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G. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

該当なし。

2. 実用新案登録該当なし。

3. その他

該当なし。

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Teo AR, Fetters MD,Stufflebam S, Tateno M, Balhara YBS, Choi TY, Kanba S, Mathews CA, Kato TA	Identification of the hikikomori syndrome of social withdrawal: Psychosocial features and treatment preferences in four countries	Int J Soc Psychiatry	61	64-72	2015
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Identification of the hikikomori syndrome of social withdrawal: Psychosocial features and treatment preferences in four countries

Alan R Teo[1], Michael D Fetters[2], Kyle Stufflebam[3], Masaru Tateno[4], Yatan Balhara[5], Tae Young Choi[6], Shigenobu Kanba[7], Carol A Mathews[8] and Takahiro A Kato[7,9]

Abstract

Background: Hikikomori, a form of social withdrawal first reported in Japan, may exist globally but cross-national studies of cases of hikikomori are lacking.

Aims: To identify individuals with hikikomori in multiple countries and describe features of the condition.

Method: Participants were recruited from sites in India, Japan, Korea and the United States. Hikikomori was defined as a 6-month or longer period of spending almost all time at home and avoiding social situations and social relationships, associated with significant distress/impairment. Additional measures included the University of California, Los Angeles (UCLA) Loneliness Scale, Lubben Social Network Scale (LSNS-6), Sheehan Disability Scale (SDS) and modified Cornell Treatment Preferences Index.

Results: A total of 36 participants with hikikomori were identified, with cases detected in all four countries. These individuals had high levels of loneliness (UCLA Loneliness Scale $M = 55.4$, $SD = 10.5$), limited social networks (LSNS-6 $M = 9.7$, $SD = 5.5$) and moderate functional impairment (SDS $M = 16.5$, $SD = 7.9$). Of them 28 (78%) desired treatment for their social withdrawal, with a significantly higher preference for psychotherapy over pharmacotherapy, in-person over telepsychiatry treatment and mental health specialists over primary care providers. Across countries, participants with hikikomori had similar generally treatment preferences and psychosocial features.

Conclusion: Hikikomori exists cross-nationally and can be assessed with a standardized assessment tool. Individuals with hikikomori have substantial psychosocial impairment and disability, and some may desire treatment.

Keywords

Social isolation, cross-national, culture

Introduction

The notion of hermits and recluses has existed in many cultures for time immemorial. However, in recent years a particularly severe syndrome of social withdrawal first identified in Japan has garnered the interest of researchers

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and the lay public alike. Called hikikomori, it has been defined as 'a phenomenon in which persons become recluses in their own homes, avoiding various social situations (e.g., attending school, working, having social interactions outside of the home, etc.) for at least six months' (Saito, 2010). Individuals with hikikomori are frequently reported to have social contact predominantly via the internet and some reports suggest overlap with heavy internet use (De Michele, Caredda, Delle Chiaie, Salviati, & Biondi, 2013; Lee, Lee, Choi, & Choi, 2013). An estimated 232,000 Japanese currently suffer from hikikomori, and 1.2% of community-residing Japanese between ages 20–49 have a lifetime history of hikikomori (Koyama et al., 2010). A combination of a shy personality, ambivalent attachment style and life experiences including rejection by peers and parents – among other factors – may promote the development of hikikomori (Krieg & Dickie, 2011). Furthermore, scientific studies point to genetic and other biological influences on sociality that, although not specific to hikikomori, could have implications for the study of the etiology of hikikomori (Meyer-Lindenberg & Tost, 2012). While researchers debate the merits of hikikomori as a psychiatric diagnosis (Teo & Gaw, 2010), practicing clinicians in Japan indicate they view hikikomori as a 'disorder' (Tateno, Park, Kato, Umene-Nakano, & Saito, 2012).

Previous reports suggest hikikomori may exist outside of Japan. For instance, case reports have described the presence of hikikomori in several other countries (Furuhashi et al., 2012; Garcia-Campayo, Alda, Sobradiel, & Sanz Abos, 2007; Sakamoto, Martin, Kumano, Kuboki, & Al-Adawi, 2005; Teo, 2013). When presented with vignettes of hikikomori, psychiatrists from nine countries around the world indicated that such cases existed in their clinical practices (Kato et al., 2012). Nonetheless, cross-national studies designed to identify hikikomori have been lacking. Reasons for the lack of recognition have included ambiguity about the features of hikikomori (Tateno et al., 2012; Watts, 2002), and inconsistent or insufficiently detailed definitions of hikikomori (Furuhashi et al., 2011; Garcia-Campayo et al., 2007; Sakamoto et al., 2005). This has caused concern that researchers may not be referring to the same phenomenon. We have previously proposed a research-grade definition of hikikomori, but this definition has not been empirically tested (Teo & Gaw, 2010). Additionally, prior reports of hikikomori have focused on assessment of psychopathology (Lee et al., 2013; Nagata et al., 2013) but fewer studies – especially outside of Japan – have examined psychosocial features more broadly, despite the common belief that sociocultural factors are important contributors to hikikomori (Kato et al., 2012). Finally, prior research has examined treatment recommendations for hikikomori by psychiatrists, but we are unaware of studies that have explored patients' treatment preferences (Kato et al., 2012).

Aims

1. To identify cases of hikikomori cross-nationally;
2. To describe the psychosocial features and treatment preferences of individuals with hikikomori;
3. To explore possible differences in psychosocial features and treatment preferences of individuals with hikikomori across countries.

In this study, we examined individuals with social withdrawal using such a standardized definition of hikikomori cross-nationally.

Method

Design

We conducted a cross-national case series in India, Japan, South Korea and the United States.

