 2 and 3) with serum IgA levels below the detection limit (<5 mg/dl), 5 partial IgAD patients (patients number 4, 5, 6, 7, and 8) with serum IgA levels above 5 mg/dl but having more

than 2 standard deviations (SDs) below the normal level, and 2 secondary IgAD patients (patients number 9 and 10) whose conditions were caused by epileptic medication. Ten controls were also included in this study. Two of the 10 controls were child volunteers under 16 years of age, and the other 8 were adult volunteers 16 years or older. We obtained informed consent from the patients, controls, and parents of minors.

CELL PREPARATION

Peripheral blood mononuclear cells (PBMCs) were collected in heparin and separated by gradient centrifugation in Ficoll-Paque (GE Healthcare BioSciences AB, Uppsala, Sweden).¹⁶ The cells were suspended at a density of 10^6 /ml and incubated for 24 hours in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mmol/l l-glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin.¹⁰ In comparison with non-cultured cells, cells cultured for 24 hours showed higher levels of $\alpha 1$ and $\alpha 2$ gene expression (unpublished data).

cDNA SYNTHESIS AND PCR AMPLIFICATION

We extracted total RNA from PBMCs using an Iso-gene kit (Nippon Gene, Tokyo, Japan), and cDNA synthesis was carried out using 1 or 2 μ g of total RNA with oligo-dT and M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). We used the following PCR primer pair, which was targeted to the common sequence area of the $\alpha 1$ and $\alpha 2$ genes: sense 5'-CCTGGTCACCGTCTCCTCA-3' (placed at the J exon; Gene Bank accession number-L20778) and antisense 5'-TCACGCTCAGGTGGTCCTTG-3' (placed at the C α CH2 exon)¹⁷ (Fig. 1A). The PCR fragments included the CH1, hinge and CH2 regions, and the size was 532 bp for the $\alpha 1$ gene and 493 bp for the $\alpha 2$ gene. The PCR program was 35 or 40 cycles of 94°C

