Journal of Dermatological Science 86 (2017) 206–211

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Targeted exome sequencing and chromosomal microarray for the molecular diagnosis of nevoid basal cell carcinoma syndrome

Yoshihiro Matsudate [a], Takuya Naruto [b], Yumiko Hayashi [c], Mitsuyoshi Minami [d], Mikiko Tohyama [e], Kenji Yokota [f], Daisuke Yamada [g], Issei Imoto [b], Yoshiaki Kubo [a,*]

[a] Department of Dermatology, Tokushima University Graduate School of Medical Science, Tokushima, Japan
[b] Department of Human Genetics, Tokushima University Graduate School of Medical Science, Tokushima, Japan
[c] Department of Child Neurology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[d] Divisions of Dermatology, Matsuyama Red Cross Hospital, Matsuyama, Japan
[e] Department of Dermatology, Ehime University Graduate School of Medicine, Ehime, Japan
[f] Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan
[g] Department of Dermatology, University of Tokyo Graduate School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 25 January 2017
Accepted 22 February 2017

Keywords:
Nevoid basal cell carcinoma syndrome
PTCH1
Targeted exome sequencing
Next-generation sequencing
Chromosomal microarray

ABSTRACT

Background: Nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder mainly caused by heterozygous mutations of *PTCH1*. In addition to characteristic clinical features, detection of a mutation in causative genes is reliable for the diagnosis of NBCCS; however, no mutations have been identified in some patients using conventional methods.
Objective: To improve the method for the molecular diagnosis of NBCCS.
Methods: We performed targeted exome sequencing (TES) analysis using a multi-gene panel, including *PTCH1*, *PTCH2*, *SUFU*, and other sonic hedgehog signaling pathway-related genes, based on next-generation sequencing (NGS) technology in 8 cases in whom possible causative mutations were not detected by previously performed conventional analysis and 2 recent cases of NBCCS. Subsequent analysis of gross deletion within or around *PTCH1* detected by TES was performed using chromosomal microarray (CMA).
Results: Through TES analysis, specific single nucleotide variants or small indels of *PTCH1* causing inferred amino acid changes were identified in 2 novel cases and 2 undiagnosed cases, whereas gross deletions within or around *PTCH1*, which are validated by CMA, were found in 3 undiagnosed cases. However, no mutations were detected even by TES in 3 cases. Among 3 cases with gross deletions of *PTCH1*, deletions containing the entire *PTCH1* and additional neighboring genes were detected in 2 cases, one of which exhibited atypical clinical features, such as severe mental retardation, likely associated with genes located within the 4.3 Mb deleted region, especially.
Conclusion: TES-based simultaneous evaluation of sequences and copy number status in all targeted coding exons by NGS is likely to be more useful for the molecular diagnosis of NBCCS than conventional methods. CMA is recommended as a subsequent analysis for validation and detailed mapping of deleted regions, which may explain the atypical clinical features of NBCCS cases.

1. Introduction

Nevoid basal cell carcinoma syndrome (NBCCS; MIM# 109400), also known as Gorlin syndrome, is an autosomal dominant disorder exhibiting multiple BCCs, palmoplantar pits, keratocystic odontogenic tumors (KCOTs), calcification of the falx cerebri, and skeletal abnormalities [1]. *PTCH1* (MIM# 601309), mapped to 9q22.32 and consisting of 23 exons encoding 1447 amino acids, has been identified as a main causative gene for NBCCS [2,3]. The

Abbreviations: NBCCS, nevoid basal cell carcinoma syndrome; KCOT, keratocystic odontogenic tumor; Shh, sonic hedgehog; PCR–SSCP, polymerase chain reaction–single strand conformation polymorphism; CNA, copy number alteration; NGS, next-generation sequencing; SNV, single nucleotide variant; indel, insertion/deletion; TES, targeted exome sequencing; CMA, chromosomal microarray; HGMD, human genome mutation database; SNP, single-nucleotide polymorphism; ChAS, Chromosome Analysis Suite; OMIM, Online Mendelian Inheritance in Man.

* Corresponding author. Tel.: +81 886 33 7154; fax: +81 886 32 0434.
E-mail address: kubo@tokushima-u.ac.jp (Y. Kubo).

http://dx.doi.org/10.1016/j.jdermsci.2017.02.282
0923-1811/

PTCH1 protein is a receptor for sonic hedgehog (Shh), and activation of the Shh pathway due to inactivation of PTCH1 protein may be relevant to NBCCS phenotypes [2].

