**Abstract.** Background/Aim: Odontogenic diseases are diagnosed based on clinical course, imaging, and histopathology. However, a definitive diagnosis is not always possible. Patients and Methods: We analyzed whole exons of SMO, BRAF, PTCH1 and GNAS using next-generation sequencing (NGS) in 18 patients. Results: Of the 6 patients with ameloblastoma, 2 patients had the same missense mutation in BRAF, and 1 patient with peripheral ameloblastoma had a missense mutation in PTCH1. Of the 7 patients with odontogenic keratocyst, 4 patients had a missense mutation in PTCH1, 2 patients had missense mutations in BRAF, and 1 patient had a missense mutation in SMO. The patient with odontoma had missense mutations in SMO, BRAF and PTCH1. One patient with cementosseous dysplasia had missense mutations in SMO and PTCH1. The patient with adenomatoid odontogenic tumor had missense mutations in SMO. Conclusion: Whole exome sequencing of the above genes by NGS would be useful for the differential diagnosis of odontogenic diseases.

Odontogenic diseases (tumors, cysts and dysplasias) are diagnosed based on clinical course, several X-ray images, and histopathological findings, but a definitive diagnosis is not always possible (1). Odontogenic tumors derive from cells involved in tooth development, and in many cases, it is difficult to make a diagnosis due to varying histological findings. Alterations in some genes in odontogenic diseases have been identified (2, 3), but a few studies have described the analysis and application of this information for diagnosis (4).

Regarding the classification of odontogenic tumors, the “WHO histological typing of odontogenic tumors, jaw cysts, and allied lesions, Geneva: World Health Organization 1971” was published by the World Health Organization (WHO) in 1971 (5), and revised in 1992 (6), 2005 (7), and 2017 (2). In the 2005 version of WHO classification of tumors (7), pathology and genetics of tumors of the head and neck, odontogenic keratocyst (OKC) and calcifying odontogenic cyst (COC) were classified as a keratocystic odontogenic tumors (KCOT) and calcifying odontogenic cyst (CCOT), respectively. In the 2017 version WHO classification of head and neck tumors (2), these diseases were re-classified from tumors to cysts (OKC and COC). However, some patients with OKC have clinically clear neoplastic characteristics and sometimes show recurrence (8). On the other hand, even when a neoplastic disease like ameloblastoma is strongly suspected based on X-ray images and clinical course, some cases might be diagnosed as a cystic disease, because tumor cells cannot be identified in the histopathological analysis.

We believe that these problems in diagnosis may be solved by performing genetic analysis, in addition to X-ray images and histopathological findings. In most genetic analyses for odontogenic tumors, only a previously reported specific site (hot spot) in a gene is analyzed (9). There have been no reports of systematic examination of whole exons of multiple genes in odontogenic diseases. In this study, we analyzed whole exons which covered the open reading frame for smoothened (SMO), BRAF, patched 1 (PTCH1) and GNAS using an in-house next-generation sequencing (NGS) panel for odontogenic diseases. Then, we attempted to examine the usefulness of genetic analysis for the diagnosis for odontogenic diseases.
Table I. Clinical, histopathological, radiographic and mutational features of odontogenic diseases.

