FTLD had a positive family history (Ratnavalli *et al.*, 2002). In a nationwide survey in the Netherlands, 38% of FTLD patients had one or more first-degree relatives with dementia before the age of 80 years, compared with 15% of the control participants (Stevens *et al.*, 1998). In a hospital-based study by the Manchester group (Snowden *et al.*, 1996), a family history was seen in all subtypes of FTLD and 50 % of their bvFTD cases had a positive family history, similar to the findings in a Swedish series (Gustafson, 1987). On the other hand, there are only few studies on heredity in Asian FTLD patients. In the two clinic-based studies from Japan, family history was either absent (Ikeda *et al.*, 2004) or reported in less than 5% of FTLD patients (Wada-Isoe *et al.*, 2012). In a study from India, only 8.3% of bvFTD patients had a first-degree relative affected with a FTLD spectrum disorder (Ghosh *et al.*, 2013). The authors of the study also suggested that there could be distinctive behavioral patterns in Asian patients with bvFTD. Most patients in that study showed florid behavioral symptoms even in the early stages.

As advances in genetics and molecular pathology usher in clinical trials with biologically driven, disease-specific therapies for individual FTLD subtypes, it becomes essential to ensure that cross-cultural clinical and genetic differences in FTLD and its related disorders are clearly recognized. With this in mind, the present study aims to look at the family history in the different FTLD spectrum disorders in Asian countries.

Methods

Patients were recruited for the study from consecutive outpatients who attended the following Asian centers between January 2010 and December 2012: (1) Cognitive Neurology Unit, Department of Neurology, Apollo Gleneagles Hospitals (India), (2) Hasan Sadikin Hospital, Faculty of Medicine, Padjadjaran University (Indonesia), (3) Department of Neuropsychiatry, Faculty of Life Science, Kumamoto University Hospital (Japan), (4) St. Lukes Medical Center (Philippines), and (5) Taipei Veterans General Hospital and Cardinal Tien Hospital (Taiwan). All patients were examined by senior neurologists or psychiatrists and were assessed by a combination of careful medical history, laboratory testing, morphological imaging of brain such as magnetic resonance imaging (MRI) or computed tomography (CT), and functional imaging such as single photon emission computed tomography (SPECT), whenever possible. In some patients with severe behavioral symptoms, it was difficult to perform functional imaging without sedation. Patients were diagnosed with FTLD (bvFTD, SD, PA), FTD/MND, PSP, and CBS according to recognized diagnostic criteria (Brooks, 1994; Litvan *et al.*, 1996; Neary *et al.*, 1998; Boeve *et al.*, 2003). The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria (McKhann *et al.*, 1984) were used to diagnose Alzheimer's disease. Only those patients who had reliable informants such as their spouses, were included in this study.

Each pedigree was investigated across five generations (first- and second-degree relatives) for any affected family member. Data obtained from interviewing patients and family members were used to make detailed family trees. The affected family members were categorized according to appropriate diagnostic criteria into one of the following disorders: bvFTD, SD, PA, FTD/MND, PSP, CBS, MND, Parkinson's disease, and other dementias. Age at onset and a medical history, whenever possible, were also obtained. When a family had more than one affected member, only a single proband was included to avoid overestimation.

All procedures followed the Clinical Study Guidelines of Ethics Committee of Kumamoto University Hospital, Apollo Gleneagles Hospitals, Taipei Veterans General Hospital, National Yang-Ming University Hospital, Hasan Sadikin Hospital, St. Lukes Medical Center, and Cardinal Tien Hospital, and were approved by the respective internal review boards. A complete description of the study procedures was provided to the patients and their caregivers and written informed consent was obtained from them.

Results

Ninety-one patients were recruited from the five institutes. Demographic data are shown in Table 1. Forty-two patients had a diagnosis of bvFTD, two patients had FTD/MND, 22 had SD, 15 had PA, one had PA/CBS, five had CBS, and four patients had PSP. One of the patients was clinically diagnosed as FTD/MND, who showed FTLD-TDP pathology by brain biopsy. This patient and two others were found to have the C9ORF72 mutation by genetic testing. The Mini-Mental State Examination (MMSE) scores, Clinical Dementia Rating (CDR) scores, average age at onset, and duration of illness at presentation are summarized in Table 2. The MMSE scores were not available in 13 patients because of their severe behavioral and/or language disturbances, while in four patients

Table 1. Demographic data for the 91 patients with frontotemporal lobar degeneration spectrum disorders

					NUMBER OF THE EACH DIAGNOSTIC GROUP						
	N	M/F	MEAN AGE AT ONSET (YEARS)	MEAN DURATION (YEARS)	BVFTD	FTD/MND	SD	PA	PA/CBS	CBS	PSP
India	39	29/10	61.4	3.2	23 (15)	1 (1)	5 (4)	3 (2)	0	4 (4)	3 (3)
Indonesia	4	0/4	55.0	5.5	3 (0)	0	0	0	1 (0)	0	0
Japan	18	11/7	62.5	5.7	5 (4)	0	9 (5)	2 (2)	0	1 (0)	1 (0)
Philippines	7	1/6	57.7	3.1	5 (1)	0	0	2 (0)	0	0	0
Taiwan	23	8/15	63.2	2.7	6 (2)	1 (0)	8 (2)	8 (4)	0	0	0
Total	91	49/42	61.5	3.7	42(22)	2 (1)	22 (11)	15 (8)	1 (0)	5 (4)	4(3)

Notes: The numbers within brackets denote the number of male patients.
bvFTD = behavioural variant frontotemporal dementia; FTD/MND = frontotemporal dementia with motor neuron disease; SD = semantic dementia; PA = progressive non-fluent aphasia; PA/CBS = progressive non-fluent dementia and corticobasal syndrome overlap; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy.

Table 2. Age, duration, MMSE scores, and CDR scores by diagnostic group

DIAGNOSIS OF PATIENTS	N	MEAN AGE AT ONSET (YEARS)	MEAN DURATION (YEARS)	MMSE SCORE	DISTRIBUTION OF CDR SCORE (0/0.5/1/2/3) (NUMBERS OF PATIENTS)
bvFTD	42	59.9	3.7	15.4	0/5/14/18/5*
FTD/MND	2	63.5	4.5	21.0	0/1/1/0/0
SD	22	59.5	1.8	14.3	0/4/12/1/3
PA	15	67.1	3.3	15.5	1/4/4/3/2
PA/CBS	1	76.0	5.0	–	0/0/0/1/0
CBS	5	64.0	1.7	18.2	0/1/2/2/0
PSP	4	61.3	3.3	21.5	0/1/2/0/0
Total	91	61.5	3.7	15.7	1/16/35/25/10

Notes: Numeral shows the number of patients in each CDR score.
Data of 13 cases in MMSE and 4 cases in CDR were could not available.
MMSE = Mini-Mental State Examination; CDR = Clinical Dementia Rating; bvFTD = behavioural variant frontotemporal dementia; FTD/MND = frontotemporal dementia with motor neuron disease; SD = semantic dementia; PA = progressive non-fluent aphasia; PA/CBS = progressive non-fluent dementia and corticobasal syndrome overlap; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy.

CDR scores were not recorded. Sixty-six patients had information of all of their first-degree relatives. They include 35 patients with bvFTD, 14 with SD, seven with PA, one with PA/CBS overlap syndrome, five with CBS and four with PSP. Data on family history in the different FTLD spectrum disorders are shown in Table 3. Family history of any FTLD spectrum disorder was found in 5.5% of all patients, 9.5% of those with bvFTD, 50% of those with FTD/MND (out of only two patients), but in none of those with SD, PA, PA/CBS, CBS, and PSP. Among the four probands with bvFTD and positive family history, two also had family history of bvFTD, one had family history of PA, and one had family history of MND.

One bvFTD proband had three family members with FTD, including first-degree relatives, although neither pathological nor genetic data were available for any of them. Each of the other probands with bvFTD had only one other family member with a FTLD spectrum disorder. One of two probands with FTD/MND had one family member with MND. Family history of other dementias including AD and undiagnosed dementias was found in 27.5% of all patients, 26.2% of bvFTD, 27.3% of SD, 50% of PA, 75% of PSP, and in the only patient with PA/CBS overlap, but in none with FTD/MND or CBS.

