厚生労働省科学研究費補助金(難治性疾患克服研究事業)
研究分担報告書

Gorlin 症候群の病態解明と治療法確立のための臨床的研究

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平成22年度　研究要旨

我々は以前より母斑基底細胞癌症候群(NBCCS)(別名 Gorlin 症候群)(常染色体優性遺伝をする神経皮膚症候群、高発癌性遺伝疾患でもある)の遺伝子解析を行なってきた。NBCCS では基底細胞癌、髄芽腫の他に、80%以上で顎骨に角化嚢胞性歯原性腫瘍(KCOT)と呼ばれる良性腫瘍を発症することが知られている。我々は NBCCS7 例において発症した 9 個の KCOT の遺伝子解析を行い、その発症機序を解析した。7 例中 3 例で NBCCS の責任遺伝子である *PTCH1* に新たな体細胞変異を見出した。そのうち 1 例は腫瘍化との関連は不明であった。1 例では上顎と下顎で異なる遺伝子型を示した。*PTCH1* プロモーターのメチル化によるエピジェネティックな不活化は認めなかった。また *PTCH1* 同様に Shh シグナル伝達の構成分子をコードする *SMO*、*SUFU* 遺伝子に変異の検出された症例はなかった。

平成23年度　研究要旨

我々は以前より母斑基底細胞癌症候群（NBCCS）（別名Gorlin症候群）（常染色体優性遺伝をする神経皮膚症候群、高発癌性遺伝疾患でもある）の遺伝子解析を行なってきた。今年度我々は通常の方法では変異が見出せなかった症例の中に、NBCCSの責任遺伝子である*PTCH1*に複数のエキソンで重複が見られた症例と、ヘッジホッグシグナル伝達経路の下流で機能する*SUFU*遺伝子に変異のある症例を経験した。前者は国内外を通じて初めての症例であり、後者は2例目であった。前者からは通常の方法で変異陰性の場合のコピー数解析の重要性が示された。後者からは文献的考察も含めて*SUFU*遺伝子に変異のある症例は高率に髄芽腫を発生することが示され、いずれも今後の本症候群の診断と経過観察に重要な示唆を与えるものであった。

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研究分担報告書

Gorlin 症候群の病態解明と治療法確立のための臨床的研究

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平成22年度　研究要旨

Gorlin 症候群における遺伝カウンセリングでは、病歴、家系図などの情報を収集し、再発率評価を行い、医療情報の提供を行うと共に、必要に応じて社会的支援の提供を行う。未成年や未発症者における遺伝カウンセリングでは、十分な配慮が大切である。Gorlin 症候群では複数の診療科、遺伝医療の専門家、ソーシャルワーカー、患者サポート組織などの連携体制の整備、チーム医療の構築が必要である。

平成23年度　研究要旨

多発性顎嚢胞を認めGorlin症候群が疑われた6家系の患者の末梢血からゲノムDNAを抽出し、*PTCH*遺伝子検査を行い、5例に*PTCH*遺伝子変異を認め確定診断を得た。遺伝カウンセリングにおいては疾患の十分な理解を促すと同時に、遺伝子変異が家系で共有され、未発症のat riskの家系員に有用な情報になることを伝える必要がある。しかし、家族関係や家系員の年齢を視野に入れながら、提供する内容、タイミングについて考慮していくことが重要である。

厚生労働省科学研究費補助金(難治性疾患克服研究事業)
研究分担報告書

Gorlin 症候群の病態解明と治療法確立のための臨床的研究

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平成22年度　研究要旨

ゴーリン症候群患者では、放射線照射により、基底細胞癌などの腫瘍が生じることが知られている。本研究では、ゴーリン患者由来のケラチノサイトを用い、放射線応答機構について基礎的な検討を行った。本研究で用いた細胞の放射線感受性を調べたところ、X線に関しては、顕著な放射線感受性は見られなかった。また、*K-ras* 癌遺伝子におけるコドン 12 の塩基置換をドットブロットハイブリダイゼーション法により検出する遺伝子レベルでの検査法にて、ゴーリン細胞では、5Gy照射後に変異が検出された。このことから、従来言われている放射線による癌の誘発が実験レベルでも再現されることが示唆された。

平成23年度　研究要旨

ゴーリン症候群での髄芽腫は、主に幼少期に合併することが多い疾患であり、それに対する免疫療法として、IFN-β投与があるがその有効率は低い。我々は、本研究前に他の細胞株を用いた検討により、IFN-βによる細胞増殖抑制にmicroRNA-431(miR-431)が深く関与していることを報告している。この結論を基に本研究では、ゴーリン症候群に合併することの多い髄芽腫ならびにIFN-β投与による免疫療法が臨床的に行われている膠芽腫の培養細胞を用いて、IFN-βによる細胞増殖抑制にmiR-431が関与していると推察し、基礎的検討を開始した。本研究で用いた培養細胞のIFN-β感受性、miR-431ならびにmiR-431のTarget GeneであるSOCS6の発現量を検討した結果、IFN-β投与による細胞増殖抑制に伴い、miR-431発現量は有意に低下した一方で、SOCS6の発現量は有意に増加していた。以上の結果より、IFN-βによる細胞増殖抑制に、miR-431とSOCS6の発現が深く関与していることが示唆された。