Various Expression Patterns of α Genes in IgAD

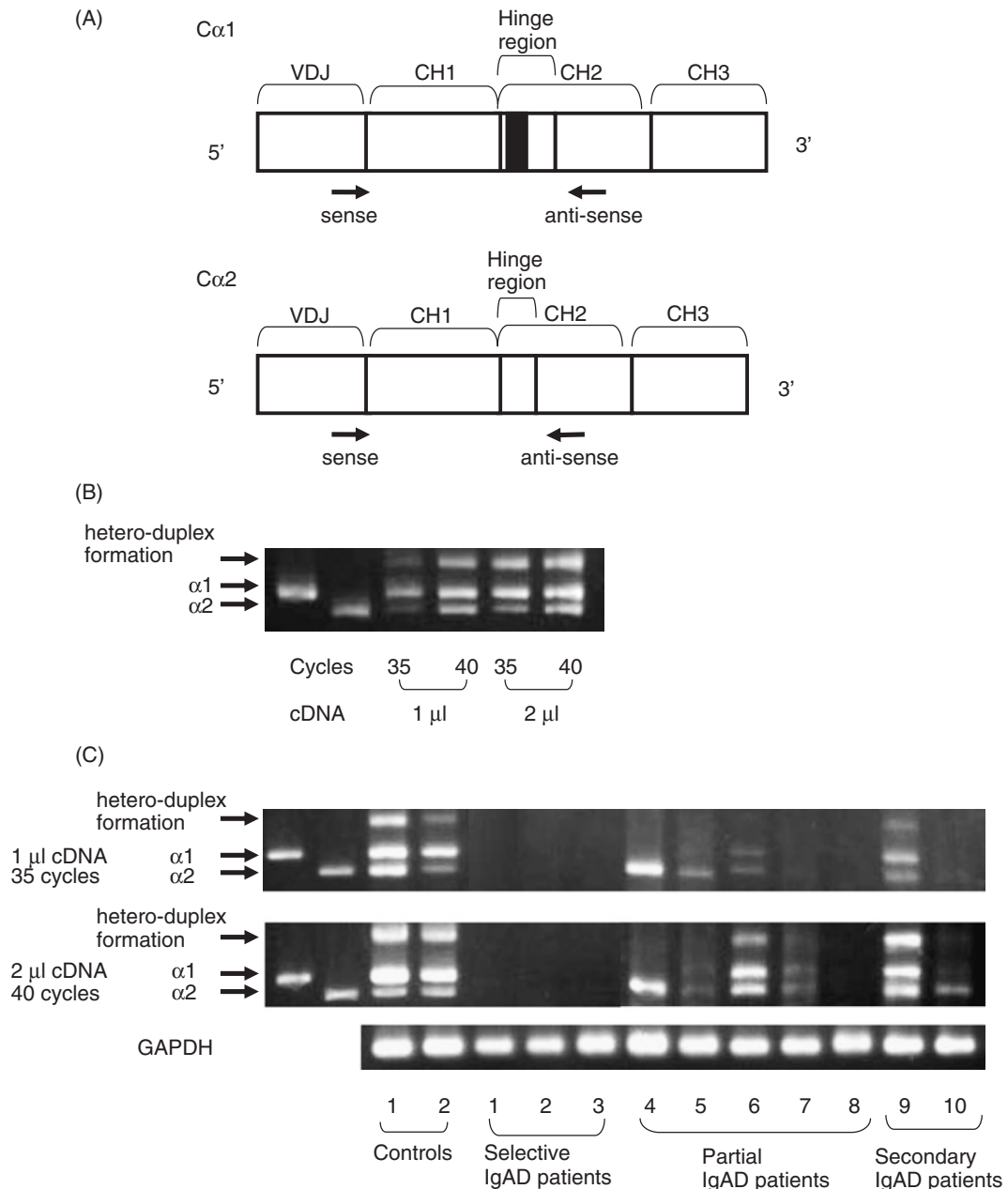


Fig. 1 Structure and expression of the $\alpha 1$ and $\alpha 2$ genes. **(A)** The primer pair was targeted to the common sequence area of the $\alpha 1$ and $\alpha 2$ genes. These figures show mature C α gene transcripts. The arrows indicate the positions of the primers. The black box indicates the deletion of 39 bases. VDJ: variable diversity joining region, CH: constant heavy chain. **(B)** RT-PCR analysis of RNA extracted from the PBMCs of a control subject, starting from 1 μ l or 2 μ l of cDNA and using 35 or 40 cycles. The two lanes at the left show PCR products amplified from the template DNA, which are T-vectors containing the IgA1 (lane 1) or IgA2 (lane 2) genes. **(C)** RT-PCR analysis of RNA extracted from the PBMCs of control subjects and IgAD patients. The $\alpha 1$ fragment, $\alpha 2$ fragment, and hetero-duplex formation are indicated by arrows. GAPDH was used as a control.

for 1 minute, 60°C for 1 minute, and 72°C for 1 minute using 1 or 2 μ l of cDNA. The PCR products were run on 4% agarose gels for 120 minutes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a

control.

DNA EXTRACTION AND PCR AMPLIFICATION

Genomic DNA was purified from polymorphonuclear

cells using Sepa Gene (Sanko Junyaku, Tokyo, Japan). We used the following PCR primer pair, which was targeted at the common sequence area of the $\alpha 1$ and $\alpha 2$ genes: sense 5'-TGACCAGCTCAGGCCATCTCT-3' and antisense 5'-CTTTGCAAACCAGAGCACTGA-3'. The PCR program was 40 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute. Four percent agarose gel electrophoresis was performed for 120 minutes. The other primer pairs were as follows: for amplification of the C ϵ gene, sense 5'-ACCTTCAGCGGTAAGAGAGGG-3' and antisense 5'-TGGGAACGTACCTGCACACTT-3', (the PCR program was 35 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute); for amplification of the C $\gamma 2$ gene, sense 5'-ATCTCTTCCTCAGCACCACCT-3' and antisense 5'-CGTGGCACTCATTTACCCGGA-3', (the PCR program was 35 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute); for the C μ gene, sense 5'-ATGTGTGTCCCCGGTGAGTGA-3' and antisense 5'-AGGGCCACCCCTGTGAACAGA-3', (the PCR program was 35 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute).