Discussion

To date, family history in FTLD, reported mostly from western European and North American populations, has been seen in up to 40% of patients, with roughly 10% of patients showing an autosomal dominant inheritance pattern (Goldman *et al.*, 2005; 2007; van Swieten and Rosso, 2008). Relevant data from Asia are sparse (Ikeda *et al.*, 2004; Ghosh *et al.*, 2013). Our study is one of the

Table 3. Family history data in each diagnostic group

DIAGNOSIS OF PATIENTS	N	FAMILY MEMBERS AFFECTED WITH FTLD AND RELATED DISEASES		FAMILY MEMBERS AFFECTED WITH OTHER OR UNDIAGNOSED DEMENTIA	
		N	%	N	%
bvFTD	42	4 (including 1 MND)	9.5	11	26.2
FTD/MND	2	1 (MND)	50.0	0	0.0
SD	22	0	0.0	6	27.3
PA	15	0	0.0	4	26.7
PA/CBS	1	0	0.0	1	100.0
CBS	5	0	0.0	0	0.0
PSP	4	0	0.0	3	75.0
Total	91	5	5.5	25	27.5

Notes: FTLD and related diseases included FTLD spectrum disorders and MND. One patient with MND among four affecting family members in bvFTD group: one affected family member with MND in the FTD/MND group.
bvFTD = behavioural variant frontotemporal dementia; FTD/MND = frontotemporal dementia with motor neuron disease; SD = semantic dementia; PA = progressive non-fluent aphasia; PA/CBS = progressive non-fluent dementia and corticobasal syndrome overlap; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy.

largest reports of FTLD in an Asian population. A positive family history of FTLD spectrum disorders was found in 5.5% of our patients. Together with the previous Asian studies, our findings, therefore, support the infrequent occurrence of family history in Asian FTLD patients. It might be due to genetic differences between western and Asian populations.

In our patients, only one proband with bvFTD showed a clear autosomal dominant inheritance pattern, whereas none of the SD patients gave a family history of any FTLD spectrum disorder. Goldman *et al.* (2005) reported autosomal dominant inheritance in 18.2% of their patients with bvFTD and 1.9% of their SD patients, while Rohrer *et al.* (2009) described this inheritance pattern in 20% of their bvFTD patients but not in their SD patients. Goldman *et al.* (2005) also showed that familial aggregation, in which there were three or more affected family members, occurred in 8.1% of bvFTD patients but not in SD patients, and that 18.2% of bvFTD patients and 15.1% of SD patients had a single affected first-degree relative. In our study, most of the bvFTD patients had sporadic disease and only 2.4% of patients had a single affected relative. Therefore, in Asia, the occurrence of familial FTLD is undoubtedly lower than that in the western countries.

In recent years, various genetic abnormalities in microtubule-associated protein tau (MAPT), progranulin (GRN), and C9ORF72 have been associated with familial FTLD. In Asia, MAPT mutations have been reported in familial SD (Ishizuka *et al.*, 2011), although typically in low frequency (Wada-Isoe *et al.*, 2012). These results suggest that genetic factors for the development of FTLD may have a less important role in the Asian population. Rohrer *et al.* (2009) demonstrated that 186 out of the 225 FTLD patients in their study had no mutations in known genes such as MAPT, GRN, valosin-containing protein (VCP), TARDP, chromatin modifying protein 2B (CHMP2B), and fused in sarcoma (FUS), and did not show strong family history. Unknown genetic defects may be associated with the development of many sporadic FTLD cases.

There are several limitations in the current study. First, although diagnosis of each proband was based on comprehensive examination including brain imaging, and followed recognized consensus criteria, the information regarding family histories were obtained by semi-structured interviews of the proband and family members. It was thereby difficult to confirm the diagnosis in many deceased or distant family members. Second, for most of our patients the diagnosis was based on clinical criteria and was not confirmed by definite pathological or genetic tests. However, going by the number of patients with FTLD spectrum disorders recruited for this study, this may be the largest research to date focusing on the family history of these disorders in Asia. This could, therefore, form the basis for future neurogenetic research in Asian countries.

Conclusion

Previous epidemiological studies have suggested that familial FTLD was rare in Asian countries. The current study, by focusing on family history in FTLD patients, demonstrated that, unlike patients from western countries, few Asian FTLD patients have a positive family history of dementia. Future research could explore possible reasons underlying these differences.

Conflict of interest

None.

Description of author's roles

R. Fukuhara participated in the study design, analyzed the data, and wrote the paper. A. Ghosh, J. Fuh, J. Dominguez, and P. A. Ong carried out clinical assessment, collected the data, and edited and revised the paper. A. Dutt and Y. Liu carried out clinical assessment and collected data. H. Tanaka carried out clinical assessment, collected the data, and assisted the analyses. M. Ikeda participated in the study design, and editing and revising the paper. All of the authors contributed to and approved the manuscript.

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本邦におけるFTDに対するoff-label処方の実態について

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原著

本邦におけるFTDに対するoff-label処方の実態について

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要　旨

全国4施設の専門外来をFTD圏の診断名で紹介された連続例87例の背景因子，紹介医の診療科および認知症症状に対する処方の内容などを調査した．紹介医は精神科医が6割で，ほか神経内科医，一般内科医，脳神経外科医などであった．約半数の例に認知症症状に対する薬剤が用いられ，コリンエステラーゼ阻害剤は様々な診療科から2割の患者に処方されていた．向精神薬は精神科医によって1/3以上の患者に処方され，抗うつ薬，抗精神病薬の処方が多かった．前頭側頭葉変性症や運動ニューロン疾患と診断されていた例には処方はなされていなかった．他の背景因子は薬剤使用には影響を与えなかった．FTDへの薬物療法ガイドラインの作成が望まれる．

キーワード：前頭側頭型認知症，off-label処方，コリンエステラーゼ阻害剤，向精神薬，薬物療法

1．はじめに

前頭側頭型認知症（frontotemporal dementia：FTD）は前頭葉や側頭葉前方部に変性の中心がある変性性認知症群であり，初老期に発症する変性性認知症の中では，アルツハイマー病（Alzheimer's disease：AD）に次いで多いとされる（Ratnavalli et al., 2002）．FTDの患者は病初期から前頭葉機能の障害に伴う社会行動の変化や人格の変化を呈することが特徴であり，臨床診断基準においても，脱抑制や無為，共感性の欠如，常同行動，食行動異常といった行動変化が主要な項目として述べられている（Rascovsky et al., 2011）．これらの特徴的な行動変化から病初期から介護者の負担が大きく（Mioshi et al., 2013），一方で精神疾患や他の認知症性疾患に誤診されていることも多いため（Woolley et al., 2011），医療現場においても対象に苦慮することが多い疾患群である．

現時点では，本邦においても，また主要な欧米諸

Off-label medication for frontotemporal dementia in Japan

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国においてもFTDに保険適応のある薬剤はない(Boxer et al., 2012b). アルツハイマー病(Alzheimer's disease: AD) に対して用いられるコリンエステラーゼ阻害剤 (Cholinesterase inhibitor: ChEI) がFTDに用いられている例も臨床場面ではみとめられるが, これらの薬剤はFTDの行動障害を悪化させることが報告されている (Mendez et al., 2007). また行動障害を抑える目的で抗精神病薬が用いられることも多いが, FTDの患者は抗精神病薬に対して錐体外路症状などの危険性が高いことも報告されている (Kerssens et al., 2008). このような疾患であるFTDに対して, 本邦における適応外処方の実態はこれまで明らかになっていない.

本研究の目的は専門医以外によってFTDと診断を受けた場合, 1) どのような処方がなされるのか, 2) 処方の内容に影響を与えるような因子があるのか, を明らかにすることである.

2. 対象と方法

2008年1月から2010年12月の期間に全国4施設 (公益財団法人浅香山病院, 愛媛大学医学部附属病院精神科神経科, 熊本大学医学部附属病院神経精神科, 東京慈恵大学医学部附属病院精神神経科) の認知症専門外来を受診した連続例から, 紹介医でFTDないしはそれに類する診断名 (ピック病, 疑い病名を含む) で紹介された患者を抽出した. そのうえで, それらの患者の年齢, 性別, 教育歴, 罹病期間, Mini mental state examination: MMSE得点 (Folstein et al., 1975) といった背景因子, 前医の診療科, 前医における認知症症状に対する処方 (ChEI, 他の認知機能障害に対する薬剤, 抗精神病薬, 抗うつ薬, 抗不安薬, 気分調整薬, 漢方薬など) の有無とその内容, 介護保険取得状況, 専門医の最終診断などを各施設の認知症データベースより調査した. ただし, 本報告は2010年末までの集計であり, ChEIとして処方されたのはDonepezilのみである. 診療科別の処方割合や紹介医の診断別の処方割合についてはχ^2検定およびFisherの正確検定にて検定を行い, 薬剤の使用に影響を与える背景因子を検討するための2群比較においては, t検定あるいはχ^2検定およびFisherの正確検定にて検討を行った.

専門医の最終診断にあたっては, 各施設に認知症学会及び老年精神医学会の専門医がおり, 画像診断および共通した認知機能バッテリーを用いて, 血液検査などで共通のプロトコールに則って除外診断を行い, 各疾患の診断基準に基づいて診断を行っている.

本研究はデータベースを用いた調査であり介入研究ではない. 患者の匿名性に関しては十分な配慮がなされており, データベースを用いる研究を行うことに関しては, 各施設の倫理委員会の承認を各々得ている.