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研究分担報告書

Gorlin 症候群の病態解明と治療法確立のための臨床的研究

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平成23年度　研究要旨

角化嚢胞性歯原性腫瘍 (KCOT) はGorlin 症候群患者・非症候群患者のいずれにも発生することが知られているが、臨床病態に著しい差があるにもかかわらず両者の細胞病理学的な差については現在までほとんど解明されていない。理由としてKCOTは良性腫瘍であり、培養細胞を用いた解析が不可能であったことが原因である。

この問題を解決するために、研究分担者はGorlin 症候群患者に生じたKCOT および非症候群患者のKCOT より腫瘍細胞の初代培養に成功し、さらに3 つの遺伝子導入により、安定した増殖能を示し、且つKCOT の腫瘍としての性質を保持したまま不死化した培養細胞を樹立した。今後は、本培養細胞を用いてGorlin 症候群患者に生じたKCOT と非症候群患者に生じたKCOT の差を細胞生物学的に解析する予定である。

Ⅲ　研究成果の刊行に関する一覧表

平成22年度分

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Ⅳ　研究成果の刊行物・別刷

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Nationwide Survey of Nevoid Basal Cell Carcinoma Syndrome in Japan Revealing the Low Frequency of Basal Cell Carcinoma

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Nevoid basal cell carcinoma syndrome (NBCCS) is characterized by developmental defects and tumorigenesis. The clinical manifestations of NBCCS have been reported in large epidemiological studies from the United States, the United Kingdom, and Australia, but not from an Asian country. We conducted a nationwide survey and identified 311 NBCCS patients in Japan. We investigated the detailed clinical manifestations of 157 patients ranging in age from 9 months to 77 years old (mean: 33.1 years). We then compared the frequency and age of onset for various tumors developed in Japanese NBCCS patients with patients from the three countries listed above in which NBCCS studies were previously conducted. Our most significant finding was the low frequency of basal cell carcinoma (BCC) in Japanese patients. Frequency of BCC in patients over 20 years of age was 51.4%, a much lower rate compared to the United States, Australia, and the United Kingdom (91%, 85%, and 73%, respectively). The mean age of BCC onset was 37.4 years of age, a much older age compared to the above-mentioned countries. These findings suggest that differences in ethnicity and/or environmental factors affect the incidence of BCC. Because the age of BCC onset is generally higher in Japanese NBCCS patients, careful skin examination over a prolonged period of time is warranted.

Key words: nevoid basal cell carcinoma syndrome; basal cell carcinoma; keratocystic odontogenic tumor; Gorlin syndrome

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INTRODUCTION

Nevoid basal cell carcinoma syndrome (NBCCS; OMIM 109400) or Gorlin syndrome is an autosomal dominant disorder characterized by developmental defects including bifid ribs, palmar or plantar pits, and tumors, such as basal cell carcinoma (BCC), medulloblastoma, or keratocystic odontogenic tumor (KCOT; formerly known as odontogenic keratocysts) [Gorlin and Goltz, 1960; Gorlin, 1987]. The estimated prevalence of NBCCS is 1 in 55,600 in the United Kingdom [Evans et al., 1993]. The gene responsible for NBCCS is the human homolog of the *Drosophila patched* gene, *PTCH1* [Hahn et al., 1996; Johnson et al., 1996]. Heterozygous loss of *PTCH1* found in certain sporadic and familial cases of BCC indicates that *PTCH1* is also a tumor suppressor gene [Gailani et al., 1996; Unden et al., 1996]. Previously, we performed the largest analysis of NBCCS patients in Japan for *PTCH1* mutations and found diverse mutations including point mutations, insertion/deletion mutations, and entire deletions of the *PTCH1* gene [Fujii et al., 2003; Nagao et al., 2005; Sasaki et al., 2010; Nagao et al., 2011]. However, to date, genotype–phenotype correlation has not been reported [Wicking et al., 1997]. Therefore, there is no correlation between *PTCH1* mutation type and the incidence of BCC or KCOT. In addition, although missense mutations tend to cluster in the transmembrane domains, no marked hot spots or common mutations have been evident in *PTCH1* [Lindström et al., 2006].

Abbreviations: NBCCS, nevoid basal cell carcinoma syndrome; BCC, basal cell carcinoma; KCOT, keratocystic odontogenic tumor.

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To date, there have been no large studies of NBCCS in Asia except one small literature-based survey from Korea [Ahn et al., 2004], which retrospectively examined 33 individuals with NBCCS. Since the incidences of various types of cancer including BCC are associated with ethnicity or geographical factors [Leiter and Garbe, 2008], it is imperative to compare the clinical manifestations of NBCCS between Asians and Caucasians. Herein, we report on the first nationwide survey of NBCCS in Asia examining 157 Japanese patients and show a markedly low frequency of BCC in Japan compared with countries in which mainly Caucasians live.