Study participants

Participants who had a history of or current social withdrawal were recruited. Indian participants were referred from psychiatric outpatient clinics. Japanese and Korean participants were referrals from either a hospital or community mental health center. At the US site, participants responded to an online advertisement. All participants were adults between the ages of 18 and 39, noninstitutionalized and fluent in the local language of their respective site (English used in India). Participants with a self-reported history of schizophrenia, dementia, mental retardation or autism spectrum disorders and participants with social withdrawal due to a chronic physical illness or injury were excluded. A total of 108 individuals were screened for eligibility, with 26 excluded for not meeting criteria for hikikomori, 18 for age, 2 for schizophrenia, 1 with an autism spectrum disorder and 6 who withdrew consent. This left 55 (51%) who met initial eligibility criteria. An additional 18 individuals did not complete consent or study measures and 1 was excluded for later reporting a history of schizoaffective disorder, leaving a final sample of 36 for analysis. Participants were compensated US$50 or equivalent in local currency. This study was approved by the institutional review boards of each participating site. All participants provided written informed consent for participation.

Measures

Assessment of hikikomori. Researchers administered an interview to assess for the presence of suspected hikikomori (see Appendix 1 for questionnaire), adapted from our earlier proposed definition (Teo & Gaw, 2010). We defined hikikomori as (1) spending most of the day and nearly every day at home (duration of at least 6 months);

(2) avoiding social situations, such as attending school or going to a workplace (duration of at least 6 months); (3) avoiding social relationships, such as friendships or contact with family members (duration of at least 6 months); and (4) significant distress or impairment due to social isolation.

Self-report measures. We administered the University of California, Los Angeles (UCLA) Loneliness Scale, the Lubben Social Network Scale-6(LSNS-6), the Sheehan Disability Scale (SDS), the Cornell Treatment Preferences Index (CTPI) and a questionnaire on sociodemographic characteristics to participants.

The UCLA Loneliness Scale is a 20-item questionnaire that assesses how often individuals endorse subjective feelings of loneliness (e.g. 'How often do you feel that you lack companionship?'). The score range is 20 to 80, with higher scores indicating greater degrees of loneliness (Russell, 1996). Each item is rated on a 4-point scale from 1 ('never') to 4 ('always'). As the Revised UCLA Loneliness Scale has been validated Korean and Japanese samples, it was used at these sites (Kim, 1997; Kudou & Nishikawa, 1983). At the United States and Indian sites, Version 3 of the UCLA Loneliness Scale was used. Version 3 is identical to the revised version, except for minor wording adjustments (Russell, 1996).

The LSNS-6 is a 6-item questionnaire that assesses the number of people in an individual's social network with whom one has social contact (e.g. 'How many relatives do you see or hear from at least once a month?') and who are a source of social support (e.g. 'How many friends do you feel close to such that you could call on them for help?'). There are two subscales for family and friends. The total score range is 0–30 (0–15 for each subscale), and a total score less than 12 is indicative of social isolation (Lubben et al., 2006). Such a score implies fewer than two social network members, on average, for each item. Each item is rated on a 6-point scale from 0 ('none') to 5 ('nine or more'). The LSNS-6 has been validated in Korean and Japanese (Hong, Casado, & Harrington, 2011; Kurimoto et al., 2011).

The SDS is a 5-item questionnaire that assesses disability or functional impairment. The first three items evaluate level of disruption in each of three domains (work/school, social life and family life/home responsibilities) with response choices on a 0 ('not at all') to 10 ('extremely') scale, while the remaining two items evaluate days lost and days unproductive (Sheehan, 1983). Higher scores indicate more disability. The word 'symptom' in the SDS was replaced with 'social isolation' for this study. The scale has been validated in Korean and Japanese (Lee & Song, 1991; Yoshida, Otsubo, Tsuchida, Wada, & Kamijima, 2004).

The CTPI is a 6-item questionnaire that evaluates several different depression treatment preferences, including treatment modality and type of treatment provider (Raue, Schulberg, Heo, Klimstra, & Bruce, 2009). We modified the CTPI to assess preferences related to social isolation (e.g. 'I wish to receive counseling or psychotherapy for my social isolation'). The response scale for the first five items is a 5-point Likert scale from 1 ('strongly disagree') to 5 ('strongly agree'), and the final item uses ranked treatment preferences. For the CTPI, as well as other instruments that lacked an existing translation in a target language, we translated the instrument and used back translation as verification of adequate adaption.

Statistical analysis

We compared variables using the t-test and chi-square for continuous and categorical variables, respectively. When any group or cell contained five or fewer participants, we replaced the t-test and chi-square with the Wilcoxon Rank-Sum test and Fisher's exact test, respectively. Linear regression models were used to examine the association between country and several outcome variables, including loneliness, social network and functional disability. Logistic regression models were similarly used for the association between country and the dichotomized treatment preferences. The regression models were adjusted for the effects of the educational level and age as these were significant in bivariate correlations with country. Sample sizes for particular analyses vary due to differences in number of responses. Significance level for all tests was set at $p < .05$ and tests were two-tailed. Data were analyzed using Stata Version 12 (Stata Corp.)

Results

Identification of hikikomori

Regarding the first aim, 36 adult participants with social withdrawal who met criteria for hikikomori were identified. The cases were found in all four countries included in this study. As seen in Table 1, the vast majority were men with varied education levels. The majority of participants lived with family members; just four (11%) lived alone. Their self-reported period of social withdrawal was on average 2.1 years.

Psychosocial features

We quantitatively described a number of features of individuals with hikikomori. Scores on the UCLA Loneliness Scale indicated a high level of loneliness among all participants ($M = 55.4$, $SD = 10.5$). By comparison, prior studies with normal controls in American, Indian and Korean samples have shown mean scores of about 40 (SD around 9) (Jayashankar, 2013; Lee & Lee, 2004; Russell, 1996), and studies with depressed participants have shown average scores of 49.8 (Grov, Golub, Parsons, Brennan, & Karpiak, 2010). Likewise, social networks for our sample were

Table 1. Sociodemographic characteristics of participants with hikikomori in four countries.

