RESEARCH LETTER

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A Susceptibility Locus on 1p32–1p34 for Congenital Macrostomia in a Chinese Family and Identification of a Novel *PTCH2* Mutation

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TO THE EDITOR:

Macrostomia, (horizontal facial cleft, lateral facial cleft, transverse facial cleft) is a rare congenital malformation. On occasion, it can be a sign in certain syndromes, such as Goldenhar syndrome or Treacher–Collin syndrome [McCarthy and West, 1977]. Rarely, it occurs as an isolated malformation. The cause has remained unknown.

We studied autosomal dominantly inherited macrosomia in a large family of Chinese Han origin (Fig. 1A and Table I). Linkage and haplotype analysis identified the macrostomia-associated locus between markers D1S193 and D1S2652 on 1p32–1p34; the maximum LOD score at D1S2797 was 4.18 ($\theta = 0.00$, Table II). At this locus, we identified a heterozygous mutation in exon 11 (NC_000001.9) of *PTCH2* (1423G → A, resulting in Val471Ile in the 4th transmembrane domain) (NP_003729) in all patients with macrosomia (Fig. 1B). No mutation was found in family members who lacked macrosomia and we found no *PTCH2* mutations in 520 unrelated controls.

Although over-expression of SMO induced GLI-dependent luciferase activity, co-expression of wild-type PTCH2 inhibited SMO-induced activity significantly, confirming the inhibitory function of PTCH2 in the SHH signaling network (Fig. 1C). In contrast, co-expression of PTCH2 Val471Ile did not inhibit SMO-induced luciferase activity. Over-expression of PTCH2 significantly inhibited cell proliferation, but over-expression of PTCH2 Val471Ile did not appear to have a major effect on the cell growth rate (Fig. 1D). Western blot analysis confirmed that the expression level of PTCH2 and PTCH2 Val471Ile is similar (Fig. 1E).

The hedgehog signaling network has been reviewed extensively elsewhere [Cohen, 2003]. *PTCH2* encodes a 1204-amino acid transmembrane protein with about a 54% overall identity to *PTCH1* [Motoyama et al., 1998; Smyth et al., 1999] and a 90% identity to *Ptch2* in mice [Carpenter et al., 1998]. In the absence of Sonic Hedgehog (SHH) stimulation, PTCH2, like PTCH1, can maintain the hedgehog signaling pathway in an inactive state by inhibiting SMO. When SHH binds to PTCH2, the inhibitory effect of PTCH2 on SMO is removed, resulting in activation of SMO, downstream signaling, and upregulation of target genes, such as the GLI family proteins [Hahn et al., 1996; Young et al., 2000; Chen et al., 2002; Vokes and McMahon, 2004; Hutchin et al., 2005]. SHH

