J Bone Miner Metab (2003) 21:1–4

Review article

Journal of
Bone and
Mineral
Metabolism

Disruption of the *C7orf2/Lmbr1* genic region is associated with preaxial polydactyly in humans and mice*

Taizo Horikoshi[1,5], Naoto Endo[1], Minoru Shibata[2], Peter Heutink[3], Robert E. Hill[4], and Sumihare Noji[5]

[1] Division of Orthopedic Surgery, Department of Regenerative and Transplant Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[2] Division of Plastic and Reconstructive Surgery, Department of Functional Neuroscience, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[3] Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands
[4] MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, UK
[5] Department of Biological Science and Technology, Faculty of Engineering, University of Tokushima, 2-1 Minami-Josanjima, Tokushima 770-8506, Japan

Key words preaxial polydactyly · translocation · Ssq · *C7orf2* · *Lmbr1*

Introduction

Preaxial polydactyly [PPD (MIM190605)] is one of the most frequently observed human developmental malformations of the limbs. Sporadic cases of isolated PPD have been described, but most show an autosomal dominant mode of inheritance. The phenotype shows large variation within families and varies from triphalangeal thumb or duplication of the thumb to tibial aplasia. Using several large families, a PPD locus was mapped to a 450-kb region on chromosome 7q36, and all families described so far are linked to this region [1–4]. Recent data suggest that the autosomal dominant form of PPD is a part of a complex locus that includes the other limb disorders. The gene responsible for complex polysyndactyly [5] and acheiropodia (MIM 200500) [6] has been mapped in the same region. Acheiropodia is a rare autosomal recessive disorder of the limb with a striking phenotype. All four limbs seem to be amputated at the level of elbow or knee, so that the hands and feet are absent except for an ectopically formed single bone at the distal end of the humerus (Bohomoletz bone). Within the human PPD critical region, five transcriptional units were identified, but finding genetic lesions within these families and the mouse mutants has remained problematic [7].

In the syntenic region in mouse, two mutations have been localized; Hemimelic extra toes (*Hx*), characterized by PPD and tibial aplasia [8], and Hammertoe (*Hm*) [9], characterized by syndactyly. Because of the significant similarity between the human and mouse phenotypes and the synteny of the chromosomal region, we consider it likely that the *Hm* and *Hx* mutations are the mouse equivalent of the human mutations linked to chromosome 7q36. Here we show that a novel polydactylous mutant called Sasquatch (*Ssq*) is an allele of *Hx*.

Offprint requests to: T. Horikoshi
Received: July 8, 2002 / Accepted: July 17, 2002
* Recipient of JSBMR Encouragement Award 2001

Identification of the breakpoint in the t (5,7)(q11, q36) patient and of the mouse *Ssq* insertion site

By using a combination of human and mouse genetic approaches, we identified mutations in a human PPD patient and in the mouse *Ssq* mutant. The patient, a 3-year-old girl, had bilateral symmetrical duplication of the triphalangeal thumb and triplication of the great toe without any associated abnormality (Fig. 1). She carries a de novo translocation with a breakpoint within the critical region for PPD. The breakpoint lies within the gene *C7orf2*, one of the five transcripts in the PPD critical region. Further analyses identified the breakpoint within intron 5 of the gene. Prediction of the secondary sequence topology [10] suggests that the *C7orf2* gene encodes a protein with multiple transmembrane domains. Thus, there is a possibility that the *C7orf2* protein products operate as receptors or as part of larger membrane-bound complexes.

The second mutation identified was in the mouse mutant *Ssq* [11]. The *Ssq* mutant arose as a result of a transgenic insertion. The insertion was found to lie within the same intron of *Lmbr1* gene, the mouse ortholog of *C7orf2*, showing that the mouse *Ssq* mutation is a model for human PPD. In addition, these disruptions occurred in a stretch of repetitive DNA. These data pinpoint the *C7orf2*/*Lmbr1* genic region as critical to the preaxial polydactyly phenotype observed in the two species.