MATERIALS AND METHODS

Study Subjects

To understand the frequencies of various clinical manifestations in Japanese NBCCS patients, we sent a questionnaire regarding the basic information of NBCCS patients including the number of patients being treated for NBCCS and their sex, age, and major clinical manifestations to the Departments of Pediatrics, Genetics, Neurosurgery, Dermatology, and Oral Surgery of University Hospitals, Regional Children's Hospitals, and large Municipal Hospitals with 500 or more beds in Japan. Out of the 1,938 medical facilities that we sent the questionnaire to, 1,107 responded, 147 of which had NBCCS cases. Then, we sent a second questionnaire regarding the detailed clinical manifestations of the patients to these facilities. We used the diagnostic criteria outlined by Kimonis et al. [1997], which have frequently been employed in previous studies (Table I). Regarding the patients enrolled in this study, they were required to have 2 of the 6 major criteria or 1 major and 2 minor criteria. This research was approved by the ethics committee of Chiba University, and conformed to the provisions of the Declaration of Helsinki in 1995 (as revised in Seoul 2008).

TABLE I. Diagnostic Criteria for NBCCS Used in Our Study

Major criteria
- More than 2 BCCs or 1 under the age of 20 years
- Odontogenic keratocysts of the jaw proven by histology
- Three or more palmar or plantar pits
- Bilamellar calcification of the falx cerebri
- Bifid, fused, or markedly splayed ribs
- First-degree relative with NBCC syndrome

Minor criteria
- Macrocephaly determined after adjustment for height
- Congenital malformations: cleft lip or palate, frontal bossing, "coarse face," moderate or severe hypertelorism
- Other skeletal abnormalities: sprengel deformity, marked pectus deformity, marked syndactyly of the digits
- Radiological abnormalities: bridging of the sella turcica, vertebral anomalies, such as hemivertebrae, fusion, or elongation of the vertebral bodies, modeling defects of the hands and feet, or flame-shaped lucencies of the hands or feet
- Ovarian fibroma
- Medulloblastoma

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 18 for Windows (SPSS Inc., Chicago, IL). Statistical differences in the frequencies of clinical manifestations between Japanese and Caucasians were evaluated using the Pearson's chi-squared test or Fisher's exact test. The distribution of the onset of the first BCC or KCOT in the affected individuals was estimated by means of Kaplan–Meier analysis. The Kaplan–Meier curves of the incidences of BCC and the KCOT in Americans reported by Kimonis et al. [1997] were scanned and plotted using Microsoft® Excel® and then compared with those of the Japanese patients. Statistical comparisons between the results of the Kaplan–Meier analyses of the Japanese and American populations were performed using the Log-Rank test. Spearman's rank-correlation coefficient was used to examine the correlation between the number of tumors and the age of onset.

RESULTS

In the first survey, we found 311 NBCCS patients in Japan. This represents a prevalence of 1 in 235,800 according to the current population estimates by the Statistics Bureau of the Ministry of Internal Affairs and Communications of Japan. After the second survey, the detailed clinical manifestations of 157 NBCCS patients were available. These patients were confirmed to include no duplications according to the date of birth, sex, and age. No significant difference in the survey questionnaire completion rate was observed among departments or types of hospitals. The patients were widely distributed throughout Japan. The ages of the 157 patients ranged from 9 months to 77 years, with their mean age being 33.1 years. There were 83 males and 74 females (ratio: 1.1:1). All but one patient, a Japanese-Brazilian boy, were Japanese. The age ranges of both sexes showed a bimodal distribution (Fig. 1), and the mean ages of the males and females were 30.09 and 36.34, respectively.

The frequencies of the various clinical features of the Japanese NBCCS patients are listed (Table II). The clinical manifestations that displayed frequencies greater than 50% were KCOT (86.3%), falx calcification (79.4%), palmar/plantar pits (69.2%), hypertelorism (68%), and a broad nasal bridge (58.4%). The major criteria that were less frequently seen included BCC (37.8%), rib anomalies (36.4%), and a relevant family history (48.4%).

We compared the frequencies of representative manifestations with those previously reported for the United States [Kimonis et al., 1997], the United Kingdom [Evans et al., 1993], and Australia [Shanley et al., 1994] (Table III). No marked differences among the populations were observed in the frequencies of clinical manifestations other than BCC and KCOT. The details of the tumors that occurred in the NBCCS patients are described below.

Basal Cell Carcinoma

Of the 148 Japanese NBCCS patients, 56 (37.8%) had BCC either as part of their clinical history or at the time of our evaluation. The frequency of BCC in patients of over 20 years was 51.4% (53/103), and that of patients of over 40 years of age was 71.7% (33/46). These frequencies were significantly lower than those described in previous reports (Table III). A less significant difference was

