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Nationwide survey of Baller-Gerold syndrome in Japanese population

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Abstract. Baller-Gerold syndrome (BGS) is a rare autosomal genetic disorder characterized by radial aplasia/hypoplasia and craniosynostosis. The causative gene for BGS encodes RECQL4, which belongs to the RecQ helicase family. To understand BGS patients in Japan, a nationwide survey was conducted, which identified 2 families and 3 patients affected by the syndrome. All the three patients showed radial defects and craniosynostosis. In one patient who showed a dislocated joint of the hip and flexion contracture of both the elbow joints and wrists at birth, a homozygous large deletion in the *RECQL4* gene was identified. This is the first reported case of BGS in Japan caused by *RECQL4* gene mutation.

Introduction

There are five human RecQ-like proteins (RECQL1, BLM, WRN, RECQL4 and RECQ5), each having 3' to 5' DNA helicase activity but little sequence similarity outside the helicase motifs (1). Three of these helicases encode causative genes for Bloom syndrome (BLM), Werner syndrome (WRN) and Rothmund-Thomson syndrome (RECQL4), respectively. These three syndromes show genomic instability and cancer susceptibility, but each also has distinctive features (2). RECQL4 is the causative gene for Rothmund-Thomson syndrome (OMIM 266280) characterized by poikiloderma and skeletal defects. Homozyogous mutations or compound heterozygous mutations of the *RECQL4* gene causes Rothmund-Thomson syndrome (3,4).

However, mutations in the *RECQL4* gene have been associated with two other recessive disorders: One is RAPADILINO syndrome (OMIM 266280) which is characterized by radial hypoplasia, patella hypoplasia and arched plate, diarrhoea and dislocated joints, little size and limb malformation, slender nose and normal intelligence (4). The other is Baller-Gerold syndrome (BGS) (OMIM 218600) characterized by radial aplasia/hypoplasia and craniosynostosis (5). Three syndromes have overlapping features, such as short stature and radial ray abnormalities (6).

In Japan there have been no reports on BGS. We have performed a nationwide questionnaire based study of BGS. Three BGS patients were identified: one was the sporadic case with BGS, and the remaining two were brothers. In this patient, we found for the first time a homozygous large deletion in the *RECQL4* gene in Japan.

Materials and methods

Patients and questionnaire. From 2012 to 2014, a preliminary questionnaire for soliciting information about BGS patients was sent to 1,407 Departments of 515 Pediatrics, 515 Dermatology Departments and 377 Cancer Hospitals in Japan. The response rate was 83, 68 and 36%, respectively. Parents of the three patients suspected of having GBS were requested to fill out more extensive questionnaire to obtain detailed information on the three patients. The analyses were approved by the ethics committee of Nagara Medical Center, and written informed consent was obtained from the patients and/or their parents.

Genetic analysis. Peripheral blood monocytes cells (PBMCs) were separated using Ficoll-Paque (Amersham Bioscience, Uppsala, Sweden). Genomic DNA from PBMCs of the patient and his parents were prepared using a Sepa Gene kit (Sankyo Jyunyaku, Tokyo, Japan). Amplification of the full-length *RECQL4* gene region was performed using PrimeSTAR

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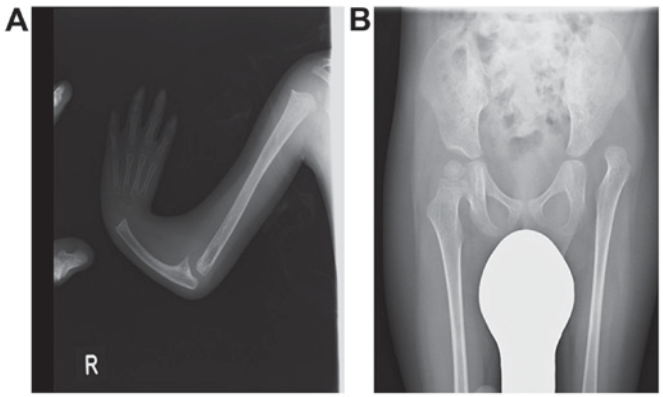


Figure 1. Skeletal anomalies of case 3 revealed by X-ray examination. Right radial ray defects; (A) absent radius and absent thumb. (B) Left hip dislocation.

GXL DNA polymerase (Takara Bio, Inc., Shiga, Japan), using primers 5'-ATTGGCTGCTTGTCCGAG-3' and 5'-GCCTGG AATATGTGATGTGC-3'. The PCR products were electrophoresed on 0.7% agarose gel, and were also sequenced using Big Dye Terminator v3.1 (Thermo Fisher Scientific, Waltham, MA, USA), using primers 5'-GGTGAGCCATATGTGAAC TGG-3' and 5'-CACTGCATCCACAGAGCAAG-3'.

Results

Three patients in two families were identified to be affected by BGS by the questionnaire-based survey in Japan. One family had a 3-year-old older brother (case 1) and a 1-year-old younger brother (case 2) with BGS. The older brother showed craniosynostosis, thumb hypoplasia, radial ray defects and imperforate anus and nasolacrimal duct malposition. Operations were performed for craniosynostosis, imperforate anus and nasolacrimal duct malposition. The younger brother showed left ptosis and was diagnosed as having BGS on the basis of his clinical features and the finding of the X-ray examination of the systemic bone. Their *RECQL4* genes were not analyzed because informed consents were not obtained.

Case 3 was a 4-year-old boy. In addition to craniosynostosis and thumb hypoplasia (Fig. 1), he showed left hip dislocation, left knee joint dislocation, bent elbows, and excessive hand abduction. His intelligence is within the normal range, as determined by a new edition K-type development inspection. At 4 years of age he was still not ambulatory but could sit.