QUANTIFICATION OF IgA SUBCLASSES IN PLASMA

The levels of the IgA subclasses in plasma were measured by enzyme-linked immunosorbent assay (ELISA). For IgA1, coating was performed with a mouse monoclonal anti-IgA1 (NI69-11), and detection of IgA1 was performed with horseradish peroxidase (HRP)-labeled goat anti-human IgA (Cappel, Organon Teknika, Turnhout, Belgium).¹⁸ For IgA2, coating was performed using goat anti-human IgA (BETHYL, Montgomery, TX, USA), and detection of IgA2 was performed using mouse anti-human IgA2-HRP (B3506B4).¹⁹ ELISA plates were coated overnight at 4°C with mouse monoclonal anti-IgA1 (diluted to 1 : 200 with 0.05 M sodium carbonate, pH 9.6) or goat anti-human IgA (diluted to 1 : 100 with 0.05 M sodium carbonate, pH 9.6). The plates were washed then incubated with standard serum and plasma dilutions. IgA1 was detected using goat anti-human IgA-HRP (diluted to 1 : 10000 with 1% bovine serum albumin [BSA] in phosphate-buffered saline [PBS]-0.02% Tween 20), and IgA2 was detected using mouse anti-human IgA2-HRP (diluted to 1 : 1000 with 1% BSA in PBS-0.02% Tween 20). The samples were tested repeatedly. The lower limits of IgA1 and IgA2 detection were 5 ng/ml and 1 μ g/ml, respectively.

RESULTS

PCR AMPLIFICATION OF $\alpha 1$ AND $\alpha 2$ GENE EXPRESSION

RT-PCR analysis was performed using primer pairs that amplified both $\alpha 1$ and $\alpha 2$ mRNAs and could distinguish $\alpha 2$ mRNA from $\alpha 1$ mRNA taking advantage of the deletion of 39 bases in the hinge region of the $\alpha 2$ gene. Various PCR conditions were tested, and

the optimal conditions were deemed to be 2 μ l of cDNA and 40 cycles of amplification (Fig. 1B). Control samples gave an intense $\alpha 1$ band and a less-intense, shorter $\alpha 2$ band in all 4 PCR conditions. Another band with less electrophoretic mobility than the $\alpha 1$ band was determined to be a hetero-duplex formation of the $\alpha 1$ and $\alpha 2$ fragments, because the subcloning of this band yielded clones of the $\alpha 1$ and $\alpha 2$ fragments. Figure 1C shows $\alpha 1$ and $\alpha 2$ gene expression in 10 IgAD patients. In 3 selective IgAD patients (patients number 1, 2 and 3), no expression of the $\alpha 1$ and $\alpha 2$ genes was detected. Three partial IgAD patients (patients number 5, 6 and 7) and 2 secondary IgAD patients (patients number 9 and 10) showed $\alpha 2$ and $\alpha 1$ gene expressions; however, patient No. 4 showed only $\alpha 2$ gene expression, but no $\alpha 1$ gene expression. One partial IgAD patient (No. 8) showed no bands in this RT-PCR analysis.

