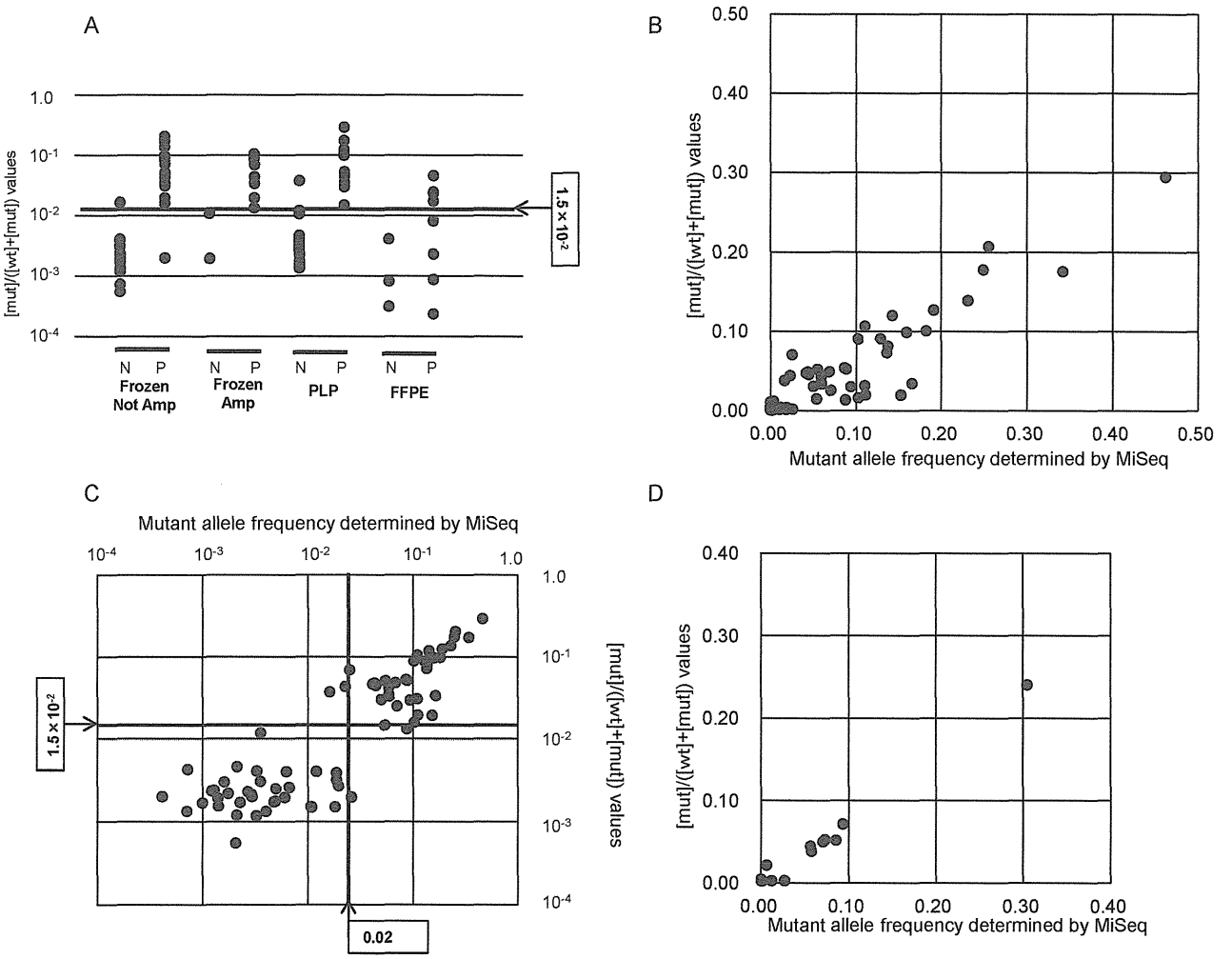

Figure 4. qAS-PCR of AITL and PTCL-NOS samples. A, Shown are [mut]/([wt]+[mut]) values for each sample. N, mutation negative determined by MiSeq; P, mutation positive determined by MiSeq; Amp, amplified; PLP, periodate/lysine/paraformaldehyde-fixed; FFPE, formalin-fixed/paraffin-embedded. B, Comparison of [mut]/([wt]+[mut]) values by qAS-PCR and mutant allele frequencies as determined by MiSeq for 95 original or whole genome-amplified DNA samples, including 43 AITL and 52 PTCL-NOS. Cut-off values were determined as 1.5×10^{-2} for [mut]/([wt]+[mut]) by qAS-PCR and as 0.02 for mutant allele frequencies as determined by MiSeq. C, Comparison of [mut]/([wt]+[mut]) values by qAS-PCR and mutant allele frequencies as determined by MiSeq for 95 DNA samples in a log scales. D, Comparison of [mut]/([wt]+[mut]) values by qAS-PCR and mutant allele frequencies as determined by MiSeq for 13 FFPE PCR-amplicon samples.
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from MiSeq-positive FFPE samples were significantly lower than those from other MiSeq-positive samples (Miseq-positive FFPE vs MiSeq-positive other samples; 1.56×10^{-2} vs. 9.38×10^{-2}, $p<0.05$, Student's t-test) (Figure 4A). Four out of all 8 MiSeq-positive FFPE samples were negative by qAS-PCR. Therefore, we excluded FFPE samples and analyzed data from 95 DNA samples that had been purified from PLP-fixed or frozen tissues.

When [mut]/([wt]+[mut]) values were compared with mutant variant allele frequencies determined by MiSeq, the rank correlation coefficient was 0.785 (Spearman's correlation $P<0.001$) (Figure 4B and C). Among the 95 samples analyzed, 38 (29 AITL and 9 PTCL-NOS) were judged positive and 57 (14 AITL and 43 PTCL-NOS) were judged negative by MiSeq. By comparison, when the cut-off level for [mut]/([wt]+[mut]) values was set at 1.5×10^{-2}, according to ROC curve (Supplemental Figure 1), 38 cases were judged positive for the G17V *RHOA* mutation, including 29 AITL and 9 PTCL-NOS. Overall, 91 of 95 specimens showed concordant results using both methods, while 4 cases showed discordant results (Figure 4B and C). If we assume that data generated by MiSeq is accurate, then the sensitivity and specificity of qAS-PCR were as high as 94.7% and 96.5%, respectively. Positive and negative concordance rates of the two methods were 94.7% and 96.5%, respectively (Table 4, Table S1 in File S1).

The four cases showing discordant results provided us with an insight into the comparison between MiSeq and aAS-PCR. Two samples were positive only based on MiSeq, and two were positive only by qAS-PCR. When we performed HISEQ2000 sequencing [12] for all these four samples, we observed $\geqq 0.02$ mutation allele frequencies in two samples. One had been deemed positive only by qAS-PCR and the other only by MiSeq. The other two samples showed <0.02 mutation allele frequencies by HISEQ2000. One of them was judged as negative only by qAS-PCR and the other only by MiSeq. Overall, accuracy with qAS-PCR and MiSeq was comparable.

Table 4. Correlation between qAS-PCR and MiSeq.