Characteristic	Total	Japan	USA	India	Korea		p
	(n = 36)	(n = 11)	(n = 11)	(n = 10)	(n = 4)		
	n (%)	n (%)	n (%)	n (%)	n (%)		
Male	29 (81)	10 (91)	7 (64)	9 (90)	3 (75)		.33
Age (years)							
18–21	11 (32)	2 (18)	2 (18)	3 (30)	4 (100)	}	.04
22–30	11 (32)	3 (27)	4 (36)	6 (60)	0 (0)		
31–49	12 (35)	6 (55)	5 (45)	1 (10)	0 (0)		
Education level							
High school graduate or less	16 (44)	7 (64)	2 (18)	3 (30)	4 (100)	}	.01
Some college or more	20 (56)	4 (36)	9 (81)	7 (70)	0 (0)		
Living situation							
Lives with others	32 (89)	10 (91)	8 (73)	10 (100)	4 (100)	}	.2
Lives with no one	4 (11)	1 (9)	3 (27)	0 (0)	0 (0)		

weak, with participants scoring a mean of 9.7 (SD = 5.7) on the LSNS-6. By comparison, prior studies with normal controls have shown average scores of 17.4 (Lubben et al., 2006). Individuals with hikikomori showed slightly higher scores on the family subscale (M = 5.4, SD = 3.0) than the friend subscale (M = 4.3, SD = 3.5). Participants with hikikomori had moderate levels of functional disability on the SDS (M = 16.5, SD = 7.9), levels comparable to patients with psychiatric disorders and more than three-fold higher than those with no mental illness in a study of a study of primary care patients (Olfson et al., 1997). Impairment was highest in terms of social life/leisure activities, compared to work/school and family life.

Treatment preferences

A total of 78% of the sample expressed a desire for treatment for their social withdrawal. In terms of modality of treatment, participants preferred psychotherapy (M = 3.6, SD = 1.5) over medication (M = 2.9, SD = 1.4); $t(31) = 2.13$, p = .04. In addition, participants also were significantly more likely to be interested in psychotherapy and medicine management delivered *in-person* compared to an option for provision by *webcam* ($p < .001$ for both comparisons). Participants ranked individual psychotherapy most as a desired treatment, with few desiring complementary and alternative treatments such as herbal remedies or exercise (Figure 1). As for treatment provider, participants preferred mental health specialists (M = 3.6, SD = 1.2) over primary care physicians (M = 2.7, SD = 1.2); $t(34) = 3.87$, $p < .001$.

Cross-national comparisons

We compared treatment preferences and psychosocial characteristics of participants across the four countries in this study as our exploratory aim: that is, to generate hypotheses about cross-national differences in hikikomori that might be tested in future studies. Across countries, results generally were similar. For comparison of treatment preferences across countries, the Korean sample was excluded from analyses due to small sample size (n = 4). In adjusted models controlling for age and level of education, there were no statistically significant differences in overall desire for treatment, desire for pharmacotherapy, desire for psychotherapy, interest in webcam-delivered medication management or psychotherapy, interest in in-person-delivered medication management or desire for treatment provided by a mental health professional. Participants in the United States were significantly less likely to desire treatment by a primary care physician compared to Japan (odds ratio (OR) = 0.04, 95% confidence interval (CI) = 0.00–0.60). Also, Indian participants had a significantly lower interest in in-person psychotherapy (OR = 0.00, 95% CI = 0.00–0.31). Table 2 illustrates psychosocial features of our sample of individuals with hikikomori. As illustrated by the beta coefficient, American participants demonstrated on average a 12-point higher score on the UCLA Loneliness Scale and a 4-point higher score on the family life subscale of the SDS, as compared with Japanese participants. Indian participants had significantly stronger social networks but higher levels of functional disability. Finally, Korean subjects had significantly higher levels of loneliness, weaker friendships in their social network and higher functional disability.

Discussion

This study bolsters evidence that hikikomori, as a phenotype of severe social withdrawal, exists cross-nationally. Strengths of our approach include use of a

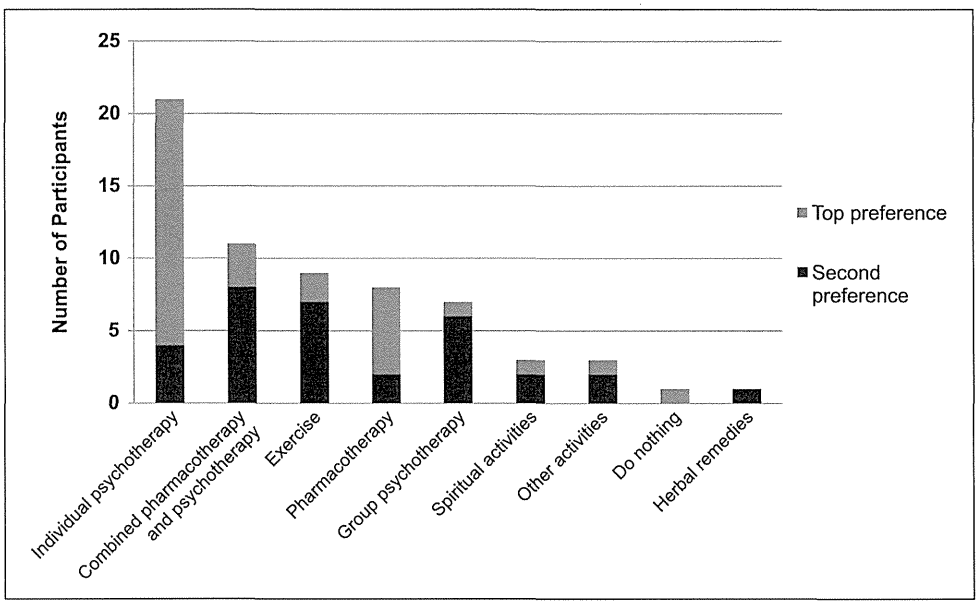

Figure 1. Top two treatment preferences of participants with hikikomori for their social withdrawal (n = 32).