3. 結果

3.1. 患者および紹介医の背景

今回対象となった患者87例の背景を表1に示す. 男女比はほぼ同等で, 平均年齢が66.9歳, 平均の初診時MMSE得点は18.4であった.

紹介医の診断はFTDおよび疑い, 前頭側頭葉変性症 (Frontotemporal lobar degeneration: FTLD) および疑い, 側頭葉優位型圏内, ピック病および疑い, 運動ニューロン疾患 (Frontotemporal dementia with motor neuron disease: FTD-MND) 圏内などであり, FTDの診断が6割以上を占めた. 紹介医の属性は精神科, 神経内科, 内科, 脳神経外科, その他であり, 精神科が6割以上を占めた.

3.2. 認知症に対する薬剤を使用していた例

87例のうち, 何らかの認知症に対する薬剤の使用を用いていた例はほぼ半数の49.4%(43例)であった. 認知機能に対する薬剤を用いていたのは23%(20例) であり, ChEIは20.7% (18例) に用いられていた. 脳代謝改善薬は2.3% (2例) に用いられていた. 一方で精神症状に対する薬剤 (以下向精神薬とする: 抗精神病薬, 抗うつ薬, 抗不安薬, 気分調整薬, 特定の漢方薬を含む) は35.6% (31例) に用いられていた (ChEIとの重複や, 向精神薬同士での重複を含む). 抗うつ薬が16.1% (14例) に, 漢方薬 (全て抑肝散) が11.5% (10例) に, 抗精

表1. demographic data of patients and referring physicians

Sex（Male : Female）	42 : 45
Age	66.9（11.6）
education（year）	11.3（2.9）
disease duration（year）	3.0（2.1）
MMSE score	18.4（9.5）
Referring physicians' diagnosis (FTD/FTLD/temporal variant/Pick's disease/FTD-MND)	55/5/9/11/7
Referring physicians' background (psychiatrist/neurologist/general physician/neurosurgeon/others)	53/17/9/6/2

MMSE : Mini-Mental State Examination
FTD : Frontotemporal dementia
FTLD : Frontotemporal Lobar Degeneration
FTD-MND : FTD with motor neuron disease
mean（SD）for Age, education, disease duration, and MMSE score

神病薬が10.3%（9例）に，抗不安薬が9.2%（8例）に，気分調整薬が1.1%（1例）に用いられていた．

3.3. 診療科別の処方

診療科によって処方の傾向が異なるかどうかも検討した．まずChEIの診療科別の処方率であるが，精神科では13/53（24.5%），神経内科では2/17（11.8%），内科では2/9（22.2%），その他は1/8（12.5%）であった．χ^2検定（Fisherの正確検定）にて有意差は認められなかった．一方で向精神薬は精神科では26/53（49.1%）に処方されていたのに対し，神経内科では1/17（5.9%），内科では2/9（22.2%），その他は2/8（25.0%）とχ^2検定（Fisherの正確検定）にて有意（P=0.003）に精神科で多く処方されていた．抗精神病薬が処方されていた9例のうち8例は精神科での処方であり，抗うつ薬は14例全例が精神科での処方であった．漢方薬（抑肝散）は10例中8例が精神科での処方であった．向精神薬については，どの種類の薬剤でも精神科での処方が多いという結果であった．

3.4. 紹介医の診断別の処方

紹介医の診断名による処方割合についても検討した．まず，ChEIが処方されていた18例ではFTDおよび疑いという診断が11例（61.1%）で最も多く，FTLDおよび疑い，FTD-MND圏内と診断された例にはChEIは処方されていなかった．向精神薬が処方されていた31例でもFTDおよび疑いが21例（67.7%）ともっと多く，FTLDおよび疑い，FTD-MND圏内と診断された例には向精神薬は処方されていたなかった．

3.5. 専門医の診断と紹介医の処方

専門医の診断は必ずしも紹介医の診断と一致しない．紹介医の過小診断や過剰診断に基づく処方も問題になりうる．そこで，専門医の診断と紹介医の処方割合についても検討した．ChEIが処方されていた18例のうち，専門医によってFTDと診断された例は4例（28.6%）であったが，ChEIが処方されていなかった69例のうち，専門医によってFTDと診断された例は20例（29.0%）であった．ほぼ類似した値であり，χ^2検定とFisherの正確検定によって有意差は認められなかった．向精神薬が処方されていた31例のうち，専門医によってFTDと診断された例は5例（16.1%）であった．一方で向精神薬が処方されていなかった56例のうち，専門医によってFTDと診断された例は19例（33.9%）であり，向精神薬が処方されていなかった例の方が専門医によってFTDと診断される割合が高い傾向にあった．しかしχ^2検定とFisherの正確検定によって有意差は認められなかった．

3.6. 薬剤の使用に影響を与える背景因子

ChEIの使用に影響を与えるような背景因子があるかどうか，ChEIの使用の有無によって2群に分け，比較を行った（表2）．しかしながら，性別，

表 2. Factors associated with ChEI use

	No ChEI use (n=69)	ChEI use (n=18)	
Sex (Male : Female)	33 : 36	9 : 9	n.s
Age	65.7 (12.1)	71.4 (8.0)	n.s
education (year)	11.3 (3.0)	11.5 (2.8)	n.s
disease duration (year)	2.8 (2.1)	3.7 (1.8)	n.s
MMSE score	19.0 (9.5)	16.0 (9.2)	n.s
care insurance use (yes : no)	20 : 49	5 : 13	n.s

ChEI : Cholinesterase Inhibitor
MMSE : Mini-Mental State Examination
mean (standard deviation) for Age, education, disease duration, and MMSE score

表 3. Factors associated with psychotropic drug use

	No psychotropic drug use (n=56)	psychotropic drug use (n=31)	
Sex (Male : Female)	26 : 30	16 : 15	n.s
Age	67.6 (11.4)	65.7 (12.0)	n.s
education (year)	11.5 (2.8)	11.1 (3.2)	n.s
disease duration (year)	2.9 (2.1)	3.0 (2.2)	n.s
MMSE score	17.7 (9.2)	19.6 (10.0)	n.s
care insurance use (yes : no)	19 : 37	6 : 25	n.s

MMSE : Mini-Mental State Examination
mean (standard deviation) for Age, education, disease duration, and MMSE score

年齢，教育年数，罹病期間，MMSE 得点，介護保険の取得状況などいずれも 2 群間の有意差はなかった．

同様に，向精神薬の使用に影響を与えるような背景因子があるかどうか，向精神薬の使用の有無によって 2 群に分け，比較を行った（表 3）．しかしながら，性別，年齢，教育年数，罹病期間，MMSE 得点，介護保険の取得状況などいずれも 2 群間の有意差はなかった．

4. 考　察

本検討は本邦で最初の FTD に対する off-label 処方の実態調査である．その結果，約半数の例に何らかの認知症症状に対する薬剤が用いられ，ChEI は 2 割の例に処方されていることが明らかになった．向精神薬は 1/3 以上に処方されており，中では抗うつ薬の処方が多かった．ChEI はさまざまな診療科の医師に処方されていが，向精神薬は主に精神科医によって処方されていた．

FTD に対する不適切な治療に関してはいくつかの問題がある．まず，FTD が他の疾患に誤診され，間違った治療を受けている可能性である．FTD は精神疾患や他の認知症性疾患に誤診されることも多く（Woolley et al., 2011），そのために不適切な治療を受ける可能性がある．しかしながら，今回の対象は，紹介医によって FTD 及び類する疾患の診断がなされている例である．その例に対して 2 割に ChEI が，1/3 以上に向精神薬が処方されていた．

この本報告の ChEI の処方率の 2 割という割合を多いと判断するか，少ないと判断するかは，意見の分かれる点と思われる．例えば，他の変性疾患によ

る認知症の例では，レビー小体型認知症（Dementia with Lewy bodies：DLB）に対してのChEIの使用は数多くの論文で有用性が示され，本邦のMoriらの多施設共同RCTにおいても，認知機能，全般機能，そして精神症状も改善したと報告された（Mori et al., 2012）．実臨床においても，ChEIは多くの例に用いられていると推測される．

その一方でFTDに対するChEIの投与の報告は多くはない（Kertesz et al., 2008；Mendez et al., 2007）．そしてほとんどで，有効性は認められなかったと報告され，また脱抑制と衝動性の悪化が認められたとの報告もある（Mendez et al., 2007）．筆者らもFTDの精神症状がChEIで悪化した例を報告している（品川ら，2009）．本報告でChEIが処方された2割のFTD例は，他に選択肢がなくChEIを使用していると推測されるが，これはなるべく避けるべきであり，今後さらなる啓発が必要と思われる．