Method	Standard	Samples			N*[1]	RCC*[2]	Sensitivity	Specificity	PPV*[3]	NPV*[4]
qAS-PCR	MiSeq	AITL and PTCL-NOS	non- FFPE	all	95	0.785	94.7	96.5	94.7	96.5
				original	66	0.735	100.0	95.5	91.7	100.0
				WGA*[5]	29	0.822	87.5	100.0	100	86.7
			FFPE *[6]		13	0.919	87.5	80.0	87.5	80.0

*[1]N, number; *[2]RCC, rank correlation coefficient; *[3]PPV, positive predictive value; *[4]NPV, negative predictive value, *[5]WGA, whole-genome amplification. *[6]FFPE, formalin-fixed/paraffin-embedded.

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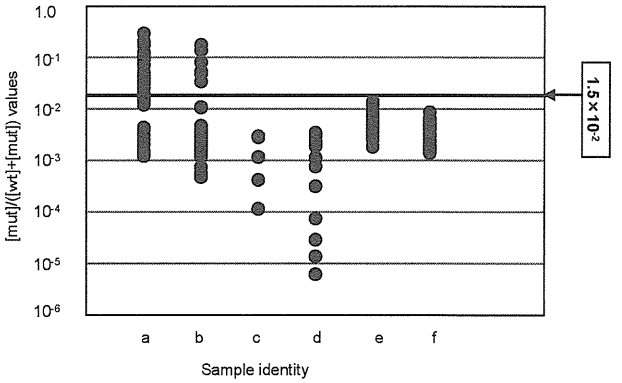

Figure 5. qAS-PCR for 275 tumor and control samples. qAS-PCR was performed for tumor samples, including 43 AITL (a), 52 PTCL-NOS (b), 5 T-cell lymphoma other than AITL and PTCL-NOS (c), 19 B-cell lymphomas (d), 129 myeloid malignancies (e) and 27 control samples (f).

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The qAS-PCR method using 50 ng of whole-genome-amplified DNA did not provide a robust correlation with the Miseq data for FFPE samples. The main reason was likely to be fragmentation of genomic DNA. To overcome this limitation, DNA prepared from the 13 FFPE samples was pre-amplified by PCR prior to performing qAS-PCR. Sensitivity and specificity for FFPE samples using amplicon was 87.5% and 80.0%, respectively, based on the mutation allele frequencies determined by MiSeq. (Figure 4D, Table S2 in File S1). Therefore, even for FFPE samples, the qAS-PCR method could robustly estimate the G17V RHOA mutation allele frequencies.

Effect of whole-genome amplification for qAS-PCR

When we divided the 95 samples into original DNA and whole-genome-amplified DNA cohort, sensitivity and specificity were 100% and 95.5% for original DNA cohort, and 87.5% and 100% for whole-genome-amplified DNA cohort, respectively (Supplemental Figure 2A-D, Table S3A and B in File S1).

In order to determine whether amplification influences the evaluation of mutation allele frequency by qAS-PCR, we compared the data for 15 pairs of original and whole-genome-amplified samples. Fourteen out of 15 pairs showed concordant results with each other (Table S3C and D in File S1, Figure S2E in File S1). One sample, which was judged positive by MiSeq, showed discordant results by qAS-PCR; positive for the original DNA and negative for the whole-genome-amplified DNA. As a summary, with some limitations, whole-genome-amplified DNA could provide robust results in most cases.

qAS-PCR for myeloid, B-cell and other T-cell malignancies

We performed qAS-PCR for buccal cells and non-tumor samples including bone marrow cells without lymphoma infiltration obtained from lymphoma patients, and confirmed that the qAS-PCR values were below the cut-off level in all samples. Then, we applied qAS-PCR for 153 tumor samples other than AITL and PTCL-NOS, including 129 myeloid, 19 B-cell, and 5 T-cell malignancies. Sanger sequencing also showed no mutant signals for any of these samples. All qAS-PCR values calculated using these samples were below the cut-off level (Figure 5).

Discussion

Our recent discovery of the highly frequent G17V *RHOA* mutation in AITL and AITL-like PTCL-NOS led us to develop a novel method to detect this mutation [12]. The results of qAS-PCR analysis described here are correlated well with those derived from deep sequencing (Table 4), while qAS-PCR is superior to deep sequencing in terms of the cost and convenience. There is a pressing clinical need for a well-validated *RHOA* testing method with optimal analytical performance using the least amount of difficult-to-obtain patient specimens. We show here that even DNA samples subjected to whole-genome amplification or low quality/concentration DNA extracted from FFPE samples can serve as reliable material for our qAS-PCR method, if appropriate PCR procedure and primers are used. Allele-specific PCR for G17V *RHOA* mutation was mentioned in other report [13], although sensitivity and specificity of the methods were not described.

In a previous study, we defined the cut-off level of mutant allele frequencies determined by MiSeq as 0.02 [12]. In this study, we defined the cut-off level as 1.5×10^{-2} for qAS-PCR, but it remains to be determined whether these cut-off levels are sufficient to detect AITL. Given our finding that the mutated *RHOA* allele frequencies distributed below 0.05 in many AITL samples [12], the tumor cell content might be very low and could be detected in some cases only when the cut-off levels of qAS-PCR and deep sequencing are lowered. If we set the cut-off value lower, the sensitivity should be improved with the increase of false-positive results, raising a dilemma common to other clinical testings.

Several hotspot mutations that reveal distinct hematologic malignancies have been identified in conditions other than T-cell lymphomas. For example, detection of the V617F *JAK2* mutation is a part of the diagnostic criteria for myeloproliferative neoplasms in the latest version of WHO classification [1], although consensus is not reached about the detection methods and cut-off levels. Methods have been developed to detect this mutation including allele-specific PCR and a PCR-restriction fragment length polymorphism (RFLP) approach utilizing mutation sequence specificity for a restriction enzyme[15–18]. More recently, a V600E *BRAF* mutation in hairy cell leukemia [19], an L265P *MYD* mutation in Waldenström macrogloblinemia [20], and several mutations in *STAT3* in large granular lymphocytic leukemia [21] have been identified as diagnostics of these tumor types. In the future, it is likely that molecular alterations, including the G17V *RHOA* mutation, will be increasingly incorporated into the diagnostic criteria for hematologic malignancies. In summary, our novel method to detect the G17V *RHOA* mutation could provide an important clinical tool to diagnose AITL and AITL-like PTCL-NOS and in the future serve as a means to classify AITL and PTCL-NOS.

Supporting Information

File S1 Figures S1–S2 and Tables S1–S4. Figure S1. ROC curve for data of qAS-PCR and MiSeq. Horizontal axis shows 1-specificity and Vertical axis shows sensitivity of qAS-PCR method compared to the data of MiSeq. Figure S2. Effect of whole-genome amplification for qAS-PCR A, Comparison of [mut]/([wt]+[mut]) values by qAS-PCR and mutant allele frequencies as determined by MiSeq for 66 original samples (linear). B, Comparison of [mut]/([wt]+[mut]) values by qAS-PCR and mutant allele frequencies as determined by MiSeq for 66 original samples (log scale). C, Comparison of [mut]/([wt]+[mut]) values by qAS-PCR and mutant allele frequencies as determined by MiSeq for 29 whole-genome amplified samples (linear). D, Comparison of [mut]/([wt]+[mut]) values by qAS-PCR and mutant allele frequencies as determined by MiSeq for 29 whole-genome amplified samples (log scale). E, Comparison of [mut]/([wt]+[mut]) values by qAS-PCR for 15 pairs of original and whole-genome amplified samples in a log scale.
(PDF)

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Author Contributions

Conceived and designed the experiments: RN-M MS-Y SC. Performed the experiments: RN-M. Analyzed the data: RN-M MS-Y KY SY Y. Shiozawa TN KS MS SO KT NN. Contributed reagents/materials/analysis tools: TE HM NO T. Kato NK YY KI YO SS T. Komeno Y. Sato TI IK. Contributed to the writing of the manuscript: RN-M MS-Y SC.

