Table 4 Comparison of Prior and Subsequent Pregnancy Outcomes in Carriers of a Reciprocal Translocation Ascertained on the Basis of Recurrent Pregnancy Loss: In Vitro Fertilization/Preimplantation Genetic Diagnosis

		Prior Pregnancies		IVF/PGD Outcomes			
	No. of Carriers	Unsuccessful	Successful	No. of Cycles	Did Not Conceive	Unsuccessful Pregnancies	Successful Pregnancies
Munné et al, 1998[19]	3	14 miscarriages	1	3	1	0	2 (twins; ongoing pregnancy)
Munné et al, 1998[21]	1	2 miscarriages*	0	1	0	1 miscarriage	0
Willadsen et al, 1999[25]	2	6 miscarriages†	0	2	1	0	1 (preterm)
Munné et al, 2000[20]	3	22 miscarriages	0	3	3	0	0
Escudero et al, 2000[16]	2	5 miscarriages	1	2	1	0	1 (monozygotic twins, hydronephrosis)
Coonen et al, 2000[15]	2	13 miscarriages, 1 fetal demise, 1 genetic termination	0	4	3	0	1 (term)
Simopoulou et al, 2003[23]	4	14 miscarriages	0	4	2	1 miscarriage (biochemical)	1 (term)
Sugiura-Ogasawara et al, 2008[4]	2	4 miscarriages	0	2	1	1 miscarriage	0
Wiland et al, 2008[24]	1	2 miscarriages	0	1	0	0	1 (triplet reduction, singleton term)
Totals	**20 carriers**	**98% total prior pregnancies** (82 miscarriages, 1 fetal demise, 1 genetic termination)	**4% total prior pregnancies** (2 successful pregnancies)	**22 cycles**	**12 did not conceive**	**3 miscarriages**	**7 successful pregnancies**

*Estimated, based on "repeated pregnancy loss."
†Estimated, based on "habitual abortion" and "recurrent miscarriage."
IVF/PGD, in vitro fertilization/preimplantation genetic diagnosis.

100% for women with primary RPL) to subsequent,[10,12] is highly biased in favor of intervention, and therefore it is inappropriate to conclude benefit. The IVF/PGD studies were often descriptive, with reporting of embryo number, chromosomal determination, and only successful transfers.[20,33] Other recent systematic reviews of IVF/PGD for carriers of a structural chromosome with a history of recurrent miscarriage[37] and unexplained recurrent miscarriage[38] have not shown benefit with this strategy, compared with medical management.

There remains concern for carriers of a reciprocal translocation of having an ongoing pregnancy or live birth with an unbalanced rearrangement. In this review, only 1 of >100 patients had a ongoing pregnancy with an unbalanced reciprocal translocation[5]; other pregnancies with unbalanced reciprocal translocations ended in miscarriage. Therefore, the reason for ascertainment appears to be important. In this systematic review, the ascertainment was RPL, which appears to have a more favorable prognosis than a history of an ongoing pregnancy or live birth with an unbalanced translocation.

Goddijn et al,[3] in a retrospective analysis of 1324 Dutch couples, identified 51 carriers of structural chromosome rearrangement, of which 63% were reciprocal translocation. A nested case-control study of 41 of the 51 couples revealed no unbalanced structural chromosome rearrangements in subsequent ongoing pregnancies. Amniocentesis was performed on 26 of the 41 ongoing pregnancies; all were euploid, with 58% 46,XX or 46,XY, and 42% balanced structural chromosome rearrangements.

This study was designed to review systematically the effectiveness of management strategies for carriers of a reciprocal translocation involving two chromosomes, ascertained on the basis of RPL. We identified a total of 129 carriers who met the entry criteria. In the medical management group, using the first pregnancy after evaluation, the subsequent live birthrate was 60% (65 of 109). Using all subsequent outcomes, the cumulative live birthrate was 74% (81 of 109 cases) in the medical management group. In the IVF/PGD group, the subsequent live birthrate per cycle started was 35% (7 of 20); the cumulative live birthrate was the same.

Unfortunately, the published data are limited, and there are differences in the reporting of the data in each group, as previously discussed. It is difficult to directly compare outcomes for these two management strategies because of the different end points reported. Understanding the differences is essential for effective counseling. Until a well-designed study comparing the two strategies is performed, or at least prospective cohort studies with strict entry criteria and definitions, the cumulative experience and success of both medical management and IVF/PGD must be used for counseling of patients who are carriers of a reciprocal translocation, ascertained on the basis of RPL.

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SYCP3 mutation may not be associated with recurrent miscarriage caused by aneuploidy

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BACKGROUND: *SYCP3* mutations have been shown to generate an aberrant synaptonemal complex in a dominant-negative manner and to contribute to abnormal chromosomal behavior that might lead to recurrent miscarriage. We examined whether *SYCP3* mutation is associated with recurrent miscarriage caused by embryonic aneuploidy.

METHODS: The *SYCP3* 657T>C mutation was examined using PCR and sequencing in 101 patients with a history of three or more unexplained recurrent miscarriages and 82 fertile controls with no history of miscarriage. The embryonic karyotype in the aborted conceptus was analyzed.

RESULTS: The 657T>C mutation of *SYCP3* was identified in one patient with a history of six recurrent miscarriages with embryonic euploidy and one fertile woman in the control group. Patients with abnormal and normal chromosome were found to repeat miscarriage with abnormal and normal chromosome, respectively.

CONCLUSIONS: The 657T>C mutation of *SYCP3* may not be associated with recurrent miscarriage caused by aneuploidy. We found no clinical significance of routine examination of the *SYCP3* mutation because only one benign mutation was ascertained in 101 patients.

Key words: SYCP3 / recurrent miscarriage / fetal chromosome / meiosis / polymorphism

Introduction

SYCP3 mutations in women were found to generate an aberrant synaptonemal complex in a dominant-negative manner and to contribute to abnormal chromosomal behavior that might lead to recurrent miscarriage (Bolor *et al.*, 2009). Bolor *et al.* (2009) found *SYCP3* mutations in 2 of 26 (7.7%) patients with recurrent miscarriage. SYCP3 is a DNA-binding protein and a structural component of the synaptonemal complex, which mediates the synapsis or homologous pairing of chromosomes during meiosis of the germ cells. Male mice homozygous for the null mutation of the *Sycp3* gene are sterile as a result of massive apoptotic cell death in the testis during meiotic prophase (Yuan *et al.*, 2000). *Sycp3*-deficient female mice are subfertile with a severely reduced oocyte pool. Although two-thirds of mouse offspring are healthy, one-third is affected by aneuploidy and succumbs during development *in utero* (Yuan *et al.*, 2002). This is consistent with the observations that in humans, a mutation in *SYCP3* was identified in two patients with azoospermia (Miyamoto *et al.*, 2003), and that the lack of *SYCP3* gene expression in human testis may have a negative effect on spermatogenesis and male fertility (Aarabi *et al.*, 2006).

The identifiable causes of recurrent miscarriage may include abnormal chromosomes in either partner (particularly translocations), antiphospholipid antibodies (aPL) and uterine anomalies (Farquharson *et al.*, 1984; Sugiura-Ogasawara *et al.*, 2004, 2010). A currently prevailing hypothesis is that recurrent miscarriage may be a polygenetic disorder associated with both genetic and environmental determinants. Polymorphisms related to thrombophilia, such as Leiden mutation and prothrombin mutation, are known to be associated with recurrent miscarriage, although the mutations are not found in the Asian population (Nelen *et al.*, 1996; Rey *et al.*, 2003; Rai and Regan, 2006; Suzumori and Sugiura-Ogasawara, 2010). However, whether Factor V Leiden and Factor II prothrombin polymorphisms are risk factors for recurrent miscarriage is controversial (Coulam *et al.*, 2006; Goodman *et al.*, 2006).

An abnormal embryonic karyotype causes not only sporadic spontaneous abortion but also recurrent miscarriage because it was found in 51% of recurrent cases (Ogasawara et al., 2000; Carp et al., 2001). Bolor et al. (2009) could not prove an association between the *SYCP3* mutations found in 7.7% of patients and embryonic aneuploidy.

