

A syndactyly type IV locus maps to 7q36

Daisuke Sato · Desheng Liang · Lingqian Wu · Qian Pan ·
Kun Xia · Heping Dai · Hua Wang · Gen Nishimura · Koh-Ichiro Yoshiura ·
Jiahui Xia · Norio Niikawa

Received: 26 March 2007 / Accepted: 2 April 2007 / Published online: 3 May 2007
© The Japan Society of Human Genetics and Springer 2007

Abstract Syndactyly occurs as an isolated abnormality or a part of a malformation syndrome. Syndactyly types I, II, III and V have been mapped to chromosomal regions 2q34–q36, 2q31–q32, 6q21–q23.2 and 2q31–q32, respectively, whereas syndactyly type IV (SD4) is extremely rare, and its gene localization has not yet been assigned. The SD4 manifests complete syndactyly of all fingers accompanied with polydactyly, and flexion of the fingers gives

the hand a cup-shaped appearance. We performed a linkage and haplotype analysis of a Chinese pedigree with autosomal dominant, non-syndromic SD4 using a set of 406 microsatellite markers. The analysis gave the maximum two-point LOD score of 1.613 at recombination fraction of 0.00 and penetrance of 1.00. Thus, the SD4 locus in the family was likely assigned to a 17.39-cM region at a segment between markers D7S3070 and D7S559 at 7q36, although the LOD score obtained was not high enough to conclude the localization. Analysis of three candidate genes, *LMBR1*, *SHH* and *ZRS*, failed to identify any pathogenic mutations. Our gene mapping may give a clue to identify the putative SD4 gene and provide a better understanding of normal human limb development.

Daisuke Sato and Desheng Liang equally contributed to this study.

D. Sato · D. Liang · L. Wu · K.-I. Yoshiura · N. Niikawa
Department of Human Genetics, Nagasaki University
Graduate School of Biomedical Sciences, Nagasaki, Japan

D. Sato · D. Liang · L. Wu · K.-I. Yoshiura · N. Niikawa
Solution Oriented Research of Science and Technology
(SORST), Japan Science and Technology Agency (JST),
Kawaguchi, Japan

D. Sato
Department of Pediatrics, Hokkaido University Graduate
School of Medicine, Sapporo, Japan

D. Liang · L. Wu (✉) · Q. Pan · K. Xia · H. Dai · J. Xia
National Laboratory of Medical Genetics, Xiangya Hospital,
Central South University, 110 Xiangya Road, Changsha,
Hunan 410078, China
e-mail: wulingqian@cnlmg.com

H. Wang
Women and Children's Hospital of Hunan Province,
Changsha, China

G. Nishimura
Department of Radiology, Tokyo Metropolitan Kiyose
Children's Hospital, Tokyo, Japan

Keywords Syndactyly type IV · Linkage analysis ·
Disease gene mapping

Introduction

Syndactyly is one of the most frequent congenital limb abnormalities and occurs as an isolated anomaly or a part of a malformation syndrome. Syndactyly falls into five major types I–V based on different combinations of affected fingers and toes and showing an autosomal dominant mode of inheritance. Syndactyly type I (OMIM 185900), type II (OMIM 186000), type III (OMIM 186100) and type V (OMIM 186300) have been mapped to chromosomal regions 2q34–q36, 2q31–q32, 6q21–q23.2 and 2q31–q32, respectively. Among them, only type III syndactyly was suggested to be an allelic disorder of oculodentodigital dysplasia (ODDD, MIM 164200) that is caused by mutations in the gene for gap junction protein alpha-1 (GJA1). Genes responsible for other types of syndactyly have not been

identified. Syndactyly type IV (SD4, OMIM 186200) is extremely rare and has been reported only twice since the first description by Haas in 1940 (Gillesen-Kaesbach and Majewski 1991; Rambaud-Cousson et al. 1991). Patients with this disease have complete syndactylism of all the fingers accompanied with polydactyly and cup-shaped hands due to flexion of the fingers. The etiology of SD4 has remained unknown, and its gene localization has not yet been mapped.

We recently encountered a Chinese pedigree with autosomal dominant SD4. Herein we report on their clinical manifestations and genetic linkage study.