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CASE REPORT

Pheochromocytoma as the first manifestation of MEN2A with RET mutation S891A: report of a case

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Abstract We report a rare case with pheochromocytoma as the first manifestation of multiple endocrine neoplasia type 2A with *RET* mutation S891A. Bilateral pheochromocytomas were identified in a 54-year-old woman. Screening for RET revealed a rare S891A mutation located in the intracellular tyrosine kinase domain. This mutation was previously recognized as one of the mutations only in cases manifesting solely medullary thyroid carcinomas (MTCs). Since calcitonin stimulation test indicated positive result, total thyroidectomy was performed 1 year after the bilateral adrenalectomy, and C-cell hyperplasia was diagnosed by histopathological examination. Our report suggests that cases with S891A mutation, akin to those with other *RET* mutations, require screening for pheochromocytoma. In addition, it is indicated that calcitonin stimulation test should be performed even in the unaffected elder cases with S891A mutation although the mutation is classified as lowest risk group on MTC in guidelines.

Keywords MEN2A · RET · Pheochromocytoma

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Introduction

Multiple endocrine neoplasia type 2 (MEN2) is an autosomal, dominantly inherited disorder manifesting various combinations of medullary thyroid carcinoma (MTC) and pheochromocytoma, with hyperparathyroidism (MEN2A) or neuromas of the enteric autonomic nerve cells (MEN2B). MEN2 is caused by the gain-of-function mutation in the *RET* protooncogene, encoding a transmembrane receptor tyrosine kinase [1, 2]. Most of the mutations, found in the cysteine-rich extracellular domain, give rise to ligand-independent receptor dimerization and cross-phosphorylation, leading to constitutive activation of the downstream signal of the receptor [3]. Mutation of the cysteine codon 634 constitutes 80–90 % of MEN2A cases, although those caused by mutations of the cysteine codon 611, 618, and 620 are also observed. Although they are a minor subset, *RET* mutations in MEN2A cases are also identified within the intracellular domain, including those originally reported as mutations of familial medullary thyroid carcinoma (FMTC). On the other hand, most cases with MEN2B carry M918T or A883F mutations in the tyrosine kinase domain, suggesting strong genotype–phenotype correlations.

The discovery of strong genotype–phenotype correlations that govern the development of MEN2A-associated endocrine neoplasia in MEN2 cases has prompted us to utilize the identified *RET* mutations for the prediction of

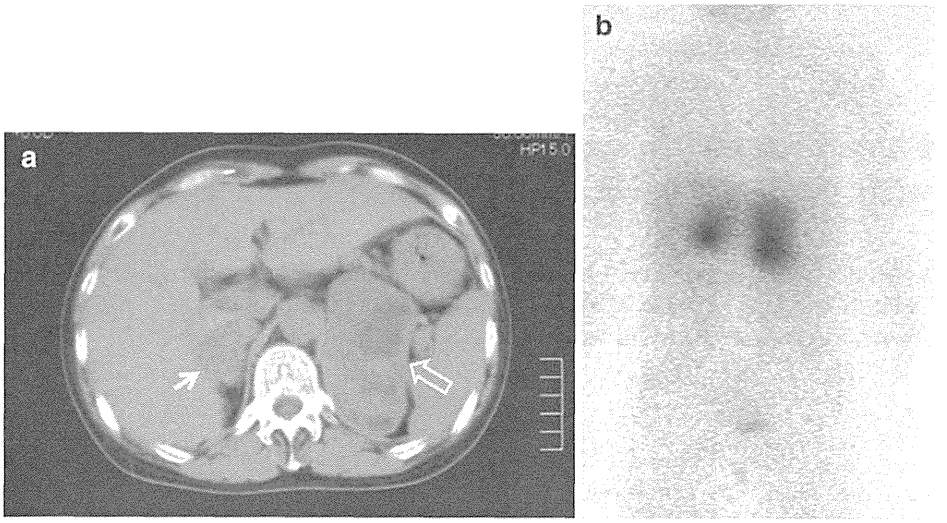

Fig. 1 **a** Computed tomography of abdomen shows *right* (*arrow*) and *left* (*open arrow*) adrenal tumors. **b** ^{131}I-MIBG scan reveals abnormal uptakes indicating bilateral pheochromocytomas

prognosis, and for the determination of surgical concept. In particular, mutations at codons 609, 768, 790, 791, 804 and 891 are classified as level 1, having the lowest risk for aggressiveness among the three levels of MTC [4]. A rare mutation, S891A, has been associated solely with intermediate-risk FMTC [5–7]. For the carriers of such FMTC mutations, intensive screening for age-related development of pheochromocytoma need not be started until they are 20 years old [7]. However, a rare case with a S891A mutation expressing MTC and pheochromocytoma was recently reported, suggesting the limitation of genotype-based predictions [8].

In this manuscript, we report a rare case of a patient who was affected by bilateral pheochromocytomas as the first manifestation of MEN2A, whose subsequent screening for *RET* mutation identified S891A.

Case report

A 57-year-old woman visited a local hospital with cough and vomiting. She had episodes of periodic headaches and paroxysmal palpitations. Bilateral adrenal tumors were identified by abdominal CT scan (maximum diameter of 4 cm for right mass and 9 cm for left mass) (Fig. 1a). Pheochromocytomas were diagnosed by ^{131}I-metaiodobenzylguanidine (MIBG) scan (Fig. 1b), along with elevated urinary catecholamine and metabolite concentrations (Table 1), and she was, therefore, transferred to the Fujita Health University Hospital for surgical management. On admission, blood pressure was 111/68 mmHg. Since her maternal aunt had adrenal disease and had died from cerebral vascular disease, MEN2A was strongly suspected. Although her basal serum calcitonin level was normal(37.0 pg/ml), elevated levels were recorded following stimulation with 2 mg/kg of Ca^{2+} infusion (330 pg/ml). However, neither ultrasonography nor ^{99m}Tc-methoxy-isobutyl-isonitrile (MIBI) scan showed any cervical lesions. Although serum PTH level was elevated (340.5 pg/ml), serum calcium and phosphorus were within normal range (9.3, 3.8 mg/dl), and urinary Ca excretion was not elevated (Ca/creatinine = 0.163). Finally, screening for the *RET* gene was performed after appropriate informed consent was obtained (approved by the Ethical Review Board for Human Genome Studies at Fujita Health University), and, unexpectedly, the S891A mutation was identified.