| Cases | Gender | Age | Histopathology    | Site             | Radiographic findings                                                                 | BRAF   | SMO  | PTCH1 |
|-------|--------|-----|-------------------|------------------|--------------------------------------------------------------------------------------|--------|------|-------|
| 1     | F      | 82  | Ameloblastoma     | Mandible         | A 30x20 mm large mass that grows outward from the inside of the left mandibular branch and extends to the outside of the maxilla and pterygium is observed. | T440P  | ND   | ND    |
| 2     | F      | 20  | Ameloblastoma     | Mandible         | A 36x23 mm large multilocular radiolucent lesion is observed surrounding the impacted lower right third molar. | T440P  | ND   | ND    |
| 3     | M      | 22  | Ameloblastoma     | Mandible         | A 81x35 mm large multilocular radiolucent lesion is observed from the lower left first premolar to the impacted lower right third molar. | ND     | ND   | ND    |
| 4     | M      | 17  | Ameloblastoma     | Mandible         | A 65x39 mm large monolocular radiolucent lesion is observed from the lower left first molar to the mandibular ramus surrounding the impacted lower left third molar. A knife edge resorption is noted at the apex of the lower left second molar. | ND     | ND   | ND    |
| 5     | F      | 89  | Ameloblastoma     | Mandible         | A 22x28 mm large multilocular mass lesion that grows from the inner surface of the right mandibular ramus to the outer surface of the right maxilla and medial pterygoid muscles is observed. A compression absorption image is noted in the right mandibular ramus and the outer surface of the right maxilla. | ND     | ND   | V582G |
| 6     | M      | 22  | Ameloblastoma     | Mandible         | A 41x28 mm large monolocular lesion is observed from the lower left second molar to the impacted lower left third molar. | ND     | ND   | ND    |
| 7     | M      | 66  | Odontogenic       | Mandible         | A 87x29 mm large well-defined multilocular radiolucent keratocyst lesion including an impacted third molar is observed from the lower right first premolar to the right lower jaw branch. A knife edge resorption is noted at the lower right second molar root. | T263P  | ND   | ND    |
| 8     | M      | 34  | Odontogenic       | Mandible         | A 74x29 mm large monolocular lesion including the lower right molar root is observed. | ND     | ND   | Y801S |
| 9     | M      | 67  | Odontogenic       | Mandible         | A 89x38 mm large multilocular radiolucent keratocyst lesion is observed from the lower right central incisor to the right lower second molar. | K51N   | ND   | V322G |
| 10    | M      | 22  | Odontogenic       | Mandible         | A 67x32 mm large multilocular radiolucent keratocyst lesion is observed from the lower right first premolar to the mandibular ramus including a right lower impacted third molar. | ND     | ND   | V588G |
| 11    | M      | 47  | Odontogenic       | Maxilla          | A 38x28 mm large monolocular radiolucent keratocyst lesion is observed from the upper right second premolar to the second molar region in the maxillary sinus. | ND     | ND   | G105A |
| 12    | F      | 20  | Odontogenic       | Maxilla          | A 54x42 mm large monolocular radiolucent keratocyst lesion is observed from the upper left second molar to the left second molar region in the maxillary sinus. The lesion contains an impacted upper left third molar. | ND     | N396T| ND    |
| 13    | M      | 76  | Odontogenic       | Maxilla          | A 47x41 mm large circular radiopaque lesion is observed from the upper left first premolar to the upper left second molars. The partial defect of the maxillary sinus wall is observed. | T263P  | Y394S| T456P |
| 14    | M      | 65  | Odontoma          | Maxilla          | A 77x28 mm large irregular shaped radiopaque lesions form the lower right second premolar to the second molar. | ND     | ND   | ND    |
| 15    | F      | 59  | Cemento-osseous   | Mandible         | A 37x17 mm large radiopaque lesion is observed at the root apex of the lower right first and second molar. A 30x20 mm large radiopaque lesion is observed at the root apex of the lower left first molar. A radiolucent area surrounding the lower left radiopaque lesion is noted. | ND     | ND   | ND    |
| 16    | F      | 74  | Cemento-osseous   | Mandible         | A 77x28 mm large irregular shaped radiopaque lesions form the lower right second premolar to the second molar. | ND     | Y394S| F715V |

Table I. Continued
Patients and Methods

Patients and samples. The subjects were 18 patients with odontogenic diseases who underwent diagnosis and treatment in the Department of Oral and Maxillofacial Surgery, Dokkyo Medical University Hospital from January 2014 to December 2018. The patients included 6 cases with ameloblastoma, 7 with OKC, 2 with cemento-osseous dysplasia (COD), 1 with adenomatoid odontogenic tumor (AOT), 1 with odontoma, and 1 with dentigerous cyst (Table I). This study was approved by the Ethics Committee of Dokkyo Medical University Hospital (No. R35-233).