ChEIが処方されていた18例においても，ChEIが処方されていなかった69例においても，専門医によってFTDと診断された例は3割弱であった．これはつまり，例えば行動・心理症状（Behavioral and psychological symptoms of dementia：BPSD）を伴うADのような例が紹介医によって多くFTDと誤診され，ChEIが処方されているわけではないことを意味する．さらにChEIが用いられている対象と，そうでない対象との間には背景因子に有意差のある項目はなく，ChEIが用いられる対象の一定の傾向は認められなかった．

本報告は2010年末までの集計であり，2011年に本邦で発売された，GalantamineやRivastagmine，Memantineは今回の検討には含まれていない．Memantineは認知機能改善目的以外にもBPSDに対して有用との報告があり（Gauthier et al., 2008），BPSDのある対象に比較的多く用いられ，Memantineが今回の調査の対象に含まれていたならば，その頻度は高かったかもしれない．しかしながら，Memantineは近年米国において大規模な無作為化試験が行われたが，プラセボに比して有意な結果を得ることはできなかった（Boxer et al., 2012a）．実際にはMemantineの投与も有用でない可能性が高い．

一方で35%という向精神薬の処方割合についてはどう考えるべきであろうか？ 2012年の「かかりつけ医による認知症者に対する向精神薬の使用実態調査に関する研究事業報告書」によれば，認知症患者に対する向精神薬の服用は95%とかなり高率であった（認知症ケア学会，2012）．ただしこの数字は医師が複数の患者に対してひとりでも向精神薬を使用している割合であり，単純な比較はできない．また2006年の報告で，精神科医が診ている認知症患者の62%にBPSDが認められ，そのうち93%が薬物療法を受け，そのうち81%に抗精神病薬が用いられていた（すなわち，精神科医が診ている認知症患者の47%に抗精神病薬が用いられていた）という報告もある（本間，2006）．それらに比べると本報告の数字は低い．他の疾患より行動症状が目立ち，それに伴う介護負担も大きいはずのFTDにおいて，何故向精神薬の処方割合が低いのであろうか？ これにはいくつかの理由があると考えられる．まず，他の認知症と異なり，FTDと診断された場合，不用意に向精神薬を処方せず，専門医への紹介を優先させている可能性がある．また，本研究の例は入院例を含まない外来例であることや，処方医の診療科の比率が前述の調査と異なるため，それが処方割合に影響している可能性もある．いずれにせよ，安易な向精神薬の処方を行っていないという点では，好ましいことと思われる．

向精神薬のなかで，抗うつ薬の使用が最も頻度が高かったのは興味深い．選択的セロトニン再取り込み阻害薬（selective serotonin reuptake inhibitor：SSRI）の強迫性障害や神経性大食症に対する有効性を背景として，最初にSwartzらがFTD患者に対するSSRIの使用を報告して以降（Swartz et al., 1997），フルボキサミンやパロキセチン，セルトラリンの有用性の報告がなされている（Ikeda et al., 2003；Mendez et al., 2005；Moretti et al., 2002）．SSRIではないが，間接的セロトニン再取り込み阻害薬であるトラドゾンを用い，興奮，焦燥，うつなどの症状に改善がみられたという報告もあり（Lebert et al., 2004），抗うつ薬はFTDの常同行動や食行動異常に対して有用である可能性が高い．その抗うつ薬が抗

精神病薬や抗不安薬より多く用いられているということは，これらの知識が普及しているということを意味する．前述の厚生労働省の統計では，認知症全般に対して最も多く用いられる向精神薬は抗精神病薬という結果であり，それに比べて好ましい結果であると言える．

抗うつ薬に比して頻度が低いとはいえ，抗精神病薬も約1割に対して用いられていた．行動障害に対して用いられている可能性があり，この数字はある程度やむを得ないと言えるかもしれない．しかし他の認知症におけるBPSD同様に，FTDにおいても過鎮静や錐体外路症状などの問題がある（Kerssens et al., 2008）．FTLDと診断された100例のうち61例に重篤なBPSDがあり，24例に抗精神病薬が投与されたが，8例（33%）に錐体外路症状が認められたとの報告もある（Pijnenburg et al., 2003）．さらに抗精神病薬の使用には注意を喚起していく必要がある．

漢方薬（抑肝散）は，や抗うつ薬に次いで多く処方されていた．抗精神病薬に比べ比較的安全に使用できる点が評価されているものと考えられた．FTDに対する漢方薬の知見は少なく，本邦の木村らの5例のケースシリーズがある程度である（Kimura et al., 2009）．今後さらなる多数例での検討が求められる．

向精神薬が処方されていた31例と，向精神薬が処方されていなかった56例では，専門医によってFTDと診断された割合は16.1%（前者）と33.9%（後者）で，向精神薬が処方されていなかった例の方が高い傾向にあったが，有意差は認められなかった．そのため断言はできないが，向精神薬が処方されている例の方が，過剰診断されやすい傾向にあると考えられた．その他の背景因子に有意差のある項目はなく，向精神薬が用いられる対象の一定の傾向は認められなかった．

FTLD，FTD-MNDといった診断名がついていた場合，ChEIも向精神薬も用いられていなかった点は興味深い．これらの診断名がつけられた場合，典型的な行動症状ではなく，言語症状や運動症状などの非定型的な症状が存在する可能性がある．そういった例に対してはChEIも向精神薬使用せずに，専門医への紹介を優先させるという対応をとると考えられた．

本研究の限界点について述べる．まず本研究では4施設という限られた施設への紹介例を対象としており，いずれも精神科の認知症専門外来への紹介であり，自然と精神科からの紹介が多く，一方でFTD-MNDのような例は比較的少ない．このようなサンプリングバイアスが結果に影響を与えた可能性はある．

その一方で専門外来受診例であり，重度な行動障害を有し，在宅での生活が困難で入院しているような例は含まれていない．このような患者を含めるとoff-label処方の割合が変わる可能性がある．また，なお各施設の専門医の診断にあたっては，画像診断および認知機能バッテリーを用い，診断基準に基づいて診断を行っている．しかしながら全例が病理診断を受けているわけではなく，病理学的な最終診断がFTDではない可能性は否定できない．また，各患者がどのような症状を有しており，紹介医がどの症状に対して処方をしたかに関しては，各施設の受診時には既に処方がなされた以降であり，症状が変化した可能性があるので，評価できない．今後は紹介医がどのような症状に対して処方をしたかという調査も必要である．

5. まとめ

本研究は，本邦ではじめてなされたFTDに対するoff-label処方の現状調査である．ChEIや抗精神病薬などが用いられている現状が明らかとなり，今後は薬剤使用に対する啓発や，非薬物療法を含めたFTDへの治療ガイドラインの整備が望まれる．

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Off-label medication for frontotemporal dementia in Japan

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In order to clearly the situation of off-label medication in Japan, we investigated the medication and demographic data of consecutive 87 subjects those were referred with the diagnosis of Frontotemporal dementia (FTD) syndrome. 60% of referring physicians were psychiatrists, followed by were neurologists, general physician, neurosurgeon, and other physicians. Half of the subjects were treated with some kind of medications for dementia. Cholinesterase inhibitor is prescribed for 20% of all subjects by various physicians, while psychotropic drugs were prescribed for 35% of all subjects mainly by psychiatrists. Antidepressant and antipsychotics are most common among them. Other background factors such as age, sex, duration, and MMSE scores are not associated with medication use. We need to establish guideline of pharmacological treatment for patients with FTD.

Key wards : Frontotemporal dementia, off-label medication, cholinesterase inhibitor, psychotropic drugs, pharmacological treatment

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Long-Term Safety Issues of iPSC-Based Cell Therapy in a Spinal Cord Injury Model: Oncogenic Transformation with Epithelial-Mesenchymal Transition

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SUMMARY

Previously, we described the safety and therapeutic potential of neurospheres (NSs) derived from a human induced pluripotent stem cell (iPSC) clone, 201B7, in a spinal cord injury (SCI) mouse model. However, several safety issues concerning iPSC-based cell therapy remain unresolved. Here, we investigated another iPSC clone, 253G1, that we established by transducing OCT4, SOX2, and KLF4 into adult human dermal fibroblasts collected from the same donor who provided the 201B7 clone. The grafted 253G1-NSs survived, differentiated into three neural lineages, and promoted functional recovery accompanied by stimulated synapse formation 47 days after transplantation. However, long-term observation (for up to 103 days) revealed deteriorated motor function accompanied by tumor formation. The tumors consisted of Nestin$^+$ undifferentiated neural cells and exhibited activation of the OCT4 transgene. Transcriptome analysis revealed that a heightened mesenchymal transition may have contributed to the progression of tumors derived from grafted cells.