Table 1 Urinary catecholamine and metabolite level

	mg/day	(Normal range)
Adrenaline	961.6	(3.4–26.9)
Noradrenaline	180.6	(48.6–168.4)
Dopamine	1247.8	(365.0–961.5)
VMA	43.1	(1.5–4.3)
HVA	7.1	(2.1–6.3)
Metanephrine	23.02	(0.04–0.19)
Normetanephrine	4.96	(0.09–0.33)

Bilateral adrenal tumors were surgically resected, and the diagnosis of pheochromocytomas was confirmed by histological examination (Fig. 2a). One year later, the patient underwent prophylactic total thyroidectomy. Histological examination demonstrated that multiple nodal lesions were scattered, indicating the presence of C-cell hyperplasia without any evidence of MTC (Fig. 2b, c). Regarding the four resected parathyroid glands (Fig. 3a), two right glands were slightly enlarged (231 mg for superior and 118 mg for inferior glands) compared with the two left glands (48 mg for superior and 15 mg for inferior glands). However, no specific histological change was

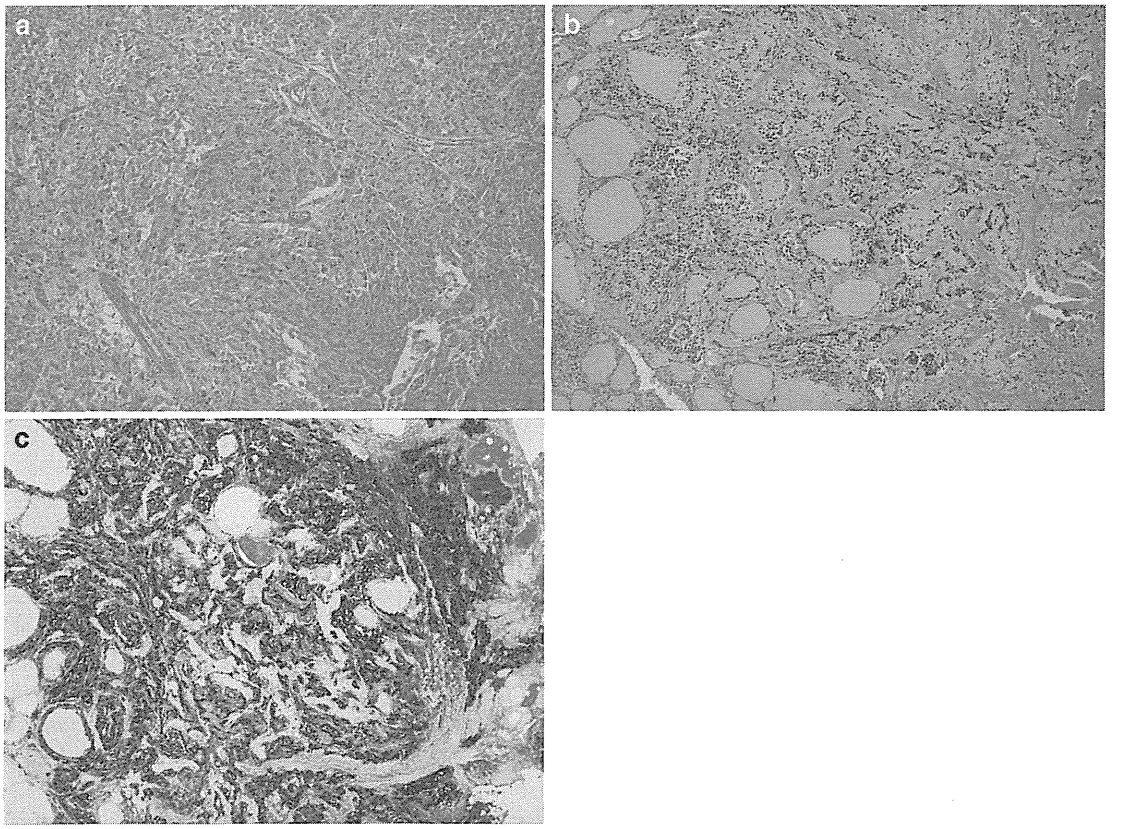

Fig. 2 **a** Microscopy of *left* adrenal tumor shows pheochromocytoma (hematoxylin & eosin, ×100). **B** Microscopy of thyroid gland shows C-cell hyperplasia (hematoxylin &eosin, ×100). **c** Microscopy of thyroid gland shows C-cell hyperplasia (calcitonin immunostaining, ×100)

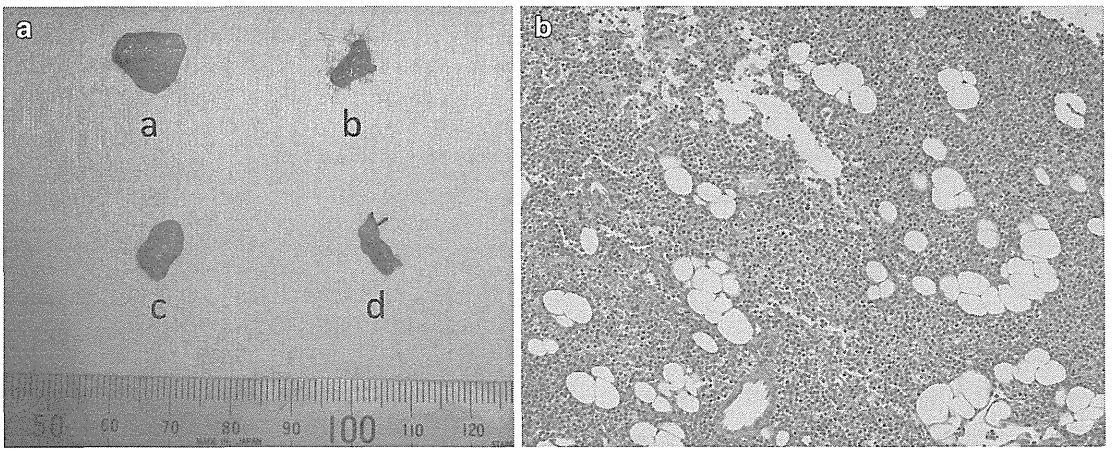

Fig. 3 **a** Macroscopy of the resected parathyroid glands shows slightly enlarged *right upper* and *lower* gland. (**a** *right upper* gland, **b** *left upper* gland, **c** *right lower* gland, **d** *left lower* gland) **b** Microscopy of right upper parathyroid gland shows no hyperplasic change (hematoxylin & eosin, ×100)

observed even in the enlarged glands (Fig. 3b). Approximately, one-third of the resected parathyroid glands were implanted into the muscle of the left forearm.

Currently, the patient's two brothers (59 and 53 years old, respectively) and three sisters (69, 64 and 61 years old, respectively) do not have any clinical symptoms associated with MEN2A, although screening for *RET* mutation has not been performed for them yet. The patient has two sons, 29 and 27 years old, neither of whom have clinical symptoms. The 27-year-old son requested *RET* mutation screening,

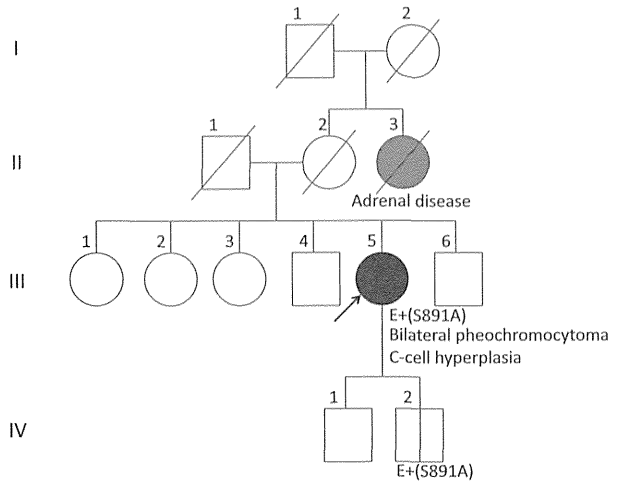

Fig. 4 Pedigree of the family. The *arrow* indicates the proband. The II-3, who was affected by adrenal disease and died from cerebral vascular disease, might possibly carry the S891A mutation

and indeed, the S891A mutation was identified (Fig. 4). Routine chemical screening of the blood, including basal serum calcitonin levels, was all normal. Ultrasonography did not detect any mass within the thyroid. He is being followed up carefully as a presymptomatic MEN2A case.

