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A new locus for postaxial polydactyly type A/B on chromosome 7q21–q34

Robert-Jan H Galjaard[*,1], Arie PT Smits[2], Joep HAM Tuerlings[2], Aagje G Bais[1], Aida M Bertoli Avella[1], Guido Breedveld[1], Esther de Graaff[1], Ben A Oostra[1] and Peter Heutink[1]

[1]Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands; [2]Department of Human Genetics, University Medical Center Nijmegen, Nijmegen, The Netherlands

Postaxial polydactyly (PAP) is the occurrence of one or more extra ulnar or fibular digits or parts of it. In PAP-A, the extra digit is fully developed and articulates with the fifth or an additional metacarpal/metatarsal, while it is rudimentary in PAP-B. Isolated PAP usually segregates as an autosomal dominant trait, with variable expression. Three loci are known for PAP in humans. PAPA1 (including PAP-A/B in one patient) on 7p13 caused by mutations in the GLI3 gene, PAPA2 on 13q21–q32 in a Turkish kindred with PAP-A only, and a third one (PAPA3) in a Chinese family with PAP-A/B on 19p13.1–13.2. We identified a fourth locus in a large Dutch six-generation family with 31 individuals including 11 affected. Their phenotype varied from either PAP-A, or PAP-B to PAP-A/B with or without the co-occurence of partial cutaneous syndactyly. We performed a whole-genome search and found linkage between PAP and markers on chromosome 7q. The highest LOD score was 3.34 obtained at D7S1799 and D7S500 with multipoint analysis.
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Introduction

Postaxial polydactyly (PAP) is the occurrence of one or more extra ulnar or fibular digits or parts of it.[1] The incidence varies from 1/3300 to 1/630 and from 1/300 to 1/100 livebirths in Caucasian- and African-Americans, respectively.[2] The phenotype of PAP is usually subdivided into types A and B. In type A, the extra digit is fully developed and articulates with the fifth or an additional metacarpal/metatarsal. In type B, it is rudimentary and mostly presents as a skin tag (pedunculated postminimus).[1]

PAP is either seen as an isolated malformation or associated with other defects. Associated defects can be restricted to the limbs. If not, they can be part of a

syndrome, or of a multiple congenital anomaly case.[3] The syndromic cases have a heterogeneous aetiology, for instance trisomy 13. Partial cutaneous syndactyly between toes 2–3, 4–5, and other, is a frequent finding in individuals with PAP.[3]

Isolated PAP usually segregates as an autosomal dominant trait, with variable penetrance and expression. Penetrance rates of 0.68 and 0.43 have been estimated for types A and B, respectively,[4] although higher estimates have been published.[5]

Currently, there are three loci for isolated PAP in humans. PAPA1 (MIM 174200) has been described in three families with PAP-A/B with a disease-causing mutation in the human transcription regulator GLI3 gene on 7p13.[6–8] PAPA2 (MIM 602085) on 13q21–q32 has been reported in a Turkish kindred with PAP-A only.[9] PAPA3 has recently been published in a Chinese family with PAP-A/B on 19p13.1–13.2.[10]

We present a fourth locus in a family with PAP-A/B and partial cutaneous syndactyly on 7q21–q34. In our family

*Correspondence: R-JH Galjaard, Department of Clinical Genetics, Erasmus Medical Center, PO Box 1738, 3000 DR Rotterdam, The Netherlands. Tel: +31 1040 87214; Fax: +31 1040 87200;
E-mail: r.galjaard@erasmusmc.nl

there are patients with three types of PAP (type A, B or A/B) with or without syndactyly.

Materials and methods

A large six-generation family with PAP and/or syndactyly was ascertained (Figure 1). After informed consent was given, 31 individuals were clinically examined and blood samples were taken from them. Genomic DNA was isolated from peripheral blood lymphocytes as described before.[11] From four affected individuals, deceased at the time of examination, anamnestic data were available (Table 1).