Table 2. Multivariable linear regression of exploratory associations between psychosocial features of hikikomori and country.

	Japan	USA		India		Korea	
	(n = 11)	(n = 11)		(n = 10)		(n = 4)	
Characteristic	β	β	(95% CI)	β	(95% CI)	β	(95% CI)
Loneliness (UCLA Loneliness Scale)	Ref	12.35**	(5.41, 19.29)	−3.78	(−10.90, 3.33)	16.31**	(6.44, 26.17)
Social network (Lubben Social Network Scale – 6)	Ref	0	(−4.69, 4.68)	5.05*	(0.24, 9.85)	−5.37	(−12.03, 1.29)
Family subscale	Ref	−0.24	(−2.77, 2.30)	3.41*	(0.81, 6.01)	−0.86	(−4.46, 2.75)
Friend subscale	Ref	0.23	(−2.73, 3.19)	1.64	(−1.40, 4.67)	−4.51*	(−8.72, −0.31)
Functional disability (Sheehan Disability Scale)	Ref	4.95	(−1.90, 11.81)	9.04*	(2.16, 15.92)	13.86*	(3.44, 24.27)
Disrupted work/school work	Ref	−0.36	(−3.40, 2.68)	2.20	(−0.85, 5.25)	1.49	(−3.13, 6.12)
Disrupted social life/leisure activities	Ref	2.04	(−0.29, 4.36)	2.86*	(0.48, 5.24)	4.67*	(1.01, 8.32)
Disrupted family life/home responsibilities	Ref	4.03**	(1.54, 6.52)	4.06**	(1.51, 6.61)	7.70***	(3.78, 11.61)

UCLA: University of California, Los Angeles.
Analyses controlled for age and level of education. Japan used as the reference group (Ref) for country comparisons.
*statistically significant at the .05 level; **statistically significant at the .01 level; ***statistically significant at the .001 level.

standardized definition and assessment tool for hikikomori across four countries with diverse cultures and operationalizing hikikomori with discrete questions about the frequency, length and quality of social withdrawal. Past approaches have relied on a single, complex question (Koyama et al., 2010; Umeda, Kawakami, & The World Mental Health Japan Survey Group, 2002–2006, 2012), an approach that may cause misunderstanding by placing a high cognitive burden on the respondent (Schwarz, 2007). Thus, this study offers a new interview tool to help assess for hikikomori. Our data showing loneliness and limited connections with social network members among study participants support the validity of our assessment approach to hikikomori as we have defined it.

Psychosocial features

Perhaps the most striking features of hikikomori participants in this study were high loneliness scores and impaired social network scores. Our descriptive data paint a picture of the average individual with hikikomori being intensely lonely and deficient in social support, apparently unable to maintain meaningful social ties. This is despite rarely living alone and indicating a desire for treatment of their social withdrawal.

Treatment preferences

In these individuals who have been avoided social contact for such a prolonged period of time, we were surprised to find a consistent preference for treatment delivered in-person, as opposed to telepsychiatry-style. We believe this is the first study to describe treatment preferences in a sample of individuals with hikikomori. Understanding treatment preferences is a valuable first step for intervention research, particularly in light of evidence that treatment response rates for hikikomori are low (Nagata et al., 2013). Individuals with hikikomori may feel ambivalent about their desire for social relationships, and a patient–provider relationship may offer an entry point into re-establishing social connections. Given these results, future intervention studies for hikikomori might consider evaluating home visitation, particularly when conducted by a mental health professional and with an aim of boosting the social support of hikikomori patients (Dickens, Richards, Greaves, & Campbell, 2011; Lee et al., 2013). Other interventions that have shown promise in populations with mental illnesses and are thought to work by bolstering social relationships, such as peer support, might be investigated (Pfeiffer, Heisler, Piette, Rogers, & Valenstein, 2011; Proudfoot et al., 2012).

Limitations

This study was designed as a case series, and therefore several limitations in interpretation of the results bear note. First, our sample was small, but we have employed statistical methods that adjust for sample size. Second, cross-national comparisons should only be regarded as exploratory because different recruitment methods were used across countries, data harmonization across cultures is always imperfect and adjustment for potential confounders was limited to basic sociodemographic variables. Third, individuals with hikikomori who are able to participate in a research study such as this are unlikely to be representative of all of those with hikikomori. In particular, individuals with hikikomori are often perceived as resistant to undergo treatment, and our sample may represent those who have milder symptoms or begun recovery. Nonetheless, this highlights a group that may represent great opportunity for intervention. Fourth, as this was primarily a descriptive study, no comparison group was included, though we have included comparisons with normative data for selected measures. Finally, the CTPI has not been validated in international samples, and therefore treatment preference data must be interpreted cautiously.