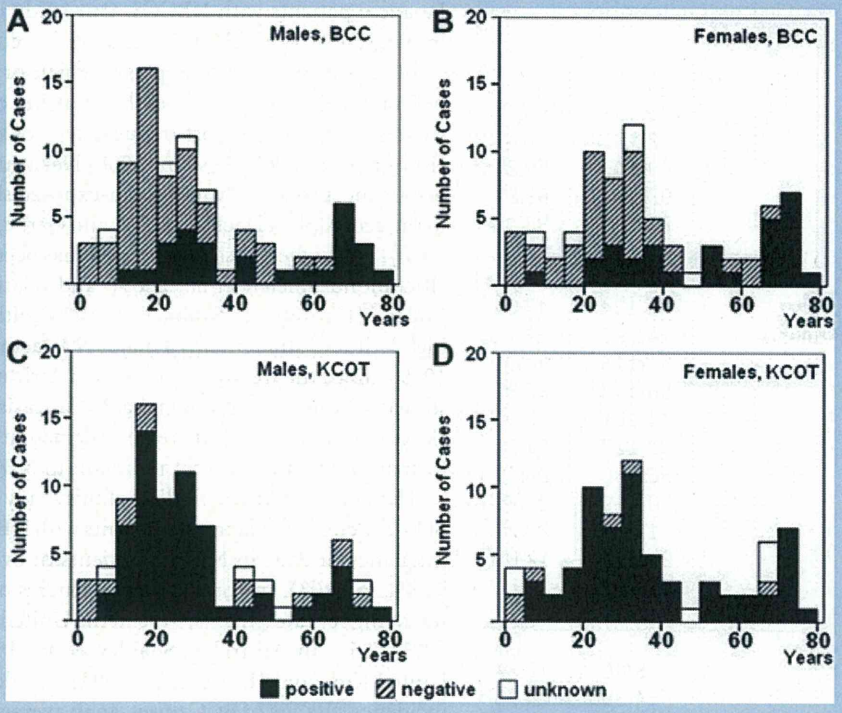

FIG. 1. Age distribution of NBCCS. Age distribution of BCC (A,B) and KCOT (C,D) cases. The presence or absence of BCC and KCOT at the time of the survey is shown. Male (A,C) and female (B,D) cases are shown separately.

detected between Japan and the United Kingdom than between Japan and the United States, which may reflect the reduced exposure of the patients in the United Kingdom to ultraviolet radiation. The age of the patients at the initial BCC diagnosis ranged from 4 to 69 years (mean: 37.4 years, median: 38 years), which was much higher than those in Western countries. Accordingly, Kaplan–Meier analysis of BCC demonstrated that its frequency in the Japanese population is markedly lower than that reported for Caucasians by Kimonis et al. [1997] (Fig. 2). As reported previously [Jones et al., 2011], the frequency of BCC was similar between males and females (34.62% vs. 42.03%). The age distributions of BCC frequency in males and females sexes are shown in Figure 1A and Figure 1B, respectively. We observed no correlation between the number of BCC and the age of onset ($r = 0.049$; data not shown).

KeratoCystic Odontogenic Tumor

One hundred twenty-six out of 146 (86.3%) patients with NBCCS had a history of KCOT, which was significantly higher than the incidences described in the previous reports, although the difference was less marked than that for BCC. The age of the patient at the onset of the tumor ranged from 5 to 77 years (mean: 32.8 years), and the number of tumors ranged from 1 to 10 (mean: 3.68). In contrast to BCC, the number of KCOT was inversely correlated with the age of onset ($r = -0.453$; Fig. 3). The frequency of KCOT in the patients aged over 20 years was 91.1% (92/101), and that in the patients aged over 40 years was 82.0% (32/39). Kaplan–Meier analysis of the frequency of KCOT in Japanese and American NBCCS patients indicated that there were no significant differences in the frequency of KCOT and the age at first KCOT between the two groups (Fig. 4). The frequency of KCOT was similar between males and females (81.82% vs. 91.18%). The age distributions of KCOT incidence in males and females are shown in Figure 1C and Figure 1D, respectively.

Medulloblastoma

Four out of 120 patients (3.4%) had medulloblastoma, and these patients were diagnosed between the ages of 0 and 4 years (mean: 1.8 years). Kimonis et al. [1997] reported that radiotherapy for medulloblastoma results in the early development of BCC and other cerebral tumors, such as ependymomas and meningiomas. In our study, all four patients had radiation therapy for medulloblastoma. However, to date, none of the four patients have had subsequent BCC on the irradiated area.

Ovarian Fibroma

Forty women with NBCCS were evaluated for the frequency of ovarian fibroma, and 5 were found to have ovarian fibromas (12.5%). Their ages ranged from 16 to 25 years (mean: 20.8 years). The frequency of ovarian fibroma in this study was somewhat lower than those described in previous reports. However, since not all women underwent abdominal ultrasound, the actual frequency might have been higher than observed.

TABLE II. Frequencies of the Clinical Manifestations in Japanese NBCCS Patients

50% or greater		
KCOT	126/146	86.3%
Falx calcification	77/97	79.4%
Palmar/plantar pits	99/143	69.2%
Hypertelorism	97/141	68.8%
Broad nasal bridge	73/125	58.4%
15–49%		
Family history	62/128	48.4%
Frontoparietal bossing	63/134	47.0%
Family members developing tumors	41/93	44.1%
Highly arched eyebrows	51/116	44.0%
Basal cell carcinoma	56/148	37.8%
Rib anomalies	44/121	36.4%
Coarse face	43/117	27.9%
Macrocephaly	36/136	26.5%
Prognathism	30/119	25.2%
Bridging of sella turcica	18/76	23.7%
Intellectual disability	24/136	17.6%
Hydrocephalus	22/136	16.2%
Vertebral anomalies	16/106	15.1%
14% or less		
Ovarian fibroma	5/40	12.5%
Short 4th metacarpals	4/38	10.5%
Cleft lip/palate	12/133	9.0%
Epilepsy	12/134	9.0%
Pectus deformity	8/118	6.1%
Medulloblastoma	4/120	3.3%
Sprengel deformity	3/102	2.7%
Flame-shaped lucencies of hands/feet	1/40	2.5%
Cardiac fibroma	2/95	2.1%
Syndactyly	3/143	2.1%
Polydactyly	2/134	1.5%
Modeling defects of hands/feet	0/49	0%