Initially, a genome search was started with 24 individuals of our family including all 11 affecteds with PAP (Figure 1). LOD scores were calculated using the MLINK and LINK-MAP programs of the LINKAGE package version 5.1.[12] The following model was used: PAP as an autosomal dominant condition with a penetrance of 0.9, a gene frequency of 0.0003, a phenocopy rate of 0.05, and equal recombination rates between males and females. In this model only individuals with PAP, including V-6, were considered to be affected (Table 1). Allele frequencies were calculated from independent individuals of this family. In total, 26 additional polymorphic markers of chromosome 7 were selected from the Center of Medical Genetics, Marshfield Medical Research Foundation (htpp://research. Marshfield-clinic.org/genetics/). The most recently published high-resolution recombination map of the human genome of Kong *et al* (htpp://genetics.nature.com) was also used. For marker GATA63F08, locus number D7S2202 has recently been assigned. The results of haplotype analysis in this family prompted us to determine if another locus could be associated with the PAP- and the syndactyly phenotype. A whole-genome screen was performed on all affecteds except for V-6, five nonaffected and five control individuals. Microsatellite markers from the Weber Human Screening Set V5 ($n = 363$) were tested, DNA pooling and shared segment analysis were performed essentially as described before.[13] DNA quantity and quality were carefully matched to ensure equal amplification.

Results
Clinical findings

The clinical findings of 17 affected individuals with PAP and/or syndactyly of our family are presented in Table 1. They do not have additional anomalies. PAP-A is the predominant polydactyly phenotype. The expression of both the PAP and syndactyly phenotypes is highly variable in our family, especially for descendants of III-7 and IV-5, as examplified in Figure 2. This variability concerns differences in involvement of upper/lower limbs, left/right side, type A and/or B regarding PAP, and the interdigital space(s) (IDS) and extent of syndactyly. However, the PAP phenotype is strikingly consistent in four preceding generations of VI-1 (Figure 3A).

Linkage analysis for PAP

A genome search was carried out with 24 individuals of our family including 11 affecteds with PAP. Evidence of linkage was found with a marker on chromosome 7q35 (data not shown). Additional markers around D7S1799 were tested to identify the smallest region in which the gene involved has to reside. With two-point linkage analysis the highest LOD score obtained was 3.18 for marker D7S1799 at $\theta = 0$ (Table 2). Using multipoint analysis, LOD scores above 3.20 were found for markers D7S1799–D7S495, so evidence for linkage is spread over a large genomic region (Figure 4).

Haplotype analysis

Only affecteds with PAP and individual VI-6, with bilateral partial cutaneous syndactyly of IDS 2, share the same haplotype for markers between D7S1799 and D7S495 (Figure 1). In addition, they share their haplotype for markers GATA63F08 and D7S2513 with the clinically normal individuals IV-19 and V-18, and individual VI-9 having only syndactyly (Figure 1). The maximum size of the genomic regions located on chromosome 7q21–q34, between D7S1799 and D7S495, and GATA63F08 and D7S2513 is about 50 and 3.7 CM, respectively.

No other locus associated with the PAP phenotype was identified in this family using the DNA pooling and shared segment analysis (data not shown).

Discussion

We describe a large family with PAP-A/B and/or syndactyly. This family is interesting because of the presence of three types of PAP with or without syndactyly. In addition, a single individual with PAP-B (V-6) does not share the haplotype shared by the other affecteds with PAP-A/B.