Preimplantation genetic screening (PGS) for aneuploidy has been performed widely; however, there is no evidence of its ability to improve delivery rates (Platteau et al., 2005; The ACOG., 2009; Harper et al., 2010). The *SYCP3* mutation might be a candidate for selection of cases for PGS if an association between the mutation and aneuploidy is established. Also, the 7.7% frequency of *SYCP3* mutation is relatively high because the frequency of translocations, aPL and major uterine anomalies is 4.5% (Sugiura-Ogasawara et al., 2004), 10.7% (Balasch et al.,1990) and 3.2% (Sugiura-Ogasawara et al., 2010), respectively. Here we investigate whether *SYCP3* mutations may be associated with recurrent miscarriage caused by aneuploidy.

Materials and Methods

Patients

All patients underwent a systematic examination, including hysterosalpingography, chromosome analysis for both partners, determination of aPL, including lupus anticoagulant and β2glycoprotein I-dependent anticardiolipin antibodies (Ogasawara et al., 1996), and blood tests for hyperthyroidism, diabetes mellitus and hyperprolactinemia before subsequent pregnancy in Nagoya City University Hospital between 2007 and 2010. A blood sample was taken at the examination and frozen at −70°C before analysis. Patients with identifiable causes of miscarriage, such as translocations, aPL and uterine anomalies, were excluded. The 81 patients for whom a previous or subsequent embryonic (or fetal) karyotype was ascertained at least one time were studied. A further 20 patients for whom the embryonic karyotype was unknown were added.

In Japan, miscarriage is defined as loss within 22 weeks gestation and stillbirth is defined as loss at 22 or more weeks of gestation. Stillbirths after 22 weeks gestation were included in the present study and shown as prior history in Table I.

A total of 101 patients with a history of three or more (3–16) unexplained consecutive first-trimester miscarriages were examined. Subsequent pregnancies were followed up until October 2010. The mean age of participants at examination was 34.4 ± 3.8 years, and the average number of previous miscarriages was 3.8 ± 2.7. Twenty-four patients had a history of live birth and two patients experienced recurrent miscarriage after changing partner, having had a live birth by a previous partner. The mean number of previous live births was 0.27 ± 0.5.

Gestational age was calculated based on basal body temperature charts. Ultrasonography was performed once a week from 4 to 8 weeks of gestation. Dilation and curettage was carried out when miscarriages were diagnosed, and the karyotypes of aborted conceptuses were determined using a standard G-banding technique.

The 82 fertile women with no history of recurrent miscarriage and complications of pregnancy were examined as controls. The fertile controls were recruited in Nagoya City University Hospital and Asamoto Women's Clinic. The mean age of women in the control group was 32.3 ± 6.2 years, and the average number of deliveries was 1.53 ± 0.6.

The study was approved by the Research Ethics Committee of Nagoya City University Graduate School of Medical Sciences. Each patient provided their written consent after full disclosure about the purpose and methods to be employed.

DNA analysis

Genomic DNA was extracted from peripheral blood samples with the Midi Blood DNA Extraction kit (Qiagen, Tokyo, Japan). Oligonucleotide primers were designed to amplify each coding sequence, as well as exon–intron boundaries of the human *SYCP3* gene, encompassing exons 7–9 (GenBank accession number NM_153694). The sense and antisense PCR and sequence primers for *SYCP3* were, respectively, 5′-GATGGCGTG TGCCTATAATCCAAG-3′ and 5′-CGTCTTTATTTAATTGACAGTGT TAG-3′. Additional direct sequence primers were 5′-GTCAT GTTGCTCAGGCTGGTC-3′, 5′-TCTGTGGATTGATAATTATCTACT G-3′, 5′-TCCAATGCTCTGAGAACC-3′ and 5′-TCACCACAGC AAGTTGTG-3′. The coding exons 7–9 and exon–intron boundaries of human *SYCP3* gene were amplified by PCR and sequenced using the Big Dye Terminator v3.1 Cycle Sequencing kit (ABI Prism, Applied Biosystems, Foster City, CA, USA) on a 3100 automated sequencer.

Results

Heterozygous 657T>C mutation in exon 8 of *SYCP3* was ascertained in one of 101 patients who had had six recurrent miscarriage and in one of the 82 fertile controls (Fig. 1). The IVS7-16_19 delACTT previously reported in one patient with recurrent miscarriage or 643delA previously reported in two patients with azoospermia was not found in any patients with recurrent miscarriage or in fertile controls. No other new mutation was found in patients with recurrent miscarriage or controls.

Thirty-two patients experienced miscarriage with a normal embryonic (fetal) chromosome karyotype, and 47 patients presented an abnormal embryonic (fetal) karyotype (Table I). Two patients had miscarriages with both normal and abnormal karyotype.

Patient No. 77 with the 657T>C mutation was 31 years old and experienced a total of six miscarriages and no live birth. Available fetal karyotypes were shown as 46, XX and 46, XY. The control with the 657T>C mutation had a history of one live birth and no miscarriage.

Nine patients (No. 39–47) had repeated miscarriage with an abnormal karyotype, and seven patients (No. 75–81) had repeated miscarriage with a normal karyotype. Only 2 out of 18 (11.1%) patients had experienced miscarriages with both abnormal and normal embryonic karyotypes.

Discussion

In the present study, we found a heterozygous 657T>C mutation in exon 8 of the *SYCP3* gene in one patient and one fertile control. We could not find the IVS7-16_19 delACTT reported in one patient with recurrent miscarriage or 643delA reported in two patients with azoospermia in any patients with recurrent miscarriage or in fertile controls (Miyamoto et al., 2003; Bolor et al., 2009). No other new mutation was found in patients with recurrent miscarriage or controls.

Bolor et al. (2009) reported that 7.7% (2 of 26) patients with unexplained recurrent miscarriage were found to carry independent heterozygous nucleotide alterations, IVS7-16_19delACTT and 657T>C in *SYCP3*, neither of which was present among a control group of 150 fertile women. They also reported that analysis of transcripts from minigenes harboring each of these two mutations revealed that both mutations affected normal splicing, possibly resulting in the

Table I Previous miscarriage, live birth and subsequent pregnancy outcome with karyotype analysis (n = 101 patients).

Pt.	Prior history			Age (year)	Subsequent pregnancy			Karyotype	Cumulative live birth
	No. of P.M.	No of L.V.	Karyotype of previous miscarriage		Outcome		Karyotype of miscarriage		
1	2	2		37	f		47,XX,+16	A	y
2	3	0	#3 47,XY,+16	34	s	s		A	y
3	4	1		39	f		47,XX,+22	A	y
4	2	1		37	f	s	47,XX,+21	A	y
5	2	0		38	f	s	48,XX,+8,+22	A	y
6	3	0		28	f	s	47,XY,+16	A	y
7	3	0	#3 69,XXY	34	s			A	y
8	4	1	#3 47,XY,−13,+i(13)(p10),+i(13)(q10)[11]/46,XY,−13,+i(13)(q10)[19]	33	no			A	y
9	4	1	#2 47,XY,+18 stillbirth 32w	32	s			A	y
10	2	0		31	f	s	47,XX,+22	A	y
11	3	0	#3 45,X	42	s			A	y
12	4	1	#4 48,XY,+10,+13[12]/47,XY,+13[8]	35	no			A	y
13	3	0	#3 47,XX,+12	40	s			A	y
14	4	0	#4 46,XY,5cenh+,add(8)(p23)[7]/46,XY,5cenh+[13]	32	s			A	y
15	4	1	#3 47,XY,+7	38	s			A	y
16	4	1	#4 47,XY,+3	34	no			A	y
17	3	1	#3 47,XY,+16	36	s			A	y
18	4	0	46,XX,del(6)(q27)[12]/46,XX,add(6)(q27)[3]/46,XX,add(6)(q27)[2]/	37	s		46,XX,der(6)t(1;6)(q11;q27)[2]/46,XX,der(6)t(6;9)(q27;q12)[1]	A	y
19	2	0		33	f	s	48,XX,+15,+20	A	y
20	3	0	#3 45,XY, −21	30	f	s	ND*	A	y
21	3	1	#3 45,X	32	no			A	y
22	2	0	#2 47,XX,+22	32	f	s		A	y
23	3	1		40	f	s	48,XX,+14,+15	A	y
24	3	0	#3 47,XY,+16	31	s			A	y