INTRODUCTION

Advances in stem-cell-based therapies may help overcome CNS disorders such as spinal cord injury (SCI). Transplantation of neural stem/progenitor cells (NS/PCs) has yielded beneficial effects and improved functional recovery in SCI animal models (Cummings et al., 2005; Hofstetter et al., 2005; Iwanami et al., 2005; Ogawa et al., 2002; Okada et al., 2005; Salazar et al., 2010; Yasuda et al., 2011). Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced PSCs (iPSCs), can differentiate into NS/PCs (Falk et al., 2012; Fujimoto et al., 2012a; Kumagai et al., 2009; Miura et al., 2009; Nori et al., 2011; Okada et al., 2004, 2008; Tsuji et al., 2010), oligodendrocyte precursor cells (OPCs) (Keirstead et al., 2005; Wang et al., 2013), and motoneuron progenitors (Erceg et al., 2010; Lukovic et al., 2014) in vitro. Previous studies demonstrated the therapeutic potential of mouse and human iPSC-derived NS/PCs for SCI in mice and non-human primates (Fujimoto et al., 2012b; Kobayashi et al., 2012; Nori et al., 2011; Tsuji et al., 2010). However, tumorigenicity remains a major concern for clinical applications of iPSCs.

Previously, we reported the safety and therapeutic potential of human iPSC-derived neurospheres (iPSC-NSs) for SCI in non-obese diabetic–severe combined immunodeficient (NOD-SCID) mice (Nori et al., 2011) using the iPSC clone 201B7 (Nori et al., 2011; Takahashi et al., 2007). Here, we aimed to characterize novel NS/PCs derived from a different iPSC clone, 253G1. We established this clone from the same adult human dermal fibroblasts used for 201B7 by transducing three reprogramming factors: *OCT4*, *SOX2*, and *KLF4* (Nakagawa et al., 2008). Grafted 253G1-derived neurospheres (253G1-NSs) survived and differentiated into three neural lineages in the injured spinal cord, and some of the resultant cells formed synapses with host neurons. Motor function in grafted mice initially recovered but then gradually declined, and tumors emerged during long-term observation. These tumors consisted of undifferentiated Nestin$^+$ cells, but not NANOG$^+$ pluripotent cells. Late-onset activation of the *OCT4* transgene (Tg) may be associated with tumor formation. Transcriptome analysis revealed altered expression of genes involved in the epithelial-mesenchymal transition (EMT), which is related to tumor invasion and progression. Moreover, canonical pathway analysis revealed upregulation of the Wnt/β-catenin signaling pathway after 253G1-NS transplantation, which played a critical role in tumor development.

Thus, although 253G1-NSs conferred temporary functional recovery in mice with SCI, they later developed into tumors and worsened the overall outcome.

RESULTS

Grafted 253G1-NSs Survive in Injured Spinal Cord and Differentiate into Three Neural Lineages

Immunodeficient (NOD-SCID) mice were used for xenograft experiments. After laminectomy, contusive SCI was induced at the Th10 level. Nine days after injury, 5×10^5 253G1-NS-derived cells, which were lentivirally transduced with the fluorescent protein Venus (an altered yellow fluorescent protein; Nagai et al., 2002) or ffLuc (Venus fused to firefly luciferase; Hara-Miyauchi et al., 2012), were injected into the lesion epicenter. Histological analyses were performed 47 days (d) after transplantation. The grafted 253G1-NSs survived, migrated into the host spinal cord (Figures 1A and 1B), and differentiated into neuronal nuclei $(NeuN)^+$ (17.2% ± 2.6%) and β-tubulin isotype III $(\beta III\ tubulin)^+$ (42.2% ± 3.1%) neurons, glial fibrillary acidic protein $(GFAP)^+$ astrocytes (15.0% ± 0.7%), and adenomatous polyposis coli CC-1 $(APC)^+$ oligodendrocytes (2.7% ± 0.3%; Figures 1C–1G). Quantitative analysis revealed that 67% of $NeuN^+$ mature neurons were $GAD67^+$ GABAergic neurons (Figure 1H). Small numbers of grafted cells differentiated into tyrosine hydroxylase $(TH)^+$ and choline acetyltransferase $(ChAT)^+$ cholinergic neurons (Figures 1I and 1J).

Grafted 253G1-NS-Derived Neurons Form Synaptic Connections with Host Neurons

We performed triple immunostaining for human nuclear protein (HNu) and two neuronal markers, βIII tubulin and the presynaptic protein Bassoon (Bsn). Because the anti-Bsn antibody selectively recognized the mouse and rat epitopes, but not the human epitopes (Figure S1), we were able to evaluate the ability of 253G1-NS-derived neurons to integrate with the host neural circuitry using this approach. Grafted $\beta III\ tubulin^+/HNu^+$ cells in parenchymal locations were contacted by synaptic boutons of host neurons (Figure 1K). Moreover, triple immunostaining for HNu, βIII tubulin, and human-specific synaptophysin (hSyn) revealed dense terminal fields of human-derived boutons apposed to host neurons (Figure 1L). Host $ChAT^+$ neurons in the ventral gray matter were contacted by the $hSyn^+$ graft-specific terminals (Figure 1M). Immuno-electron microscopy also revealed $Venus^+$ human pre- and post-synaptic structures, as well as synapse formation between host neurons and $Venus^+$ 253G1-NS-derived neurons (Figure 1N).

Grafted 253G1-NSs Promote Motor Function Recovery after SCI

We assessed motor function recovery using the Basso mouse scale (BMS) score, Rotarod test, and DigiGait system. According to the BMS score, the 253G1-NS-grafted group exhibited significantly better functional recovery than the PBS-injected control group ≥12 days after transplantation (BMS score = 3.2 ± 0.1 at 12 days post-transplantation and 3.3 ± 0.2 at 47 days post-transplantation; Nori et al., 2011; Figure 1O). In the Rotarod test, 253G1-NS-grafted mice remained on the rod significantly longer (61.1 ± 7.1 s) than the control group (33.0 ± 7.3 s; Nori et al., 2011) at 47 days post-transplantation (Figure 1P). Gait performance was evaluated using the DigiGait image analysis system. All 253G1-NS-grafted mice could walk on the treadmill at 8 cm/s, whereas some control mice (4/16) could not. Stride length was significantly longer in the 253G1-NS-grafted group (4.2 ± 0.1 cm) than in the control group (2.2 ± 0.1 cm; Nori et al., 2011; Figure 1Q).

Tumors Form after 253G1-NS Transplantation, Resulting in Deteriorated Motor Function

We extended the follow-up period to 103 days post-transplantation to investigate the long-term safety of the grafted 253G1-NSs. Although recovery of motor function persisted for up to 47 days post-transplantation, 253G1-NS-grafted mice exhibited gradual deterioration of hind limb motor function thereafter (Figure 2A). To monitor the survival and growth of the grafted cells in the mouse spinal cord, we lentivirally transduced 253G1-NSs with ffLuc, which allowed us to identify grafted cells by their bioluminescent luciferase signals and fluorescent Venus signals. The photon count of grafted 253G1-NSs decreased within the first week post-transplantation, but gradually increased at 14 days post-transplantation and thereafter, demonstrating the survival and growth of the grafted cells. Between 42 and 70 days post-transplantation, the photon counts sharply increased (Figures 2B and 2C), consistent with the deterioration of the BMS score shown in Figure 2A. At 103 days post-transplantation, the photon count of the grafted 253G1-NSs increased more than 10-fold from its initial value (Figure 2C).

Histological analyses revealed tumors in 253G1-NS-grafted spinal cords. These tumors were divided into three groups based on the diameter of the lesion (small-tumor group, $\phi < 200\ \mu m$; medium-tumor group, $200 < \phi < 700\ \mu m$; large-tumor group, $700\ \mu m < \phi$). Some of the tumors (12/22) exhibited microcystic masses consisting of HNu/Nestin double-positive human-derived bipolar cells with hair-like processes. These masses were observed in all tumors from the large-tumor group (7/7; Figures 2D and 2E show representative images of microcystic masses from the large-tumor group). Such masses were also present

