FIG. 2. Preoperative rotational panoramic dental radiography showing overgrowth of the right mandibular body, angle, ramus, and caudally deviated right mandibular condyle with flattened head with normal dentition.

fused. There was no apparent fusion between the bones of the skull and the cervical vertebrae.

Three-phase whole-body Technetium-99m hydroxymethylene diphosphonate (99mTc-HMDP) bone scintigraphy showed increased accumulation in bones with apparent hyperostosis, including the right side of the skull, the mandible, and the upper cervical vertebrae (Fig. 4). Accumulation in the other bones was symmetric and within normal limits.

Laboratory examination was unremarkable. Thyroid stimulating hormone, free T3, free T4, luteinizing hormone, follicular stimulating hormone, prolactin, adrenocorticotropic hormone, growth hormone, somatomedin C, and alkaline phosphatase were within normal limits.

The patient thus underwent hyperostosis reduction of the frontal and frontoparietal bones, and of the right side of the mandibular body and angle. The hyperostotic areas were easily exposed through a coronal incision and a lower gingivobuccal incision. The hyperostotic region appeared normal in color and texture. The bulging bone tissue was resected without significant bleeding or dural injury. The patient followed an uneventful postoperative course. Unfortunately, CT performed 2 years postoperatively showed recurrence of the bony bulges. A microscopic examination of the excised bone tissue, stained with hematoxylin–eosin, demonstrated bony overgrowth consisting of mature lamellar bone and normal periosteum. No malignant changes were observed.

AKT-1 Mutation Analysis

Under the approval of the ethical committee of Chiba University, DNA sequencing and fragment assay of the oncogene *AKT1* were performed. Genomic DNA was extracted from the formalin-fixed paraffin-embedded (FFPE) section of bulging frontal bone and mandibular angular bone using the NucleoSpin® FFPE DNA kit (Takara Bio, Inc., Shiga, Japan).

Standard Polymerase Chain Reaction (PCR) and Sanger sequencing

The extracted DNA was amplified by PCR using MightyAmp® for FFPE (Takara Bio, Inc.). PCR was performed by annealing at 98°C for 2 min, then performing 40 cycles at 98°C for 10 sec to 68°C for 30 sec. The outer primers used were as follows: forward, 5′-GGCA-CATCTGTCCTGGCACAC-3′; reverse, 5′-AGCCTCACGTTGGT CCACATC-3′. The PCR product was gel purified with Sephadex (GE Healthcare, Little Chalfont, UK). Semiautomated sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit and the ABI3730xI sequencer (Applied Biosystems, Foster City, CA), as described in the manufacturer's protocols. Both the outer and the inner primers were used during sequencing. The inner primers used were as follows: forward, 5′-CCTGGCTGCCTGGCG AGGGTC-3′; reverse, 5′-GTCCACATCCTGCGGCCGCTC-3′. Standard PCR and Sanger sequencing of the DNA from the
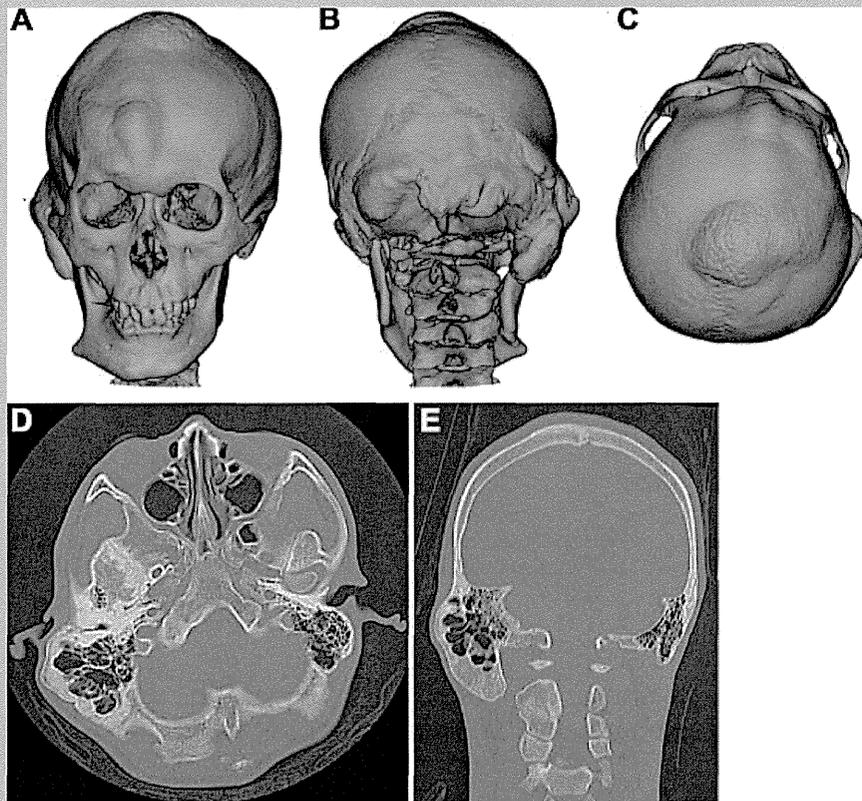
FIG. 3. Preoperative volume-rendered computed tomography (CT) shows hyperostoses of the right paramedian area of the frontal bone, from the mid- to right-side of the frontoparietal bones along the coronal suture, the bilateral nuchal line and the posterior tubercle of the occipital bone, the right lambdoid suture and occipitotemporal suture area, the right side of the mandile, the right side of the cervical spine (A–C). Axial computed CT without contrast showing enlarged mastoid cells with preserved pneumatization on the right, and stenosis of the right external auditory canal caused by hyperostoses (D). The coronal view shows hyperostoses of the right side of the cervical vertebrae from C2 to C5 and fusion of the right side of the cervical vertebrae between C2 and C3 are evident (E).