DNA extraction. DNA was obtained from 14 frozen specimens collected from the central area of a tumor and 4 formalin-fixed paraffin-embedded (FFPE) sliced specimens. For the frozen specimens, hematoxylin and eosin staining was performed to confirm that the specimen included the tumor. DNA was extracted with Isogen (Qiagen, Hilden, Germany) from approximately 50 mg of frozen specimens (cases 1 and 6-18) or 10 slices of FFPE specimens cut at 10 μm thickness (cases 2-5) in 250 μl of lysis buffer. Phenol extraction and ethanol precipitation were performed. DNA samples were dissolved in Tris-EDTA buffer (pH 7.4) for quantitative determination. For our NGS analysis, at least 646 ng of DNA was required. Sufficient DNA was obtained from all 18 of the above specimens.

Next generation sequencing. NGS analysis was performed using an in-house panel. Sequences of whole exons which covered the open reading frame of SMO, BRAF, PTCH1, and GNAS were analyzed. First, 646 ng of genomic DNA were fractionated into pieces of several hundred base pairs, and the library was constructed using TruSeq Custom Amplicon Low Input (Illumina, San Diego, CA, USA) and individual custom oligonucleotides for BRAF, SMO, PTCH1, and GNAS. Using TruSeq Custom Amplicon Index Kit (Illumina), an identification sequence and two types of adapters were added. The concentration of the library was adjusted for each specimen, and sequencing was performed with a next-generation sequencer (MiSeq, Illumina) using a MiSeq Reagent Kit v2 (300 Cycles) (Illumina) after mixing of the library. Data were uploaded to Illumina Variant Studio 3.0 to extract mutations.

Results

Clinicopathological characteristics of the patients. The 18 patients with odontogenic disease who underwent NGS were diagnosed according to the 2017 WHO classification of head and neck tumors (2). Histopathological diagnosis was finally determined in consultation with an oral pathologist (Prof. Naozumi Ishimaru, Department of Oral Molecular Pathology, Tokushima University Graduate School of Biomedical Sciences) after provisional diagnosis by general pathologists at our hospital. Gender, age, histopathological diagnosis, site of onset, and X-ray findings are shown in Table I.

Patients 1 and 5 had peripheral ameloblastoma, and patient 1 showed follicular type-dominated histopathological findings (Figure 1A). Patients 2-6 had plexus type ameloblastoma. All ameloblastoma in the study developed in the mandible. Two patients (cases 2, 3) had multilocular cyst, 4 patients (cases 1, 4, 5, 6) had monolocular cyst, and 1 patient (case 4) showed a knife cutting image of the dental root.

Patients with OKC analyzed in this study were diagnosed as KCOT based on the 2005 WHO classification (cases 7-13, case 9: Figure 1B, case 12: Figure 1C). OKC developed in the maxilla of 3 patients (cases 11, 12, 13: Figure 2E, F, and G) and in the mandible of 4 patients (cases 7, 8, 9, 10: Figure 2A, B, C, and D). Three patients with OKC had multilocular cyst (cases 7, 9, 10: Figure 2A, C, and D), 4 patients had monolocular cyst (cases 8, 11, 12, 13: Figure 2B, E, F, and G), and 1 patient showed a knife cutting image of the dental root (case 7: Figure 2A).

The patient with odontoma (case 14) was an extraordinal case in which hard tissues occupying the maxilla and soft tissues around the hard tissues were mixed. The soft tissues were mainly inflammatory maxillary sinus mucosa with no clear odontogenic epithelium (Figure 1D). A patient with COD (case 15) had a typical X-ray image of a lesion in the bilateral posterior mandible, and a family history was also confirmed. The other patient with COD (case 16) also had lesions in the bilateral posterior mandible. The left lesion gave a typical X-ray image, but the right lesion had a cementum/bone-like radiopaque image surrounded by a soft tissue-like radiolucent image. Thus, for the right lesion, genetic analysis of the squamous epithelium-like soft tissues connecting to hard tissues was performed (Figure 1E).
In the patient with AOT (case 17), the maxillary canine was pushed to the upper part of the maxillary sinus, and the cyst occupied the entire maxillary sinus (Figure 1F). A patient with dentigerous cyst (case 18) was also analyzed as a negative control. During postoperative follow up of 11 months to 4 years, there was no recurrence in any patients.