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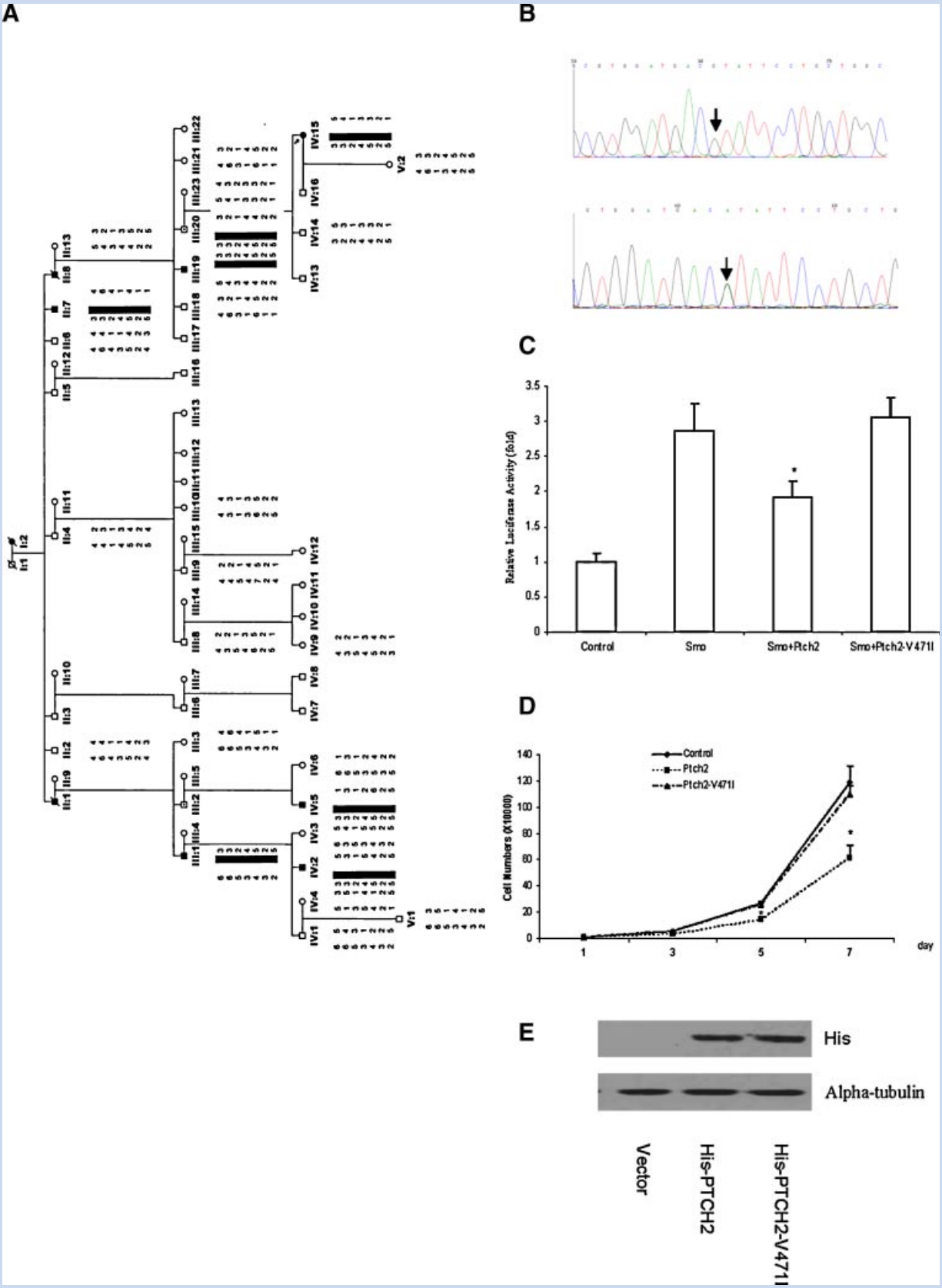
A
B
C
Relative Luciferase Activity (fold)
3.5
3
2.5
2
1.5
1
0.5
0
Control
Smo
Smo+Ptch2
Smo+Ptch2-V471I
D
Control
Ptch2
Ptch2-V471I
Cell Numbers (X10000)
140
120
100
80
60
40
20
0
1
3
5
7
day
E
His
Alpha-tubulin
Vector
His-PTCH2
His-PTCH2-V471I

TABLE I. Clinical and Genetic Data for the Chinese Macrostomia Family

Pedigree No.[a]	Age	Sex	Macrostomia[b]	V471I mutation	Evaluation	Accessory fagi	Other malformations
I:2	Dead	Female	Yes	Unknown	History	Unknown	Unknown
II:1	Dead	Male	Yes	Unknown	History	Unknown	Unknown
II:7	67	Male	Yes	Yes	Clin. exam	Yes	No
II:8	Dead	Male	Yes	Unknown	History	Unknown	Unknown
III:1	65	Male	Yes	Yes	Clin. exam	Yes	Hypoplasia of mandible
III:2	62	Male	No	Yes	Clin. exam	No	No
III:19	35	Male	Yes	Yes	Clin. exam	Yes	Hypoplasia of mandible
III:20	65	Male	No	Yes	Clin. exam	No	No
IV:2	28	Male	Yes	Yes	Clin. exam	Yes	No
IV:5	31	Male	Yes	Yes	Clin. exam	Yes	Hypoplasia of mandible
IV:15	28	Female	Yes	Yes	Clin. exam	Yes	Hypoplasia of mandible

[a]Designation of individual corresponds to Figure 1.
[b]The distance from the angle of cleft to middle line was 46 5 mm (n = 7). The distance from contralateral normal mouth angle to the middle line was 30 3 mm (n = 5). Clin. Exam, clinical examination.