FFPE frontal bone and the mandibular angular bone showed no mutation in the oncogene *AKT1*.

Modified PCR and Restriction Enzyme Assay for the c.49G>A Mutation of the AKT1

PCR restriction enzyme assay for detecting the c.49G>A mutation of *AKT1* was applied to the extracted genomic DNA [Lindhurst et al., 2011; Wieland et al., 2013]. The extracted DNA was amplified by PCR using MightyAmp® for FFPE (Takara Bio, Inc.). PCR was performed by annealing at 98°C for 2 min, followed by 40 cycles of 98°C for 10 sec to 68°C for 60 sec. The primers used were as follows: FAM labeled forward, 5′-FAM-CAGGCATCCCAGGCACATCTG TCC-3′; unlabeled reverse, 5′-AGTAGCGTGGCCGCCAGGTCTT GATGTTCT-3′. Unfortunately, we were unable to obtain the desired PCR product with this method, and the PCR restriction enzyme assay was thus aborted.

DISCUSSION

Hyperostosis is characterized by a proliferation of histologically normal bone tissue. Cranial hyperostosis can be associated with a wide variety of syndromes and diseases [Kannu et al., 2011]. Proteus syndrome was first described as a clinical entity by Temtamy and Rogers in 1976 and was named by Wiedemann et al. several years later [Temtamy and Rogers, 1976; Wiedemann et al., 1983]. Wiedemann et al. [1983] chose to name the syndrome Proteus after the sea god of Greek mythology who is able to assume many forms [Opitz and Jorde, 2011]. Proteus syndrome is characterized by segmental or patchy, aggressive, disproportionate postnatal overgrowth of multiple tissues and organs [Biesecker, 2006]. Proteus syndrome is extremely rare; less than 100 cases of true Proteus syndrome have been reported [Turner et al., 2004]. Cohen [2005] warned against misdiagnosis, stating that many patients reported to have Proteus syndrome did not have the true syndrome according to the consensus diagnostic criteria. Turner et al. [2004] reported that only 47% of patients (97 of 205) as having Proteus syndrome in the literature met the consensus diagnostic criteria. Our patient meets the general diagnostic criteria of Proteus syndrome, given the mosaic distribution of lesions, sporadic occurrence, progressive course, and he meets one of the specific diagnostic criteria categories with asymmetric, disproportionate overgrowth by hyperostosis of the skull and the external auditory canal (specific diagnostic

FIG. 4. Preoperative whole-body Technetium-99m hydroxymethylene diphosphonate bone scintigraphy showing increased tracer accumulation at the same sites as the hyperostoses of the head and neck. There is otherwise no abnormal uptake.

type 1, Maffucci syndrome, isolated hemimegalencephaly, hypomelanosis of Ito, hemihyperplasia-multiple lipomatosis syndrome, encephalocraniocutaneous lipomatosis, Gardner variant of familial adenomatous polyposis, tuberous sclerosis, Cowden syndrome, or Bannayan–Riley–Ruvalcaba syndrome [Cohen, 2005; Kannu et al., 2011].