Cardiac Fibroma

Two patients (1 male and 1 female) among 95 evaluated patients were found to have cardiac fibromas (2.1%). Their age at diagnosis ranged from 6 to 7 years. The size of the cardiac fibromas in these patients did not increase over time, and no functional deficits were observed.

Other Tumors

The other tumors recognized in this study included meningioma (4 cases), ovarian tumors other than fibromas (3 cases), and thyroid tumors (2 cases).

DISCUSSION

In this study, we first performed a nationwide survey of NBCCS in Japan. To date, there have been three large epidemiological studies of NBCCS, which were performed in the United States [Kimonis et al., 1997], the United Kingdom [Evans et al., 1993], and Australia [Shanley et al., 1994], and 105, 84, and 118, patients, respectively, were enrolled in these studies. In this study, we enrolled 157 Japanese patients with NBCCS. Our study is the first large epidemiological survey of NBCCS in an Asian country.

BCC is a malignant neoplasm which originates from a pluripotential cell in the basal cell layer of the epidermis or follicular structure, and is the most frequent type of skin cancer in humans [Rubin et al., 2005; Epstein, 2008; Nakayama et al., 2011]. BCC occurs most frequently in the sun-exposed skin area, and is related to the geographical factors [Leiter and Garbe, 2008; Dessinioti et al., 2010]. The highest incidence of BCC has been reported in Australia [Richmond-Sinclair et al., 2009], and relatively low incidence in Finland [Hannuksela-Svahn et al., 1995], although sunlight even at high latitudes still remains a major risk factor of BCC [Moan et al., 1989]. Since the frequency of BCC in African-Americans has been shown to be much lower than that in Caucasians in the United States [Kimonis et al., 1997], increased pigmentation may prevent BCC formation from ultraviolet radiation in NBCCS families.

The most significant finding of this study was the low frequency of BCC detected in Japanese patients with NBCCS. In our study, the frequency of BCC in NBCCS patients of over 20 years of age was 51.4% (53/103). In contrast, other studies of NBCCS have shown much higher rates; that is, 91% in the United States [Kimonis et al., 1997], 85% in Australia [Shanley et al., 1994], and 73% in the United Kingdom [Evans et al., 1993]. In addition to the low BCC incidence, the age of BCC onset, at an average of 37.4-year old, was markedly higher in the Japanese population than those in Western countries. Considering previous reports mentioned above, the lower frequency and the higher onset age of BCC in Japanese NBCCS population are probably due to geographical factor and/or ethnicity. However, since it is not uncommon that BCC develops in the less sun-exposed area in NBCCS patient, UV-exposure is obviously not the only factor for BCC development. Therefore, especially in Japan, careful skin examination over a prolonged period of time is warranted, even after middle age, for the early diagnosis and treatment of late onset BCC.

KCOT, which was formerly known as multiple jaw cysts, is sometimes the first manifestation of NBCCS, and early surgical intervention for tumors is clinically required for both the maintenance of jaw function and the prevention of subsequent bacterial infections. KCOTs are basically benign cystic lesions, but they often show locally destructive behavior and high recurrence rates [Morgan et al., 2005]. Recently, they were re-classified as benign neoplasms of odontogenic origin in the World Health Organization classification 2005 [Madras and Lapointe, 2008]. In this study, the mean age at KCOT onset was 19.8 years, which was similar to those reported in two of the other large studies; that is, 17.3 years in the United States [Kimonis et al., 1997] and 15.5 years in Australia [Shanley et al., 1994]. Accordingly, Kaplan–Meier analysis found that the frequency of KCOT among Japanese NBCCS patients was similar to that in the United States. However, the frequency of KCOT in patients over 20 years of age was 91.1%, which was slightly but significantly higher than those found in other studies; that is, 81% in the United States [Kimonis et al., 1997], 80% in Australia [Shanley et al., 1994], and 82% in the United Kingdom [Evans et al., 1993]. These findings also suggest that Japanese NBCCS patients should receive careful periodical examinations for the jaw cysts. It remains to be elucidated whether ethnicity and/or environmental