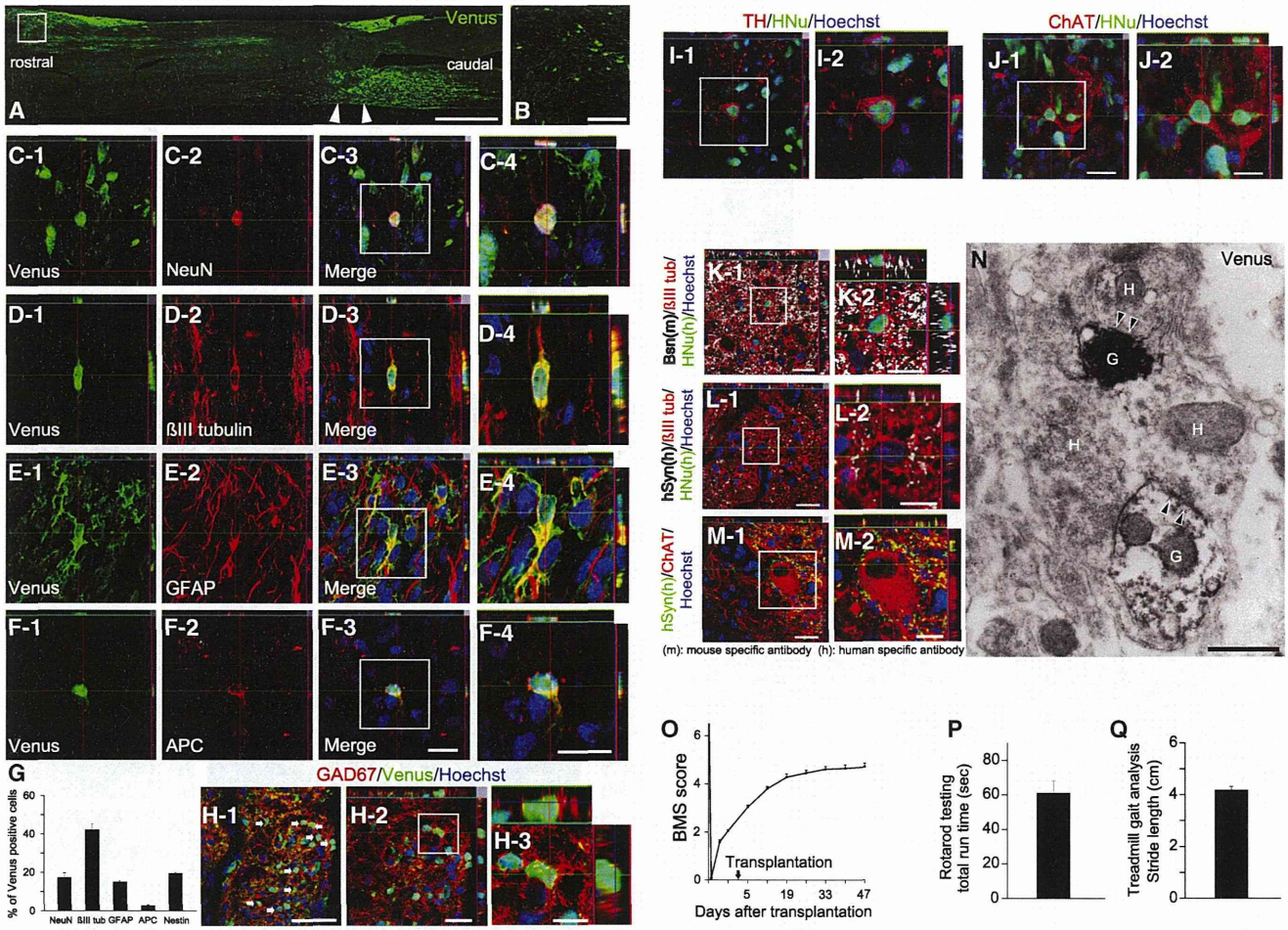

Figure 1. Grafted 253G1-NSs Mainly Differentiate into Neurons and Form Synapses with Host Spinal Cord Neurons

(A and B) Venus$^+$ 253G1-NSs integrated into the mouse spinal cord. Arrowheads indicate the lesion epicenter.

(C–F) Representative images of Venus$^+$ grafted cells immunostained for the markers NeuN (mature neurons) (C), βIII tubulin (all neurons) (D), GFAP (astrocytes) (E), and APC (oligodendrocytes) (F).

(G) Percentages of cell-type-specific marker-positive cells among Venus$^+$ grafted cells at 47 days post-transplantation. Values are expressed as the mean ± SEM (n = 4 mice).

(H) Most 253G1-derived neurons differentiated into GAD67$^+$ (GABAergic) neurons.

(I and J) TH$^+$/HNu$^+$ neurons and ChAT$^+$/HNu$^+$ neurons were observed, but were rare.

(K) Sections were triple stained for HNu (green), βIII tubulin (red), and the presynaptic marker Bassoon (Bsn, white). The Bsn antibody recognized the mouse, but not the human, protein.

(L) Sections triple stained for HNu (green), βIII tubulin (red), and the human-specific presynaptic marker hSyn (white). βIII tubulin$^+$/HNu$^-$ neurons represented host mouse neurons. The hSyn antibody recognized the human, but not the mouse, protein.

(M) Large numbers of somatic and dendritic terminals from graft-derived nerve cells were present on host ChAT$^+$ motor neurons at the ventral horns.

(N) Electron microscopy (EM) images show synapse formation between host mouse neurons and graft-derived Venus$^+$ (black) human neurons. Pre- and post-synaptic structures indicate transmission from a graft-derived neuron to a host neuron, and from a host neuron to a graft-derived neuron. H, host neuron; G, graft-derived neuron; arrowheads, post-synaptic density.

(O) Motor function in the hind limbs was assessed weekly using the BMS score until 47 days post-transplantation. Values are expressed as the mean ± SEM (n = 32 mice).

(P) Rotarod test 47 days after transplantation. Graph shows total run time. Values are expressed as the means ± SEM (n = 10 mice).

(Q) Treadmill gait analysis using the DigiGait system 47 days post-transplantation. Graph shows stride length. Values are expressed as the means ± SEM (n = 19 mice). Behavioral analyses were performed by two observers who were blinded to the treatment conditions.

Scale bars, 1,000 μm in (A); 100 μm in (B); 50 μm in (J-1); 20 μm in (F-3), (F-4), (H-1), (J-2), (K-1), (L-1), and (M-1); 10 μm in (H-2), (J-3), (K-2), (L-2), and (M-2); 0.5 μm in (N). See also Figure S1.

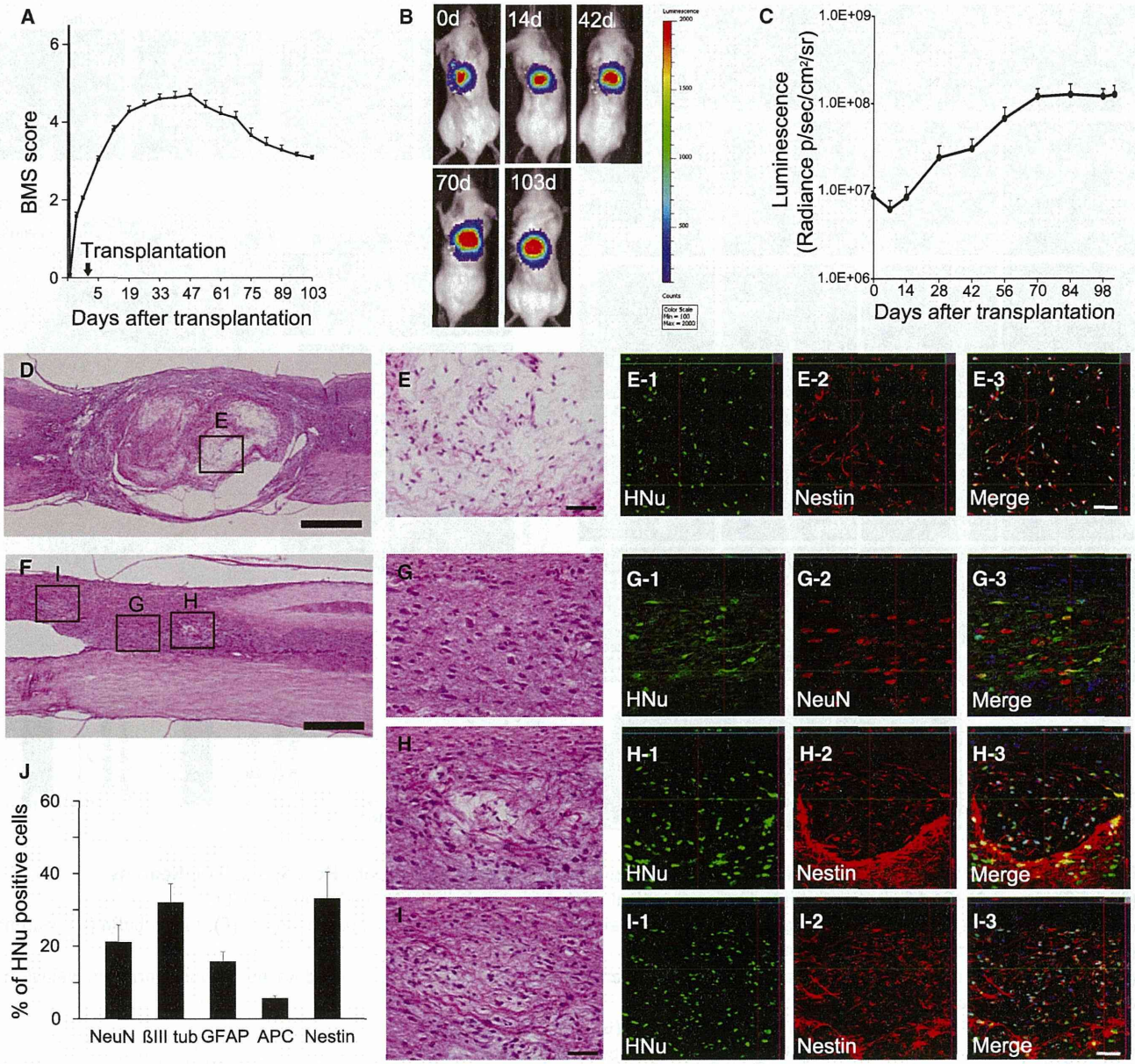

Figure 2. Tumor Formation during Long-Term Observation after 253G1-NS Transplantation

(A) Up to 103 days post-transplantation, motor function in the hind limbs was assessed weekly using the BMS score. Values are expressed as the means ± SEM (n = 32 mice up to 47 days post-transplantation; thereafter, n = 22 mice until 103 days post-transplantation).