PLASMA IgA LEVELS IN CONTROLS AND IgAD PATIENTS

Plasma IgA1 and IgA2 levels were assayed separately (Table 2). The IgA1 levels were much higher than the IgA2 levels in the 10 controls. In the 3 selective IgAD patients, plasma IgA1 and IgA2 levels were very low. One partial IgAD patient (patient No. 4) had a normal IgA2 level, but no detectable IgA1. This finding is in accordance with the result of this patient showing no $\alpha 1$ gene expression in PBMCs. Although the IgA2 level of patient No. 5 was below the threshold, other patients with partial IgAD (patients number 6, 7, 8) showed various IgA1 and IgA2 levels although these levels were much lower than that of the control group.

PCR AMPLIFICATION OF THE IMMUNOGLOBULIN GENES OF IgAD PATIENTS

In patient No. 4, $\alpha 1$ gene expression was not detected and no IgA1 protein was detected in his plasma. As shown in Table 1, the plasma levels of the IgG subclasses showed that IgG2 and IgG4 levels were below the detection limits. Hence, we carried out PCR amplification of the genomic $\alpha 2$ and $\alpha 1$ genes, together with the μ , $\gamma 2$ and ϵ genes. As shown in Figure 2, no PCR products were detected for the $\alpha 1$, $\gamma 2$ and ϵ genes in patient No. 4, whereas they were clearly detected in 2 control and other IgAD patients. A large genomic deletion of the A1-GP-G2-G4-E genes can be proposed as the molecular basis of IgAD in patient No. 4.

LONGITUDINAL CHANGE IN THE SERUM IgA LEVEL OF PATIENT NO. 4

The immunological data of 2 families of patients with IgG2-IgG4-IgA1-IgE deficiency, including patient No. 4, are shown in Table 3. The serum IgA levels of patient No. 4 over time are shown in Table 4. The patient's IgA levels remained at a level more than 2 SDs

Table 2 The levels of IgA subclasses and IgA1/IgA2 ratios in plasma as measured by ELISA

Patient No.	Serum IgA levels (mg/dl)	Plasma IgA1 and IgA2 levels (mg/dl)		IgA1/IgA2 ratio	mRNA expression		Genes		
		IgA1	IgA2		α 1	α 2	α 1	α 2	
Selective IgA deficiency									
1	< 5	2.48 \pm 0.26	ND [†]	NC [‡]	— [¶]	—	+ [§]	+	
2	< 5	ND	ND	NC	—	—	+	+	
3	< 5	2.16 \pm 0.74	ND	NC	—	—	+	+	
Partial IgA deficiency									
4	17	ND	13.67 \pm 1.39	NC	—	+	—	+	
5	15	2.71 \pm 0.36	ND	NC	+	+	+	+	
6	27	8.28 \pm 0.52	0.91 \pm 0.25	8.40	+	+	+	+	
7	45	29.29 \pm 6.83	4.88 \pm 0.54	6.97	+	+	+	+	
8	8	6.28 \pm 0.67	0.86 \pm 0.45	8.87	—	—	+	+	
Secondary IgA deficiency									
9	12	8.59 \pm 1.77	1.17 \pm 0.36	6.56	+	+	+	+	
10	9	0.56 \pm 0.09	0.18 \pm 0.03	3.03	+	+	+	+	
Controls (n = 10)		128.02 \pm 36.98	19.39 \pm 8.93	7.96 \pm 4.14					

[†] ND : undetected, [‡] NC : not calculated.

[§] + : detected, [¶] — : undetected.