Although we had performed polymerase chain reaction–single strand conformation polymorphism (PCR–SSCP) analysis and Sanger sequencing in 20 cases of NBCCS following the previous report [4], we failed to detect any *PTCH1* mutations in 8 of 20 cases (40%) of NBCCS by the analyses. As the detection rates of germline *PTCH1* mutations in cases of NBCCS were reported to be 40–75% by conventional methods, such as PCR–SSCP analysis, PCR-based direct sequencing of all exons, or denaturing high performance liquid chromatography [5–7], our detection rate of 60% was similar to those results. This failure to detect mutations may be due to copy number alterations (CNAs) of the regions over one exon of *PTCH1* [8,9], the presence of mutations located outside exons and exon-intron boundaries of *PTCH1*, such as mutations within introns or regulatory regions of *PTCH1*, or mutations in other candidate genes associated with the Shh pathway, such as *PTCH2*, *SUFU*, *SHH*, *GLI1/2/3*, *SMO*, *HHIP*, *KIF7*, and *STK36*. Among them, *PTCH2* (MIM# 603673) and *SUFU* (MIM# 607035) have been identified as causative genes with heterozygous mutations in rare cases of NBCCS [10–12].

Recently, next-generation sequencing (NGS) has been adopted in clinical testing for patients with congenital diseases suspected to be genetic in origin including NBCCS, because single-nucleotide variations (SNVs) and small insertions/deletions (indels) as well as gross CNAs involved in a defect of disease-causing genes can be simultaneously screened using whole exome sequencing or targeted exome sequencing (TES) in a time- and cost-effective manner [9]. In this study, we performed a comprehensive TES-based genetic analysis using a multi-gene panel, including *PTCH1*, *PTCH2*, *SUFU*, and other shh-related genes except *SMO* and *STK36* in 8 cases with no mutations detected by previous PCR–SSCP analysis and 2 recently enrolled cases of NBCCS (Fig. 1), gross deletions detected in *PTCH1* by TES were examined in detail by chromosomal microarray (CMA) analysis.

2. Materials and methods

2.1. Cases analyzed by TES analysis

We analyzed 10 Japanese cases with a clinical diagnosis of NBCCS based on the presence of diagnostic criteria of Kimonis (Table 1, Supplementary Fig. S1) [13]. In 8 cases, no *PTCH1* mutations were detected by our previous PCR–SSCP analysis (Fig. 1). In the remaining 2 recent new cases, we performed TES analysis without PCR–SSCP analysis in advance. The molecular diagnosis was performed using genomic DNA extracted from the patient's whole blood after obtaining informed consent. This study was approved by the ethical committees of Tokushima University.

2.2. TES by NGS and data analyses

We used a MiSeq bench-top sequencer (Illumina, San Diego, CA, USA) to perform NGS with a TruSight One Sequencing Panel (Illumina), which provides for the simultaneous targeted sequencing of the exon regions of 4813 clinically relevant genes. The alignments of sequencing reads to the human reference genome (GRCh37/hg19), duplicate read removal, local realignment around indels, base quality score recalibration, variant calling and annotation were performed as previously described [14]. To identify single nucleotide variants (SNVs), we excluded sequence variants with minor allele frequencies (>0.01) included in various human genome variation databases, as previously described [14] and an integrative Japanese Genome Variation Database (iJGVD, https://ijgvd.megabank.tohoku.ac.jp/) [15]. To complement the SNVs and indel analyses, detection of CNAs using TES data with a resolution of a single exon to several exons, depending on the size of exons, was performed using DNAcopy (R/Bioconductor; http://bioconductor.org) [16] and eXome-Hidden Markov Model v1.0 (XHMM, https://atgu.mgh.harvard.edu/xhmm/) as previously described [17].

Candidates for pathogenic variants were confirmed by Sanger sequencing. The pathogenicity of missense variants was assessed using tools for prediction of possible impact of amino acid substitution on the structure and function of human protein such as MutationTaster (http://www.mutationtaster.org/index.html), PolyPhen-2 version 2.2.2r398 (http://genetics.bwh.harvard.edu/pph2/index.shtml), and PROVEAN tool (http://provean.jcvi.org.). Identified alterations were also evaluated by comparison with known alterations reported in mutation databases such as the Human Genome Mutation Database (HGMD) professional 2016.1 (http://www.hgmd.cf.ac.uk/ac/index.php) and ClinVar (http://www.ncbi.nlm.gov/clinvar).