TABLE III. Comparison of NBCCS Anomalies Among Four Large Studies

	Evans et al. (United Kingdom)	Shanley et al. (Australia)	Kimonis et al. (United States)	Our study (Japan)
Number of cases	84	118	105	157
Number of families	29	64	26	139
Mean age (years)	N.A.	35	34.5	33.1
Sex ratio M:F	1:1.3	1:1.3	1:1.2	1.1:1
Number with BCC-total (%)	33/70 (47)	90/118 (76)**	71/90 (80)[a],**	56/148 (37.8)
Age >20 years (%)	33/45 (73)*	71/84 (85)**	58/64 (91)[a],**	53/103 (51.4)
Age >40 years (%)	19/21 (90)	35/37 (95)*	34/35 (97)[a],**	33/46 (71.7)
Mean age first BCC (years)	N.A.	20.3	21.4	37.4
Number with KCOT/total (%)	46/70 (66)**	85/113 (75)*	78/105 (74)*	126/146 (86.3)
Age >20 years (%)	37/45 (82)	66/82 (80)*	60/74 (81)	92/101 (91.1)
Age >40 years (%)	19/21 (90)	25/35 (71)	29/38 (76)	32/39 (82.0)
Range of total number of KCOT (mean)	N.A.	1–28 (6)	1–28 (5.1)	1–10 (3.68)
Mean age at first jaw cyst (years)	N.A.	15.5	17.3	19.8
Pitting-palms/soles (%)	50/70 (71)	82/103 (80)	89/102 (87)**	86/143 (60.1)
Cleft lip/palate (%)	4/70 (5)	4/107 (4)	3/103 (3)	12/133 (9.0)
Calcification of falx cerebri (%)	NA	81/89 (92)*	53/82 (65)*	78/98 (79.6)
Medulloblastoma (%)	3/84 (4)	1/118 (1)	4/105 (4)	4/120 (3.3)
Ovarian fibroma (%)	6/25 (24)	9/63 (14)	9/52 (17)	5/40 (12.5)

N.A., not applicable.
[a]Restricted to Caucasians.
*$P < 0.05$.
**$P < 0.001$.

factors are responsible for this difference. Alternatively, the difference may be due to the comparative data collection bias, that is, types of clinics and physicians solicited for patients. In any case, since the difference was relatively small, more cases need to be examined in order to conclude that the frequency of KCOT in Japanese NBCCS is higher than that in Western countries.

In this study, the frequency of medulloblastoma among NBCCS patients was 3.4%, which was similar to those described in previous reports [Evans et al., 1993; Shanley et al., 1994; Kimonis et al., 1997]. Medulloblastoma is the most common malignant brain tumor in childhood. In NBCCS patients, medulloblastomas are reported to occur earlier than sporadic medulloblastomas. The mean ages of onset of syndromic and sporadic medulloblastomas are 28 months and 6 years, respectively [Amlashi et al., 2003]. In our study, the mean age was 1.8 years, which was comparable to those of previous reports. Although the frequency of

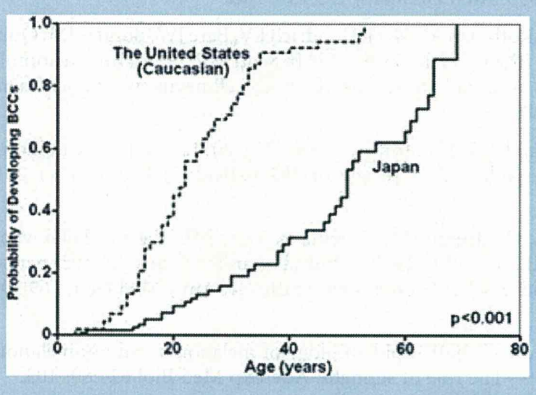

FIG. 2. A Kaplan–Meier curve of the risk of NBCCS patients developing BCC at different ages; Japanese versus Caucasians. The data for Caucasians in the United States were derived from a previous report [Kimonis et al., 1997].

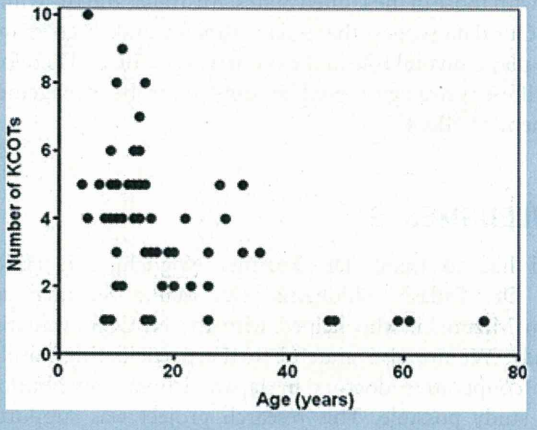

FIG. 3. Scatter plot showing the correlation between the number of KCOTs and the patient's age at onset.

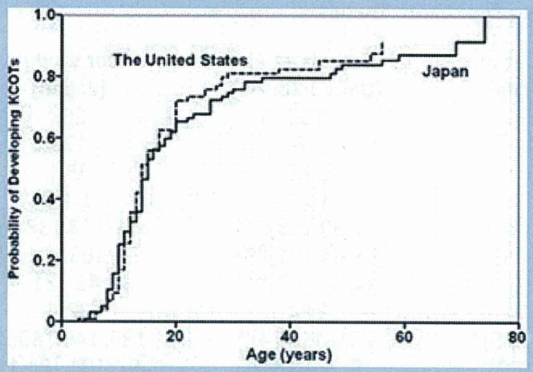

FIG. 4. A Kaplan–Meier curve of the risk of NBCCS patients developing KCOT at different ages; Japanese versus Americans. The data for Americans were derived from the previous report [Kimonis et al., 1997].

medulloblastoma is low in NBCCS, careful investigation is still required in young NBCCS patients since medulloblastoma is one of the most malignant tumors that can develop in NBCCS patients [Evans et al., 1991], and radiation therapy for medulloblastoma might cause subsequent BCC in the irradiated skin area [Smucker and Smith, 2006]. The incidences of other tumors; that is, ovarian and cardiac fibromas, are also similar to those in Western countries.