Materials and methods

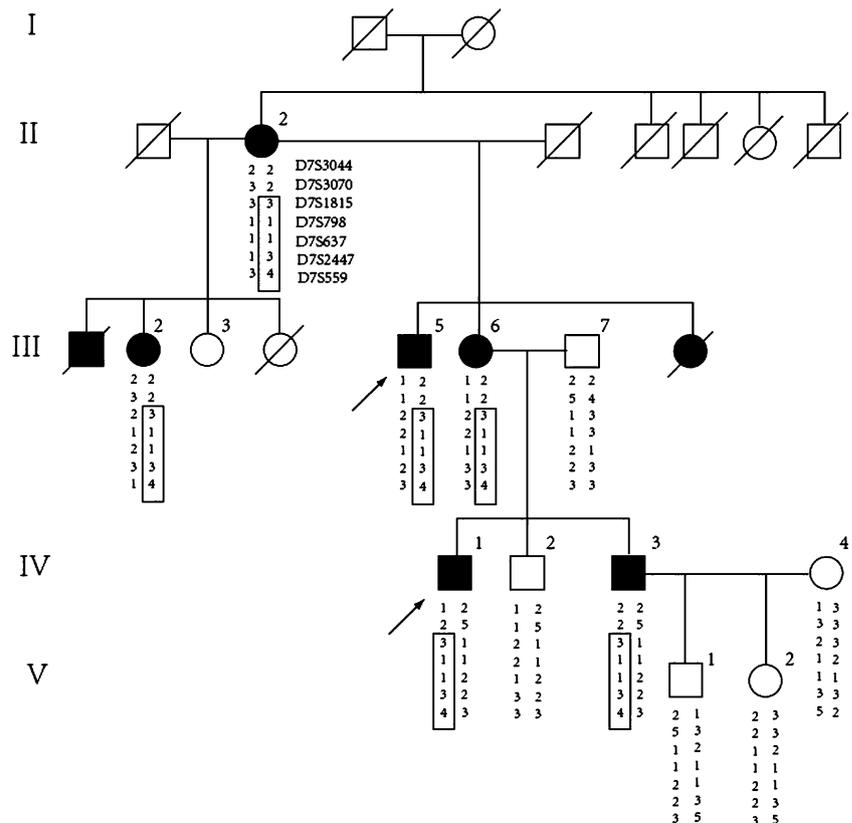
This study was approved by the Committee for Ethical Issues on Human Genome and Gene Analysis, Nagasaki University. We ascertained a five-generation non-consanguineous Chinese family with autosomal dominant, non-syndromic syndactyly (Figs. 1, 2). The family consisted of 23 members, including 8 affected individuals (4 females and 4 males). A total of 11 family members (6 affected and 5 unaffected individuals) were available for clinical evaluations and linkage and haplotype analyses.

All the patients examined were mentally normal, but had hand and/or foot anomalies. Individuals II-2, III-2 and III-6

had bilateral complete syndactyly with flexion of fingers, cup-shaped hands, polydactyly with two additional small and non-functional fingers at the edge of both hands, six metacarpals (Fig. 2a, b) and normal feet. In addition to the hand polysyndactyly, individuals III-5 and IV-1 had foot anomalies such as seventh and eighth toes on the left and right foot, respectively, and partial cutaneous syndactyly between toes two and three. Their excess toes existed on the tibial side of both feet, and lower extremities were bent and tubby, and showed tibial hemimelia. Knee and ankle joint malformations were present in III-5 and IV-1. Radiograph of the hands and left foot of III-5 showed six metacarpals (Fig. 2g), seven metatarsals and tibial hypoplasia (Fig. 2h), leading to a diagnosis of the disease in the family as Haas type (type IV) mirror-image polydactyly of hands and feet with tibial hypoplasia. III-5 (Fig. 2c–f) also had two triphalangeal thumbs bilaterally. Individual IV-3 presented with syndactyly of fingers two to six and toes five to six, stiffness of proximal interphalangeal joints in all his fingers and two additional small and non-functional fingers bilaterally. None of the five patients showed bone fusion radiologically.