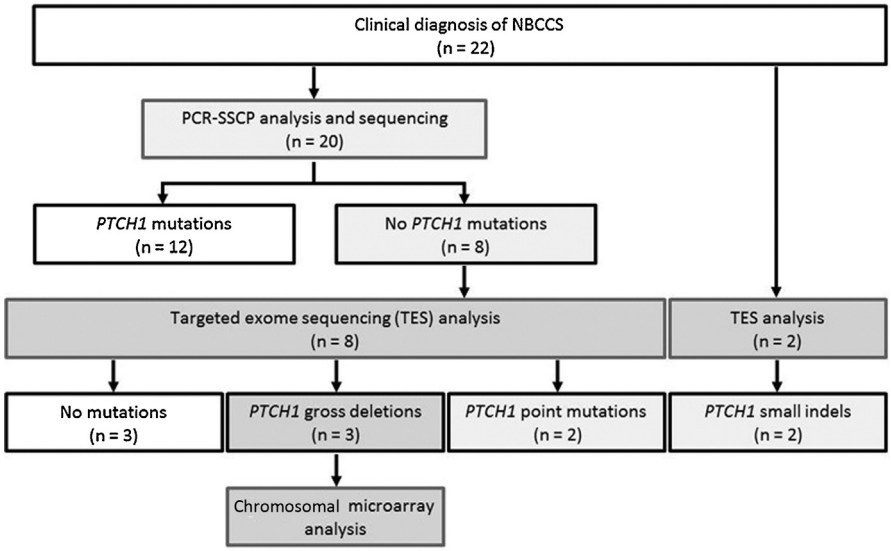

Fig. 1. Flow chart of this study.

Table 1
Clinical features of NBCCS cases in this study.

Case no.	Age (yr)	Gender	Major criteria						Minor criteria	Other clinical features
			BCC		Multiple KCOTs	Palmo-plantar pits	Calcification of falx cerebri	Rib anomalies	Family history		
			Numbers	Locations							
1	31	M	No		Yes	Yes	Yes	No	No	No	No
2	21	F	3	Face	Yes	Yes	Yes	No	No	No	West syndrome, MR, hydro-cephalus, autism
3	60	F	Dozens	Head, face	Yes	Yes	Yes	No	Yes (son)	Coarse face, scoliosis	No
4	64	M	16	Face, abdomen, back	Yes	Yes	Yes	No	No	No	No
5	17	F	No		Yes	Yes	Yes	Yes	No	No	No
6	31	F	1	Forehead	No	Yes	Yes	No	No	Cleft palate, coarse face	Hydro-cephalus
7	62	F	6	Forehead, axilla, upper chest	No	Yes	Yes	No	Yes (son, daughter)	No	No
8	41	F	No		Yes	No	Yes	No	No	Macro-cephaly, frontal bossing, hyper-telorism	No
9	35	M	1	Nose	Yes	No	Yes	No	No	Frontal bossing, hyper-telorism	No
10	21	M	No		Yes	No	NT	No	No	Hyper-telorism	Skin cysts

BCC, basal cell carcinoma; KCOT, keratocystic odontogenic tumor; MR, mental retardation; NT, not tested.

2.3. Chromosomal microarray (CMA) analysis

CNA validation and detailed mapping of altered regions were performed using the CytoScan HD array platform (Affymetrix, Santa Clara, CA, USA), which provides 750,000 polymorphic (single-nucleotide polymorphism, SNP) and 1950,000 non-polymorphic (CNA) markers as described previously [9,17]. The raw data were analyzed using Affymetrix Chromosome Analysis Suite (ChAS) Software and the output data were interpreted with the UCSC Genome Browser (http://genome.ucsc.edu). The disease-related genes were selected based on the information from Online Mendelian Inheritance in Man (OMIM, http://www.omim.org/).

3. Results

All 10 cases satisfied clinical diagnostic criteria of NBCCS (Table 1). Out of 10 cases, specific SNVs or small indels of *PTCH1* causing inferred amino acid changes were identified in 4 cases, whereas CNAs within or around *PTCH1* were found in 3 cases (Fig. 1, Table 2). SNVs or CNAs were detected by TES in 5 of 8 cases, in which possible causative SNVs were not detected by previous PCR–SSCP analysis (Fig. 1, Table 2). In the remaining 3 cases, however, no possible disease-causing genetic alterations were detected in *PTCH1*, *PTCH2*, *SUFU*, and other shh-related genes except *SMO* and *STK36*, which are not included in TruSight One

Table 2
Results of targeted exome sequencing analysis.