There is a longstanding debate in the literature if the PAP-A and PAP-B phenotypes are genetically heterogeneous. Differences between PAP-A and PAP-B concerning incidence, penetrance and side preference have been used to argue that the phenotypic distinction between them is also genetically determined.[1,2,4,14] Until now, only families with individuals with the PAP-A or PAP-A/B phenotype have been used for genotyping; none of them includes the PAP-B only phenotype. In our family individual V-6 with PAP-B only does not share the haplotype with other affecteds with PAP-A or PAP-A/B (Figure 1). In addition, both of his alleles are transmitted to his offspring, who are clinically normal. This indicates his phenotype is probably caused by a genetic defect elsewhere on the genome, or less likely, by a nongenetic factor. This is corroborated by the finding that no other locus, associated with PAP-A/B, was found in this family after we performed a whole-genome search. In order to strengthen our findings we also tested
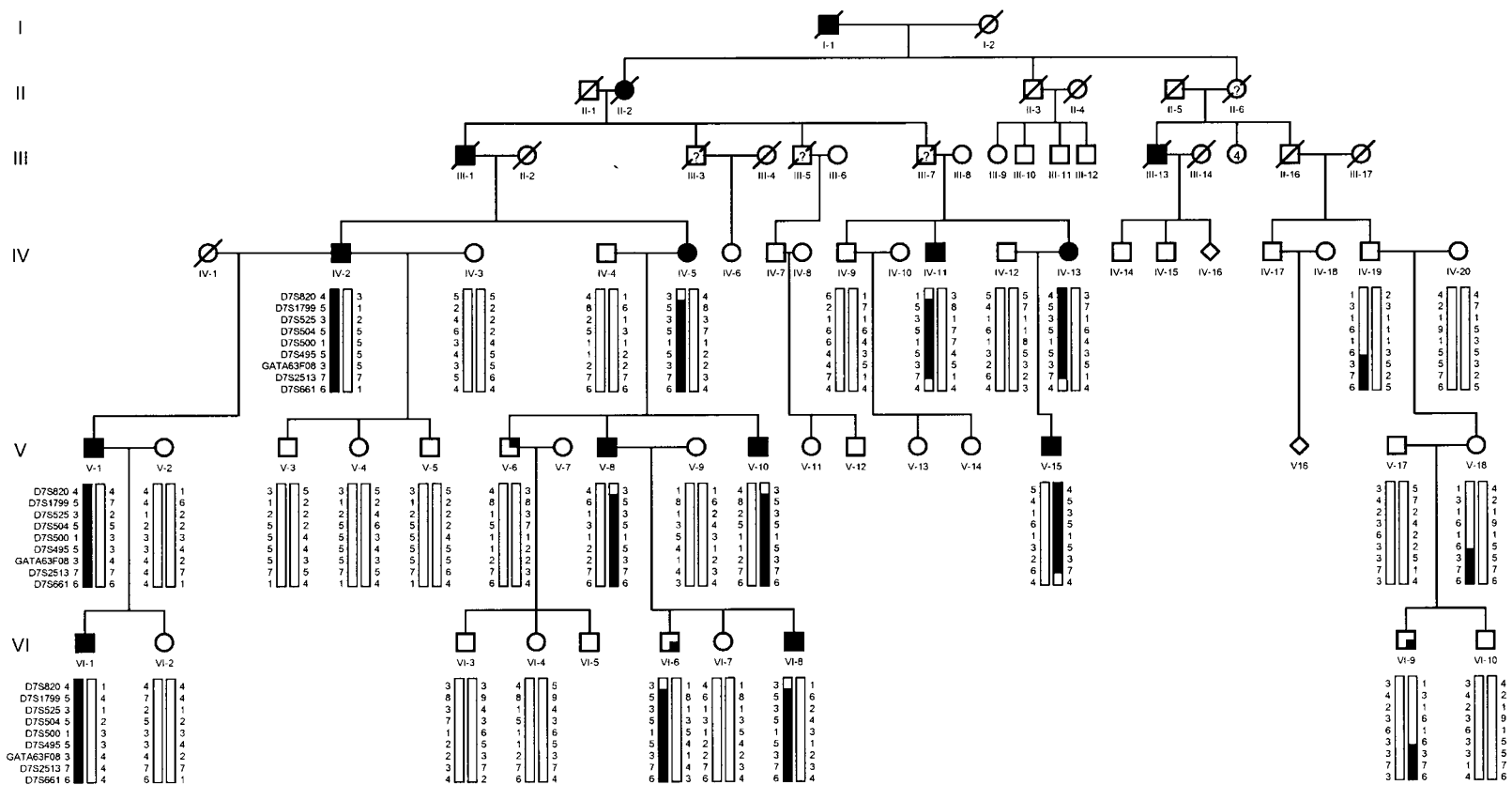
Figure 1 Pedigree diagram of a subset of a family with PAP. Pedigree symbols: black = PAP-A/B with or without cutaneous syndactyly. Black right upper quadrant = PAP-B only. Black right lower quadrant = cutaneous syndactyly digit 2–3 feet only. Haplotypes for eight chromosome 7 markers for 31 individuals. Shared haplotype for markers D7S1799–D7S495 between affecteds with PAP/syndactyly, except for V-6 (phenocopy) and VI-6 (nonpenetrance).

Table 1 Clinical findings of affecteds with PAP/syndactyly

Patient No.	PAP type left hand	PAP type right hand	Syndactyly left hand	Syndactyly right hand	PAP type left foot	PAP type right foot	Syndactyly left foot	Syndactyly right foot
I-1*	P	P	?	?	P	P	?	?
II-2*	A	A	?	?	A	A	?	?
III-1*	N	A	IDS 4–5	N	A	A	N	N
III-13*	N	N	?	?	P	P	?	?
IV-2	A	A	N	N	A	A	N	N
IV-5	A	A	N	N	N	N	N	IDS 4–5
IV-11	N	N	N	N	A	A	N	IDS 5–6
IV-13	N	A	N	IDS 4–5	A	N	IDS 4–6	N
V-1	A	A	N	N	A	A	N	N
V-6	B	B	N	N	N	N	N	N
V-8	A	A	N	N	A	A	N	N
V-10	A	A	N	N	A	A	N	IDS 5–6
V-15	A	B	N	IDS 2–3	A	A	N	N
VI-1	A	A	N	N	A	A	N	N
VI-6	N	N	N	N	N	N	IDS 2–3	IDS 2–3
VI-8	B	B	N	N	A	A	IDS 2–3	IDS 2–3
VI-9	N	N	N	N	N	N	IDS 2–3	IDS 2–3