Conclusion

In sum, this study suggests that hikikomori exists cross-nationally, can be assessed with a brief interview tool and is associated with substantial loneliness, impaired social networks, disability and desire for treatment. Results of our study suggest several possible directions for future research. First, we believe future cross-national studies of hikikomori should obtain larger samples, which could be achieved by focusing on just two locations for comparison. Another approach would be to compare hikikomori participants to a control group such as participants with social anxiety disorder to help tease out differences between hikikomori and other conditions. Although it was beyond the scope of this study to conduct formal psychometric testing on our hikikomori assessment tool, future research on the reliability and validity of the hikikomori diagnostic interview would be helpful. Furthermore, development and testing of a hikikomori scale could help with conceptual clarity (e.g. constructs associated with hikikomori) and distinction from related conditions such as social anxiety disorder. Once validated, a hikikomori scale or diagnostic interview could then be applied to research on the prevalence and detection of hikikomori. To reach a more representative sample including individuals unable to leave their residence under any circumstance, Internet-based surveys on hikikomori should be developed. Finally, interventions that account for patient preference might be tested.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix 1

esdate **Date of Screening:** _____ _____ _____
Month Day Year

Step 1: Give explanation of study (suggested script below)
'First, I will give you a little background information. This is a research study about social isolation. The purpose of this study is to find out how common social isolation is and difficulties are associated with it. In order to participate in the study, all interested individuals must participate in an interview and complete several questionnaires. No treatment will be provided as part of the study. There is compensation of $50 for time and costs to you. Your participation is completely voluntary. Whether you choose to participate or not does not have any effect on the medical services you receive. Next, I will ask you some background questions.'

Step 2: Collect demographic information

es1 Gender: $Female_0$ $Male_1$

es2 Ethnicity *(circle all that apply)*: $Asian_1$ $Black_2$ $Hispanic_3$ $White_4$ $Other_5$

es3 Age: ___ ___ **[If age is <18 or >39 years, mark 'Ineligible' in Step 6]**

Step 3: Collect contact information (on separate document)

Step 4: Review inclusion criteria

es4 1. Do you currently spend most of the day and nearly every day at home?
_____Yes_1 _____No_0

es5 *a (If yes)* When did it start?
_____ _____ _____
Month Day Year

es6 2. Have you ever in the past spent most of the day and nearly every day at home?
_____Yes_1 _____No_0

es7,es8 *a (If yes)* When did the longest past period start and end?
Start: _____ _____ _____ End: _____ _____ _____
Month Day Year Month Day Year

STOP AND ASSESS:
[If 1 is 'NO' and 2 is 'NO' mark 'Ineligible' in Step 6. If 1(a) is less than 6 months ago and period in 2(a) is less than 6 months, mark 'Ineligible' in Step 6]

es9 3. Do you currently avoid social situations, such as attending school or going to a workplace?
_____Yes_1 _____No_0

a (If yes) What are a couple (two) examples?
_____
_____
_____

es10 *b (If yes)* When did it start?
_____ _____ _____
Month Day Year

es11 4 Have you ever in the past avoided social situations?
_____Yes_1 _____No_0

es12,es13 *a (If yes)* When did the longest past period start and end?
Start: _____ _____ _____ End: _____ _____ _____
Month Day Year Month Day Year

STOP AND ASSESS:
[If 3 is 'NO' and 4 is 'NO', mark 'Ineligible' in Step 6. If 3(a) is less than 6 months ago and period in 4(a) is less than 6 months, mark 'Ineligible' in Step 6]

es14 5 Do you currently avoid social relationships, such as friendships or contact with family members?
_____Yes_1 _____No_0

a (If yes) What are a couple (two) examples?
_____
_____
_____

Appendix 1 (Continued)

es15 b *(If yes)* When did it start?

_____ _____ _____
Month Day Year

es16 6 Have you ever in the past avoided social relationships?
_____Yes$_1$ _____No$_0$

es17,es18 a *(If yes)* When did the longest past period start and end?
Start: _____ _____ _____ End: _____ _____ _____
Month Day Year Month Day Year

STOP AND ASSESS:
[If 5 is 'NO' and 6 is 'NO' mark 'Ineligible' in Step 6. If 5(a) is less than 6 months ago and period in 6(a) is less than 6 months, mark 'Ineligible' in Step 6]

es19 7 Considering your most severe period of social isolation, *(did/does)* it do any of the following:
a) interfere significantly with your normal routine;
b) interfere significantly with your ability to work or attend school;
c) interfere significantly with social activities or relationships; or
d) bother you a lot?
_____Yes$_1$ _____No$_0$
[If NO, mark 'Ineligible' in Step 6]

8 Briefly, what is/was the reason you started being socially isolated?
_____
_____
_____
[If all episodes due to a chronic physical illness or injury, mark 'Ineligible' in Step 6]

Step 5: Review of exclusion criteria

es20 1. Are you comfortable speaking and reading English*?
_____Yes$_1$ _____No$_0$ **[If NO, mark 'Ineligible' in Step 6]**

es21 2. Do you live in a group home or institution?
_____Yes$_1$ _____No$_0$ **[If YES, mark 'Ineligible' in Step 6]**

3. Do you have a history or have you been told you have any of the following conditions:
es22a _____ Schizophrenia
es22b _____ Dementia (any type)
es22c _____ Mental Retardation
es22d _____ Asperger Syndrome
es22e _____ Autistic Disorder (Autism)
[If any of the above conditions checked, mark 'Ineligible' in Step 6]

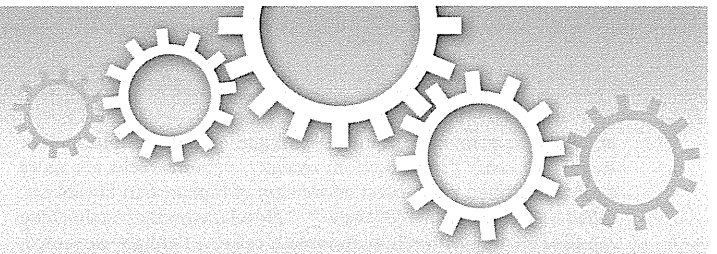
Step 6: Determine eligibility screening outcome

es23 1. _____$_0$ Ineligible for study participation.
'I'm sorry to say that you are not eligible to participate in this study. Thank you for your interest. Do you have any questions or concerns?'