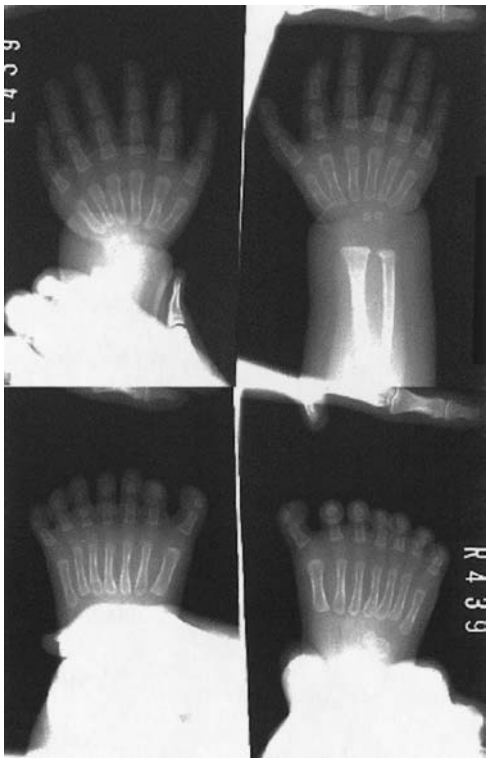

Fig. 1. Limb phenotype of preaxial polydactyly (PPD) patient carrying a de novo translocation t(5,7)(q11,q36). Radiograms of hands showing bilateral duplication of the thumb (*upper panels*) and radiograms of feet show bilateral triplication of the great toe (*lower panels*)

Mutation analyses of PPD families

Analysis of transcription in the translocation case and *Ssq* shows that the translocation breakpoint or the transgenic insertion appears to confer partial transcriptional stop activity, resulting in transcripts that terminate in the intron between exons 5 and 6. However, sequence analyses of *C7orf2* in PPD families indicate that no exons are skipped in patients. Thus, truncation of the *C7orf2* protein product between exon 5 and 6 may not be a general feature of the familial PPD cases.

Discussion

Our data suggest that PPD is associated with mutations in the *C7orf2* genic region. Then, how are they acting? Studies of large chromosomal deletions on human 7q36 and mouse chromosome 5 revealed that a loss of a single copy of *C7orf2* does not result in preaxial supernumerary digits or other limb defects [12,13], which suggests that the mutations are not haploinsufficiencies but rather act as dominants. The *C7orf2* gene encodes a putative multipass membrane-associated protein and may operate at the cell surface as a receptor or part of a receptor complex. Preaxial polydactyly mutations may result from truncated, activated forms of this protein. However, contradictory evidence from the study of five different families suggests that the explanation may be more complicated. These patients showed no nonsense mutations or other mutations predicted to yield truncated proteins and, in addition, RT-PCR failed to detect premature transcriptional termination within the *C7orf2* gene. These data cast some doubt on the suggestion that a specific truncated product is the predominant cause of PPD in families and that this disorder may result from a complex series of mutational events.

Studies in the mouse models for PPD do suggest a close relationship with *Sonic Hedgehog* (*Shh)* expression. *Shh* is expressed in a posterior domain referred to as the zone of polarizing activity (ZPA) and is fundamental to anteroposterior patterning of the limb [14]. Normally Shh expression in the anterior part of the limb bud is actively repressed by transcriptional factors such as *Gli3* [15]. In *Ssq* and *Hx*, as in other unrelated preaxial polydactylous mutants [16–20], *Shh* is detected in an additional anterior ectopic site outside the ZPA. Generally, an ectopic site of signaling activity is predicted to be essential in the formation of extra digits. The relationship between *Ssq* and PPD suggests that in humans the supernumerary preaxial digits correspondingly result from ectopic expression of *Shh*. Thus, the mutations within the *C7orf2* gene reported here appear to have a role in the misexpression of the *Shh* gene. Given that extra digits result from *Shh* misexpression, then the converse loss of limb expression may be expected to effect severe distal limb truncations as seen in acheiropodia patients. Recently, genomic deletion including exon 4 of C7orf2 was identified in acheiropodia patients. There are no mouse models for acheiropodia; however, in limbs of the mouse model for *Shh*-targeted deletions, truncation of long bones, complete loss of feet, and sometimes an ectopic single bone at the distal end of the humerus/femur, similar to the Bohomoletz bone, were seen [21]. We suggest that, in contrast to *Ssq* and PPD, acheiropodia results from a limb-specific loss of *Shh* expression. Thus, we predict that *C7orf2* mutations in this region correspond very closely to limb-specific expression of *Shh*.