Discussion

S891A mutation constitutes 2 % of all *RET* mutations identified in MEN2/FMTC cases [9]. Early reports stress the association of this mutation with FMTC, but accumulating evidence shows the mutant's capacity to induce a wider spectrum of MEN2A [10]. S891A mutation causes MTC in 63.5 % of cases, pheochromocytoma in 4.1 % of cases and parathyroid hyperplasia in 4.1 % of cases [10]. Compilation of MEN2A-related clinical manifestation in patients with RETS891A mutation in previous reports [5, 6, 8, 10–17] is described in Table 2. Indeed, the management guideline of medullary thyroid cancer by the American Thyroid Association categorizes pheochromocytoma in S891A mutation as 'rare' [18]. None of ten Japanese cases with S891A mutation reported in a recent study had pheochromocytoma [17], but our case report combined with previous data indicates that S891A patients as well as other MEN2A patients require early detection of subclinical pheochromocytoma to prevent a potential hypertensive crisis In MEN2 patients, the gain-of-function mutation in the *RET* receptor tyrosine kinase gene constitutively activates the downstream signals, leading to transformation of the cells [3]. Although the mutations in the cysteine-rich extracellular domain all target cysteine codons, inducing ligand-independent RET dimerization, the mutations located in the intracellular tyrosine kinase domain do not target cysteine codons. These mutations, including S891A, are considered to give rise to structural changes in the protein facilitating the access of adenosine triphosphate and substrate to the catalytic site [19]. The less constitutive RET kinase activation relative to mutations in the cysteine-rich extracellular domain might result in less neoplastic transforming capacity [20, 21].

The S891A mutation is classified as level 1 [4] or level A [18], the lowest risk group among the three (level 1–3) or the four (level A–D) *RET* codon mutation stratification

Table 2 MEN2A-related clinical manifestation in patients with RETS891A mutation in previous reports

	Total patients	No. affected patients	No. asymptomatic gene carriers	Mean age at Dx (year)	MTC	CCH	PH	PHEO
Schulte et al. [10]	36	33	3	41	25	23	3	1
Jimenez et al. [8]	6	3	3	45	2	2	0	1
Hofstra et al. [5]	5	3	2	47	3	1	0	0
Dang et al. [6]	3	3	0	ND	3	ND	ND	ND
Elisei et al. [11]	14	6	8	44	6	ND	ND	ND
Yip et al. [12]	3	3	0	ND	3	DC	0	0
Asari et al. [13]	1	1	0	ND	1	0	0	1
Paszko et al. [14]	2	2	0	ND	2	0	0	0
Wohllk et al. [15]	4	2	2	49	2	2	0	0
Frank-Raue et al. [16]	5	5	0	ND	1	4	ND	ND
Imai et al. [17]	10	ND	ND	ND	ND	ND	ND	0
Total	89	61	18		48	32	3	3

Modified Table 2 in [10]

DC data clustered, *Dx* diagnosis, *ND* no data, *MTC* medullary thyroid carcinoma, *CCH* C-cell hyperplasia, *PH* primary hyperplasia, *PHEO* pheochromocytoma

categories. The penetrance and aggressiveness of MTC arising in cases with S891A are variable, but MTC develops at a later age and grows more slowly than with the higher risk mutations. There has been little consensus concerning the management of patients with level 1/level A mutations. According to the guidelines, cases with S891A mutation still need prophylactic resection, and some experts recommended thyroidectomy by the age of 5 years, while others suggest that thyroidectomy by the age of 10 years is appropriate with careful follow-up and periodic calcitonin testing [4, 14, 18]. Since the timing of thyroidectomy should be determined considering the earliest finding of MTC in asymptomatic carriers, prophylactic thyroidectomy at an early age is generally recommended even in cases with level 1/level A mutations [14, 16, 18, 22]. In this regard, two S891A cases, whose MTCs were diagnosed at 17 years old, might be of help in determining the timing of surgery [10].

In this patient, serum levels of PTH were mildly elevated, although the urinary excretion of Ca^{2+} was not increased. One possibility for this is that the patient has primary hyperparathyroidism due to parathyroid gland hyperplasia or adenoma, as is observed in 20–30 % of MEN2A cases [23]. Since the resected right parathyroid glands of the patient were found to enlarge, we carefully examined the histology of the four resected parathyroid tissues, but there was no evidence of hyperplasia or adenoma. Alternatively, it is possible that the elevated serum PTH levels were due to secondary to decreased serum Ca^{2+} levels induced by excess calcitonin excretion from the C-cell hyperplasia. Furthermore, this hypothesis implies that the patient with the S891A mutation carried slow-growing C-cell hyperplasia for a long period, during which time the decreased Ca^{2+} was compensated for by the secondary hyperparathyroidism. These data lend support to the concept that C-cell hyperplasia in the patient with S891A mutation, in spite of its slow growth speed, has a high chance of obtaining another hit for malignant transformation. The patient's two brothers and three sisters, all of whom are more than 50 years old, do not have any clinical symptoms associated with MEN2A at this moment. This implies that early surgical intervention is not required for S891A cases, although it is still possible that none of the brothers and sisters carries the S891A mutation. Careful follow-up of these older relatives is required even though they do not yet have any MEN2-related symptom.

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DEVELOPMENT AND VALIDATION OF A SHORT SCALE TO MEASURE HOW SOCIAL RELATIONSHIPS SUPPORT THE CONTINUOUS AND CONSCIOUS ENDEAVOUR TO LOSE WEIGHT

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Summary. This paper reports the development of a short scale (ten items) entitled 'Social Relationships to Prevent Obesity' (SRPO), which examines how social relationships support the continuous and conscious endeavour to lose weight. The construct and criterion validity of this scale were ascertained in this study. Factor structure and reliability were examined using data from a randomized controlled trial. A confirmatory factor analysis of the SRPO revealed three relevant factors. The results suggest that the SRPO has both validity and clinical utility and can thus be used as a screening tool in weight-loss interventions and to assess the degree of, and trends in, self-control for weight loss in individuals. The scale can also be used to examine the environmental and self-control problems faced by obese people – factors that should be considered when conducting weight-loss interventions.

Introduction

Obesity is typically prevented through dieting and exercise regimens, and these two measures are related to the individual's surroundings (Sallis *et al.*, 1987). Moreover, on theoretical grounds, lifestyle-related obesity (henceforth, obesity) – like most social and personal problems (smoking, substance abuse, etc.) – seems to involve a substantial component of deficient self-control (Tangney *et al.*, 2004). In the context of the present study, 'self-control' means a continuous and conscious endeavour to lose weight

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that leads to a modification of the daily habits that are responsible for current obesity and the failure of previous weight-loss efforts (Kan, 2007). This term is synonymous with what Frank (1988) termed 'commitment'. Effective weight-loss interventions should enable participants to acquire social support through a social network (Sallis *et al.*, 1987). Such support should promote the participants' self-control and commitment to weight loss, and aid in the elimination of habits that lead to obesity.