*Figure 1. Histopathological findings of representative patients with odontogenic diseases. A) (case 1): Ameloblastoma follicular type, B) (case 9): Odontogenic keratocyst, C) (case 12): Odontogenic keratocyst, D) (case 14): Odontoma, E) (case 16) Cement-osseous dysplasia, F) (case 17): Adenomatoid odontogenic tumor.*

Mutations of *SMO, BRAF, PTCH1, and GNAS* in odontogenic diseases. Of the 6 patients histopathologically diagnosed with ameloblastoma in the mandible, 2 patients (case 1: Figure 1A, case 2) had a missense mutation (T440P) in *BRAF* (Table I). T440P is an activating mutation that has been reported in patients with lung cancer (10). The patient (case 5) with peripheral ameloblastoma in the mandible which showed the invasion in the masticatory space had a missense mutation (V582G) in *PTCH1*. The remaining 3 patients (cases 3, 4, 6) had no mutation in the four analyzed genes (Table I).

Of the 7 patients histopathologically diagnosed with OKC (Figure 2A-G), 4 patients (case 8: Figure 2B, case 9: Figure 1B, 2C, case 10: Figure 2D, case 11: Figure 2E) had a missense mutation in *PTCH1* (Table I). Among the 7 patients, two patients each with a large lesion in the right posterior mandible (case 7: Figure 2A, case 9: Figure 2C) had missense mutations in *BRAF* (T263P in case 7, and K51N and Y647D in case 9). The patient with a large lesion in the left posterior maxillary, which pushed the wisdom tooth to the upper area and occupied the maxillary sinus (case 12: Figures 1C and 2F) had a missense mutation in *SMO* (N396T). Only 1 patient histopathologically diagnosed
with OKC (case 13: Figure 2G) did not carry a mutation in the examined genes. The patient histopathologically diagnosed with odontoma (case 14: Figure 1D) had missense mutations in SMO (Y394S), BRAF (T263P), and PTCH1 (T456P and D461N) (Table I). No mutations of the examined genes were found in a patient with COD (case 15), which was thought to be a familial case. However, missense mutations in SMO (Y394S) and PTCH1 (F725V) were found in patient 16 (Figure 1E) with COD, in which soft tissue with squamous cell like cells was found around a hard tissue lesion. The patient with AOT in the maxilla (case 17: Figure 1F) had missense mutations of SMO (Y394S and Y399S). The patient with a dentigerous cyst (case 18) had no mutations as we expected. Among all 18 subjects in the study, 7 had a V860G mutation in GNAS, which is considered to be a missense variant of single nucleotide polymorphism.

Figure 2. Panoramic X-ray images of patients with odontogenic keratocyst. A: case 7, B: case 8, C: case 9, D: case 10, E: case 11, F: case 12, G: case 13. Arrow heads show the area of the disease. Odontogenic keratocyst developed in the mandible of 4 patients (cases 7, 8, 9, 10), and in the maxilla of 3 patients (cases 11, 12, 13). Three patients (cases 7, 9, 10) had multilocular cyst, 4 patients (cases 8, 11, 12, 13) had monolocular cyst. One patient (case 7) showed a knife cutting image of the dental root.
Discussion

For NGS analysis of SMO, BRAF, PTCH1 and GNAS in odontogenic disease, DNA was initially extracted from 33 samples (16 frozen specimens, 17 FFPE specimens). However, we were able to extract the required amount of >646 ng of DNA for NGS from only 18 samples (14 frozen specimens, 4 FFPE specimens). Thus, we successfully extracted the sufficient amounts of DNA for NGS analysis from 14 of 16 frozen specimens (87.5%), but from only 4 of the 17 FFPE specimens (23.5%). However, we were not able to extract the sufficient amounts of DNA for NGS analysis from any of decalcification FFPE specimens.