DNA samples were extracted from peripheral blood leukocytes of the 11 members of the family after obtaining written informed consents. We carried out a whole-genome search except for the chromosome X. These individuals

Fig. 1 Pedigree of a Chinese SD4 family with haplotypes at seven marker loci on chromosome 7. The number in the box depicts haplotype common to affected individuals



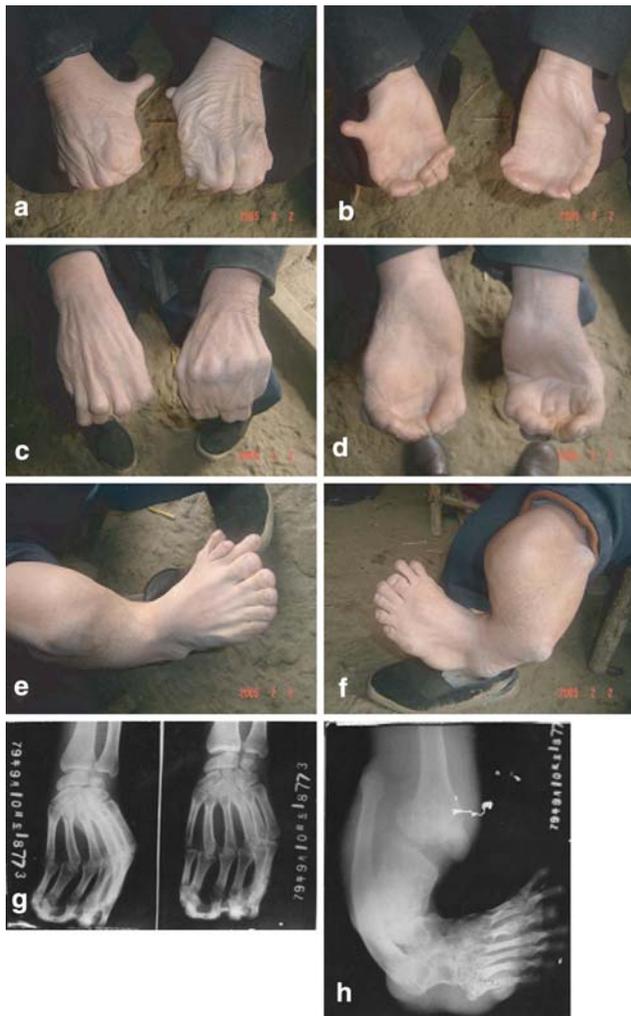


Fig. 2 Hand/foot malformations of affected individuals. **a, b** Polysyndactyly of fingers in III-6. **c–f** Polysyndactyly of fingers and/or toes in III-5. **g, h** Radiograph of hands and left leg/foot in III-5, showing six metacarpals and seven metatarsals without bone fusion, and tibial hypoplasia

were genotyped at 406 microsatellite marker loci that are distributed with an average of 10-cM intervals over the whole genome. Two-point LOD scores were calculated using the MLINK program of the FASTLINK package, assuming that the disease in the family is inherited in an autosomal dominant mode with complete penetrance (penetrance = 1.00), the disease-allele frequency is 0.001 and allele frequencies are equal at all the marker loci.

A mutation screening in three candidate genes, *LMBR1* for limb region 1 protein, *SHH* for sonic hedgehog and *ZRS* for an *SHH* regulator, was performed in six affected individuals and two unaffected members of the family. All exon and flanking intron sequences of *LMBR1* and *SHH* and a 774-bp highly conserved sequence of *ZRS* were amplified by PCR for direct sequencing. PCR conditions were set at 40 cycles of 94°C for 30 s, 62°C for 30 s and at

72°C for 45 s in a 15- μ l mixture containing 1 \times PCR buffer with 1.5 mM MgCl₂, 0.2 mM each of dNTP, 1 μ M each primer and 0.4 units ExTaq DNA polymerase (TaKaRa, Otsu, Japan). PCR products were treated with ExoSAP-IT (AmershamBiosciences, Piscataway, NJ), and both strands of DNA were sequenced with BigDye Terminator Sequencing kit version 3.1 according to the supplied protocol (AppliedBiosystems, Foster City, CA). The reaction mixture was purified using Sephadex G-50 superfine (AmershamBiosciences) and analyzed on the ABI Genetic Analyzer 3100 (AppliedBiosystems) with the Sequence-Analysis software (AppliedBiosystems) and aligned with the AutoAssembler version 2.1.1 software (AppliedBiosystems) to find DNA alterations.