Continued

Table I *Continued*

Pt.	Prior history			Age (year)	Subsequent pregnancy			Karyotype	Cumulative live birth
	No. of P.M.	No of L.V.	Karyotype of previous miscarriage		Outcome		Karyotype of miscarriage		
25	4	0	#3 47,+22	39	s			A	y
26	2	0		37	f		47,XY,?	A	n
27	3	0		34	f		47,XY,+16	A	n
28	2	0		39	f		47,XX,+16	A	n
29	2	0		32	f		47,XY,+16	A	n
30	5	0	#5 46,X,+16	27	f		Bio Misc	A	n
31	3	0		28	f		47,XX,+13	A	n
32	5	0		46	f		47,XX,+22	A	n
33	3	0	#3 47,XX,add(2)(q37),+20	42	no			a	n
34	4	0	#1 stillbirth 16w; #4 47,XY,+15	39	no			a	n
35	3	0		41	f		47,XX,+16	a	n
36	4	0		43	f		48,XY,+16,+21	a	n
37	4	0		38	f		46,X,+3	a	n
38	6	0	#1 stillbirth 33w; #4 45,X	37	no			a	n
39	7	0	#5 47,XX,+16; #7 45,X	30	s			aa	y
40	6	0	#6 47,XY,+16	32	f	s	47,XX,+13	aa	y
41	2	1	47,XY,+21***	30	f		47,XX,+5	aa	y
42	3	0	#3 47,XX,+16	29	f	s	47,XX,+3	aa	y
43	6	1	#6 47,XX,+16	41	f		47,XX,+12	aa	y
44	2	0	#2 46,XY,add(8)(p23)	33	f		47,XY,+16	aa	n
45	4	0	#2 45,X; #4 47,XX,+idic(8)(q?21.2)	35	no			aa	n
46	2	0		30	f	f	47,XX,+15;45,X	aa	n
47	2	0	#2 47,XX,+15	35	f		45,X	aa	n
48	14	2	#12 47,XX,+16 #14 46,XY, 2 children with previous husband	45	no			an	y
49	4	1	#3 47,XY,+16;#4 46,XX	30	f		46,XY	ann	y
50	4	0	#4 46,XY	35	s	s		n	y
51	4	0	#1 Stillbirth 28w; #4 46,XX	28	s		On-going preg.EDC06/27/11	n	y
52	4	0	#3 Stillbirth 18w	35	f	s	46,XY stillbirth 33w	n	y
53	3	0	#3 46,XY	33	s			n	y
54	5	1	#5 46,XY	36	s			n	y
55	4	1	#3 46,XY	36	no			n	y

56	3	0	#3 46,XX	30	s				n	y
57	3	0	#3 46,XX	32	s				n	y
58	2	0		34	f	s		46,XX	n	y
59	3	1	#3 46,XX	35	no				n	y
60	3	1	#3 46,XX	33	s				n	y
61	4	0	#4 stillbirth 15w	37	f	s		46,XX stillbirth 13w	n	y
62	3	0	#3 46,XX	33	s				n	y
63	3	1	#3 46,XY	37	s				n	y
64	3	0		26	f			46,XX	n	n
65	3	0		34	f			Normal Karyotype	n	n
66	5	0	46,XY	40	no				n	n
67	2	0		32	f			46,XX	n	n
68	3	0	#3 46,XY	35	f			NT**	n	n
69	2	0		39	f			46,XX	n	n
70	3	0	#3 46,XX	28	no				n	n
71	2	0		36	f			46,XX	n	n
72	2	0		30	f			46,XY	n	n
73	3	0	#3 46,XX	34	no				n	n
74	2	0		31	f			46,XY	n	n
75	3	0	#2 46,XX; #3 46,XY	31	s				nn	y
76	2	0	#2 46,XY	22	f	s		46,XY; On-going preg.EDC 05/27/11	nn	y
77	5	0	#5 46,XY	31	f			46,XY	nn	n
78	5	0	#3 46,XX; #4 46,XY; #5 46,XY	36	f	f	s	46,XX,t(11;19)(q21;q13.1) [4]/46,XX[26], Bio Misc	nnnn	y
79	6	0	#3#,4#,6 46,XX,inv(9); #5 46,XY,inv(9)	31	s			On-going preg.EDC 02/23/11	nnnn	y
80	9	0	#5 46,XX;#6 46,XX;#8 46,XY	38	f			46,XX	nnnn	n
81	13	0	#4 46,XY; 46,XX; 46,XX; 46,XX	33	f	f	f	46,XX; 46,XX; 46,XX	Nnnnnnn	n
82	3	0		31	s					y
83	3	0		39	s					y
84	3	0		26	s					y
85	3	0		38	s					y
86	23	0		33	s					y
87	3	1		35	f			Bio Misc		y
88	3	0		28	s					y
89	3	0	#2 stillbirth 15w	30	s					y
90	3	1		35	no					y

Continued

Table I *Continued*

Pt.	Prior history			Age (year)	Subsequent pregnancy		Karyotype	Cumulative live birth
	No. of P.M.	No of L.V.	Karyotype of previous miscarriage		Outcome	Karyotype of miscarriage		
91	3	2	2 children with previous husband	34	s			y
92	3	1		38	s	On-going preg.EDC03/24/11		y
93	3	0		36	s			y
94	5	0		34	f	ND*		n
95	2	0		28	f	Bio Misc		n
96	4	0		41	no			n
97	4	0		42	no			n
98	3	0		33	no			n
99	4	0		31	no			n
100	3	0	#1 stillbirth 18w; #3 stillbirth 23w	32	no			n
101	4	0		37	f	Bio Misc		n
	3.782178	0.267327		34.347				
	2.681805	0.507762		4.4033				

*ND, not detected; **NT, not tested; ***Live birth, He is 8 years old; Pt., patient; P.M., previous miscarriage; L.V., Live Birth; Bio Misc, biochemical miscarriage, decreasing hCGs < 1500 mIU/ml; age, age at examination karyotype; a, aneuploidy; n, normal karyotype (euploidy) from Pt. 1–81, karyotype were knowed in proir history or subsequent pregnancy; 'Outcome' reflect the conclusion of subsequent pregnancy; 's' means success in live birth delivery; 'f' means failure, miscarriage.