Case no.	*PTCH1* mutation detected by PCR–SSCP analysis	Altered gene(s) detected by TES	Mutation of *PTCH1*							
			Type	Nucleic acid change	Amino acid change	Exon	Mutation-Taster prediction (score)	PolyPhen-2 prediction (score)	PROVEAN tool prediction (score)	Reported as pathogenic alteration
1	No	No								
2	No	50 genes including *PTCH1*	Gross deletion			All				No
3	No	*PTCH1*	Nonsense	c.2198C>G	p.S733X	14	Disease causing (1)	NA	Neutral (−2.287)	No
4	No	No								
5	No	*PTCH1*	Gross deletion			12–15				No
6	No	No								
7	No	*PTCH1*	Missense	c.1313G>T	p.S438I	9	Disease causing (1)	Probably damaging (0.996)	Deleterious (−5.048)	No
8	No	25 genes including *PTCH1*	Gross deletion			All				No
9	NT	*PTCH1*	2-bp deletion and 8-bp insertion	c.3487_3488delinsAATGGGCC	p.G1163delinsNGP	21	Disease causing (0.999)	NA	Deleterious (−12.476)	No
10	NT	*PTCH1*	Frame-shift	c.1670_1671del	p.T557Sfs*69	12	Disease causing (1)	NA	NA	Takahashi et al. [18]

PCR–SSCP, polymerase chain reaction–single strand conformation polymorphism; NT, not tested; NA, not available.

sequencing panel, even though these 3 cases satisfied the same diagnostic criteria as the other 7 cases (Table 1).

3.1. Causative SNVs and indels identified in cases with NBCCS by TES

3.1.1. Case 3

In case 3, we identified a nonsense mutation in *PTCH1* (p.S733X) caused by a 1 base substitution at nucleotide 2198, which was predicted to be a loss-of-function mutation, likely leading to an aberrant mRNA targeted for degradation via nonsense-mediated decay (NMD) or resulting in a truncated protein.

3.1.2. Case 7

In case 7, we identified a missense mutation in *PTCH1* (p.S438I) caused by a 1 base substitution at nucleotide 1313 in exon 9. This mutation was predicted to be "deleterious" and "damaging" by PROVEAN tool and PolyPhen-2, respectively. The same alteration was detected in the affected daughter, although genetic analysis has never been performed in the affected son (Supplementary Fig. S1). Therefore, we concluded this alteration to be responsible for NBCCS, even though this mutation has not been reported in HGMD or ClinVar.

3.1.3. Case 9

In case 9, we identified an in-frame indel, a 2-bp deletion and 8-bp insertion (c.3487_3488delinsAATGGGCC) in exon 21, resulting in the inclusion of two amino acids (p.G1163delinsNGP) within the transmembrane domain. This mutation was predicted as "deleterious" by PROVEAN tool. Therefore, we concluded this alteration to be responsible for NBCCS, even though this mutation has not been reported in HGMD or ClinVar.

3.1.4. Case 10

In case 10, we identified a possibly deleterious frameshift mutation (p.T557Sfs*69) creating a new stop codon at codon 625 by a 2-bp deletion at nucleotides 1670-1671 in exon 12. As this mutation was previously reported to be causative for NBCCS by Takahashi et al. [18], we concluded this alteration to be responsible for NBCCS.

3.2. Causative CNAs identified in cases with NBCCS by TES

In 3 cases, we were unable to identify any causative SNVs or indels within the possible target genes, but rather detected entire or intragenic *PTCH1* deletions resulting in loss-of-function of this gene by TES analysis (Table 2, Fig. 2). All these deletions failed to be detected by previous PCR–SSCP analysis. As TES is able to detect CNAs only on targeted exons and unable to correctly determine an altered copy number, CMA analysis was subsequently performed to validate these deletions and to determine copy number and detailed genomic location of the deleted regions (Fig. 3A–C).

3.2.1. Case 2 and case 8

In cases 2 and 8, we detected gross hemizygous deletions including the entire *PTCH1* gene. In case 2, we identified an approximately 4.06 Mb deletion (chr9: 97,848,293–101,911,452) containing 90 target exons including all exons of *PTCH1* by TES. Hemizygous deletion was confirmed by CMA, and the precise size of the deletion determined by CMA was approximately 4.3 Mb (chr9:97,637,037–101,936,873) containing 50 RefSeq genes (24 disease-related genes) from *C9orf3* to *TGFBR1* (Fig. 3A). In case 8, we identified an approximately 1.22 Mb deletion (chr9:97,848,293–99,064,223) containing 50 target exons including all exons of *PTCH1* by TES. Hemizygous deletion was confirmed by CMA, and the precise size of the deletion determined by CMA was approximately 2.22 Mb (chr9:97,470,295–99,685,607) containing 25 RefSeq genes (9 disease-related genes) from *C9orf3* to *LOC441454* (Fig. 3B). These alterations have not been reported in HGMD or ClinVar. Array-based karyotype results of cases 2 and 8 were arr[hg19] 9q22.32(97,637,037_101,936,873) × 1 and arr[hg19] 9q22.32(97,470,295_99,685,607) × 1, respectively.