The data suggest an intriguing relationship between *C7orf2* and *Shh* and raise the question, is *C7orf2* a regulator of the *Shh* gene? The physical linkage of *Shh* and *C7orf2*, which map apart at an approximate 1-Mb distance in both mouse and human, make this difficult to discern. The role for *C7orf2* in limb morphogenesis remains to be determined. As it stands, we cannot rule out the previously suggested possibility that the *C7orf2* genic region is dominated by *Shh* long-range, limb-specific regulatory elements (Fig. 2).

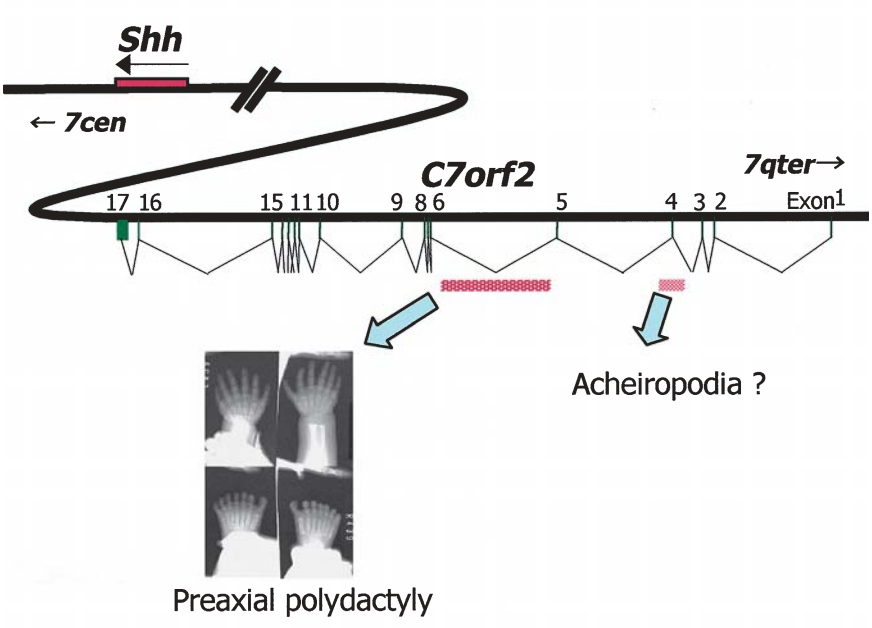

Fig. 2. Diagram of relationship between *SHH* and putative limb-specific *cis*-acting regulatory elements that reside at least 800 kb away. It is hypothesized that mutation within intron 5 of *C7orf2* removes the repressor of anterior ectopic *SHH* expression, whereas deletion around exon 4 inactivates limb-specific *SHH* expression

The inability to resolve all mutations is not unique to this genetic region. Mutations that lie outside the coding region are notoriously difficult to identify. In future investigations, however, these mutations may pinpoint unidentified isoforms or regulatory regions.

Acknowledgments. We thank the PPD patients and their families for participation. We are grateful to H.E. Takahashi of Niigata University of Health and Welfare for his continued support. This work was supported partly by grants from the Japan Society for Promotion of Science (Research for the Future, (S.N. and T.H.) and from the Ministry of Education, Sports, and Culture (S.N.), and the Netherlands Organization for Scientific Research (NWO) (P.H.).

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