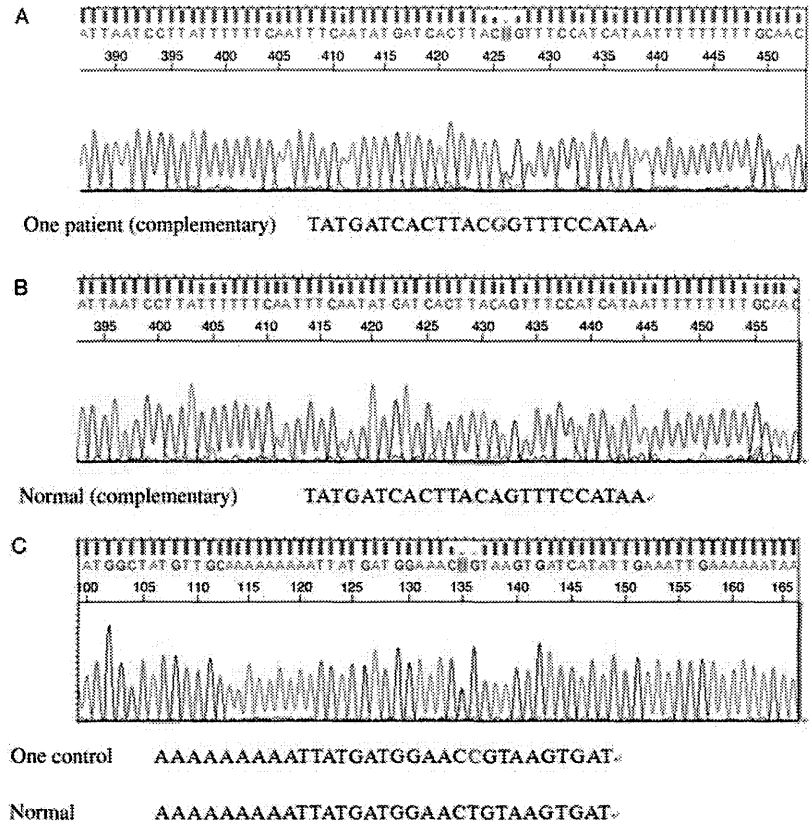

Figure 1 Nucleotide sequence results for the *SYCP3* gene in women with recurrent miscarriage and a control. (**A**) Heterozygous 657T>C mutation in exon 8 of the *SYCP3* gene of one patient with a history of six recurrent miscarriages. (**B**) Normal genotype in one patient with recurrent miscarriage. (**C**) Heterozygous 657T>C mutation in exon 8 of the *SYCP3* gene in one control with a history of one live birth and no miscarriages. From (A–C) sequences are all of the same region, and (A) and (B) sequences are complementary.

production of proteins that were mutated at the C-terminus. However, the effects of the *SYCP3* mutations on non-disjunction or the function of the synaptonemal complex have not been clear in mammals so far.

Our patient with 657T>C had repeated miscarriages with euploidy. It may be that the 657T>C mutation is a polymorphism without the specific function ascertained in the Bolor *et al.* (2009) study in humans. Further studies with larger numbers and a wide range of cases are needed to define whether the *SYCP3* mutations can be a cause of recurrent miscarriage.

Sycp3-deficient mice show complete meiotic arrest leading to male infertility (Yuan *et al.*, 2000). Miyamoto *et al.* (2003) identified in two azoospermia patients a 1 bp deletion of the *SYCP3* gene (643delA) that results in a premature stop codon and truncation of the C-terminal, coiled-coil-forming region of the SYCP3 protein. The mutant protein showed greatly reduced interaction with the wild-type protein *in vitro* (Miyamoto *et al.*, 2003). Reynolds *et al.* (2007) suggested that azoospermia associated with a decrease in the *DAZ* gene function in humans might, in part, be the consequence of failure at synapsis caused by reduced levels of the SYCP protein. However, no female patient with the 643delA mutation of SYCP3 has been reported. The infertile women might have the mutation because embryos with trisomy or monosomy except 45,X are frequently seen by PGD.

Our data showed that among the normal fetal karyotypes, XX and XY were found at a similar frequency, indicating that fetal rather than maternal karyotypes were obtained. In this study, about 90% of patients with abnormal and normal embryonic (fetal) karyotype tended to have repeat miscarriages with abnormal and normal karyotypes, respectively. The results suggest that unexplained recurrent miscarriage should be grouped as two types: one is miscarriage caused by abnormal embryonic karyotype and the other is 'real' unexplained recurrent miscarriage.

Moreover, the prognosis of a successful pregnancy for patients with an abnormal embryonic karyotype was better than for patients with a normal embryonic karyotype (Ogasawara *et al.*, 2000). No therapeutic approach to improve the rate of live birth could be found at this time (Kaandorp *et al.*, 2010). Thus, the gene associated with unexplained recurrent miscarriage with normal embryonic karyotype is more important (Suzumori and Sugiura-Ogasawara, 2010).

The results of our study suggest no clinical significance of routine screening for the presence of the *SYCP3* mutation in women with recurrent miscarriage because we detected only one benign mutation in 101 such patients. Future studies in mammalian animal models are likely to accelerate our understanding of the molecular mechanisms involved in recurrent miscarriage and will provide additional candidate genes to be screened in recurrent miscarriage patients and embryos with genetic factors.

Authors' roles

N.S., Y.O., M.N. and M.S.-O. were involved in conception and design; E.M., K.O. and C.Y-N. conducted data analysis; E.M., N.S. and M.S.-O. were involved in drafting the article. All authors agreed final approval of the version to be published.

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Efficacy of the Antiphospholipid Score for the Diagnosis of Antiphospholipid Syndrome and Its Predictive Value for Thrombotic Events

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Objective. **To define the antiphospholipid score (aPL-S) by testing multiple antiphospholipid antibodies (aPL) and to evaluate its efficacy for the diagnosis of antiphospholipid syndrome (APS) and predictive value for thrombosis.**

Methods. **This study comprised 2 independent sets of patients with autoimmune diseases. In the first set of patients (n = 233), the aPL profiles were analyzed. Five clotting assays for testing lupus anticoagulant and 6 enzyme-linked immunosorbent assays (IgG/IgM anticardiolipin antibodies, IgG/IgM anti-β_2-glycoprotein I, and IgG/IgM phosphatidylserine-dependent antiprothrombin antibodies) were included. The association of the aPL-S with a history of thrombosis/pregnancy morbidity was assessed. In the second set of patients (n = 411), the predictive value of the aPL-S for thrombosis was evaluated retrospectively. Two hundred ninety-six of these patients were followed up for >2 years. The relationship between the aPL-S and the risk of developing thrombosis was analyzed.**

Results. **In the first set of patients, the aPL-S was higher in those with thrombosis/pregnancy morbidity than in those without manifestations of APS ($P < 0.00001$). For the aPL-S, the area under the receiver operating characteristic curve value was 0.752. In the second set of patients, new thromboses developed in 32 patients. The odds ratio (OR) for thrombosis in patients with an aPL-S of ≥30 was 5.27 (95% confidence interval [95% CI] 2.32–11.95, $P < 0.0001$). By multivariate analysis, an aPL-S of ≥30 appeared to be an independent risk factor for thrombosis (hazard ratio 3.144 [95% CI 1.383–7.150], $P = 0.006$).**

Conclusion. **The aPL-S is a useful quantitative index for diagnosing APS and may be a predictive marker for thrombosis in autoimmune diseases.**

Antiphospholipid antibodies (aPL) are a heterogeneous group of circulating immunoglobulins related to diverse clinical phenomena including arterial and venous thrombosis, pregnancy complications, livedo reticularis, valvular disease, nonthrombotic neurologic disorders, and thrombocytopenia. The term antiphospholipid syndrome (APS) is used to link thrombosis and/or pregnancy morbidity to the persistence of aPL as one of the most common causes of acquired thrombophilia (1).

In particular, anticardiolipin antibodies (aCL), anti-β_2-glycoprotein I (anti-β_2GPI), and lupus anticoagulant (LAC) are associated with APS. Assays for LAC are the most traditional laboratory method used to detect aPL. Lupus anticoagulants are immunoglobulins (IgG, IgM, IgA, or their combination) that interfere with in vitro phospholipid-dependent tests of coagulation (activated partial thromboplastin time [APTT], kaolin clotting time [KCT], dilute Russell's viper venom time [dRVVT]).

In the early 1980s, radioimmunoassays and enzyme-linked immunosorbent assays (ELISAs), which

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directly detected circulating aCL, were devised (2,3). Those aCL cross-reacted with negatively charged phospholipids, such as phosphatidylserine and phosphatidylglycerol (4). Thus, the term aCL was expanded to aPL. Further studies showed the requirement of a cofactor for the binding of autoimmune aCL to solid-phase phospholipids (5–7); β_2GPI was identified as that cofactor. Beta$_2$-glycoprotein I bears the epitopes for aCL binding that are exposed when β_2GPI binds to negatively charged phospholipids (8,9).