Factors influencing the maintenance of a healthy weight and lifestyle

Obesity researchers recognize the importance of not only biological but also environmental and behavioural factors in sustaining a healthy lifestyle. Thus, future policies aimed at addressing obesity should involve interventions that mitigate unhealthy behavioural and environmental influences (Cutler & Glaeser, 2005; Christakis & Fowler, 2007; Eid *et al.*, 2008; Hill *et al.*, 2008; Li *et al.*, 2009; Yakusheva *et al.*, 2011). Specifically, people with deficient self-control require support from others and the environment in their continuous and conscious efforts to adopt and maintain the behaviours that prevent obesity. These behaviours include consuming an optimal amount of food; paying attention to the amount of fat and salt in one's diet, particularly while preparing one's own meals; and avoiding a sedentary lifestyle (Sallis *et al.*, 1992; Komlos *et al.* 2004; Cutler & Glaeser, 2005). In order for the participants of a weight-loss programme to develop strong self-control, the intervention needs to be targeted accurately and demonstrate clearly to recipients the factors involved in successful weight loss.

Studies using behavioural modification theories have demonstrated the considerable influence of social relationships and social support – for example, from one's spouse and friends – on dieting and exercising (Sallis *et al.*, 1987, 1992; Prochaska *et al.*, 1992; Marcus & Simkin, 1994; Unger & Johnson, 1995; Deforche & De Bourdeaudhuij, 2000). The peer effect, in terms of social interactions with friends, family and acquaintances within a social network, has been shown to influence weight problems (Wallston *et al.*, 1978; Cutler & Glaeser, 2005; Christakis & Fowler, 2007; Cohen-Cole & Fletcher, 2008; Fowler & Christakis, 2008; Renna *et al.*, 2008; Trogdon *et al.*, 2008; Ali *et al.*, 2011; Fortin & Yazbeck, 2011; Yakusheva *et al.*, 2011; Dale *et al.*, 2012).

In recent years, the importance of peer support through expansive social networks for weight loss has been enthusiastically examined in light of the obesity pandemic. At least three dimensions of support have been identified: the existence and quantity of social relationships, the structure of those relationships and their functional content. The first two dimensions are more correctly conceived of as aspects of a social network, while the last dimension captures the behaviours by which one person actually supports another (Sallis *et al.*, 1987; Manski, 1993; McDowell & Newell, 1996; Steptoe & Ayers, 2005). Social relationships and support have a long-standing association with health. Instrumental, emotional and ongoing support have been shown to be important to sustained behaviour change and health in research involving people living with chronic conditions, such as diabetes, cancer, cardiovascular diseases, mental illness and HIV/AIDS (Boothroyd & Fisher, 2010). Conversely, social isolation has been shown to predict mortality and morbidity (House *et al.*, 1988; Brummett *et al.*, 2001; House, 2001). In particular, peer support can provide assistance with daily management tasks, provide social and emotional support to stay motivated and deal with the stress chronic disease

often brings, and help people stay connected to clinical care and improve outcomes in self-management (Boothroyd & Fisher, 2010).

An important mediator in the relationship between self-control and individual behaviour is 'locus of control' (LOC). Locus of control refers to generalized expectations about the determinants of one's circumstances. On the basis of their experiences and learning history, individuals come to expect that future outcomes will be determined by either internal factors such as their own actions or characteristics (i.e. internal locus of control) or by external factors and opportunities not dependent on their own efforts or abilities (i.e. external locus of control) (Rotter, 1954, 1966; Contrada & Goyal, 2005). This theory forms the basis for an important idea in the field of behaviour modification: an individual's health behaviour might successfully be modified by promoting an internal LOC (Nir & Neumann, 1995). Researchers have found strong and consistent correlation between an external LOC and failure to comply with healthful behaviours (Wallston *et al.*, 1978; Macgregor *et al.*, 1997; Fujita & Noguchi, 2009).

The need for a short scale to measure social support in the weight-loss context

Many interventions have been conducted using these basic social support and LOC theories, but research findings have not always been consistent (Sallis *et al.*, 1987; Gorin *et al.*, 2008; Bahr *et al.*, 2009; Finnerty *et al.*, 2010). With regard to social relationships, for instance, some people lose weight if supported in terms of diet and exercise by co-operative spouses, while others, naturally, are influenced by a shared environment of obesity-promoting habits and traditions. Dieters may be unaware of these influences and may not understand why they fail to lose weight despite their best efforts. This inconsistency is primarily due to the shortcomings of the instruments used to measure the influences of social relationships on obesity (Brownell & Stunkard, 1981; Black & Lantz, 1984; McLean *et al.*, 2003; Yakusheva *et al.*, 2011). The instruments used in these previous studies were not designed to directly address the self-control problems of people with excess weight. Further, these instruments typically have an excessively large number of questions (Funch *et al.*, 1986; Sallis *et al.*, 1987; Karlsson *et al.*, 1995; Yata *et al.*, 2003; Gruber, 2008; Sherrill-Mittleman *et al.*, 2009). Consequently, respondents might have submitted unreliable or inappropriate responses to questions on self-control, because people with poorly regulated self-control often show impatience and destructive patterns of persistence when confronted with a large number of questions (Stunkard & Messick, 1985; Baumeister & Heatherton, 1996; Kan, 2007; Tangney *et al.*, 2004; Wills *et al.*, 2007). An additional shortcoming of these instruments is that they fail to adequately measure psychological adjustment to, and compliance with, healthy behaviour, both of which need to be examined in a weight-loss study to assess participants' degree of self-control (Tangney *et al.*, 2004; Carver, 2005).

In order to overcome these shortcomings, a short scale titled 'Social Relationships to Prevent Obesity' (SRPO) was developed in this study. Through items that place as little burden on respondents as possible, the scale examines the social relationships that support self-control with regard to weight loss, taking into consideration family- and community-based physical activities and dietary habits and the social-communication environment. This short scale is one of the first scales developed to measure the degree

of, and trends in, social support for self-control by using a minimum number of items related to weight-control interventions.

The objective of the present study was to develop and evaluate the validity and reliability of the SRPO. For this purpose, it uses secondary data from Takada *et al.* (2011). The SRPO is a refined and extended version of a previous scale titled 'Social Support to Counter Obesity' (SSCO; Takada *et al.*, 2010); the SRPO differs from the SSCO in that the latter does not explicitly measure social support that promotes self-control.