2. _____$_1$ Eligible for study participation.
'I'm pleased to say that you are eligible to participate in this study.'

*English replaced by the local language for non-English-speaking sites.

SCIENTIFIC REPORTS



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SUBJECT AREAS:
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Direct induction of ramified microglia-like cells from human monocytes: Dynamic microglial dysfunction in Nasu-Hakola disease

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Microglia have been implicated in various neurological and psychiatric disorders in rodent and human postmortem studies. However, the dynamic actions of microglia in the living human brain have not been clarified due to a lack of studies dealing with *in situ* microglia. Herein, we present a novel technique for developing induced microglia-like (iMG) cells from human peripheral blood cells. An optimized cocktail of cytokines, GM-CSF and IL-34, converted human monocytes into iMG cells within 14 days. The iMG cells have microglial characterizations; expressing markers, forming a ramified morphology, and phagocytic activity with various cytokine releases. To confirm clinical utilities, we developed iMG cells from a patient of Nasu-Hakola disease (NHD), which is suggested to be directly caused by microglial dysfunction, and observed that these cells from NHD express delayed but stronger inflammatory responses compared with those from the healthy control. Altogether, the iMG-technique promises to elucidate unresolved aspects of human microglia in various brain disorders.

Microglia, immune cells in the brain, play major immunological/inflammatory roles as brain macrophage in the central nervous system (CNS). The origin of resident microglia have been proven to be from primitive myeloid progenitors (primitive macrophage) that arise in the yolk sac before embryonic day 8[1]. Resident microglia form as a ramified type (called ramified microglia), whose branches constantly move and survey the microenvironment under physiological conditions in the CNS[2], and once activated, shift to an ameboid type, phagocytose, and release various mediators such as pro-inflammatory cytokines[3–5]. Microglia are suggested to contribute to the pathophysiology of various neurological and psychiatric disorders[6–8]. Nasu–Hakola disease (NHD) which is a very rare autosomal recessive disorder, initially reported in Finland and Japan[9,10], is believed to be caused by microglial dysfunction. Until now, only about 200 cases have been reported worldwide and the majority of cases are in the Finnish and Japanese populations[11]. NHD is characterized by formation of multifocal bone cysts and progressive early-onset dementia with various psychiatric symptoms including personality changes[11,12], caused by mutations of DNAX-activation protein 12 (DAP12)[13] or triggering receptor expressed on myeloid cells 2 (TREM2)[14], both of which are expressed in human microglia. A rodent brain study showed that DAP12 is expressed only in microglia and deletion of DAP12 induces synaptic impairments possibly due to microglial dysfunction[15]. A human postmortem study has revealed the absence of DAP12 expression on ramified microglia in the brains of NHD patients[16].

The above-mentioned reports have strongly supported the theory that human microglia maladaptively contribute to a variety of neurological and psychiatric disorders including NHD, while dynamic analysis of microglial dysfunction in the human brain has yet to been clarified. The most significant limitation in human brain research is the difficulty in obtaining living brain cells including microglial cells from living human brains due to ethical and technical considerations. To solve this limitation, alternative methods have long been warranted. Presently, human neuronal cells can be established from somatic cells (not from the brain) such as skin fibroblasts by

utilizing the gene-modification technique of induced pluripotent stem (iPS) cells[17,18]. In addition, recently, neuronal cells are more easily established from direct conversion of human skin fibroblasts, called induced neuronal (iN) cells[19–21]. Novel methods of establishing ramified microglia from human somatic cells are strongly warranted, based on iPS or direct conversion techniques, while none have yet been reported. Herein, we show a novel technique for developing induced microglia-like (iMG) cells easily and quickly from adult human peripheral blood cells. In addition, by utilizing this iMG-technique, we present the first translational analysis of the dynamic actions of microglia from a patient of NHD.

Results

Inducing ramified microglia-like cells. To determine what cytokines induce ramified microglia from human peripheral monocytes, we selected and tested the effects of the following candidate cytokines; granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and interleukin (IL) -34, all of which are suggested to be essential for developing and maintaining ramified microglia[22–25]. Untreated monocytes showed round shapes (Fig. 1A). Macrophages, induced by GM-CSF (10 ng/ml), shifted to an ameboid morphology on DAY 14 (Fig. 1B). On the other hand, treatment of M-CSF (10 ng/ml) alone or IL-34 (100 ng/ml) alone showed a spindle morphology (Fig. 1C and D), and the cocktail of both cytokines induced more complicated morphologies than the single treatment (Fig. 1E).

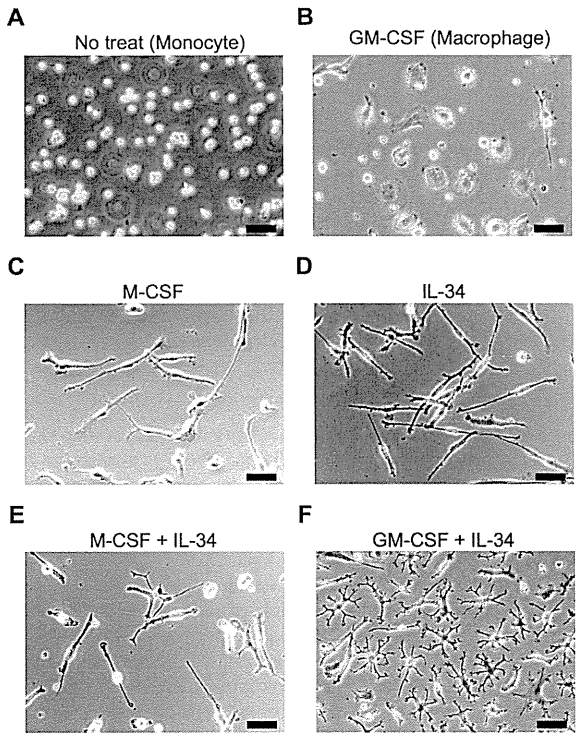

Figure 1 | Inducing ramified microglia from human peripheral monocytes. The monocytes on the day of isolation (A) were incubated with the following candidate cytokines; GM-CSF (10 ng/ml; B), M-CSF (10 ng/ml; C), IL-34 (100 ng/ml; D), M-CSF + IL-34 (E) and GM-CSF + IL-34 (F) for 14 days. The optimal cytokine conditions were tested by morphological changes using phase-contrast microscopy. The cocktail of both GM-CSF and IL-34 induced small soma body bearing numerous branched collaterals, which expressed the specific morphology of ramified microglia (F). Scale bar, 50 μm.