Prothrombin, another main phospholipid binding protein, has been reported to be a probable cofactor for LAC (10–13). An ELISA for the detection of antiprothrombin antibodies (APT) using prothrombin alone as the antigen coated onto irradiated plates (APT-alone assay) was described in 1995 (14). However, the association between APT alone and clinical manifestations of APS remains controversial (15). Our group (16) and other investigators (17,18) established an ELISA to detect antibodies against the phosphatidylserine/prothrombin complex (anti-PS/PT) and observed that IgG anti-PS/PT were highly prevalent in patients with APS compared with patients with other diseases (16). We also showed that the detection of anti-PS/PT strongly correlated with the clinical manifestations of APS and with the presence of LAC.

In consideration of this historical background and, moreover, the heterogeneity of the properties of aPL, we have performed multiple aPL assays, not only for research purposes but also as routine clinical practice in our autoimmune disease clinic. In the current study, we first tried to represent the aPL profile of each patient, using a quantitative score defined as the "antiphospholipid score" (aPL-S), and analyzed the value of the aPL-S for the diagnosis of APS. We then retrospectively analyzed the predictive value of the aPL-S for thrombotic events in patients with autoimmune diseases.

PATIENTS AND METHODS

Patients. This retrospective study included 2 sets of patients from our database. The first group comprised 233 consecutive patients with systemic autoimmune diseases who were examined at the Rheumatic and Connective Tissue Disease Clinic at Hokkaido University Hospital in 2006 (study 1).

Plasma and serum samples were obtained from the patients, and all testing for aPL was performed in our laboratory. The historical profiles, clinical manifestations, and diagnoses were carefully obtained by review of the medical records or by interviewing the patients (Table 1). Arterial thrombotic events comprised stroke, myocardial infarction, and iliac artery occlusion, as confirmed by computed tomography (CT) scanning, magnetic resonance imaging, or conventional angiography. Deep vein thrombosis and pulmonary thrombosis were defined as venous thrombosis and were confirmed by CT scanning, angiography, or scintigraphy. Pregnancy morbidity was defined by the revised Sapporo criteria for APS (1).

Table 1. Characteristics of the 233 patients in study 1*

Diagnosis and manifestations	No. men/ no. women	Total
APS	5/32	37
Primary APS	1/12	13
APS with SLE	3/13	16
APS with other collagen disease	1/7	8
SLE	4/73	77
Rheumatoid arthritis	7/24	31
Sjögren's syndrome	0/18	18
Systemic sclerosis	5/9	14
Vasculitis syndrome	3/8	11
Polymyositis/dermatomyositis	0/8	8
Behçet's disease	1/5	6
Others	10/21	31
Clinical manifestations of APS	6/40	46
Thrombosis	6/32	38
Arterial thrombosis	3/24	27
Venous thrombosis	5/10	15
Pregnancy morbidity	0/14	14
Total	35/198	233

* APS = antiphospholipid syndrome; SLE = systemic lupus erythematosus.

The second group comprised 411 consecutive patients who were examined at the Rheumatic and Connective Tissue Disease Clinic between January 1, 2002 and December 31, 2003 (study 2). Among these 411 patients, those who were followed up for <2 years were excluded from the study. The final population eligible for analysis of thrombosis risk comprised 296 patients. The median followup period for the eligible patients was 72 months. The clinical profiles of these patients are described in Table 2. The study was performed in accordance with the Declaration of Helsinki and the Principles of Good Clinical Practice.

Table 2. Characteristics of the 296 patients in study 2*

Diagnosis and manifestations	No. men/ no. women	Total
APS	3/40	43
Primary APS	2/17	19
APS with SLE	1/23	24
SLE without APS	10/79	89
Rheumatoid arthritis	8/42	50
Sjögren's syndrome	0/16	16
Systemic sclerosis	4/21	25
Vasculitis syndrome	3/2	5
Polymyositis/dermatomyositis	2/8	10
Behçet's disease	4/7	11
Others	9/39	48
Newly developed thrombosis	6/26	32
Arterial thrombosis	2/20	22
Venous thrombosis	4/10	14
Total	43/253	296

* APS = antiphospholipid syndrome; SLE = systemic lupus erythematosus.

Plasma samples. Venous blood was collected into tubes containing a one-tenth volume of 0.105*M* sodium citrate and was centrifuged immediately at 4°C. Plasma samples were depleted of platelets by filtration and then stored at −80°C until used.

Determination of LAC. Three clotting tests were performed for LAC determination, using a semiautomated hemostasis analyzer (STart 4; Diagnostica Stago) according to the guidelines recommended by the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis (19). For measurement of the APTT, a sensitive reagent with a low phospholipid concentration (PTT-LA test; Diagnostica Stago) was used for screening and mixing tests, and the results were confirmed with the use of a StaClot LA kit (Diagnostica Stago). The dRVVT was used to screen for the presence of LAC, and the results were confirmed with a Gradipore LAC test. The KCT was measured using a kaolin solution (Dade-Behring) according to a standard protocol. The cutoff level of positivity for the LAC tests was previously established as above the 99th percentile of levels in 40 healthy subjects, as used for our routine laboratory assays. For defining the aPL-S, the results of the 3 mixing procedures and the 2 confirming tests were used.

Anticardiolipin antibody–anti-β_2GPI ELISA. IgG and IgM aCL were assayed according to a standard aCL ELISA (20). Normal ranges for IgG aCL (>18.5 IgG phospholipid units) and IgM aCL (>7.0 IgM phospholipid units) were previously established, using the 99th percentile of the levels in 132 healthy controls as the cutoff level of positivity.

IgG and IgM anti-β_2GPI antibodies were determined by ELISA, as previously reported (21). Purified human β_2GPI was purchased from Yamasa. Irradiated microtiter plates (MaxiSorp; Nunc) were coated with 4 μg/ml of purified β_2GPI in phosphate buffered saline (PBS) at 4°C and washed twice with PBS. To avoid nonspecific binding of proteins, wells were blocked with 150 μl of 3% gelatin (BDH Chemicals). After 3 washes with PBS containing 0.05% Tween 20 (PBS–Tween 20; Sigma), 50 μl of serum diluted with PBS containing 1% bovine serum albumin (PBS–1% BSA; Sigma) in a 1:50 dilution was added in duplicate. Plates were incubated for 1 hour at room temperature and washed 3 times with PBS–Tween 20. Fifty microliters per well of the appropriate dilution of alkaline phosphatase–conjugated goat anti-human IgG and IgM (Sigma) in PBS–1% BSA was added. After 1 hour of incubation at room temperature and after 4 washes in PBS–Tween 20, 100 μl/well of 1 mg/ml of *p*-nitrophenyl phosphate disodium (Sigma) in 1*M* diethanolamine buffer (pH 9.8) was added. Following color development, optical density at 405 nm was measured by a Multiskan Ascent plate reader (ThermoElectron Corporation). Normal ranges for IgG (>2.2 units/ml) and IgM (>6.0 units/ml) anti-β_2GPI were established, using the 99th percentile of the levels in 132 nonpregnant healthy controls as the cutoff level of positivity.

ELISA for the detection of anti-PS/PT. Anti-PS/PT antibodies were detected by ELISA, as previously described (16). Briefly, nonirradiated microtiter plates (Sumilon Type S; Sumitomo Bakelite) were coated with 30 μl of a 50-μg/ml preparation of phosphatidylserine (Sigma) and dried overnight at 4°C. To avoid nonspecific binding of proteins, wells were blocked with 150 μl of Tris buffered saline (TBS) containing 1% fatty acid–free BSA (catalog no. A6003; Sigma) and 5 m*M* $CaCl_2$ (BSA–$CaCl_2$). After 3 washes in TBS containing 0.05% Tween 20 (Sigma) and 5 m*M* $CaCl_2$, 50 μl of a 10-μg/ml preparation of human prothrombin (Diagnostica Stago) in BSA–$CaCl_2$ was added to half of the wells in the plates, and the same volume of BSA–$CaCl_2$ alone (as sample blank) was added to the other half.