3.2.2. Case 5

In case 5, we detected an approximately 8.92 kb intragenic deletion of *PTCH1* from exon 12 to exon 15 (chr9:98,229,388–98,238,306) by TES. Hemizygous deletion within *PTCH1* of approximately 7.93 kb (chr9: 98,227,309–98,235,234) was confirmed by CMA. Array-based karyotype results of case 5 was arr[hg19] 9q22.32(98,227,309_98,235,234) × 1, although the location and size of the deleted region may be underestimated or overestimated due to uneven coverage of probes on CMA (Fig. 3C). These alterations have not been reported in HGMD or ClinVar.

4. Discussion

In this study, we applied TES using a TruSight One Sequencing Panel based on NGS technology to simultaneously investigate the

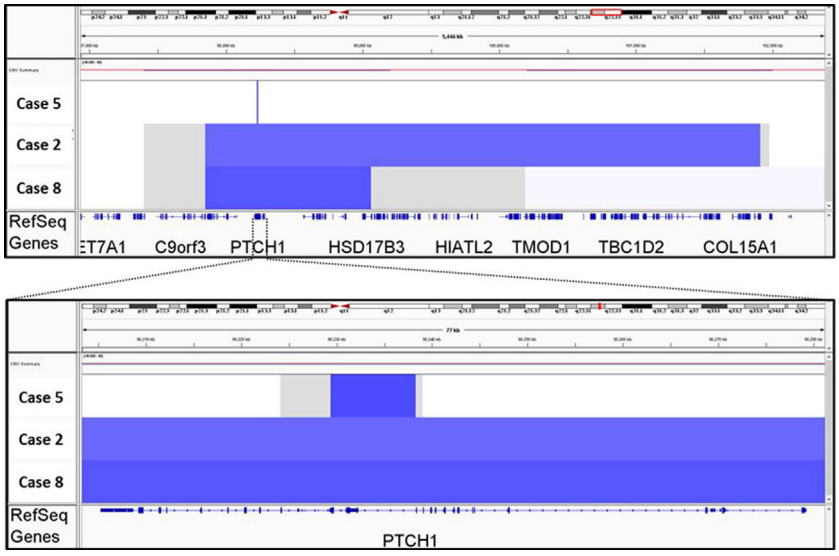

Fig. 2. Graphical representation of DNA copy number alterations in *PTCH1* regions by targeted exome sequencing analysis.