Results and discussion

The disease in the Chinese family was clinically diagnosed as syndactyly type IV (SD4), because one (III-5) of the affected members had complete syndactylism of all fingers and toes accompanied with polydactyly. Flexion of fingers together with cutaneous syndactyly gave his hands a cup-shaped appearance. SD4 in the family was inherited as an autosomal dominant mode as was reported in the family with SD4 (Gillesen-Kaesbach and Majewski 1991; Rambaud-Cousson et al. 1991).

At an initial genotyping at the 406 marker loci, only seven members [five affected and two unaffected members (II-2, III-2 and 5–7, and IV-2 and 3)] were available. We obtained a possible linkage of the disease locus to five markers (*D2S2152*, *D7S559*, *D12S1052*, *D16S3039* and *D17S1822*) on chromosomes 2, 7, 12, 16 and 17 with LOD scores higher than 1.00. However, haplotype analysis excluded four of the five loci and retained *D7S559* as a candidate region for SD4 at 7q36.3. We then performed a second analysis by the use of more markers around 7q36.3 and by adding four more members (one patient IV-1 and three unaffected members IV-4, V-1 and V-2) who participated in the study later. The maximum two-point LOD score within the locus was 1.613 at recombination fraction of 0.00 and penetrance of 1.00 (Table 1). Haplotype analysis showed that all the six affected members had the same haplotype ‘‘3-1-1-3-4’’ for five marker loci, *D7S1815*, *D7S798*, *D7S637*, *D7S2447* and *D7S559* (Fig. 1). From these findings, it is most likely that SD4 in the Chinese family was assigned to a 17.39-cM region at 7q36, although the LOD score obtained was not high enough to conclude a concrete linkage. Direct sequencing of patients’ DNA for exon and flanking intron sequences of *LMBR1* and *SHH* and the conserved sequence of *ZRS* revealed no pathogenic mutation.

To a similar region where we assigned a SD4 locus, several forms of limb abnormalities have been mapped.

Table 1 Two-point LOD score of chromosome 7q markers at various recombination fractions

Marker	Position (cM)	LOD score at theta (penetrance = 1.00)						
		0.00	0.05	0.10	0.15	0.20	0.25	0.30
D7S3044	153.7	-2.75	-0.480	-0.225	-0.096	-0.021	0.024	0.047
D7S3070	165.6	0.68	0.714	0.700	0.657	0.594	0.517	0.430
D7S1815	167.1	1.380	1.276	1.166	1.050	0.928	0.798	0.660
D7S798	169.9	0.861	0.796	0.728	0.657	0.581	0.500	0.415
D7S637	174.0	0.519	0.479	0.437	0.393	0.347	0.298	0.246
D7S2447	175.5	1.192	1.013	0.900	0.784	0.663	0.540	0.415
D7S559	183.0	1.613	1.446	1.271	1.090	0.902	0.712	0.526

They include preaxial polydactyly (Hing et al. 1995; Heus et al. 1999; Zguricas et al. 1999), complex polysyndactyly (Tsukurov et al. 1994), triphalangeal thumb (Heutink et al. 1994; Radhakrishna et al. 1996; Balci et al. 1999) and acheiropodia (Ianakiev et al. 2001). Among genes in the region, *LMBR1*, *SHH* and *ZRS* merit comments. It was shown that five point mutations residing in the highly conserved sequence of *ZRS* were associated with congenital preaxial polydactyly (Lettice et al. 2003; Gurnett et al. 2007), mutations in the chicken *Lmbr1* are linked to chicken polydactyly (Huang et al. 2006), and *Shh* was responsible for the digit duplication activity in chick embryos (Riddle et al. 1993). Unfortunately, we failed to identify any pathogenic mutations of these genes in the SD4 patients from the Chinese family.

In conclusion, we have mapped the SD4 locus in the Chinese family to 7q36. This may become a clue to identify the gene responsible for this rare disease and provide a better understanding of normal human limb development.

Acknowledgments We are grateful to the patients and relatives for their participation in this study. N. Niikawa was supported in part by a Grant-in-Aid for Scientific Research (Priority Areas for Applied Genomics, no. 17019055) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by SORST from the Japan Science and Technology Agency (JST). L. Wu was supported by the Research Grant (30571201) from National Natural Science Foundation of China.