After 1 hour of incubation at 37°C, the plates were washed, and 50 μl of serum diluted 1:100 in BSA–$CaCl_2$ was added to duplicate wells. Plates were incubated for 1 hour at room temperature, followed by the addition of alkaline phosphatase–conjugated goat anti-human IgG or IgM and substrate. The anti-PS/PT antibody titer of each sample was derived from the standard curve according to dilutions of the positive control. Normal ranges for IgG (>2.0 units/ml) and IgM (>9.2 units/ml) anti-PS/PT antibodies were established, using the 99th percentile of the levels in 132 nonpregnant healthy controls as the cutoff level of positivity.

Statistical analysis. Statistical analysis was performed by Mann-Whitney U test, Fisher's exact test, or chi-square test, as appropriate. *P* values less than 0.05 were considered significant. The diagnostic accuracy of the aPL-S was assessed by receiver operating characteristic (ROC) curve analysis. The Kaplan-Meier approach was used to estimate the probability of thrombosis developing after aPL testing was performed. The risk of thrombosis was evaluated using multivariate Cox regression analysis. All statistical analyses were performed using SPSS software.

RESULTS

Definition of the aPL-S. To define the aPL-S, we used the first group of patients (n = 233) with autoimmune disease. In this population, the relative risks (approximated by odds ratios [ORs]) of having clinical manifestations of APS (thrombosis and/or pregnancy morbidity) were calculated for each aPL test. Furthermore, in each test, the specificity and sensitivity for the diagnosis of APS were calculated (Table 3). To define the aPL-S, we devised an original formula in which the aPL-S was determined by the OR, as follows: aPL-S = $5 \times \exp([\text{OR}] - 5)/4$. Consequently, an OR of 5 corresponds to an aPL-S of 5. The upper limit of the score for each aPL test was determined as 20.

In the aCL, anti-β_2GPI, and anti-PS/PT ELISAs, a second cutoff level was defined to separate patients with high antibody levels from those with medium or low levels of antibodies. The definition of high titers was established as more than the median levels of antibody-positive patients in each of the tests in the entire population studied. We observed that high levels of IgG aCL, anti-β_2GPI, and anti-PS/PT antibodies were closely related to the clinical manifestations of APS. In contrast, no relationship between clinical manifestations and titers of antibodies was observed in the IgM ELISAs.

Table 3. Relative risk of clinical manifestations of APS for each aPL test*

Test	Cutoff	Sensitivity, %	Specificity, %	OR (95% CI)	aPL score
APTT mixing	>49 sec.	39.1	89.3	5.36 (2.53–11.4)	5
Confirmation test, ratio	>1.3	19.6	95.2	4.81 (1.79–12.9)	2
	>1.1	30.4	90.9	4.38 (1.96–9.76)	1
KCT mixing	>29 sec.	45.6	88.8	6.64 (3.17–13.9)	8
dRVVT mixing	>45 sec.	28.2	90.9	3.93 (1.74–8.88)	4
Confirmation test, ratio	>1.3	17.4	94.7	3.72 (1.38–10.1)	2
	>1.1	28.3	90.4	3.7 (1.65–8.27)	1
IgG aCL, GPL					
High titers	>30	15.2	98.4	11 (2.72–44.5)	20
Medium/low titers	>18.5	19.5	94.6	4.31 (1.63–11.3)	4
IgM aCL, MPL	>7	6.52	96.3	1.79 (0.45–7.22)	2
IgG anti-β_2GPI, units					
High titers	>15	23.9	98.4	19.3 (5.11–72.7)	20
Medium/low titers	>2.2	30.4	92.5	5.4 (2.35–12.4)	6
IgM anti-β_2GPI, units	>6	8.7	91.4	1.02 (0.32–3.20)	1
IgG anti-PS/PT, units					
High titers	>10	19.6	97.8	11.1 (3.25–38.1)	20
Medium/low titers	>2	28.3	95.7	8.81 (3.39–22.9)	13
IgM anti-PS/PT, units	>9.2	6.52	98.9	6.45 (1.05–39.8)	8

* APS = antiphospholipid syndrome; aPL = antiphospholipid antibody; OR = odds ratio; 95% CI = 95% confidence interval; APTT = activated partial thromboplastin time; KCT = kaolin clotting time; dRVVT = dilute Russell's viper venom time; aCL = anticardiolipin antibody; GPL = IgG phospholipid units; MPL = IgM phospholipid units; anti-β_2GPI = anti-β_2-glycoprotein I; anti-PS/PT = anti-phosphatidylserine/prothrombin complex.

Therefore, the aPL scores for the IgG aCL, anti-β_2GPI, and anti-PS/PT antibody tests were separately defined.

For the determination of LAC, APTT, dRVVT, and KCT mixing tests were performed. In case of a positive APTT or dRVVT result, a complementary confirmation test was carried out, and an additional score was given. If the result of the confirmation test was >1.3, a score of 2 was added, and if the result was >1.1, a score of 1 was added. The aPL-S for each patient was calculated as the total scores for positive aPL tests and represents the complete aPL-S.

The partial aPL-S was defined using aPL tests that were included in the updated classification criteria for APS (1) and according to the guidelines recommended by the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis (19) and included tests for IgG/IgM aCL, IgG/IgM anti-β_2GPI, and LAC (only the APTT and dRVVT).

Correlation between the aPL-S and clinical manifestations. Among the first group of 233 patients, the aPL-S ranged from 0 to 86. Forty-six patients had experienced at least 1 of the clinical manifestations of APS (thrombosis and/or pregnancy morbidity), and the scores for these patients were higher than the scores for patients who did not have such manifestations (Figure 1A).

The prevalence of APS manifestations increased in accordance with increasing antiphospholipid scores. Patients were subdivided into 5 groups according to the aPL-S as follows: score of 0, scores of 1–9, scores of 10–29, scores of 30–59, and scores of ≥60. The prevalence of APS manifestations in the 5 groups was 10%, 26%, 29%, 56%, and 89%, respectively.

The partial aPL-S was also evaluated in the same population of patients and ranged from 0 to 56. When patients were subdivided into groups according to the partial aPL-S, the prevalence of APS manifestations was 13%, 23%, 36%, 44%, and 88% for a score of 0, scores of 1–9, scores of 10–19, scores of 20–39, and scores of ≥40, respectively.

Diagnostic value of the aPL-S for APS. The ROC curves for the aPL-S, the partial aPL-S, and the revised Sapporo criteria for APS showed a hyperbolic pattern, implying that the aPL-S is a potential quantitative marker for diagnosing APS (Figure 1B). The area under the curve (AUC) values were 0.752 for the aPL-S, 0.692 for the partial aPL-S, and 0.686 for the revised Sapporo criteria. ROC analysis was performed for each of the clinical manifestation of APS. ROC curves for either arterial thrombosis, venous thrombosis, or pregnancy