*=anamnestic data, P=present, N=normal, ?=unknown, A=PAP-A, B=PAP-B, IDS=interdigital space.
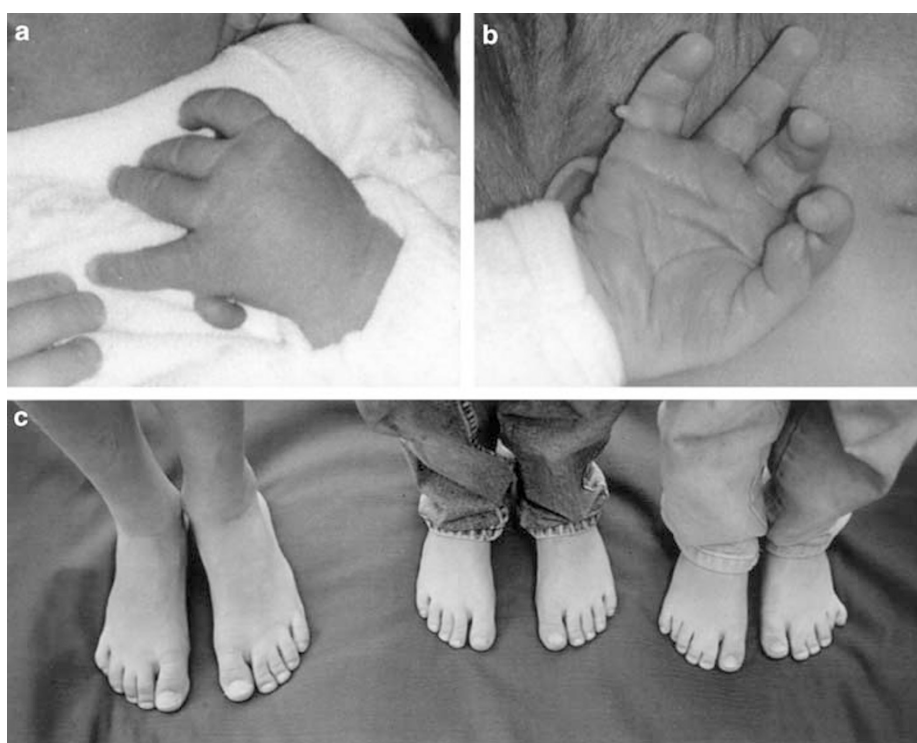

Figure 2 (**a**, **b**) Left and right hand, respectively, of VI-8 with PAP-B. (**c**) From left to right: feet of VI-6 with cutaneous syndactyly digits 2–3 only, normal feet of VI-7, feet with PAP-A/syndactyly digits 2–3 of VI-8.

10 other small families with PAP, but none of them was linked to this 7q region. These findings are indicative of further genetic heterogeneity of PAP, except for the locus harbouring the GLI3 gene. In our family, the PAP-A/B phenotype is genetically homogeneous as described before, but the PAP-A/B and PAP-B only phenotypes are genetically heterogeneous.