The estimated prevalence of NBCCS in the Japanese population was 1/235,800, which was lower than those reported in the United Kingdom (1/55,600) [Evans et al., 1993] and Australia (1/164,000) [Shanley et al., 1994]. However, since a considerable fraction of NBCCS patients are treated at local dermatologists or dentists, this estimate represents a minimum figure.

In conclusion, we investigated the clinical manifestations of 157 Japanese NBCCS patients and found a low frequency of BCC compared with those in the United States, Australia, and the United Kingdom. Our data suggest that Asian ethnicity and/or geographical factors play a pivotal role in the occurrence of BCC. Therefore, in Asia, a different strategy should be adopted for the management and treatment of NBCCS.

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Splicing Aberration in Naevoid Basal Cell Carcinoma Syndrome

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Pre-RNA splicing is a transcriptional process whereby introns are removed from a primary transcript and exons are joined to generate a mature protein-coding mRNA transcript. The reaction is initiated by transcripts binding to spliceosomal protein complexes that recognize splice site signals at exon/intron boundaries (1). The flexibility of splice sites in individual genes facilitates complex gene regulation and protein diversity by alternative splicing (2). Alterations in splice site selection, can be affected by gene mutations and may cause splicing modifications. A new "cryptic" splice site can replace the original because of a change in spliceosome affinity (3) and has pathogenic consequences if there is an amino acid change or a protein truncation.

Naevoid basal cell carcinoma syndrome (NBCCS) is caused by germline mutations in the *patched protein homolog 1* (*PTCH1*) (4, 5). NBCCS is characterized by a predisposition to neoplasms and a range of developmental anomalies (6), including multiple basal cell carcinomas (BCCs), odontogenic keratocysts, palmoplantar pits and bifid ribs. *PTCH1* is located on chromosome 9q22.3 (7), where it encodes a receptor protein for the hedgehog signalling pathway (8) that plays an important role in developmental processes such as cell polarity and pattern formation (9).

This study tested the benefits of additional mRNA analysis in a NBCCS patient in whom a mutation was identified by genomic sequencing as splicing aberration.

CASE REPORT

A 58-year-old Japanese man with a history of congenital hydrocephaly and previous extirpation of a jaw cyst during his teenage years noticed black nodules on his face and thighs, which were excised after they were diagnosed as BCCs. He had no family history of NBCCS, but his face presented the characteristic manifestation of hypertelorism, and multiple pits were observed on his palms and soles. Pantomography identified multiple odontogenic keratocysts in his mandible, while a skull MRI indicated communicating hydrocephalus. The patient was diagnosed with NBCCS after satisfying 3 major diagnostic criteria (10), namely, the presence of multiple BCCs, palmoplantar pits and odontogenic keratocysts. Mutational analysis (see http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1332) for details including references to (11) and (12).

Prior to commencing this study, written informed consent was obtained from the patient and permission was obtained from the ethical committee of Tsukuba University Hospital.

A mutation analysis of *PTCH1* was made to investigate the cause of the patient's symptoms. We extracted genomic DNA from the patient's peripheral blood and performed genomic sequencing, which revealed a mutation site where the 584th guanine (G) was replaced by adenine (A) (c.584G>A) on exon 3 (as per GenBank entry NM_000264) (Fig. 1A). This mutation was identified in a previous study (11, 13), but this point mutation suggested a missense mutation whereby the 195th amino acid arginine was replaced by lysine (p.R195K). However, the mutation could affect splicing because the mutation was located at the 3′ end of exon 3. Therefore, we established an immortalized cell line from the patient's lymphoblastoid cells to obtain mRNA, which was then subjected to RT-PCR. Electrophoresis of the product revealed an additional large band (Fig. 1C). Sequencing of the additional product indicated the activation of a cryptic 5′ splice site in intron 3 and the insertion of a 37-bp intronic sequence, which included a termination signal, resulting in premature termination of translation (Fig. 1E). This demonstrated the presence of a splicing aberration and suggested the formation of a truncated PTCH1 protein as a result of the c.584G>A mutation. Thus, we concluded that this NBCCS case was attributable to a splicing aberration.

DISCUSSION

The *PTCH1* mutation identified in the present case was located at an exon/intron boundary, where it could disrupt the splicing signal, so it was reasonable to predict a splicing aberration. However, aberrant splicing can also be caused by silent mutations in which single base substitutions do not change specific amino acids. Indeed, a mutation in *LAMB3* of a junctional epidermolysis bullosa patient initially appeared to be a silent mutation, but it actually resulted in the aberrant splicing of a cryptic splice site (14). A comparative study of mRNA and genomic DNA in neurofibromatosis patients showed that 50% of the patients had mutations resulting in splicing aberration, which was confirmed by mRNA analysis, although 37% of the patients had splice site mutations that were identified without cDNA sequencing (15). Therefore, these previous studies and the present case clearly illustrate the significance of additional cDNA sequencing after the identification of a genomic mutation. An aggregate analysis of NBCCS patients with genomic mutations revealed that two out of 28 patients (7.1%) had splice site mutations (16). However, our study indicates that the actual incidence of splicing aberrations among NBCCS patients might be higher.