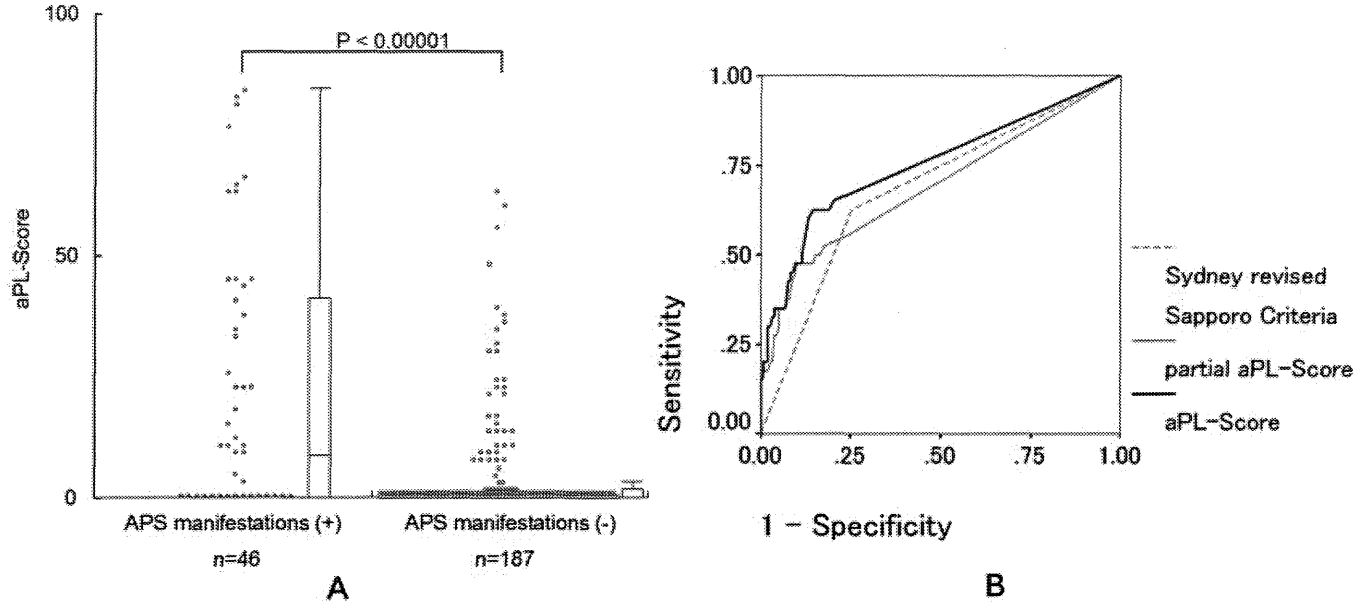

Figure 1. A, Distribution of the antiphospholipid scores (aPL-S) for patients in study 1 (n = 233). The aPL-S was defined as described in Patients and Methods. Data are shown as individual results as well as box plots, where each box represents the 25th to 75th percentiles; lines inside the box represent the median. The whisker represents the highest data still within 1.5 times the upper interquartile range. The scores for patients with antiphospholipid syndrome (APS) manifestations were significantly higher than those for patients without APS manifestations ($P < 0.00001$ by Mann-Whitney U test). **B,** Receiver operating characteristic (ROC) curves for the aPL-S, the partial aPL-S, and the revised Sapporo criteria for APS. Sensitivity and the specificity were calculated according to the presence of a history of clinical manifestations of APS. The area under the ROC curve values for the aPL-S and the partial aPL-S were 0.752 (95% confidence interval [95% CI] 0.656–0.849) and 0.692 (95% CI 0.588–0.795), respectively.

morbidity showed a hyperbolic pattern, and the AUC for each of them was larger than that for the revised Sapporo criteria (data not shown).

When the cutoff levels for the aPL-S and the partial aPL-S were defined as 30 and 20, respectively, the OR for the aPL-S (13.6 [95% confidence interval (95% CI) 4.81–38.7]) was higher than that for the revised Sapporo criteria (4.91 [95% CI 2.36–10.2]) and the partial aPL-S (7.85 [95% CI 2.99–20.7]). The sensitivity and specificity of an aPL-S of <30 were 35% and 96%, respectively, compared with 26% and 95%, respectively, for a partial aPL-S of <20 and 63% and 75%, respectively, for the revised Sapporo criteria.

Development of new thrombotic events. In the second group of patients, we retrospectively evaluated the relationship between the aPL-S and the risk of new thrombosis. This analysis included all thrombotic events that developed since the day the aPL-S was determined until the last followup in 2009.

During the followup period, new thromboses developed in 32 patients (22 arterial thrombotic events and 14 venous thrombotic events; some patients had both events). The aPL-S among patients in whom thromboses developed was significantly higher than that among those without thrombotic events during the followup (median score 5.5 versus 0; $P = 0.012$ by Mann-Whitney U test). This was also the case for the partial aPL-S (median score 5 versus 0; $P = 0.001$ by Mann-Whitney U test).

Predictive value of the aPL-S for APS manifestations. Patients with a higher aPL-S had a stronger risk of thrombosis compared with patients with lower scores. The ORs for newly developed thrombosis in patients with an aPL-S of ≥10, ≥30, and ≥50 were 2.86 (95% CI 1.33–6.6, $P = 0.006$), 5.27 (95% CI 2.32–11.95, $P < 0.0001$), and 5.31 (95% CI 1.81–15.53, $P = 0.0008$). The positive predictive values of an aPL-S of ≥10, ≥30, and ≥50 were 20%, 31%, and 35%, respectively, whereas the negative predictive values were 92%, 92%, and 91%, respectively. For the partial aPL-S, the positive predictive values of scores ≥10, ≥20, and ≥40 were 21%, 16%, and 25%, respectively, and the negative predictive values were 92%, 91%, and 91%, respectively (Figure 2A).

Effect of antithrombotic therapy. The effect of treatment on the aPL-S was evaluated in patients with an aPL-S of ≥30. This group included 39 patients (14 with primary APS, 15 with APS and SLE, and 10 with other autoimmune diseases), and 34 (87%) received

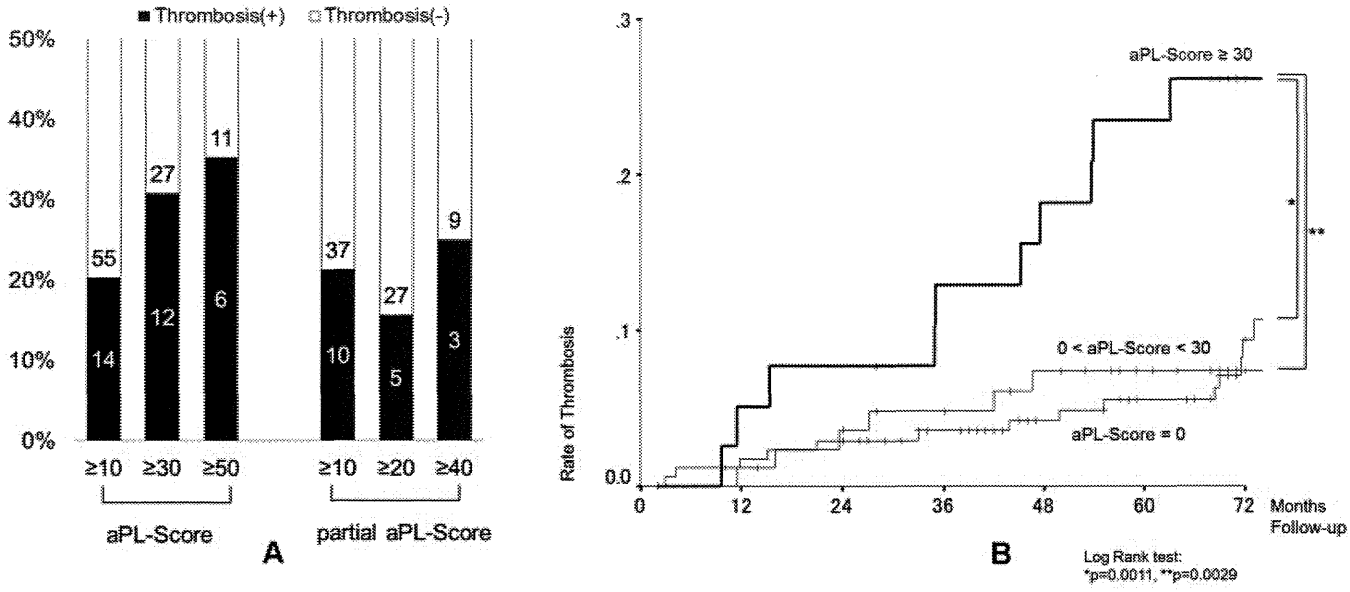

Figure 2. A, Positive predictive values (PPVs) of the antiphospholipid score (aPL-S) and the partial aPL-S for thrombosis in study 2. The numbers inside the bars represent the numbers of patients. The PPVs of an aPL-S of ≥10, ≥30, and ≥50 were 20.3%, 30.8%, and 35.3%, respectively. The PPVs of the aPL-S gradually increased in accordance with the cutoff value of the aPL-S, though the PPVs of the partial aPL-S did not. The negative predictive values of the aPL-S and the partial aPL-S were similar (90.5–92.2%). **B,** Kaplan-Meier analysis of the rate of thrombosis among patients in study 2, according to the aPL-S.

some antithrombotic medications. In 12 (31%) of these 39 patients, 15 new thromboses developed during the followup period despite antithrombotic therapy. The prevalence of thromboses among patients with an aPL-S of ≥30 was higher than that among those with a lower aPL-S (OR 5.40, 95% CI 2.38–12.23, $P = 0.00015$). The incidence rate of thrombosis among patients with an aPL-S of ≥30 was 5.144/100 person-years, whereas the rate among those with an aPL-S of 0 (no aPL) was 1.455/100 person-years. The rate of thrombosis among patients with an aPL-S of ≥30 was significantly higher than that among those with lower scores ($P = 0.0011$ for patients with an aPL-S of 0 and $P = 0.0029$ for patients with an aPL-S of 1–29, by log-rank test) (Figure 2B). In contrast, the partial aPL-S did not show significant correlation with the development of thrombosis.