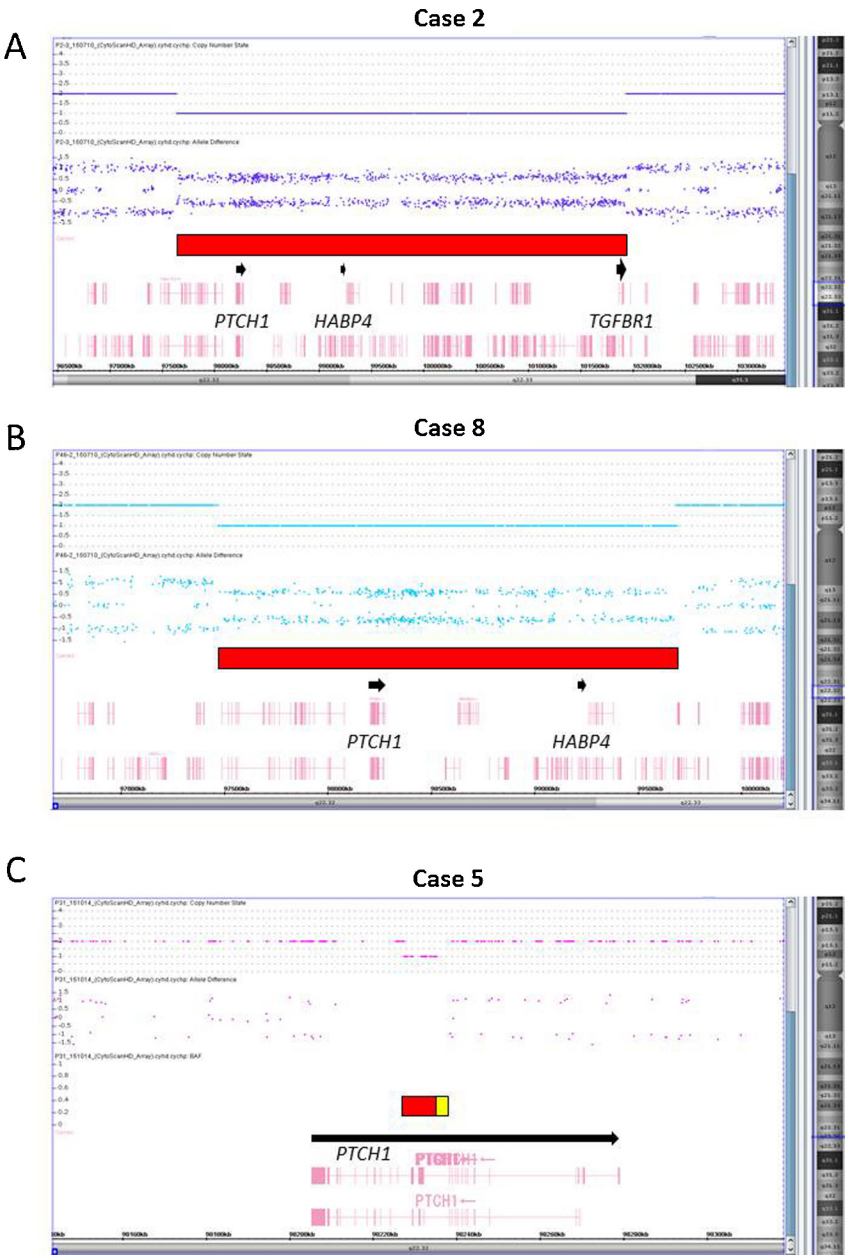

Fig. 3. Image of deleted regions detected by chromosomal microarray analysis. (A) An approximately 4.3 Mb deletion containing genes from *C9orf3* to *TGFBR1*, including full-length *PTCH1*, was detected in case 2. (B) An approximately 2.22 Mb deletion containing genes from *C9orf3* to *LOC441454*, including full-length *PTCH1*, was detected in case 8. (C) The deletion size was approximately 7.93 kb within *PTCH1* in case 5.

sequence and copy number status of all exons of candidate disease-causing genes, including known three causative genes, *PTCH1*, *PTCH2*, and *SUFU*, in 10 cases with a clinical diagnosis of NBCCS. In 2 cases (cases 9 and 10) that have not been analyzed by PCR–SSCP analysis, we detected small *PTCH1* indels. In 8 cases (cases 1–8) where we were not able to detect any *PTCH1* mutations by previous PCR–SSCP analysis and Sanger sequencing, *PTCH1* small deletions/insertions were identified by NGS and Sanger sequencing in 2 cases (cases 3 and 7). This is because the causative exons have not been checked due to no obvious pattern differences by PCR–SSCP analysis. In 3 cases (cases 2, 5 and 8), we identified gross deletions, which were not detected by conventional methods. However, no mutation was detected within possible disease-causing genes in shh pathway even by TES analysis in 3 cases (cases 1, 4 and 6), although *SMO* and *STK36*, which have never been identified as causative genes for NBCCS, are not included in our sequencing panel. In these cases, mutations may exist outside of the analyzed regions such as those within introns or regulatory regions of the candidate genes. In addition, it is difficult to exclude the possibility of somatic mosaicism occurred in causative genes, although only one case of possible type 2 mosaicism of *PTCH1* in NBCCS has been reported [19] and none of our mutation-negative cases showed signs of segmental distribution of BCCs, pits, and so on. Further examinations using additional techniques, such as whole genome sequencing and ultra deep sequencing or digital PCR, are needed to detect variations in these mutation-negative cases.

Although there was no significant difference in the major clinical features of NBCCS among the 10 cases, many atypical clinical features were observed in case 2 out of 2 cases (cases 2 and 8) in which entire *PTCH1* deletions were detected. The clinical features of case 2 included severe mental retardation, WEST syndrome (epilepsy), autism, hydrocephalus, hypotonia, inguinal

hernia, epicanthic folds, low set ears, short neck, scoliosis, and strabismus. On the other hand, case 8 presented typical clinical features of NBCCS and no atypical phenotype.

As *C9orf3* at the proximal site of the deletion was common to both case 2 and case 8, the distal region of the deletion that consisted of approximately 2.25 Mb (chr9: 99,685,608–101,936,873) containing 25 RefSeq genes (15 disease-related genes) from *NUTM2G* to *TGFBR1* may have contributed to the development of the atypical phenotype only observed in case 2. Similar phenotypes with interstitial 9q22.3 microdeletion have been reported as microdeletion 9q22.3 syndrome [20–22]. Among them, 2 cases reported by Redon et al. [20] closely resembled case 2 in terms of both clinical features and deletion sites, suggesting that genes involved in the genotype-phenotype correlation in these two reported cases and case 2 locate within this 2.25 Mb deleted region.