An association between Proteus syndrome and a somatic activating mutation in the oncogene *AKT1* has been reported [Lindhurst et al., 2011]. The mutation activates the PI3K-AKT signaling pathway that regulates cell proliferation and apoptosis. The asymmetrical hyperostoses seen in three different areas in our patient (in the calvarium, mandible, and cervical spine) suggested the presence of somatic mutation mosaicism. However, standard PCR and Sanger sequencing of DNA extracted from FFPE frontal bone and mandibular angular bone showed normal *AKT1* sequence. Modified PCR and restriction enzyme assay was aborted because we could not obtain the desired PCR products. Detecting a mutation in patients with suspected Proteus syndrome can be challenging because blood samples lack the mutation. Furthermore, a number of patients with Proteus syndrome had mutations detected in some samples but not others, and in those sites that are positive for the mutation, only a low percentage of mutant *AKT1* alleles can be found [Lindhurst et al., 2011; Wieland et al., 2013]. Lindhurst et al. [2011] and Wieland et al. [2013] used fresh or frozen tissue or cultured cells for *AKT1* mutation evaluation. Though the cause of our patient's disorder remains unclear, it is possible that his disease is a member of a large family of disorders caused by dysfunction of the PI3K-AKT pathway. Intrapatient exome sequencing has the potential to reveal new information about genic mutations in our patient [Opitz and Jorde, 2011].

We suspect that the patient's disease was caused by low level, late occurring mesodermal somatic mutation mosaicism. Among the wide variety of overgrowth syndromes, our patient's syndrome bears a similarity to Proteus syndrome, yet his *AKT1* gene sequence was normal. The sporadic occurrence and localized overgrowth in three different areas (calvaria, mandible, and cervical spine) strongly supports the idea of somatic mutation mosaicism. Somatic mutation can occur in all stages of embryogenesis. Mutations occurring in the very early stages of embryogenesis cause generalized severe, early-onset phenotypes. In contrast, mutations occurring in the late stages of differentiation cause localized, mild, late-onset phenotypes. It is difficult to distinguish somatic mutations that occur in very early stages of embryogenesis from germline mutations by phenotype alone. Recent studies revealed that even patients with clinically generalized neurofibromatosis (NF) 1 can have genetic mosaicism of the *NF1* gene [Ruggieri and Huson, 2001; Messiaen et al., 2011]. A relatively small number of mutated mosaic cells can cause obvious manifestations [Lindhurst et al., 2011; Poduri et al., 2013; Wieland et al., 2013]. Certainly, there is a possibility that our patient's disease was caused by a sporadic germline mutation of limited expressivity. Patients with a very mild subtype of germline NF2 develop symptoms only after the third decade of life resulting in few tumors other than vestibular schwanomas [Parry et al., 1996]. Phenotypic differences in patients with germline mutations can be caused by various factors, such as the type of mutation, gene modifications, gene-gene interactions, or gene-environmental interactions.

criterion B-2) [Biesecker, 2006]. However, he does not fulfill the necessary criteria for diagnosing Proteus syndrome because two from specific diagnostic category B is required whereas he meets only one from specific diagnostic category B [Biesecker, 2006].

A wide variety of rare overgrowth syndromes other than Proteus syndrome exist and phenotypes often overlap [Turner et al., 2004]. Biesecker et al. [2001], however, reported that the term "Proteus-like syndrome" can be confusing because the patient group described by this term is heterogeneous, hence the term "Proteus" is not always useful in clinical or scientific settings [Biesecker et al., 2001]. There is a risk that overdiagnosis of Proteus syndrome may result in missed diagnoses of other rare, poorly described overgrowth syndromes. Our patient did not show evidence of any other disease typically considered in the differential diagnosis of Proteus syndrome, including Klippel–Trénaunay–Weber syndrome, neurofibromatosis

TABLE I. Cases Reports of Craniofacial Hyperostosis Without Other Generalized Overgrowth

Publication	n	Patient sex	Pattern of cranial hyperostosis	Mandibular or cervical spinal involvement	Dysregulated adipose tissue	Associated features
Pagon et al. [1986]	3	Male	Symmetric	No	No	X-linked inheritance pattern; mental retardation in one patient; vacuolated histiocytes on bone histology
Smeets et al. [1994]	1	Male	Asymmetric	Mandible	No	Reported as regional Proteus syndrome
Nishimura and Nishimura [1997]	1	Female	Symmetric	No	Cardiac	Mature bone with irregular osseous tissue and sparse fibrous tissue on bone histology
Kannu et al. [2011]	1	Male	Symmetric	Cervical spine	Cardiac	Facial asymmetry
Adolphs et al. [2011]	1	Male	Asymmetric	Mandible	No	Father of two healthy children, 5 and 7 years old
Present report	1	Male	Asymmetric	Both	No	Normal AKT1 gene sequence

Phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome encompasses a group of disorders caused by a *PTEN* gene mutation and includes Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome [Marsh et al., 1999]. We did not investigate this possibility in our patient because his clinical features were quite different from those seen in disorders caused by the *PTEN* gene mutation. Cowden syndrome is a rare, autosomal dominant disorder characterized by hamartomas and the risk of carcinoma in multiple organs. Trichilemmomas are the hallmark hamartoma, occurring in 99% of patients with Cowden syndrome. Bannayan–Riley–Ruvalcaba syndrome is a rare autosomal dominant disease, with patients demonstrating the triad of macrocephaly, lipomatosis, and pigmented macules of the glans penis. Our patient's clinical features differ from those of patients with Proteus-like syndrome and *PTEN* gene mutations [Zhou et al., 2001]. Zhou et al. [2001] described three such patients, using the following diagnostic criteria for Proteus-like syndrome: the presence of lipomas, any single hamartoma, and overgrowth; but not meeting the diagnostic criteria of Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome, or Proteus syndrome.

We performed whole-body 99mTc bone scintigraphy in our patient to find hyperostoses that might not be apparent on physical or radiological examination. We, however, noted increased accumulation only in areas with apparent hyperostoses. Adolphs et al. [2011] reported similar increased accumulation in hyperostosis sites in a patient with cranial and mandibular hyperostoses who did not fit the diagnostic criteria of Proteus syndrome, despite certain similarities [Adolphs et al., 2011]. However, Khangembam et al. [2013] reported no abnormal tracer uptake in the underlying bones of asymmetric, disproportionate overgrowth sites in a patient with Proteus syndrome.

Only seven patients with cranial hyperostosis in the absence of other abnormalities have been reported in the literature (Table I). Pagon et al. [1986] reported three such patients who were related

and demonstrated an X-linked inheritance pattern. The other five patients, including ours, represent sporadic occurrences and unknown inheritance patterns. Our patient showed multiple asymmetrical hyperostoses, whereas symmetrical hyperostoses along the frontozygomtic, frontosphenoidal, and frontoparietal sutures are the most pronounced features in some patients reported [Pagon et al., 1986; Nishimura and Nishimura, 1997; Kannu et al., 2011]. Contouring craniectomy was performed in all patients, except for two patients Pagon's report [Pagon et al., 1986]; recurrence was noted in all patients who underwent surgery.

In summary, we report the unusual presentation of a patient with late-onset asymmetrical craniofacial and cervical spinal hyperostoses. Although the patient's clinical manifestations are partially shared with Proteus syndrome, he does not fulfill the consensus diagnostic criteria for Proteus. Mutation of the oncogene *AKT1* could not be detected by standard PCR and Sanger sequencing of DNA extracted from FFPE frontal bone and mandibular angular bone. Our patient's phenotype suggests the presence of a low level, late occurring mesodermal somatic mutation in mosaic form. We hope that sharing this report helps to shed light on the nature of a rare, heretofore unclassified overgrowth syndrome.

REFERENCES

Adolphs N, Menneking H, Hoffmeister B, Tinschert S. 2011. Asymmetric, disproportionate craniofacial hyperostoses of mosaic pattern in a patient not meeting Proteus syndrome criteria. J Craniofac Surg 22: 2135–2140.

Biesecker L. 2006. The challenges of Proteus syndrome: Diagnosis and management. Eur J Hum Genet 14:1151–1157.

Biesecker LG, Rosenberg MJ, Vacha S, Turner JT, Cohen MM. 2001. PTEN mutations and Proteus syndrome. Lancet 358:2079–2080.

Cohen MM Jr. 2005. Proteus syndrome: An update. Am J Med Genet Part C 137C:38–52.

Kannu P, Perry D, Rees M, O'Donnell C, Aftimos S. 2011. Another case of multiple juxtasutural hyperostoses, cervical exostoses, and fatty infiltration of myocardium. Am J Med Genet Part A 155A:589–594.

Khangembam BC, Karunanithi S, Sharma P, Agarwal KK, Singhal A, Dhull VS, Bal C, Kumar R. 2013. Proteus syndrome: A case report with bone scintigraphy findings. Diagn Interv Radiol 19:240–243.