Regarding the functions of the protein products of the genes analyzed in the study, SMO encodes a 7-transmembrane domain protein, which activates the Gi family of transcription factors. The function of SMO is suppressed by PTCH1, a membrane receptor of sonic hedgehog (SHH), and the suppression of SMO is released when SHH binds to PTCH1, which results in SMO activation and increased cell proliferation (11). An activating mutation in SMO has been reported to occur at a high rate in ameloblastoma developed in the maxilla (8). PTCH1 encodes for a 12-transmembrane domain protein (11) and is the gene responsible for the development of basal cell nevus syndrome (12). A PTCH1 mutation has been found in sporadic OKC (KCOT in the 2005 WHO classification) (13). However, PTCH1 is a large gene (24 exons with an open reading frame of 4,341 bp) with no hot spots of mutations, and studies on the sequences of whole exons have rarely been performed (14). BRAF directly binds to RAS protein, activates the MEK-ERK pathway, and is involved in cell proliferation and survival (15). Activating mutations in BRAF, such as V600E, are observed in various cancers (15, 16) and at a high rate in ameloblastoma in the mandible (9). GNAS encodes for a Gs protein, and GNAS mutation is frequently found in fibrous dysplasia. This gene is also known as a gene responsible for McCune-Albright syndrome (17).

In this study, mutations in SMO, BRAF, and PTCH1 were detected in several patients with odontogenic disease. The read depth was set at 200 (SMO and PTCH1) or 500 (BRAF) to extract mutations, which gives results with high specificity. Some patients showed suspected frameshift mutations, but mainly missense mutations were found in the study. The V600E mutation in BRAF, which has been reported to be a strong activating mutation (15, 16), was not found in our patients, but mutation sites located close to the activation domain were found, and it is likely that these caused functional changes. The T440P mutation in BRAF has been found in patients with lung cancer (10) and is considered to be an activating mutation. Among the SMO mutations, V394S was found in 3 patients and has not been recorded in SNP databases. Thus, if it is an activating mutation, it may be a new hot spot. Similarly, Y399S in SMO has not been reported as a SNP, but N396T may be a SNP. There was no mutation hot spot in PTCH1 and mutations were observed over a wide sequence. The Y1316S mutation in PTCH1 in case 9 has been found in basal cell nevus syndrome (18).

Based on the genetic mutations found by NGS, the pathological diagnosis requires a re-evaluation. Of the diseases which could not be histopathologically diagnosed as ameloblastoma (such as those diagnosed as OKC), those with a mutation in SMO in the maxilla (cases 12, 14, 17) and those with a mutation in BRAF in the mandible (cases 7, 9) may have characteristics of ameloblastoma or a related tumor. It was surprising that a mutation in PTCH1 was found in 4 of the 7 patients diagnosed with OKC (KCOT in the 2005 WHO classification). Furthermore, a patient with a lesion in the mandible (case 7: Figure 2A) had a BRAF mutation, and another patient with a lesion in the maxilla (case 12: Figures 1C and 2F) had a SMO mutation. In these cases, differential diagnosis from ameloblastoma might be required; no neoplastic ameloblastic cells were identified, and thus, it could not be histopathologically diagnosed as ameloblastoma. In addition, although a patient (case 10) was strongly suspected to have ameloblastoma based on X-ray images (Figure 2D) and clinical findings, there were no histopathological findings for ameloblastoma. Then, we diagnosed the disease in this patient as OKC. Since a PTCH1 mutation was found in this patient, the diagnosis of OKC might be appropriate.