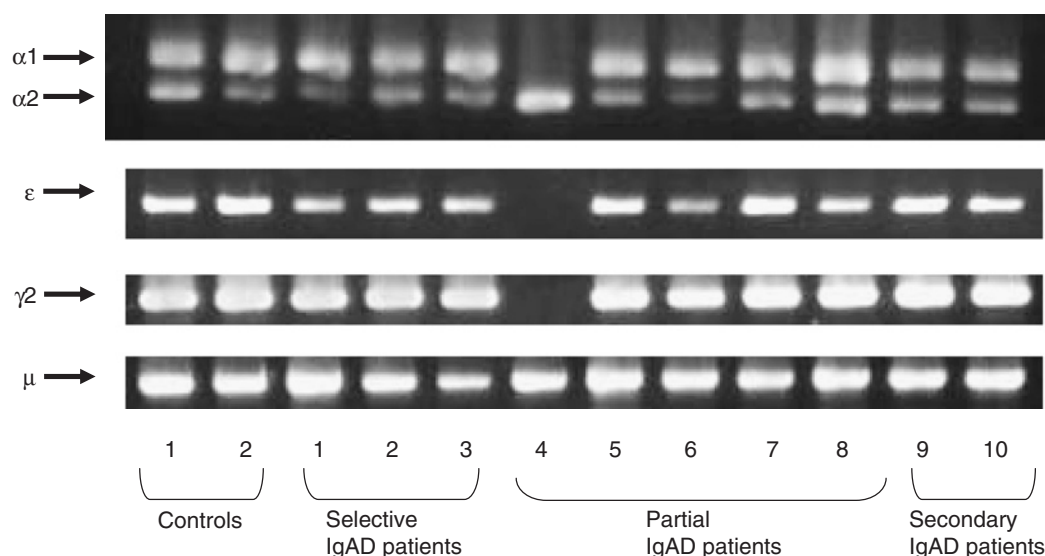


Fig. 2 PCR analysis of immunoglobulin genes in IgAD patients. PCR analysis of α 1, α 2, ϵ , γ 2 and μ genes was performed in control subjects and IgAD patients. Two different sized bands for the α gene appeared, corresponding to the α 1 and α 2 genes, as indicated by arrows.

below the normal value, but above 5 mg/dl. The immunological data of his family members are shown in Family 1 (Table 3). The patient's older brother was also found to have the same gene deletion. His serum IgA level was 21 mg/dl, and he also showed a pattern of partial IgAD. The serum IgA levels of another patient with IgG2-IgG4-IgA1-IgE deficiency and her family members are shown as Family 2 (Table 3).⁸ Both the patient and her older sister had the same deletions of the A1-GP-G2-G4-E genes. The serum IgA level of the patient's sister was 18 mg/dl and she

also showed a pattern of partial IgAD.

DISCUSSION

The serum IgA subclass levels of IgAD patients, particularly selective IgAD patients, are very difficult to measure by ELISA; thus, there have been few reports on the levels of serum IgA subclasses in IgAD patients. The α 1 and α 2 gene expression levels are low in most IgAD patients, and there have been no previous reports on the gene expression levels of the IgA subclasses in these patients.²⁰⁻²³ Hummelshoj *et al.*

Table 3 Immunological data of patient no. 4 and 2 families of patients with IgG2-IgG4-IgA1-IgE deficiency

Family 1							
	Age	Serum level					
		mg/dl					IU/ml
		IgA	IgG1	IgG2	IgG3	IgG4	IgE
Patient No. 4	4	17	933	< 8.0	22.8	< 3.0	< 2.0
Father	45	119	659	451	58.0	19.0	70.0
Mother	41	201	730	224	25.4	14.2	190.0
Brother	11	21	952	< 8.0	23.3	< 3.0	< 2.0
Family 2							
	Age	Serum level					
		mg/dl					IU/ml
		IgA	IgG1	IgG2	IgG3	IgG4	IgE
Patient	1	< 5	996	< 8.0	10.4	< 3.0	< 5.0
Father	27	161	720	281	13.7	14.1	214.8
Mother	26	180	794	248	16.7	8.2	4.2
Sister	3	18	645	< 8.0	15.1	< 3.0	< 5.0

Table 4 Longitudinal change in the serum IgA levels of patient no. 4

	20m	25m	30m	38m	56m	57m
Serum IgA level (mg/dl)	11	12	11	11	20	17
Normal value [†]	1year	2y	3y	4y		
Mean ($\pm 2SD$)	49 (16–128)	60 (20–149)	74 (25–174)	89 (31–202)		