Syndactyly is the most frequent associated anomaly in individuals with PAP.[3] In syndactyly type II or synpolydactyly (SPD, MIM186000), the characteristic phenotype is syndactyly between fingers 3–4, and toes 4–5 with or without an extra finger/toe in the syndactylous web.[15] Polydactyly does not occur without syndactyly. SPD is caused by a polyalanine tract expansion in the HOXD13
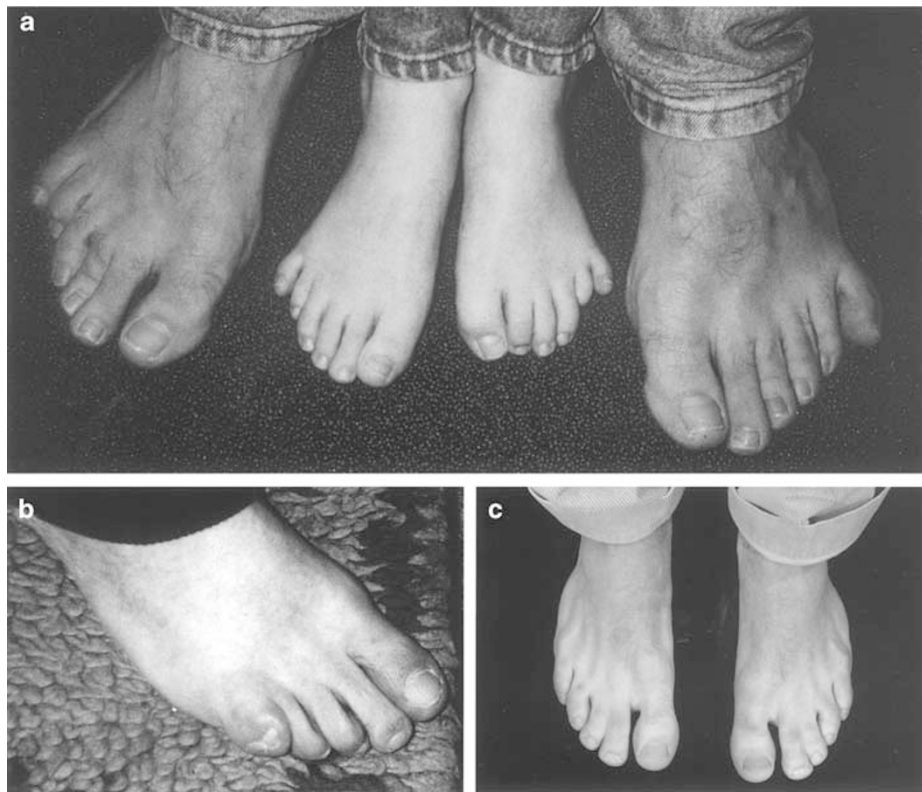
Figure 3 (**a**) Feet of V-1 and VI-1 with PAP-A. (**b**) Right foot of IV-11 with PAP-A and complete cutaneous syndactyly digits 5–6. (**c**) Feet of VI-9 with cutaneous syndactyly digits 2–3 only.

Table 2 Two-point linkage analysis between PAP/B and chromosome 7 markers at different recombination fractions

	Recombination fraction θ						
Markers	0	0.01	0.05	0.1	0.2	0.3	0.4
D7S820	−1.97	−1.69	−1.02	−0.57	−0.13	0.03	0.04
D7S1799	3.18	3.15	3.00	2.76	2.15	1.43	0.65
D7S525	2.44	2.43	2.33	2.15	1.66	1.06	0.44
D7S504	1.26	1.24	1.17	1.05	0.75	0.41	0.14
D7S500	2.64	2.59	2.38	2.11	1.54	0.94	0.39
D7S495	1.08	1.07	1.02	0.94	0.72	0.48	0.24
GATA63F08	2.00	1.96	1.81	1.60	1.13	0.65	0.24
D7S513	0.45	0.44	0.39	0.34	0.23	0.14	0.07
D7S661	−0.84	−0.51	−0.09	0.06	0.11	0.05	−0.01

gene on chromosome 2q31. This phenotype can be differentiated from that in patients of our family. Their phenotype include an extra digit 5 without syndactyly, or with syndactyly between fingers/toes not typically present in patients with SPD (Table 1). Isolated syndactyly IDS 2–3 of the feet has an incidence of 1.17/10 000 individuals.[3] We looked if PAP and syndactyly could be genetically homo or heterogeneous in our family. PAP and syndactyly are present in seven individuals (Table 1). Four individuals have PAP type A or A/B. There are two individuals (VI-6,

VI-9) with only cutaneous syndactyly IDS 2–3 of both feet (Figures 2c and 3c). All patients with PAP-A/B, share the same haplotype between markers GATA63F08 and D7S2513 (Figure 1). This includes the clinically normal mother (V-18) and maternal grandfather (IV-19) of the individual with bilateral syndactyly IDS 2–3 of his feet (VI-9). There are two possible explanations for these observed phenotypes/genotypes.