(B) Representative in vivo images of mice at 0, 14, 42, 70, and 103 days after 253G1-NS transplantation.

(C) Quantitative analysis of photon counts derived from grafted cells. Values are expressed as the means ± SEM (n = 20 mice up to 42 days post-transplantation; thereafter, n = 14 mice until 103 days post-transplantation).

(D) Representative hematoxylin and eosin (H&E) image of a large tumor (700 μm < ϕ).

(E) Boxed area in (D).

(E1–E3) Immunohistochemistry showing that most grafted cells in the microcystic area were Nestin$^+$.

(F) Representative H&E image of a medium tumor (200 < ϕ < 700 μm).

(G) Boxed area in (F). Immunohistochemistry shows that some grafted cells exhibited normal neural differentiation.

(H and I) Boxed area in (F). Some grafted cells formed microcystic masses that were positive for Nestin.

(J) Percentages of cell-type-specific, marker-positive cells among HNu$^+$ grafted cells at 103 days post-transplantation. Values represent the means ± SEM (n = 4 and 10 mice for 47 and 103 days post-transplantation, respectively). *p < 0.05, **p < 0.01.

Scale bars, 500 μm in (D) and (F), 50 μm in (E) and (G–I). See also Figure S2.

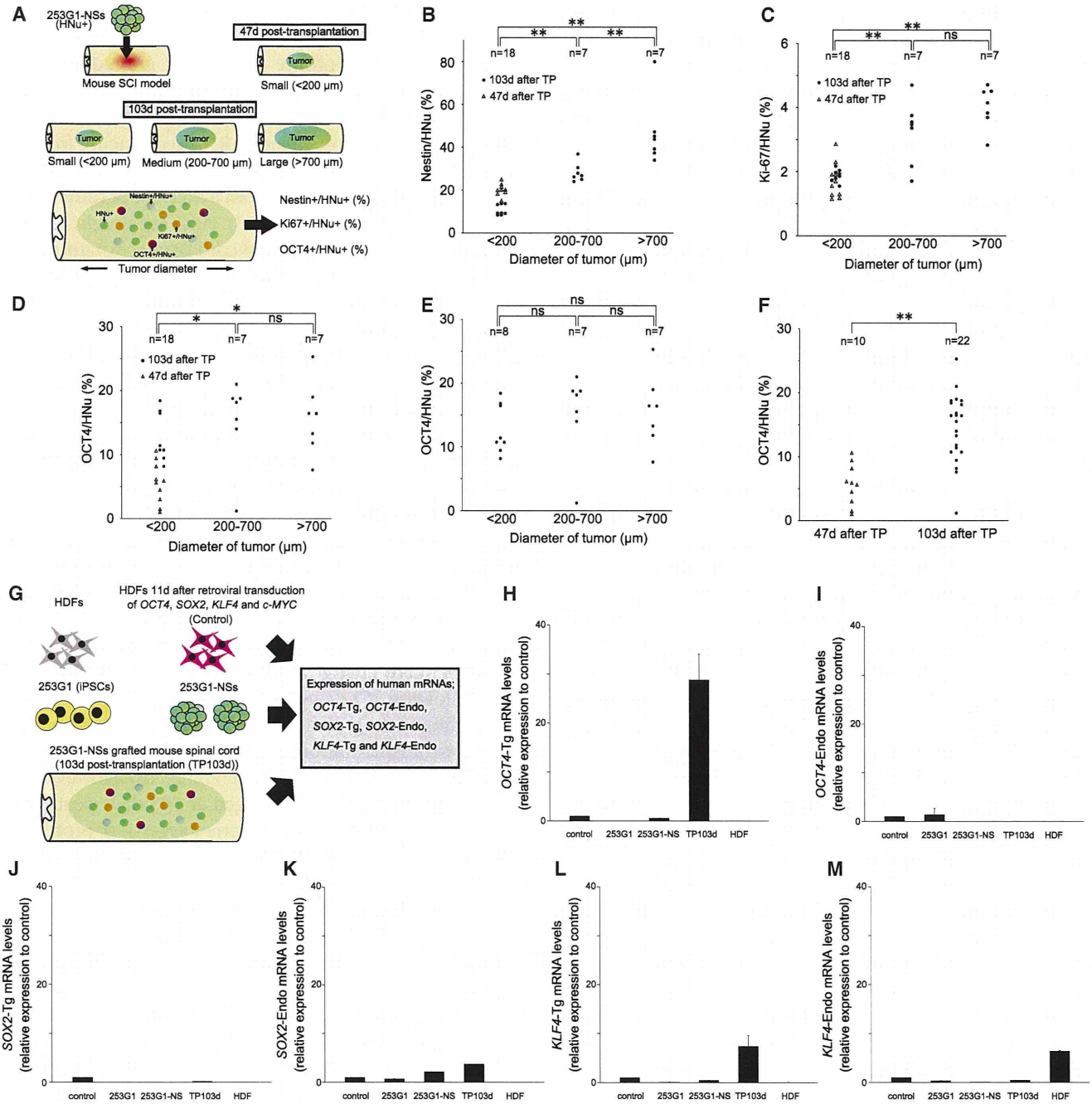

Figure 3. Histological and Gene-Expression Analyses of Tumors

(A) Schematic of histological analyses of tumors.

(B) Correlation between tumor diameter and the proportion of grafted cells that were Nestin$^+$ at 47 and 103 days post-transplantation (TP).

(C) Correlation between tumor diameter and the proportion of grafted cells that were Ki-67$^+$.

(D) Correlation between tumor diameter and the proportion of grafted cells that were OCT4$^+$.

(E) Correlation between the tumor diameter and the proportion of grafted cells that were OCT4$^+$/HNu$^+$ at 103 days after TP.

(F) Correlation between the number of days after TP (47 or 103 days after TP) and the proportion of grafted cells that were OCT4$^+$. In (B)–(F), n indicates the number of mice.

(G) Schematic of mRNA expression analyses of tumors.

(H–M) The expression of human *OCT4*-Tg, *OCT4*-Endo, *SOX2*-Tg, *SOX2*-Endo, *KLF4*-Tg, *KLF4*-Endo, c-*MYC*-Tg, and *c-MYC*-Endo mRNA in 253G1 cells, 253G1-NSs, 103-day post-transplant 253G1-NSs (TP 103d), and adult human dermal fibroblasts (HDFs) was analyzed by RT-PCR. Data

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in the majority of the medium tumors (5/7), whereas none were found in the small-tumor group (0/8). In medium tumors that exhibited microcystic masses, some grafted cells underwent normal neural differentiation (Figures 2F and 2G). However, a portion of the grafted cells formed microcystic masses positive for Nestin (Figures 2H and 2I), and Nestin$^+$ cells were also observed outside the microcystic mass. Significantly higher percentages of Nestin$^+$ cells (34.2% ± 7.3%) and APC$^+$ oligodendrocytes (5.4% ± 0.7%), as well as significantly lower percentages of βIII tubulin$^+$ neurons (32.1% ± 5.1%), were observed at 103 days relative to 47 days (Figure 2J). In addition, 15.8% ± 2.7% of grafted cells had differentiated into astrocytes at 103 days. Taken together, these data indicate that approximately 87.5% of grafted cells differentiated toward neural lineages. We observed no NANOG$^+$ pluripotent cells in grafted 253G1-NSs at 103 days post-transplantation (Figure S2).

Next, we examined the correlation between tumor diameter and the percentages of Nestin$^+$, Ki-67$^+$, or OCT4$^+$ cells among HNu$^+$ grafted cells (Figure 3A). Statistical analysis revealed a significant correlation between tumor diameter and the percentage of Nestin$^+$/HNu$^+$ cells (Figure 3B). Compared with the small-tumor group, the large- and medium-tumor groups contained significantly higher percentages of Ki-67$^+$/HNu$^+$ cells and OCT4$^+$/HNu$^+$ cells (Figures 3C and 3D). However, there was no significant correlation between tumor diameter and the percentage of OCT4$^+$/HNu$^+$ cells at 103 days post-transplantation (Figure 3E). Meanwhile, significantly more OCT4$^+$/HNu$^+$ cells were observed at 103 days than at 47 days (Figure 3F); thus, the percentage of OCT4$^+$/HNu$^+$ cells correlated positively with post-transplant duration.