It was also difficult to make a histopathological diagnosis in patient 14 (Figure 1D), and the disease was clinically diagnosed as odontoma because of the dentine-like hard tissues, which were similar to odontoma. Since mutations in BRAF, SMO, and PTCH1 were all found in soft tissues around the hard tissues in this case, a tumor related to ameloblastoma may be considered. However, since odontogenic epithelium could not be found in the tissue, a mutation in the odontogenic mesenchymal tissue might be present. Patient 16 was diagnosed with cemento-osseous dysplasia, and mutations in SMO and PTCH1 were found in soft tissues (Figure 1E), including squamous epithelium-like cells around hard tissues, suggesting the possibility of odontogenic tumor. Patient 17 (Figure 1F) showed typical images of AOT in the maxillary sinus, but a SMO mutation was also found. AOT has been shown to have mutations in RAS and beta-catenin (19), but there have been no reports of a mutation in SMO in this tumor.

In this study, NGS analysis of whole exons was shown to be useful for differential diagnosis of odontogenic diseases, although the specificity of mutations extracted from NGS should be confirmed. A re-consideration of KCOT as a potential neoplastic disease is required in diseases that are currently diagnosed as OKC. Integration of histopathological
and genetic data is needed for more definitive diagnosis of odontogenic diseases.

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**Conflicts of Interest**

The Authors have no conflicts of interest to declare.

**Authors’ Contributions**

Collection of the clinical information: Michiko Shimura, Yuta Sawatani, Tomonori Hasegawa, Ryota Kaminura, Sayaka Izumi, Chonji Fukumoto, Shuma Yagisawa, Erika Yaguchi; Next generation sequencing: Michiko Shimura, Koh-ichi Nakashiro, Daisuke Uchida; Collection of X-ray and histopathological information: Masayo Hitomi-Koide, Toshiki Hyodo; Data analysis: Michiko Shimura, Yuske Komiyama, Hitoshi Kawamata; A draft of the manuscript: Michiko Shimura, Yuske Komiyama, Hitoshi Kawamata.

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**References**

1. Robinson RA and Vincent SD: Epithelial odontogenic neoplasms. *In: Tumors and cysts of the jaws* AFIP atlas of tumor pathology. Fourth series. Silver spring. American Registry of Pathology, pp. 49-81, 2012.

2. ElNaggar AK, Chan JKC, Grandis JR, Takata T and Slootweg Chonji Fukumoto, Shuma Yagisawa, Erika Yaguchi; Next generation sequencing: Michiko Shimura, Koh-ichi Nakashiro, Daisuke Uchida; Collection of X-ray and histopathological information: Masayo Hitomi-Koide, Toshiki Hyodo; Data analysis: Michiko Shimura, Koh-ichi Nakashiro, Daisuke Uchida, Hitoshi Kawamata; A draft of the manuscript: Michiko Shimura, Yuske Komiyama, Hitoshi Kawamata.

3. Perdigão PA, Carvalho VM, DE Marco L and Gomez RS: Mutation of ameloblastin gene in calcifying epithelial odontogenic tumor. *Anticancer Res* 29(8): 3065-3067, 2009. PMID: 19661317.

4. Bilodeau EA, Weinreb I, Antonescu CR, Zhang L, Dacic S, Muller S, Barker B and Seethala RR: Clear cell odontogenic carcinomas show EWSR1 rearrangements: a novel finding and a biological link to salivary clear cell carcinomas. *Am J Surg Pathol* 37(7): 1001-1005, 2013. PMID: 23715163. DOI: 10.1097/PAS.0b013e31828a6727

5. Pindborg JJ, Kramer IRH and Torloni H: WHO International histological classification of tumours: Histological typing of odontogenic tumours, jaw cysts and allied lesions. Geneva: World Health Organization, pp. 18-19, 1971.

6. Kramer IRH, Pindborg JJ and Shear M: WHO Histological typing of odontogenic tumours. Histological typing of odontogenic tumours. 2nd ed. Berlin: Springer-Verlag, pp. 7-9, 1992.