In addition, we extracted a sample from the patient's peripheral blood, because peripheral blood monocytes express the PTCH1 protein. Moreover, analysis of *PTCH1* mRNA and genomic DNA can be performed using samples obtained from patients' peripheral blood. Although splicing patterns may differ depending on the tissues examined, our method was less invasive and more appropriate for multiple subjects with NBCCS compared with a procedure that requires disease tissue.

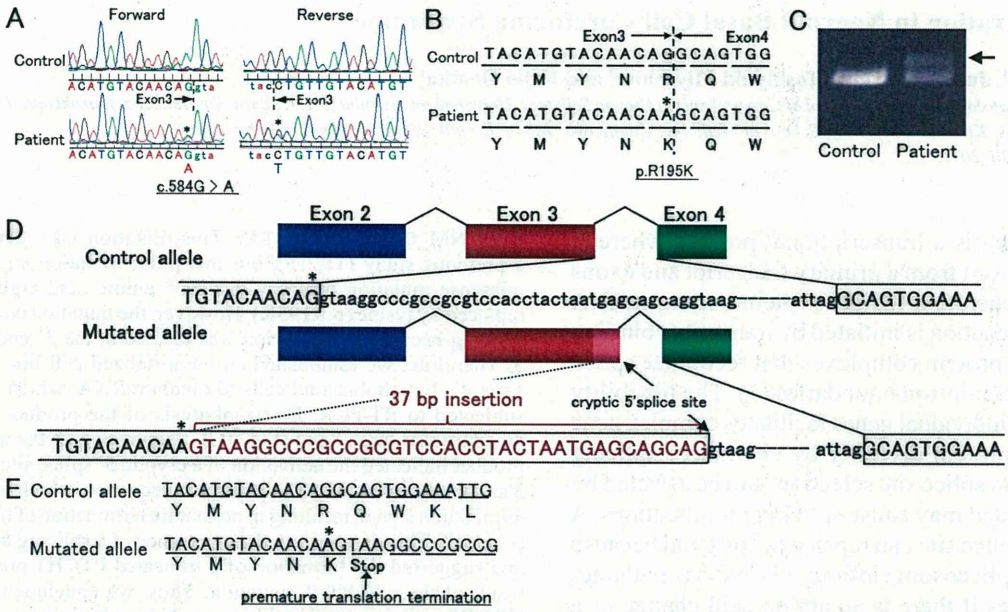

Fig. 1. The mutation identified in the patient and its effect on the splicing event. In parts (A) and (D), the exonic sequence is denoted by uppercase letters, while the intronic sequence is denoted by lowercase letters. A sample from a healthy donor was used as a control. (A) Genomic sequence of *PTCH1*. The 584th guanine (G) was replaced by adenine (A) in the patient's DNA. Note the double peaks at the last base in exon 3 (*green and red*). (B) The patient's amino acid sequence predicted from the genomic mutation c.584G>A. The 195th amino acid, arginine (R), would be substituted with lysine (K). (C) Electrophoresis of cDNA obtained from the mRNA, which was extracted from the patient's Epstein-Barr virus (EBV)-immortalized lymphoblastoid cells. Note the additional large band in the patient's lane (*arrow*). (D) Schematic representation of the splicing aberration found in the patient. A 37-bp sequence (*red letters*) was inserted following a mutation located in the 3′ end of exon 3 (*asterisks*). Because of the base substitution at the 3′ end of exon 3 (G → A), a cryptic 5′ splice site was produced in intron 3 (*double-headed arrow*), rather than the original site at the intron/exon border. This resulted in a splicing aberration on activation. (E) Results of cDNA sequencing. In addition to the amino acid alteration, a frameshift mutation due to the splicing aberration caused a termination signal (TAA) that resulted in the truncation of the PTCH1 protein.

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Mutational analysis: DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen, MD, USA). Genomic DNA samples were amplified using primers for each exon, as previously described (11). Amplified products were gel-purified using a QIAEX II Gel Extraction Kit (Qiagen). Sequencing reactions were performed using a GenomeLab DTCS Quick Start Kit (Beckman Coulter, CA, USA) with a cycle sequencing protocol, while sequencing reactions were separated on a CEQ 8000 Genetic Analysis System (Beckman Coulter). Splicing aberrations were tested using an immortalized cell line that was established from the patient by infecting with Epstein-Barr virus (EBV) (12) obtained from B95-8 cells. Total RNA was extracted from the EBV-immortalized lymphoblastoid cell line using a QIAamp RNA Blood Mini Kit (Qiagen) and subjected to reverse transcription (RT)-PCR using a Long-Range 2-Step RT-PCR Kit (Qiagen) with oligo dT. The forward and reverse primers for exons 2 and 6 were 5′-GCTGAGAGCGAAGTTTCAGA-3′ and 5′-CCAGGAATTCCAAAGGGTCGAAG-3′, respectively.