TABLE II. LOD Score of Markers Between D1S2722 and D1S2742

Marker	$\theta = 0.0$	$\theta = 0.1$	$\theta = 0.2$	$\theta = 0.3$	$\theta = 0.4$	$\theta = 0.5$
D1S2722	−3.70	−0.89	−0.25	−0.03	−0.01	0.00
D1S193	3.08	2.57	1.95	1.25	0.52	0.00
D1S2733	2.50	2.08	1.55	0.96	0.35	0.00
D1S2797	4.18	3.43	2.60	1.68	0.69	0.00
D1S197	2.82	2.34	1.77	1.14	0.48	0.00
D1S2661	0.26	0.40	0.35	0.22	0.07	0.00
D1S417	2.47	2.05	1.56	1.01	0.43	0.00
D1S2652	1.09	1.83	1.56	1.08	0.49	0.00
D1S200	−4.82	0.12	0.28	0.20	0.06	0.00
D1S2742	−10.73	−1.22	−0.47	−0.16	−0.06	0.00

FIG. 1. Pedigree of the macrostomia family, *PCTH2* heterozygous mutation and PTCH2-V471 lacks inhibitory activity. A: In the pedigree diagram of the macrostomia family, the arrow indicates the proband of this family, ■ indicates patients with macrostomia, ⊡ indicates non-penetrant heterozygotes, □ indicates the normal family members. All patients with macrostomia and non-penetrant heterozygotes had the same haplotype of genetic markers from D1S193 to D1S2652 (black bar). There was no recombination in this chromosome region. B: Exon 11 sequencing results of gene *PTCH2*. Upper: the results of a normal control; (lower) the result of the patient in this family. The arrowhead indicates the heterozygous 1423G>A mutation. C: Mutant PTCH2-V471I proteins did not inhibit SMO signaling. C3H10T1/2 cells were transiently co-transfected with the GLI1 luciferase reporter and the expression vectors for SMO and PTCH2 or PTCH2-V471I. Luciferase activity was measured 48 hr after transfection. Results are expressed as the mean standard deviation from three separate experiments. The t-test was used to assess the significance. Luciferase activity in SMO+PTCH2-V471I cells was significantly higher than that in SMO+PTCH2 cells (*$P < 0.05$). D: PTCH2-V417I did not inhibit cell proliferation. Stable cell lines expressing PTCH2, PTCH2-V417I, or containing empty vector were plated in six-well plates. Cell numbers were counted every 2 days for 1 week. The results represent average values standard deviation from three independent experiments. The *t*-test was used to assess the significance. Wells with cells expressing PTCH2-V471I had a significantly higher number of cells than those with cells expressing PTCH2 (*$P < 0.05$). E: The expression of empty vector, PTCH2 and PTCH2-V417I was confirmed by Western blot analysis using anti-His-tag monoclonal antibodies. As an internal control, the membrane was stripped and re-probed with anti-α-tubulin. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mutations and inhibitors, such as cyclopamine and jervine cause holoprosencephaly [Belloni et al., 1996; Chen et al., 2002; Cordero et al., 2004]. *PTCH2* loss-of-function mutations are associated with basal cell carcinomas and medulloblastoma [Smyth et al., 1999; Cohen, 2003]. Recently, our group identified a *PTCH2* germline mutation in the nevoid basal cell carcinoma syndrome in a Chinese family [Fan et al., 2008].

In conclusion, our results suggest that the novel germline *PTCH2* mutation (1423G → A, resulting in Val471Ile) mutation may be associated with macrostomia.

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