We also evaluated the expression of human *OCT4*-Tg, *OCT4*-endogenous (Endo), *SOX2*-Tg, *SOX2*-Endo, *KLF4*-Tg, and *KLF4*-Endo mRNAs in 253G1 cells, 253G1-NSs, and spinal cord tissues of the 253G1-NS-grafted group, which were harvested at 103 days post-transplantation (253G1-NS/transplantation [TP]-103d group; Figures 3G–3M). Compared with 253G1-NSs, *OCT4*-Tg expression was significantly higher in 253G1-NS/TP-103d spinal cords (Figure 3H), whereas *OCT4*-Endo expression was only observed in the 253G1 iPSCs (Figure 3I). *SOX2*-Endo was expressed in both 253G1-NSs and the 253G1-NS/TP-103d group, and levels of *SOX2*-Endo slightly increased after transplantation (Figure 3K). *KLF4*-Tg expression was also elevated in 253G1-NSs and the 253G1-NS/TP-103d group (Figure 3L).

Transcriptomic Differences between 253G1-NSs and 201B7-NSs Post-Transplantation

Comparative transcriptome analyses of grafted cells and surrounding host cells can reveal information regarding the differentiation status of the grafted cells and the effects of the graft on the host tissue. mRNA sequencing (mRNA-seq) enables one to analyze the global expression of individual human and mouse genes from a mixture of human and mouse cells (Bradford et al., 2013). Here, we sought to measure expression in mouse spinal cord tissue containing human cells derived from grafted human iPSC-NSs. To analyze mRNA expression in both grafted human iPSC-NSs and host spinal cord tissue, we analyzed the mRNA from NSs of 253G1 and 201B7 cells, as well as mouse spinal cord tissues containing grafted 253G1-NSs and 201B7-NSs, which were harvested at 5 and 103 days post-transplantation (PBS-5d and 103d, 253G1-NS/TP-5d and 103d, and 201B7-NS/TP-5d and 103d). The ratio of human and mouse mRNA-seq reads derived from epicenter segments (8 mm in length) of iPSC-NS-grafted spinal cord tissue was considered to reflect the ratio of human and mouse cells (Table S1). The global gene-expression patterns of these tissues were hierarchically clustered into 5-day (PBS-5d, 253G1-NS/TP-5d, and 201B7-NS/TP-5d) and 103-day groups (PBS-103d, 253G1-NS/TP-103d, and 201B7-NS/TP-103d), which may reflect time-dependent changes in the spinal cord microenvironment following SCI (Figure 4A). Similarly, the gene-expression profiles of the grafted iPSC-NSs clustered on the basis of time post-transplantation (NS, 5 and 103 days) rather than clonal (253G1 and 201B7) origin (Figure 4B). However, the profiles of the two clones diverged at 103 days post-transplantation. Furthermore, the gene-expression profiles of 253G1-NS/TP-103d and 201B7-NS/TP-103d differed significantly (Figures 4C and 4D).

Next, we identified human genes that were upregulated in the mouse spinal cord at 103 days post-transplantation relative to iPSC-NSs before transplantation (fold change > 5.0). As shown in the Venn diagram in Figure 4E, we identified 692 genes in the 253G1-NS/TP-103d group, 335 genes in the 201B7-NS/TP-103d group, and 1,023 genes in both the 253G1-NS/TP-103d and 201B7-NS/TP-103d groups that were expressed at higher levels than in 253G1-NSs and 201B7-NSs. Gene Ontology (GO) analysis of the 335- and 1,023-gene groups indicated that synaptogenesis was occurring in both the 253G1- and 201B7-NS/TP-103d groups (Tables 1 and S2). GO analysis of the 692 genes that were exclusively activated in 253G1-derived cells at 103 days post-transplantation identified a

are presented as expression levels relative to the control (HDFs) 11 days after retroviral transduction of *OCT4*, *SOX2*, *KLF4*, and *c-MYC*. Values represent the means ± SEM (n = 3 independent experiments).

The p values shown in (B)–(F) were calculated using Scheffe's test, and p values to determine significance were calculated using the Kruskal-Wallis non-parametric test: (B) 5.00E-06, (C) 7.20E-06, (D) 0.01, and (F) 1.33E-04. *p < 0.05, **p < 0.01. ns, non-significant.

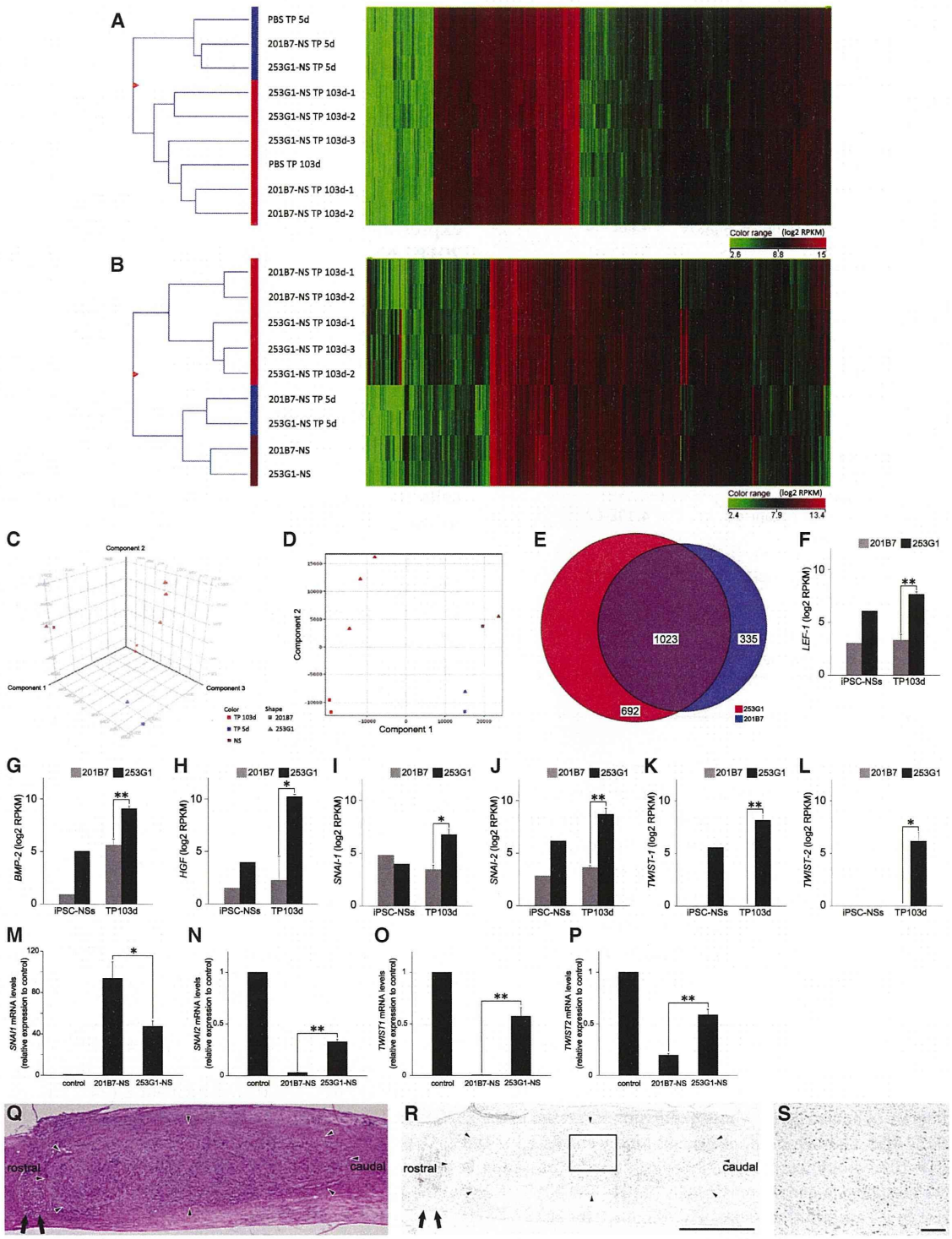

Figure 4. Global Human Gene-Expression Analysis

(A) Hierarchical clustering analysis of mouse gene-expression data from spinal cord tissues of the PBS-5d and -103d, 253G1-NS/TP-5d and -103d, and 201B7-NS/TP-5d and -103d groups.

(B) Hierarchical clustering analysis of human gene-expression data: 253G1-NSs and 201B7-NSs, as well as spinal cord tissues of the 253G1-NS/TP-5d and -103d, and 201B7-NS/TP-5d and -103d groups. In (A) and (B), the signal intensity of each gene is displayed as a heatmap colored according to the expression level.

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