Surprisingly, the cocktail of both GM-CSF (10 ng/ml) and IL-34 (100 ng/ml) induced small soma bodies bearing numerous branched collaterals (Fig. 1F), which expressed the specific morphology of ramified microglia- small soma with extensive radial ramifications. The viability of these cells post 14 days was 16.7% ± 4.2 (n = 3, mean ± SEM) as compared to the initial cell number (DAY 0). Interestingly, the earliest branched cells were observed on DAY 3 after GM-CSF and IL-34 treatment (Supplementary Fig. S1A). In addition, we confirmed that these cells survive at least one month when medium change was performed once a week (Supplementary Fig. S1B).

Phenotyping of the induced microglia-like (iMG) cells. Next, we tested whether the ramified microglia-like cells, named *induced microglia-like (iMG) cells*, have microglial characterization. Generally, it is difficult to distinguish between macrophage and microglia, because useful and specific microglial markers are very limited. Traditionally, CD11b and CD45 are used as a distinction marker between macrophage and microglia[26]. Recently, the phenotype of human microglial cells, isolated from the fresh postmortem brain, has been revealed to have lower expression of CD14 and CD200R compared to macrophage[27]. Thus, we compared the expression level of surface markers between iMG cells and induced macrophage using flow cytometry. The expression level of CD11b on iMG cells did not differ from that on macrophage, while that of CD45 decreased on iMG cells (Fig. 2A). The expression levels of CD14 and CD200R were also decreased on iMG cells compared to those on macrophage (Fig. 2B), which support that iMG cells have the specific phenotype of microglia[27]. Furthermore, Mizutani et al.[28] recently reported a clear-cut distinction between monocytes ($CCR2^{high}$, $CX3CR1^{low}$) and resident microglia ($CCR2^{low}$, $CX3CR1^{high}$) using $CX3CR1^{+/GFP}CCR2^{+/RFP}$ knockin fluorescent protein reporter mice. Therefore, we compared the expression pattern of CCR2 and CX3CR1 between monocytes and iMG cells. Monocytes were stained with bright red fluorescence (CCR2) bearing round or elliptic morphology (Fig. 2C), and iMG cells were stained with bright green fluorescence (CX3CR1) bearing highly branched forms (Fig. 2, C and D). In addition, we confirmed that the expression ratio (CX3CR1/CCR2) of iMG cells is significantly higher than that of monocytes by flow cytometry (Fig. 2E). These results indicate that the iMG cells induced by GM-CSF and IL-34 show the essential characteristics of resident microglia[28].

Melief et al.[27] have also revealed that IL-4 and dexamethasone alter specific gene expressions in fresh human microglial cells ([IL-4] HLA-DR, CCR7, CCL18, and CD200R are upregulated, and CD45 and TNF-α are downregulated; [dexamethasone] CCL18 is upregulated, and HLA-DR, CCR7, CD45, TNF-α, and CD200R are downregulated). Therefore, we assessed the above gene expression patterns in iMG cells incubated with IL-4 and dexamethasone using quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). Except for CCL18 (treated with dexamethasone), almost all gene expression patterns (HLA-DR, CCR7, CD200R, CD45 and TNF-α) in the iMG cells were in agreement with the above data using fresh human microglia[27] (Fig. 2F, G).

Functional analysis of the iMG cells. Microglia reside as a ramified form, and various molecules activate microglia into an ameboid form, phagocytizing and releasing various cytokines[3], and overactivation of microglia induces neuronal damage and various brain pathologies via pro-inflammatory cytokines such as tumor necrosis factor (TNF) -α[6,7]. To examine whether iMG cells have these dynamic functions, we tested the phagocytosis ability and the following TNF-α secretion. Interestingly, the iMG cells showed the ability of phagocytosis with morphological changes into an ameboid form (Fig. 3A). Next, we tested the ability for TNF-α production during phagocytosis on the iMG cells, and revealed that the mRNA expression and protein level of TNF-α on