From his clinical features and bone X-ray examination findings he was suspected of having BGS. Therefore, his *RECQL4* gene was analyzed. His *RECQL4* gene showed a 1,614 bp homozygous deletion and 1 bp (G) insertion (NC_000008.10:g.145737562_145739175delinsC) (Fig. 2A). Deleted DNA spanned from intron 12 to the former part of exon 18, which contained the helicase motif (Fig. 3). Agarose gel analysis (Fig. 2B) showed that his father and mother carried the wild type and mutant *RECQL4* genes, respectively.

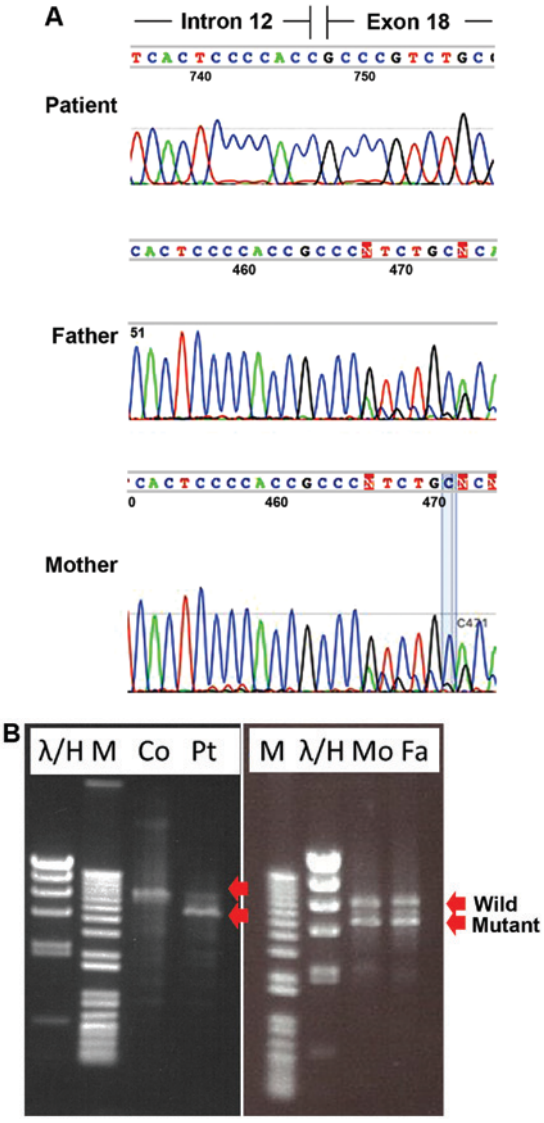


Figure 2. (A) Chromatogram of DNA sequences at DNA break point (NC_000008.10:g.145737562_145739175delinsC). (B) Large homozygous deletion of *RECQL4* gene in case 3 detected by agarose gel electrophoresis. λ/H, *Hin*dIII digested DNA marker, M, DNA marker, Co, control, Pt, case 3, Mo, Mother, Fa, Father.

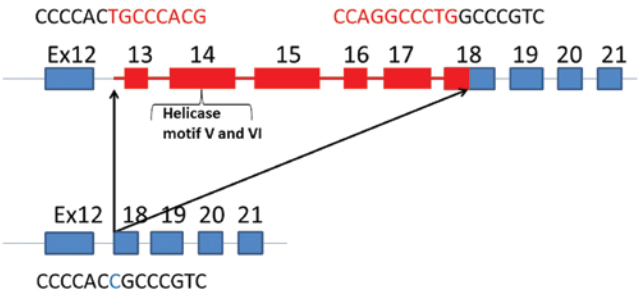


Figure 3. In case 3, detected homozygous deletion of the *RECQL4* gene from intron 12 to the former part of exon 18, resulted in the deletion of amino acids after Ala687. Red boxes indicated the deleted exons and introns. Exon 14 encodes the helicase motif V and VI (1).

Discussion

The prevalence of BGS is unknown; it is probably less than 1:1,000,000 (7). This rarity of BGS makes its diagnosis difficult in some cases. Piard *et al* (8). Showed that the mean age at referral for BGS was 7 years. In our nationwide survey only two families were identified to have members affected

by BGS. All of the affected members had craniosynostosis and radial ray defects. On the other hand Rothmund-Thomson syndrome and RAPADILINO syndrome are two recessively inherited syndromes whose clinical features overlap those of BGS. Rothmund-Thomson syndrome is characterized by poikiloderma. Radial ray hypoplasia or absent thumbs occur in a minority of cases. RAPADILINO syndrome is characterized by radial ray defects. BGS is characterized by craniosynostosis in association with radial hypoplasia. Our cases showed radial ray defect with carniosyonostosis without poikiloderma which is consistent with the characteristic of BGS.

The *RECQL4* gene in our genetically analyzed patient showed homozygous deletion from intron 12 to the former part of exon 18 resulting in the deletion of amino acids after Ala687. To our knowledge this large deletion of *RECQL4* gene has not been reported in other BGS patients. This deletion spans the RecQ helicase motif from IV to VI. The deleted RECQL4 protein in the patient might not be functional. To the best of our knowledge, this deletion in the *RECQL4* gene has not been reported. Piard *et al* reported that no RECQL4 mutations were found in their BGS group without poikiloderma. However, our patient had café-au-lait-like spots but not poikiloderma (8). The relationship between poikiloderma and *RECQL4* gene mutation should be further examined.

In our patient the parents had heterozygous *RECQL4* gene mutation without evident consanguinity. A molecular study-based diagnosis is powerful tool for genetic counselling of individuals affected by BGS. Cao *et al* Reported the case of BGS prenatally diagnosed (9). The cases of BGS diagnosed on the basis of molecular genetics should be accumulated.

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