Among 25 RefSeq genes (15 OMIM genes) from *NUTM2G* to *TGFBR1*, we take note of *TGFBR1* with particular interest. *TGFBR1* encodes the type 1 transforming growth factor beta (TGF-β) receptor. TGF-β superfamily signaling pathways are ubiquitous and essential regulators of cellular and physiological processes [23]. Heterozygous loss-of-function mutations in *TGFBR1* are reported to cause multiple developmental anomalies referred to as Loeys–Dietz syndrome [24]. This syndrome exhibits widespread perturbations in cardiovascular, craniofacial, neurocognitive and skeletal development, although cases with this syndrome having *TGFBR1* deletion have not developed aortic aneurysms to date, suggesting that at least some mutant protein needs to be present for cardiovascular features of this disease [24–26]. Scoliosis and strabismus are frequently observed in this disease, and mental retardation (developmental delay) and hydrocephalus are observed in a minority of affected individuals [26]. Although a rare case of NBCCS with autism due to a deletion of 22 base pairs in *PTCH1* has also been reported [27] and *HABP4*, which locates within the commonly deleted region between cases 2 and 8 (Fig. 3), has been proposed as a cause of mental retardation in a NBCCS patients [9], we speculated that heterozygous loss-of-function in both *PTCH1* and *TGFBR1* may contribute to some of atypical clinical features in NBCCS observed in case 2.

In conclusion, TES analysis using NGS technology demonstrated superior sensitivity compared with our previous methods in both mutation and CNA detection of candidate disease-causing genes for NBCCS. Therefore, TES-first approach followed by CMA may be cost- and time-effective for the molecular diagnosis of NBCCS.

Funding sources

None.

Conflict of interest statement

None declared.

Acknowledgment

The authors thank Mr. Hideaki Horikawa (the Support Center for Advanced Medical Sciences, Institute of Biomedical Sciences, Tokushima University) for his technical assistances.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2017.02.282.