Methods

Source of secondary data: the Takada et al. *(2011) study*

In a previous study, a tele-care intervention for weight loss was assessed through a randomized controlled trial. The participants were registered members of a community health club in Kyoto, Japan, recruited through a public advertisement. They were all obese but otherwise healthy men and women between 20 and 70 years old who met the study's strict eligibility requirements (for more details, see Takada *et al.*, 2011). There were 118 participants at baseline, and 21 dropped out before the intervention started. The participants were administered a questionnaire in person at a health check-up conducted as a part of the study. The questionnaire included the SRPO and questions on dietary habits, such as (1) number of meals, snacks, instances of eating out, and alcoholic drinks consumed; (2) regular eating of breakfast; and (3) late-night meals. The questionnaire also requested information about descriptive variables, including sex, age, body mass index, education, job status, marital status, income and value of property owned (see Table 1). The remaining participants were randomized into two groups – a tele-care group and a self-help group – matched by age, sex and body mass index. Of the 97 participants, only 66 completed the SRPO because some participants who were retired could not respond to some questions about level of support from employers (response rate: 55.9% [66/118]). The data from the 66 participants who completed the SRPO were used in the present study. The reliability and validity of the SRPO were examined using the participants' baseline data before the tele-care or self-help interventions. The research was approved by the ethics committee of the Graduate School of Medicine, Kyoto University. Analyses were conducted using SPSS v. 15.0 (SPSS Inc., Chicago, IL, USA).

Composition of the SRPO Scale

To develop this short scale, existing instruments were reviewed and items were chosen that addressed peer support for weight loss and social interaction that promotes constructive behaviour and adjustment. Items were chosen from the following instruments, all of which have been shown to have good reliability and validity.

Social Adjustment Scale. Two questions were taken from the 54-item Social Adjustment Scale (Weissman & Bothwell, 1976), which is one of the few scales designed to measure adjustment to community living among both psychotherapy patients and

Table 1. Summary statistics of respondents: 20- to 70-year-old obese men and women, Kyoto, Japan[a]

Variable	*n*	%
Sex	($n = 66$)	
Male	23	(34.8)
Female	43	(65.2)
Age (years)	($n = 66$)	
<40	24	(36.4)
≥40	42	(63.6)
BMI (kg/m^2)	($n = 66$)	
<25	28	(42.4)
≥25	38	(57.6)
Education	($n = 65$)	
Junior high school	1	(1.5)
High school	19	(29.2)
Vocational school	7	(10.8)
Junior collage	11	(16.9)
University (literature)	20	(30.8)
University (science)	3	(4.6)
Graduate school	4	(6.2)
Job status	($n = 65$)	
None	15	(23.1)
Current (with wages)	50	(76.9)
Marital status	($n = 65$)	
Single, divorced, widowed	19	(29.2)
Married	46	(70.8)
Income (JPY)	($n = 59$)	
None	10	(16.9)
<100 million	10	(16.9)
101–200 million	14	(23.8)
201–400 million	10	(16.9)
>400 million	15	(25.5)
Property value (JPY)	($n = 53$)	
None	10	(18.9)
<500 million	14	(26.4)
501–1000 million	7	(13.2)
1001–1500 million	4	(7.5)
>1500 million	18	(34.0)

[a] Data source: Takada *et al.* (2011).

healthy individuals (McDowell & Newell, 1996). The first question was 'How many times in the last two weeks have you gone out socially? For example, visited friends, gone to movies, bowling, church, restaurants, etc.?' The available response options ranged from 1 (None) to 5 (More than three times). The second question was 'How much time have you spent on hobbies or spare time interests during the last two weeks? For example, bowling, sewing, gardening, sports, reading?' The response options

ranged from 1 (I did not spend any time on hobbies or watching TV) to 5 (I spend a lot of time on hobbies almost every day).

Rand Social Health Battery. Two questions were adapted from the eleven-item Rand Social Health Battery (Donald & Ware, 1984), which is one of the few social health scales not designed for use with patients. This scale records social interactions and resources for social support but does not evaluate the subjective experience of support (McDowell & Newell, 1996). The first question was 'To how many volunteer groups or organizations do you belong (e.g. church, temple or shrine groups; clubs in the community; or parent groups)?' The response options ranged from 1 (None) to 5 (More than three groups or organizations). For the second question – 'How active are you in the affairs of the groups or clubs to which you belong?' – the response options ranged from 1 (Do not belong to any groups or attend any meetings) to 4 (Very active, attend most meetings).

Social Support and Exercise Survey and Social Support and Eating Habits Survey. Two questions were taken from the Social Support and Exercise Survey and two from the Social Support and Eating Habits Survey. These instruments are two of four separate scales (with 43 total items) designed to assess social support for diet and exercise (Sallis *et al.*, 1987). These scales were developed using a behavioural modification theory known as the ecological model (an approach characterized by its focus on levels of influence from the individual to the community; Sallis *et al.*, 1987; Ståhl *et al.*, 2001; Uechi, 2006). Two originally separate items – 'My family or friends exercised with me' and 'gave me helpful reminders to exercise' – were combined in the SRPO. Three other items were adopted verbatim: (1) 'My family or friends helped plan activities around my exercise', (2) 'My family or friends reminded me not to eat high fat, high salt foods', and (3) 'discussed my eating habit changes with me'. The response options ranged from 1 (Strongly disagree/Not at all) to 5 (Strongly agree/Very often).

Medical Outcomes Study Social Support Survey. Two questions were taken from the twelve-item Medical Outcomes Study Social Support Survey (Sherbourne & Stewart, 1991), which, though designed for use in chronically ill patients, is also universally applicable owing to its sound validity and reliability, despite being relatively short (McDowell & Newell, 1996). This instrument attempts to determine how often various kinds of support are available to the respondent. The following two items were chosen: '[How often do you have] someone to get together with for relaxation?' and '[How often do you have] someone to prepare your meals if you were unable to make them yourself?' The response options ranged from 1 (None of the time) to 5 (All of the time).

Since this was an exploratory study, a simple re-translation of the above items was employed, taking Japanese culture into consideration. It was explained to participants that 'family' was defined as 'relatives who were also members of the household'. 'Environment' was defined to include both people and physical surroundings: siblings, family members, colleagues, co-workers, neighbours and the shared environment (Rotter, 1966; Manski, 1993; Christakis & Fowler, 2007, Cohen-Cole & Fletcher, 2008; Yakusheva *et al.*, 2011).

Table 2. Items and response options for the SRPO scale ($N = 66$)

Items	Response options	*n*	%
Factor I: Family and environmental support			
My family or friends exercised with me and gave me helpful reminders to exercise			
	Strongly disagree/not at all	17	25.8
	Disagree	12	18.2
	Undecided	14	21.2
	Agree	19	28.8
	Strongly agree/very often	4	6.0
My family or friends helped plan activities around my exercise			
	Strongly disagree/not at all	17	25.8
	Disagree	23	34.8
	Undecided	18	27.3
	Agree	6	9.1
	Strongly agree/very often	2	3.0
My family or friends reminded me not to eat high-fat, high-salt foods			
	Strongly disagree/not at all	11	16.6
	Disagree	14	21.2
	Undecided	17	25.8
	Agree	17	25.8
	Strongly agree/very often	7	10.6
My family or friends discussed my eating habit changes with me			
	Strongly disagree/not at all	16	24.2
	Disagree	13	19.7
	Undecided	18	27.3
	Agree	13	19.7
	Strongly agree/very often	6	9.1
Factor II: Social interaction			
To how many volunteer groups or organizations do you belong to, like church, temple, shrine groups, clubs in the community or parent groups etc.?			
	None	31	47.0
	One	16	24.2
	Two	12	18.2
	Three	4	6.0
	More than three groups or organizations	3	4.6
How active are you in the affairs of the groups or clubs to which you belong?			
	Do not belong to any groups or attend any meetings	32	48.5
	Not active, belong but hardly ever go	0	0.0
	Fairly active, attend fairly often	15	22.7
	Very active, attend most meetings	19	28.8
[How often do you have] someone to get together with for relaxation?			
	None of the time	3	4.6
	A little of the time	19	28.8
	Some of the time	28	42.4
	Most of the time	14	21.2
	All of the time	2	3.0