Conflict of interest The authors of this manuscript declare that they have no competing interests.

References

- Balci S, Demirtas M, Civelek B, Piskin M, Sensoz O, Akarsu AN (1999) Phenotypic variability of triphalangeal thumb-polysyndactyly syndrome linked to chromosome 7q36. *Am J Med Genet* 87:399–406
- Gillessen-Kaesbach G, Majewski F (1991) Bilateral complete polysyndactyly (type IV Haas). *Am J Med Genet* 38:29–31
- Gurnett CA, Bowcock AM, Dietz FR, Morcuende JA, Murray JC, Dobbs MB (2007) Two novel point mutations in the long-range *SHH* enhancer in three families with triphalangeal thumb and preaxial polydactyly. *Am J Med Genet* 143:27–32
- Haas SL (1940) Bilateral complete syndactylism of all fingers. *Am J Surg*:363–366
- Heus HC, Hing A, van Baren MJ, Joesse M, Breedveld GJ, Wang JC, Burgess A, Donnis-Keller H, Berglund C, Zguricas J, Scherer SW, Rommens JM, Oostra BA, Heutink P (1999) A physical and transcriptional map of the preaxial polydactyly locus on chromosome 7q36. *Genomics* 57:342–351
- Heutink P, Zguricas J, van Oosterhout L, Breedveld GJ, Testers L, Sandkuijl LA, Sniijders PJLM, Weissenbach J, Lindhout D, Hovius SER, Oostra BA (1994) The gene for triphalangeal thumb maps to the subtelomeric region of chromosome 7q. *Nat Genet* 6:287–291
- Hing AV, Helms C, Slaugh R, Burgess A, Wang JC, Herman T, Downton SB, Donis-Keller H (1995) Linkage of preaxial polydactyly type 2 to 7q36. *Am J Med Genet* 58:128–135
- Huang YQ, Deng XM, Du ZQ, Qiu X, Du X, Chen W, Morisson M, Leroux S, Ponce de Leon FA, Da Y, Li N (2006) Single nucleotide polymorphisms in the chicken *Lmbr1* gene are associated with chicken polydactyly. *Gene* 374:10–18
- Ianakiev P, van Baren MJ, Daly MJ, Toledo SPA, Cavalcanti MG, Correa Neto J, Lemos Silveira E, Freire-Maia A, Heutink P, Kilpatrick MW, Tsipouras P (2001) Acheiropodia is caused by a genomic deletion in *c7orf2*, the human orthologue of the *Lmbr1* gene. *Am J Hum Genet* 68:38–45
- Lettice LA, Heaney SJH, Purdie LA, Li L, de Beer P, Oostra BA, Goode D, Elgar G, Hill RE, de Graaff E (2003) A long-range *Shh* enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. *Hum Mol Genet* 12:1725–1735
- Radhakrishna U, Blouin JL, Solanki JV, Dhoriani GM, Antonarakis SE (1996) An autosomal dominant triphalangeal thumb: polysyndactyly syndrome with variable expression in a large Indian family maps to 7q36. *Am J Med Genet* 66:209–215
- Rambaud-Cousson A, Dudin AA, Zuaite AS, Thalji A (1991) Syndactyly type IV/hexadactyly of feet associated with unilateral absence of the tibia. *Am J Med Genet* 40:144–145
- Riddle RD, Johnson RL, Laufer E, Tabin C (1993) Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell* 75:1401–1416
- Tsukurov O, Boehmer A, Flynn J, Nicolai JP, Hamel BCJ, Trill S, Zaleske D, Mankin HJ, Yeon H, Ho C, Tabin C, Seidman JG, Seidman C (1994) A complex bilateral polysyndactyly disease locus maps to chromosome 7q36. *Nat Genet* 6:282–286
- Zguricas J, Heus H, Morales-Peralta E, Breedveld G, Kuyt B, Mumcu EF, Bakker W, Akarsu N, Kay SPJ, Hovius SER, Heredero-Baute L, Oostra BA, Heutink P (1999) Clinical and genetic studies on 12 preaxial polydactyly families and refinement of the localisation of the gene responsible to a 1.9 cM region on chromosome 7q36. *J Med Genet* 36:32–40