Lindhurst MJ, Sapp JC, Teer JK, Johnston JJ, Finn EM, Peters K, Turner J, Cannons JL, Bick D, Blakemore L, Blumhorst C, Brockmann K, Calder P, Cherman N, Deardorff MA, Everman DB, Golas G, Greenstein RM, Kato BM, Keppler-Noreuil KM, Kuznetsov SA, Miyamoto RT, Newman K, Ng D, O'Brien K, Rothenberg S, Schwartzentruber DJ, Singhal V, Tirabosco R, Upton J, Wientroub S, Zackai EH, Hoag K, Whitewood-Neal T, Robey PG, Schwartzberg PL, Darling TN, Tosi LL, Mullikin JC, Biesecker LG. 2011. A mosaic activating mutation in AKT1 associated with the Proteus syndrome. N Engl J Med 365:611–619.

Marsh DJ, Kum JB, Lunetta KL, Bennett MJ, Gorlin RJ, Ahmed SF, Bodurtha J, Crowe C, Curtis MA, Dasouki M, Dunn T, Feit H, Geraghty MT, Graham JM, Hodgson SV, Hunter A, Korf BR, Manchester D, Miesfeldt S, Murday VA, Nathanson KL, Parisi M, Pober B, Romano C, Tolmie JL, Trembath R, Winter RM, Zackai EH, Zori RT, Weng L-P, Dahia PLM, Eng C. 1999. PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. Hum Mol Genet 8:1461–1472.

Messiaen L, Vogt J, Bengesser K, Fu C, Mikhail F, Serra E, Garcia-Linares C, Cooper DN, Lazaro C, Kehrer-Sawatzki H. 2011. Mosaic type-1 NF1 microdeletions as a cause of both generalized and segmental neurofibromatosis type-1 (NF1). Hum Mutat 32:213–219.

Nishimura G, Nishimura J. 1997. Multiple, juxtasutural, cranial hyperostoses and cardiac tumor: A new hamartomatous syndrome? Am J Med Genet 71:167–171.

Opitz JM, Jorde LB. 2011. Hamartoma syndromes, exome sequencing, and a protean puzzle. N Engl J Med 365:661–663.

Pagon RA, Beckwith JB, Ward BH. 1986. Calvarial hyperostosis: A benign X-linked recessive disorder. Clin Genet 29:73–78.

Parry DM, MacCollin MM, Kaiser-Kupfer MI, Pulaski K, Nicholson HS, Bolesta M, Eldridge R, Gusella JF. 1996. Germ-line mutations in the neurofibromatosis 2 gene: Correlations with disease severity and retinal abnormalities. Am J Hum Genet 59:529–539.

Poduri A, Evrony GD, Cai X, Walsh CA. 2013. Somatic mutation, genomic variation, and neurological disease. Science 341:43–51.

Ruggieri M, Huson SM. 2001. The clinical and diagnostic implications of mosaicism in the neurofibromatoses. Neurology 56:1433–1443.

Smeets E, Fryns JP, Cohen MM Jr. 1994. Regional Proteus syndrome and somatic mosaicism. Am J Med Genet 51:29–31.

Temtamy SA, Rogers JG. 1976. Macrodactyly, hemihypertrophy, and connective tissue nevi: Report of a new syndrome and review of the literature. J Pediatr 89:924–927.

Turner JT, Cohen MM Jr, Biesecker LG. 2004. Reassessment of the Proteus syndrome literature: Application of diagnostic criteria to published cases. Am J Med Genet Part A 130A:111–122.

Wiedemann HR, Burgio GR, Aldenhoff P, Kunze J, Kaufmann HJ, Schirg E. 1983. The Proteus syndrome. Partial gigantism of the hands and/or feet, nevi, hemihypertrophy, subcutaneous tumors, macrocephaly or other skull anomalies and possible accelerated growth and visceral affections. Eur J Pediatr 140:5–12.

Wieland I, Tinschert S, Zenker M. 2013. High-level somatic mosaicism of AKT1 c.49G>A mutation in skin scrapings from epidermal nevi enables non-invasive molecular diagnosis in patients with Proteus syndrome. Am J Med Genet Part A 161A:889–891.

Zhou X, Hampel H, Thiele H, Gorlin RJ, Hennekam RC, Parisi M, Winter RM, Eng C. 2001. Association of germline mutation in the PTEN tumour suppressor gene and Proteus and Proteus-like syndromes. Lancet 358:210–211.