References

[1] R.J. Gorlin, Nevoid basal cell carcinoma syndrome, Dermatol. Clin. 13 (1995) 113–125.
[2] H. Hahn, C. Wicking, P.G. Zaphiropoulos, M.R. Gailani, S. Shanley, A. Chidambaram, et al., Mutations of the human homolog of drosophila patched in the nevoid basal cell carcinoma syndrome, Cell 85 (1996) 841–851.
[3] R.L. Johnson, A.L. Rothman, J. Xie, L.V. Goodrich, J.W. Bare, J.M. Bonifas, et al., Human homolog of patched, a candidate gene for the basal cell nevus syndrome, Science 272 (1996) 1668–1671.
[4] M. Minami, Y. Urano, T. Ishigami, H. Tsuda, J. Kusaka, S. Arase, Germline mutations of the PTCH gene in Japanese patients with nevoid basal cell carcinoma syndrome, J. Dermatol. Sci. 27 (2001) 21–26.
[5] K. Fujii, Y. Kohno, K. Sugita, M. Nakamura, Y. Moroi, K. Urabe, et al., Mutations in the human homologue of drosophila patched in Japanese nevoid basal cell carcinoma syndrome patients, Hum. Mutat. 21 (2003) 451–452.
[6] A. Marsh, C. Wicking, B. Wainwright, G. Chenevix-Trench, DHPLC analysis of patients with nevoid basal cell carcinoma syndrome reveals novel PTCH missense mutations in the sterol-sensing domain, Hum. Mutat. 26 (2005) 283.
[7] K. Fujii, S. Ishikawa, H. Uchikawa, D. Komura, M.H. Shapero, F. Shen, et al., High-density oligonucleotide array with sub-kilobase resolution reveals breakpoint information of submicroscopic deletions in nevoid basal cell carcinoma syndrome, Hum. Genet. 122 (2007) 459–466.
[8] K. Nagao, K. Fujii, K. Saito, K. Sugita, M. Endo, T. Motojima, et al., Entire PTCH1 deletion is a common event in point mutation-negative cases with nevoid basal cell carcinoma syndrome in Japan, Clin. Genet. 79 (2011) 196–198.
[9] K. Morita, T. Naruto, K. Tanimoto, C. Yasukawa, Y. Oikawa, K. Masuda, et al., Simultaneous detection of both single nucleotide variations and copy number alterations by next-generation sequencing in Gorlin syndrome, PLoS ONE 10 (2015) e0140480.
[10] K. Fujii, H. Ohashi, M. Suzuki, H. Hatsuse, T. Shiohama, H. Uchikawa, et al., Frameshift mutation in the PTCH2 gene can cause nevoid basal cell carcinoma syndrome, Fam. Cancer 12 (2013) 611–614.
[11] C. Kijima, T. Miyashita, M. Suzuki, H. Oka, K. Fujii, Two cases of nevoid basal cell carcinoma syndrome associated with meningioma caused by a PTCH1 or SUFU germline mutation, Fam. Cancer 11 (2012) 565–570.
[12] M.J. Smith, C. Beetz, S.G. Williams, S.S. Bhaskar, J. O'Sullivan, B. Anderson, et al., Germline mutations in SUFU cause Gorlin syndrome-associated childhood medulloblastoma and redefine the risk associated with PTCH1 mutations, J. Clin. Oncol. 32 (2014) 4155–4161.
[13] V.E. Kimonis, A.M. Goldstein, B. Pastakia, M.L. Yang, R. Kase, J.J. DiGiovanna, et al., Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome, Am. J. Med. Genet. 69 (1997) 299–308.
[14] N. Okamoto, T. Naruto, T. Kohmoto, T. Komori, I. Imoto, A novel PTCH1 mutation in a patient with Gorlin syndrome, Hum. Genome Var. 1 (2014) 14022.
[15] Y. Yamaguchi-Kabata, N. Nariai, Y. Kawai, Y. Sato, K. Kojima, M. Tateno, et al., iJGVD: an integrative Japanese genome variation database based on whole-genome sequencing, Hum. Genome Var. 2 (2015) 15050.
[16] A.B. Olshen, E.S. Venkatraman, R. Lucito, M. Wigler, Circular binary segmentation for the analysis of array-based DNA copy number data, Biostatistics 5 (2004) 557–572.
[17] M. Watanabe, Y. Hayabuchi, A. Ono, T. Naruto, H. Horikawa, T. Kohmoto, et al., Detection of 1p36 deletion by clinical exome-first diagnostic approach, Hum. Genome Var. 3 (2016) 16006.
[18] C. Takahashi, N. Kanazawa, Y. Yoshikawa, R. Yoshikawa, Y. Saitoh, H. Chiyo, et al., Germline PTCH1 mutations in Japanese basal cell nevus syndrome patients, J. Hum. Genet. 54 (2009) 403–408.
[19] A. Torrelo, A. Hernández-Martín, E. Bueno, I. Colmenero, I. Rivera, L. Requena, et al., Molecular evidence of type 2 mosaicism in Gorlin syndrome, Br. J. Dermatol. 169 (2013) 1342–1345.
[20] R. Redon, G. Baujat, D. Sanlaville, M.L. Merrer, M. Vekemans, A. Munnich, et al., Interstitial 9q22.3 microdeletion: clinical and molecular characterisation of a newly recognised overgrowth syndrome, Eur. J. Hum. Genet. 14 (2006) 759–767.
[21] K. Shimojima, M. Adachi, M. Tanaka, Y. Tanaka, K. Kurosawa, T. Yamamoto, Clinical features of microdeletion 9q22.3 (pat), Clin. Genet. 75 (2009) 384–393.
[22] E.A. Muller, S. Aradhya, J.F. Atkin, E.P. Carmany, A.M. Elliott, A.E. Chudley, et al., Microdeletion 9q22.3 syndrome includes metopic craniosynostosis, hydrocephalus, macrosomia, and developmental delay, Am. J. Med. Genet. A 158A (2012) 391–399.
[23] K.J. Gordon, G.C. Blobe, Role of transforming growth factor-β superfamily signaling pathways in human disease, Biochim. Biophys. Acta 1782 (2008) 197–228.
[24] B.L. Loeys, J. Chen, E.R. Neptune, D.P. Judge, M. Podowski, T. Holm, et al., A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFB1 or TGFBR2, Nat. Genet. 37 (2005) 275–281.
[25] B.L. Loeys, H.C. Dietz, Loeys–Dietz syndrome, in: R.A. Pagon, M.P. Adam, H.H. Ardinger, S.E. Wallace, A. Amemiya, L.J.H. Bean, et al. (Eds.), GeneReviews, University of Washington, Seattle, 1993–2016, [Internet], 2008 Feb 28, Updated 2013 July 11.
[26] M. Lindsay, H. Dietz, Lessons on the pathogenesis of aneurysm from heritable conditions, Nature 473 (2011) 308–316.
[27] H. Delbroek, J. Steyaert, E. Legius, An 8.9 year old girl with autism and Gorlin syndrome, Eur. J. Paediatr. Neurol. 15 (2011) 268–270.