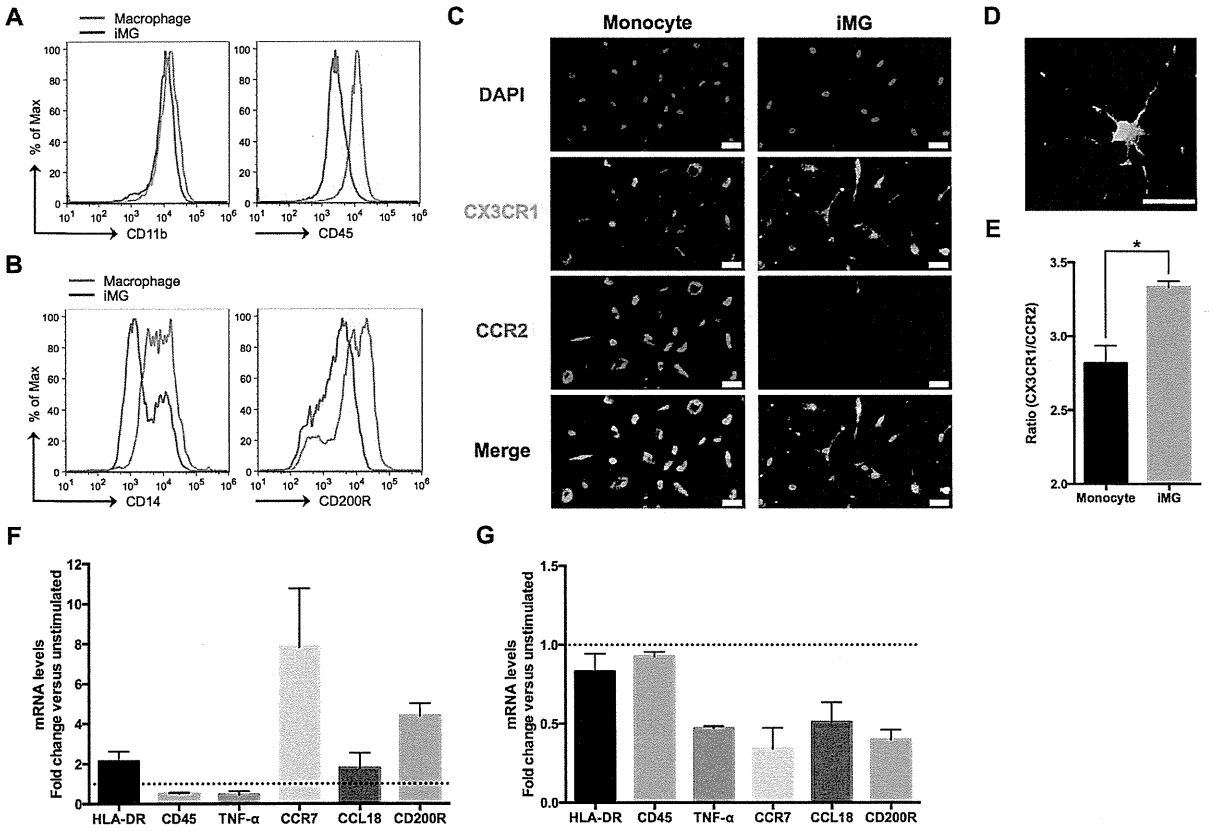

Figure 2 | The iMG cells show the character of human resident microglia. (A and B) The expression levels of surface markers on the iMG cells and induced macrophage were performed by flow cytometer. Peripheral monocytes were incubated with GM-CSF (macrophage) or cocktail of GM-CSF and IL-34 (iMG cells) for 14 days. The iMG cells showed the specific phenotypes of microglia compared to macrophage. (C to E) The expression pattern of CCR2 and CX3CR1 between monocytes and iMG cells were observed by immunocytochemistry. The monocytes and iMG cells were cultured for 14 days, and stained with specific antibodies. (C and D) The iMG cells were stained with bright green fluorescence (CX3CR1) bearing highly branched forms. Scale bar, 50 μm. (E) The expression ratio (CX3CR1/CCR2) of iMG cells was significantly higher than that of monocytes by flow cytometry (n = 3). The iMG cells were incubated with IL-4 (F) or dexamethasone (G) for 72 hours, and extracted RNA was analyzed by qRT-PCR (n = 6). Fold changes were depicted in mRNA levels after stimulation compared with unstimulated cells. $*P < 0.05$. Error bars, standard error of the mean (SEM).

the iMG cells during phagocytosis are significantly higher compared to those on non-treated cells (Fig. 3, B and C).

Analysis of the iMG cells from a patient of NHD. The above results demonstrated that the iMG cells have the dynamic functions of human microglia, and we suggest that iMG cells have the possibility to be utilized for analyzing the underlying microglial pathophysiology of brain disorders. As the initial step, we conducted the first translational analysis of the iMG cells derived from a patient of NHD. NHD is believed to be caused by microglial dysfunction, while no investigation exists using living human microglial cells from patients of NHD. We analyzed the dynamic functions of microglia using the iMG cells from a patient of NHD (141delG in DAP12 gene), after obtaining informed consent (under the permission of the Institutional Review Board of Kyushu University and Osaka University). In agreement with genetic diagnosis, the iMG cells from the NHD patient showed significantly lower expression of DAP12 than those from a healthy control, and there was no difference in TREM2 expression (Fig. 4A). Interestingly, the production of pro-inflammatory cytokines (TNF-α and IL-6) was delayed in the iMG cells from the NHD patient as compared to those from the healthy control after 24 hours. Furthermore, the iMG cells from the patient showed a significantly lower level of anti-inflammatory cytokine (IL-10) than those from the healthy control. The production levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β and IL-8) had no significant differences between the NHD and healthy control after 72 hours (Fig. 4B). Next, we examined whether it is possible for iMG cells from the healthy control to silence the target gene with siRNA (DAP12). DAP12 was successfully downregulated in the iMG cells from the healthy control (Supplementary Fig. S2). We assessed the cytokine production of iMG cells treated with siRNA. Predictably, the production of pro-inflammatory cytokines (TNF-α and IL-6) was delayed in the DAP12-silenced iMG cells as compared to the control (Fig. 4C).

Discussion

We have shown a novel technique for developing directly induced microglia-like cells from human peripheral blood cells. GM-CSF and IL-34 converted human monocytes into iMG cells within 14 days. The iMG cells have microglial characterizations; expressing markers, forming a ramified morphology, and phagocytic activity with various cytokine releases. Until now, some attempts to induce microglia-like cells from hematopoietic cells have been performed using an astrocyte co-culture system or astrocyte-conditioned media[29,30], and GM-CSF and IL-34 are known to be derived from astrocytes[30,31].