Table 2. *Continued*

[How often do you have] someone to prepare your meals if you were unable to make them yourself?		
None of the time	13	19.7
A little of the time	25	37.9
Some of the time	11	16.7
Most of the time	15	22.7
All of the time	2	3.0
Factor III: Social adjustment		
How many times in the last two weeks have you gone out socially (visited friends, gone to movies, churches, restaurants etc.)?		
None	1	1.5
Once	6	9.1
Twice	8	12.1
Three times	9	13.7
More than three times	42	63.6
How much time have you spent on hobbies or items of interests during the last two weeks?		
I did not spend any time on hobbies or watching TV	2	3.0
I usually did not spend any time on hobbies but did watch TV	12	18.2
I spent a little time on hobbies	26	39.4
I spent some time on hobbies on most days	17	25.8
I spent a lot of time on hobbies almost every day	9	13.6

Further, to ensure that the participants faced no inconvenience in giving their responses, the questions and response options were modified to be as brief as possible. The researchers attempted to use as few items as possible in the scale by combining related items and eliminating redundancies. Furthermore, the original scale used response options ranging from 1 (None) to 8 (Does not apply); the number of options was reduced to make the scale easier for participants to complete. The responses of all items were summed. It was hypothesized that a higher score on the SRPO indicates a greater amount of social support and social interaction that promotes self-control.

The exploratory version of the SRPO originally contained fouteen questions. Four questions concerned the number of athletic facilities within a convenient distance and the level of health support from the participant's employer. These items were prepared by referring to the Social Functioning Schedule (Remington & Tyrer, 1979), which is intended to assess the problems experienced in normal social functioning (such as work problems and problems in relationships with others at work, home and elsewhere) and the SLOTH model (Pratt *et al.*, 2004), which is intended to enhance health through public announcements, health promotion programmes, worksite interventions and the like. However, since these items had poor response rates, they were omitted from the scale.

The final version of the SRPO contained ten items that were carefully chosen to effectively capture the key aspects of the scales from which they were taken (for more details, see Table 2).

Results

Factor analysis

Confirmatory factor analysis was used to examine whether the data fitted the model previously hypothesized by the researchers (French *et al.*, 2005). To confirm construct validity, factor loading was calculated. A value of 0.40 or greater is generally considered acceptable for this purpose. Confirmatory factor analysis revealed that the ten items of the SRPO can be clustered into three factors. Factor I (covering the four questions from the Social Support and Exercise Survey and the Social Support and Eating Habits Survey) was called 'family and environmental support'. Factor II (covering the two questions from the Rand Social Health Battery and the two questions from the Medical Outcomes Study Social Support Survey) was called 'social interaction'. Factor III (covering the two questions from the Social Adjustment Scale) was called 'social adjustment'. The items, final factor loadings, explained variances and eigenvalues are presented in Table 3.

Table 3. Results of factor analysis of the SRPO scale[a]

Items	Factor loading
Factor I: Family and environmental support	
1. My family or friends exercised with me and gave me helpful reminders to exercise	0.76
2. My family or friends helped plan activities around me to ensure more time for exercise	0.81
3. My family or friends reminded me not to eat high-salt, high-fat foods	0.87
4. My family or friends discussed my eating habit changes with me	0.82
Factor II: Social interaction	
5. To how many volunteer groups or organizations do you belong (e.g. church, temple, shrine groups, clubs in the community or parent groups)?	0.91
6. How active are you in the affairs of the groups or clubs to which you belong?	0.89
7. [How often do you have] someone to get together with for relaxation?	0.43
8. [How often do you have] someone to prepare your meals if you were unable to make them yourself?	0.38
Factor III: Social adjustment	
9. How many times in the last two weeks have you gone out socially (visited friends, gone to movies, churches, restaurants etc.)?	0.83
10. How much time have you spent on hobbies or items of interest in the last two weeks?	0.75
Factor I. Eigenvalue[b]: 3.4; % variance explaind: 32.6; Cronbach's α: 0.85	
Factor II. Eigenvalue[b]: 2.2; % variance explaind: 20.1; Cronbach's α: 0.70	
Factor III. Eigenvalue[b]: 1.2; % variance explaind: 13.4; Cronbach's α: 0.51	

[a] A higher score on the SRPO indicates a higher degree of social support.
The Kaiser–Meyer–Olkin measure of sampling adequacy = 0.66. Bartlett's test of sphericity = 264.03; $p < 0.01$.

[b] Eigenvalues are the variances of the factors; a value over 1 indicates that factor analysis can be performed.

Table 4. Means, standard deviations and coefficients of the SRPO scores across groups

	Total ($n = 66$)			Tele-care group ($n = 36$)			Self-help group ($n = 30$)		
	Mean	SD	Cronbach's α	Mean	SD	Cronbach's α	Mean	SD	Cronbach's α
SRPO score	27.9	6.5	0.75	27.6	6.7	0.77	28.3	6.2	0.73

High factor loading values indicate high consistency among the items of the scale. The Kaiser–Meyer–Olkin measure of sampling adequacy is used to test whether the partial correlations among variables are small. The results of this test can vary between 0 and 1, and values closer to 1 indicate that factor analysis is appropriate. The Kaiser–Meyer–Olkin value was 0.66 so factor analysis was undertaken. Bartlett's test of sphericity indicated that the factor model was appropriate ($p < 0.01$) (Table 3).

Reliability

Reliability was assessed by examining the scores of the tele-care and self-help groups using Cronbach's α, the generalized formula used to express the internal consistency of a test. Higher internal consistency can also mean higher test–retest reliability (McDowell & Newell, 1996), and a value of 0.70 or above is generally considered adequate. The Cronbach's α values for the SRPO were 0.77 and 0.73 for the tele-care and self-help groups, respectively. In terms of internal consistency, Cronbach's α coefficients were high for the tele-care and self-help groups (Table 4).

Validity

One of the ways of establishing a scale's validity is to determine whether its scores are positively related to scores obtained on other scales that measure related or similar constructs. As the tele-care intervention utilized in the previous study was based on the Transtheoretical Model (which is effective in designing behaviour modification interventions), various original scales and theories related to this model were utilized to assess the validity of the SRPO: the Stages of Change Theory, the level of Motivation for Exercise (Prochaska & DiClemente, 1983; Marcus *et al.*, 1992), Decisional Balance for Exercise Scale (Marcus & Simkin, 1994) and the Self-Efficacy for Exercise Scale (Prochaska *et al.*, 1992). In the previous study, the participants were administered these scales along with the main questionnaire at the health check-up (see Takada *et al.*, 2011, for details).

Construct validity. Marital status and improvements on the Motivation for Exercise Scale were used to confirm construct validity. The stages of change and the level of motivation for exercise theories are significant core factors of the Transtheoretical Model, which describes stages along a continuum of behavioural change and the participant's motivation at each stage (1: not intending to exercise, 2: intending to exercise within 6