[†] data on normal values according to reference 28.

reported on the expression of germline transcripts of the $\alpha 1$ and $\alpha 2$ genes in IgAD patients.²⁴ The germline transcripts were induced during stimulation with TGF- β , however their levels were lower than those of control subjects. Our report shows the expression of mature transcripts of the $\alpha 1$ and $\alpha 2$ genes in partial IgAD patients. In the 3 selective IgAD patients, no expression of $\alpha 1$ and $\alpha 2$ genes was detected, presumably because the expression level was too low. In 2 of them, only plasma IgA1 levels could be measured, and in 1 of them, neither IgA1 nor IgA2 levels were unable to be measured. Patient No. 8 showed no mRNA expression of IgA subclasses, but exhibited detectable levels of plasma IgA1 and IgA2 levels. It might be assumed that the pathogenesis of partial IgAD in patient No. 8 is similar to that in selective IgAD patients. As shown in Table 2, patients number 1, 2, 3 and 8 showed no $\alpha 1$ or $\alpha 2$ gene expression, while patients number 5, 6, 7, 9 and 10 showed both $\alpha 1$ and $\alpha 2$ gene expression. There is a possibility that the mechanism underlying IgA deficiency may be different between these 2 groups. The plasma IgA2 levels were unable to be measured in 1 partial IgAD patient; however, $\alpha 2$ gene expression was seen in this patient using our method. This method is effective for determining whether partial IgAD patients lack ex-

pression of the $\alpha 1$ and $\alpha 2$ genes when their plasma IgA, IgA1 and IgA2 levels are under the detection limit.

Asano *et al.* reported detectable levels of the C α mature transcripts by RT-PCR using the same sense primer we employed and an antisense primer located in the C α CH1 region.¹⁰ Using our method, no $\alpha 1$ or $\alpha 2$ gene expression was detected in 3 selective IgAD patients. There was a difference in the annealing temperatures used (59°C in the study of Asano *et al.*), and different PCR conditions may result due to changes in the sensitivity of the assay.

IgAD patients are often associated with IgG subclass deficiency. In our study, 2 IgAD patients (patients number 4 and 8) had IgG subclass deficiency in the serum. Patient No. 4 showed deletions of the $\gamma 2$ and ϵ genes, and was considered to have a large genomic deletion of the C $\alpha 1$, $\Psi C\gamma$, C $\gamma 2$, C $\gamma 4$ and C ϵ genes, showing the same observations reported previously in a patient.⁸ In Japan, only 1 case has ever been reported. Beard *et al.*²⁵ reported that IgA-IgG2-IgG4 deficiency occurs in 4% of 73 IgAD patients. Deletions of the A1-GP-G2-G4-E genes are the most common deletions of the Ig heavy chain locus.²⁶ There could be more patients with an $\alpha 1$ gene deletion who might have partial IgAD. Partial IgAD is

often transient,²⁷ however, patients who have a deletion of the α gene will not show transient IgAD and will exhibit continuous partial IgAD.

We also classified the IgAD patients on the basis of the expression of $\alpha 1$ and $\alpha 2$ genes. Selective IgAD patients showed no $\alpha 1$ or $\alpha 2$ mRNA expression and extremely low protein levels, while secondary IgAD patients showed both mRNA and protein expressions of IgA subclasses. Partial IgAD can be classified into 3 patterns as follows: cases showing reduced levels of $\alpha 1$ and $\alpha 2$ gene expression; cases showing no $\alpha 1$ gene expression but normal $\alpha 2$ gene expression; and cases showing no $\alpha 1$ or $\alpha 2$ gene expression.

It is sometimes difficult to determine the pathogenic mechanisms operative in IgAD patients on the basis of serum IgA levels. Variations in the pathogenesis of IgAD patients might become better understood by examining the protein and mRNA expression levels of IgA subclasses.

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