To analyze the risk of thrombosis, multivariate Cox regression tests were conducted using the following data: aPL-S ≥30, age, sex, treatment with glucocorticoids, and the presence of hypertension, hyperlipidemia, diabetes, systemic lupus erythematosus, or rheumatoid arthritis at the time the aPL assays were performed. An aPL-S of ≥30 appeared to be an independent risk factor for thrombosis (hazard ratio [HR] 3.144, 95% CI 1.383–7.150, $P = 0.006$) (Table 4). A partial aPL-S of ≥20 was also analyzed using the same statistics but was not revealed to be an independent risk factor for thrombosis (HR 1.525, 95% CI 0.581–4.007, $P = 0.391$).

Table 4. Risk factors for thrombosis in autoimmune disease, as determined using multivariate analysis

Risk factor	Hazard ratio (95% CI)*
Glucocorticoid treatment	1.979 (0.809–4.842)
History of thrombosis	1.401 (0.640–3.068)
Hypertension	1.621 (0.750–3.504)
Hyperlipidemia	1.917 (0.927–3.966)
Diabetes	0.963 (0.394–2.355)
Age	1.017 (0.987–1.047)
Male sex	1.002 (0.385–2.606)
Systemic lupus erythematosus	1.052 (0.480–2.303)
Rheumatoid arthritis	0.470 (0.101–2.181)
Antiphospholipid score ≥30	3.144 (1.383–7.150)†

* 95% CI = 95% confidence interval.
† $P = 0.006$.

DISCUSSION

In this study, we demonstrated that the profile of aPL can be successfully quantitated as the aPL-S. The aPL-S level correlated with a history of thrombosis or pregnancy morbidity, suggesting that the aPL-S is a potential quantitative marker of APS. Therefore, the

current aPL-S can be unified and become a marker of the probability of having APS. Furthermore, we confirmed that the aPL-S had predictive value for recurrence and/or new onset of thrombotic events in the autoimmune disease setting. This fact suggests that treatment of APS can be modified considering the aPL-S.

Although aPL, as a group of autoantibodies sharing their properties in the phospholipid-associated molecules or reactions (22–27), have a strong link to thrombosis/pregnancy morbidity, the value of each aPL determination as a marker of APS is still not elucidated (28–32). Antiphospholipid antibodies are significantly prevalent in patients with infectious diseases, autoimmune diseases, malignant diseases, or hepatic diseases and even in healthy elderly individuals (33–37). One of the major issues involving the classification of APS has been avoiding overdiagnosis of APS by not accepting a positive result of a nonspecific aPL test as diagnostic (38). According to the APS criteria, aPL must be detected on 2 occasions not less than 12 weeks apart to determine that the presence of aPL is not transient. A low titer of aCL is not considered to be a marker of APS, although a "low positive" titer is a statistically abnormal laboratory phenomenon. However, efforts have not been successful enough, because aPL are found in many settings other than APS. In addition, updated diagnostic algorithms for catastrophic APS have been proposed, but no particular aPL has been proven to be associated with that syndrome (39).

In addition, standardization of each aPL assay has been extremely difficult. The presence of aPL defines the APS; thus, the greatest efforts have been made since the mid 1980s, when aCL were described (40,41). However, a number of variables in the assay, such as techniques, reagents, and standards, have hampered achievement of consensus (2), as described by de Groot et al in their article "Twenty-two years of failure to set up undisputed assays to detect patients with the antiphospholipid syndrome" (42). Considering the history of standardization, the establishment of a single aPL to define APS is unlikely in the near future.

In contrast, the premise that aPL represent the risk of thrombotic events and/or pregnancy morbidity either in the past or in the future would not be disputed (38,43–45). Accordingly, it would be more sensible to use aPL tests to establish an aPL profile as a marker of thrombotic risk rather than using these tests for diagnosis. Furthermore, combining multiple aPL tests would compensate for or reduce the disadvantage of each single aPL. From this point of view, our definition of the aPL-S has been proven to represent the "probability" or "likelihood" of having APS, depending on the level of the score; higher antiphospholipid scores were associated with higher risks of thrombotic events or pregnancy morbidity.

In the second part of the study, we retrospectively evaluated the value of the aPL-S for predicting the development of APS-related events in patients with autoimmune diseases. Despite receiving standard antithrombosis prophylaxis, many patients developed thrombosis during the followup period. In this cohort, the aPL-S showed a positive correlation with the risk of thrombotic events and had a significant predictive value. Those data would lead to a potential therapeutic strategy in which the intensity of antithrombotic treatment could be determined according to the aPL-S.

In clinical practice, all aPL tests are not available to all physicians. Therefore, we also defined a partial aPL-S that corresponds to the total score for the aPL tests included in the classification criteria for definite APS (1). For calculation of the partial aPL-S, the KCT mixing test and the anti-PS/PT IgG and IgM tests were excluded. The results for the complete aPL-S derived from the full battery of tests were compared with those for the partial aPL-S. A partial aPL-S seems to be a useful tool with which to evaluate the risk of thrombosis in patients with aPL (diagnostic value). However, although the aPL-S showed a positive predictive value for thrombosis that gradually increased in accordance with increasing scores, this increasing tendency was not observed with the partial aPL-S. None of the combinations of aPL tests used to define the aPL-S showed better relevance for the diagnosis of APS or for the prediction of thrombosis than the original complete aPL-S (data not shown). Inclusion of anti-PS/PT antibodies in the battery of aPL tests allows better quantification of the thrombosis risk.

Recently, Pengo et al (46) reported that in their cross-sectional study, patients with triple positivity for aCL, LAC, and anti-β_2GPI had a greater risk of thrombotic events than those who were positive for only 1 or 2 of these antibodies, which supports, in part, our findings. In the Pengo study, triple positivity was categorical (i.e., either present or absent), but our criteria were more quantitative, as proven by the ROC curves. Further, in the study of Pengo et al, anti-PS/PT antibodies were not considered. In their analysis, patients with prothrombin-dependent LAC and anti-PS/PT antibody positivity could be classified as single-positive for LAC, although this group of patients had a higher risk of APS than those with aPL positivity alone (47,48). In any case, the

combination of aPL tests should be considered when discussing the risk of thrombosis/pregnancy morbidity.

In the current study, we proved the efficacy of the aPL-S as a marker of the "probability" of APS and its value for predicting thrombosis in the setting of autoimmunity. This study is the first to attempt scoring the aPL profile, and the aPL-S successfully correlated with the risk of thrombotic events. However, the score could have other definitions, according to the population, and obviously the "true" predictive value should be validated in prospective studies. Higher accuracy of the aPL-S is obtained when all aPL tests are included. However, in clinical practice and trials, if all of the tests are not accessible, a partial aPL-S will provide important information regarding the thrombosis risk for each patient and consequently will help clinicians in making decisions about the therapeutic approach.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Atsumi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Otomo, Atsumi, Amengual, Fujieda, Kato, Oku, Horita, Yasuda, Koike.

Acquisition of data. Otomo, Atsumi, Amengual, Fujieda, Kato, Oku, Horita, Yasuda, Koike.

Analysis and interpretation of data. Otomo, Atsumi, Amengual, Fujieda, Kato, Oku, Horita, Yasuda, Koike.

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