7. Barnes L, Eveson JW, Reichart P and Sidansky D: WHO histological classification of odontogenic tumors. *In: World Health Organization classification of tumours: pathology and genetics of head and neck tumours*. 3rd ed. Lyon: IARC Press, pp. 283-327, 2005.

8. Wright JM, Odell EW, Speight PM and Takata T: Odontogenic tumors, WHO 2005: where do we go from here? Head Neck Pathol 8(4): 373-382, 2014. PMID: 25409849. DOI: 10.1007/s12105-014-0585-x

9. Sweeney RT, McClary AC, Myers BR, Biscocho J, Neahring L, Kwei KA, Qu K, Gong X, Ng T, Jones CD, Varma S, Odegaard JI, Sugiyama T, Koyota S, Rubin BP, Troxell ML, Pelham RJ, Zehnder JL, Beachy PA, Pollack JR and West RB: Identification of recurrent SMO and BRAF mutations in ameloblastomas. *Nat Genet* 46(7): 722-725, 2014. PMID: 24593540. DOI: 10.1038/ng.2986

10. Brose MS, Volpe P, Feldman M, Kumar M, Rishi I, Gerrer R, Einhorn E, Herlyn M, Mimna J, Nicholson A, Roth JA, Albelda SM, Davies H, Cox C, Brignell G, Stephens P, Futreal PA, Wooster R, Stratton MR and Weber BL: BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res* 62(23): 6997-7000, 2002. PMID: 12460918.

11. Skoda AM, Simovic D, Karin V, Kardum V, Vranic S and Serman L: The role of the Hedgehog signaling pathway in cancer: A comprehensive review. *Bosn J Basic Med Sci* 18(1): 8-20, 2018. PMID: 29274272. DOI: 10.17305/bjbms.2018.2756

12. Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG, Myers RM, Cox DR, Epstein EH Jr and Scott MP: Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* 272(5268): 1668-1671, 1996. PMID: 8658145. DOI: 10.1126/science.272.5268.1668

13. Guo YY, Zhang JY, Li XF, Luo HY, Chen F and Li TJ: PTCH1 gene mutations in Keratocystic odontogenic tumors: a study of 43 Chinese patients and a systematic review. *PLoS One* 8(10): e77305, 2013. PMID: 24204797. DOI: 10.1371/journal.pone.0077305

14. Qu J, Yu F, Hong Y, Guo Y, Sun L, Li X, Zhang J, Zhang H, Shi R, Chen F and Li T: Underestimated PTCH1 mutation rate in sporadic keratocystic odontogenic tumors. *Oral Oncol* 51(1): 40-45, 2015. PMID: 25458233. DOI: 10.1016/j.oraloncology.2014.09.016

15. Dankner M, Rose AAN, Rajkumar S, Siegel P and Watson IR: Classifying BRAF alterations in cancer: new rational therapeutic strategies for actionable mutations. *Oncogene* 37(24): 3183-3199, 2018. PMID: 29540830. DOI: 10.1038/s41388-018-0171-x

16. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woolfenden H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Haves R, Hughes J, Kosmidou V, Menzies A, Moule C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C.
Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cosso A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR and Futreal PA: Mutations of the BRAF gene in human cancer. Nature 417(6892): 949-954, 2002. PMID: 12068308. DOI: 10.1038/nature00766

17 Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E and Spiegel AM: Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. N Engl J Med 325(24): 1688-1695, 1991. PMID: 1944469. DOI: 10.1056/NEJM199112123252403

18 Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF and den Dunnen JT: LOVD v.2.0: the next generation in gene variant databases. Hum Mutat 32(5): 557-563, 2011. DOI: 10.1002/humu.21438

19 Harnet JC, Pedeutour F, Raybaud H, Ambrosetti D, Fabas T and Lombardi T: Immunohistological features in adenomatoid odontogenic tumor: review of the literature and first expression and mutational analysis of b-catenin in this unusual lesion of the jaws. Am Assoc Oral Maxillofac Surg J Oral Maxillofac Surg 71: 706-713, 2013. PMID: 23265580. DOI: 10.1016/j.joms.2012.10.006

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