First, the region shared by all of them harbours the gene(s) causing the PAP and the syndactyly phenotype, so
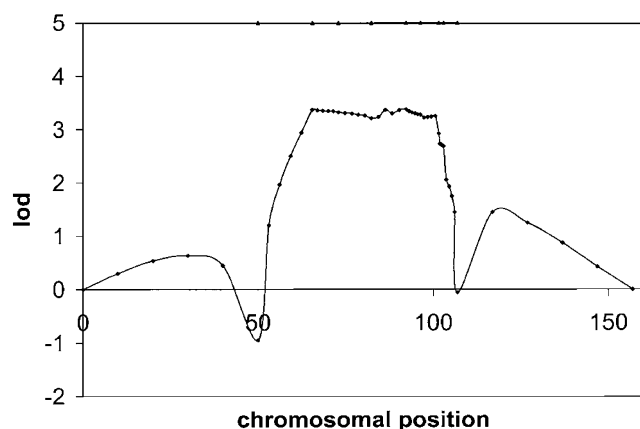
Figure 4 Results of multipoint linkage analysis between PAP-A/B and chromosome 7 markers. Triangles indicate markers used also in the two-point linkage analysis. From left to right: D7S820, D7S1799, D7S525, D7S504, D7S500, D7S495, GATA63F08, D7S2513, D7S661.

the PAP and syndactyly phenotypes are genetically homogeneous and the region would be about 3.7 cM. If so, four individuals with PAP-A/B only are nonpenetrant for syndactyly. In addition, VI-6 and VI-9 with syndactyly only do not show penetrance for the polydactyly phenotype. Moreover, the mother, the maternal grandfather and the great-grandfather of VI-9 are nonpenetrant for both PAP and syndactyly. The PAP phenotype is highly penetrant in offspring of II-1 and II-2. Nonpenetrance of this phenotype in three successive generations in offspring of II-5 and II-6 is an unlikely occurrence.

Besides, the syndactyly phenotype of VI-6 could be a variable expression of the syndactyly phenotype in this branch of the family, with a different locus than that harbouring the gene mutation causing syndactyly of VI-9, taking the relatively high incidence of syndactyly IDS 2–3 of feet in the normal population into account.

Alternatively and more likely, the PAP and syndactyly phenotypes are genetically heterogeneous in our family. In that case, the critical region is defined by the haplotype shared by patients with PAP-A/B only (Figure 1). If so, VI-6 is the only case of nonpenetrance for the PAP phenotype in this family. This is in accordance with rates of penetrance for PAP from other families reported in the literature.[4,8,14,16] Penetrance is probably determined by modifier genes, analogous to the observation of Fawcett *et al*.[17] They described CRABPII knockout mice with an extra bone of digit V and found that the penetrance of this phenotype varies according to their genetic background.[17]

We hypothesize that genetic background also affects the phenotypic expression of PAP-A and PAP-A/B. There are two affected in our family with PAP-A/B (V-15, VI-8 (Figure 2)). PAP types A and B in one individual has been described before.[8,14,18–20] Radhakrishna *et al*.[8] suggested

that GLI3 mutations cause PAP-A. In individuals with PAP-A/B, these mutations and environmental or stochastic factors could cause the PAP-B phenotype. In our view, it is difficult to envisage a major impact of the same environmental factor on patterning causing PAPA/B in V-15 and VI-8 in different generations excluding a genetic susceptibility for such a factor, although a stochastic factor could cause the phenotypic expression of PAP-A/B in V-15 and VI-8.

We identified the fourth locus containing one or more genes involved in the PAP and syndactyly phenotype. This is based on our results of the genome scan, the linkage and haplotype analyses. The maximum size of the critical region between D7S820 and GATA63F08 is about 50 cM. This chromosome 7 region excludes genes previously known to be involved in the polydactyly phenotype like the sonic hedgehog- and GLI3 genes. We looked for candidate genes and we found many genes with unknown functions which could be potential candidates, genes coding for zinc-finger containing proteins from which members are known to be involved in patterning in embryonic development, and two members of the Wnt gene family. Wnt7a, located elsewhere, is shown to be involved in dorsal–ventral patterning in the developing limb.[21] Wnt2 (MIM147870) and two variants of Wnt16 (MIM606267) are located on 7q31. There are no data available on expression in limb since this is relatively rarely looked for. Besides, we found a Wnt receptor frizzled homolog1 Drosophila (FZD1), mapped on 7q21. Frizzled1 (fz1) has distinct D–V domains of expression in mesenchyme of developing footpads.[22] Given the large number of candidate genes and the fact that none of these is an obvious candidate, we did not perform mutation analysis in our family. At this point we are pursuing other families with PAP linked to this region in order to be able to narrow down the region containing the gene associated with PAP and syndactyly in this family.

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