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Mouse Disp1 is required in sonic hedgehog-expressing cells for paracrine activity of the cholesterol-modified ligand

Hua Tian, Juhee Jeong, Brian D. Harfe, Clifford J. Tabin and Andrew P. McMahon

There was a reanalysis of data required to support the findings in *Development* **132**, 133-142.

We have repeated the facial analysis reported in Fig. 2 to provide the data required to support some of the original findings of this study (see Publisher's note). Our findings substantiate the original conclusions drawn from Fig. 2 of a dose-related genetic interaction between Disp1 and *Shh* alleles, and of the function of *Disp1* within Shh-producing cells. Some differences are reported below, which might reflect slight differences in embryonic staging or increased sensitivity of the whole-mount in situ hybridization procedure here. Importantly, they do not alter the key conclusion that reducing *Disp1* levels in Shh-producing cells results in a phenotype similar to that of genetically matched embryos with reduced *Disp1* activity throughout the embryo. These data support the overall conclusions of the paper, and, together with other data in the same report, support a model in which the principal requirement for Disp1 activity is in Shh-producing cells.

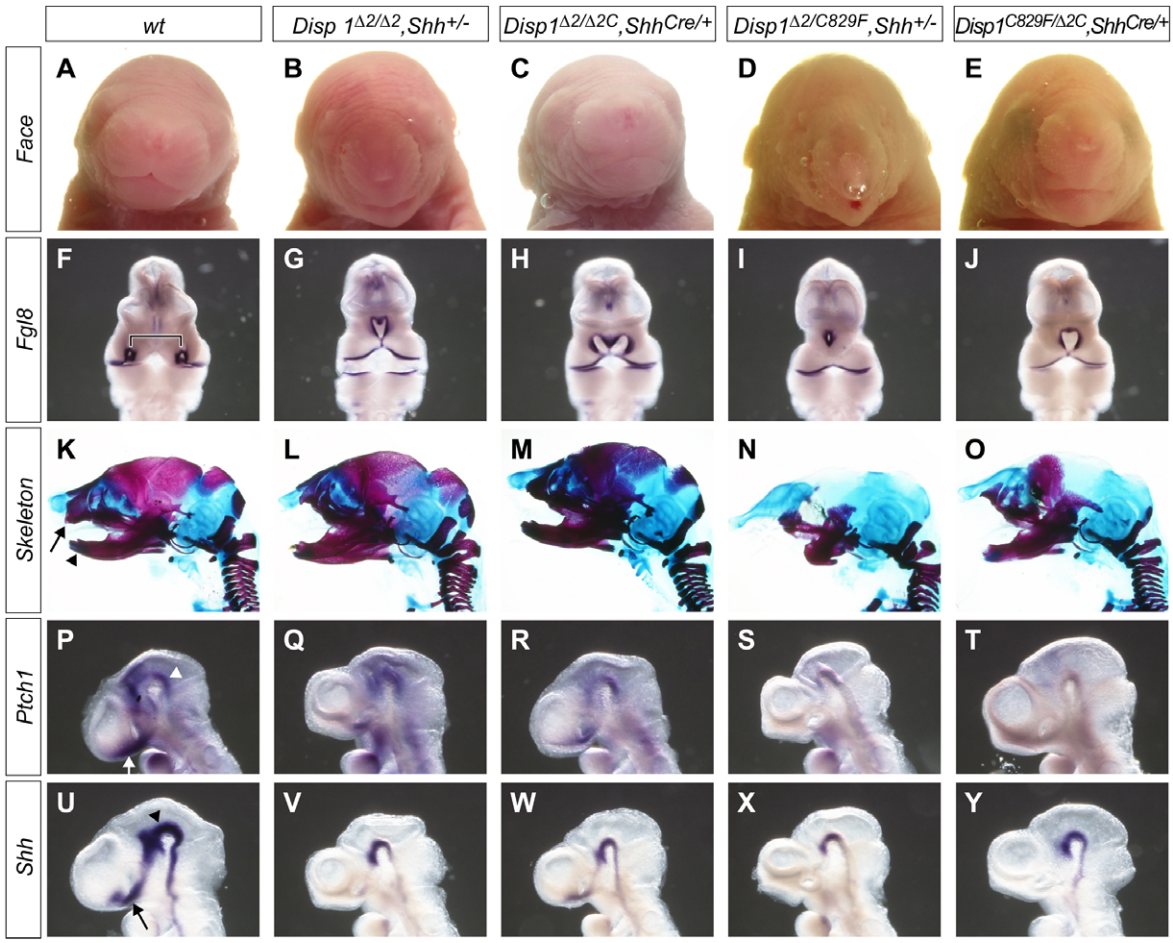

Fig. 2. Attenuating *Disp1* activity specifically in Shh-producing cells phenocopies *Disp1* hypomorphic mutants. Attenuating *Disp1* activity specifically in Shh-producing cells produced facial phenotypes that resembled genetically matched embryos with *Disp1* reduction throughout the embryo. (**A-E**) External facial views of E18.5 embryos with the indicated genotypes. (**F-J**) Whole-mount in situ hybridization with *Fgf8* probes to E10.5 embryos of the indicated genotypes. The separation of *Fgf8* expression domains in the frontal nasal processes of wild-type embryos (bracket in F) reflects the normal development of midline structures that were lost to varying degrees in embryos with attenuated *Disp1* and *Shh* activity (G-J). (**K-O**) Alcian Blue (non-mineralized cartilage)-stained and Alizarin Red (mineralized cartilage and bone)-stained head skeletal preparations of E18.5 embryos with indicated genotypes. A variable loss was observed in both upper (arrow in K) and lower (arrowhead in K) jaw structures, including the midline incisors. (**P-Y**) Whole-mount in situ hybridization with (P-T) *Ptch1* and (U-Y) *Shh* probes to E9.5 embryos of the indicated genotypes. *Ptch1* and *Shh* expression was evident in midline cell populations rostral to the optic lobes in wild-type embryos (arrows in P and U). Their expression was either markedly reduced or lost, depending on the specific combination of *Disp1* and *Shh* alleles (Q-T and V-Y). *Ptch1* and *Shh* expression was detected in the midbrain region (indicated by arrowheads in P and U) of all genotypes, albeit at reduced levels.

The following text replaces the paragraph running from p. 135 to p. 137 ('In both genotypes…in the *Disp1* mutant background.')

Disp1$^{\Delta 2/\Delta 2C}$; *Shh*$^{Cre/+}$ embryos in which *Disp1* activity was specifically knocked down in Shh-producing cells have a facial phenotype with a narrowing of the face and reduction of the premaxilla. However, the length of the snout is similar to wild type and the mandibular incisors are not fused (Fig. 2A,C,K,M). Thus, the phenotype is, as expected, generally less severe than that of the *Disp1*$^{\Delta 2/\Delta 2}$; *Shh*$^{+/-}$ embryos (Fig. 2B,L). The severity of the conditional phenotype is enhanced when *Disp1* activity is further lowered in *Disp1*$^{\Delta 2C/C829F}$; *Shh*$^{Cre/+}$ mice (Fig. 2E,O) but the phenotype is slightly weaker than that in *Disp1*$^{\Delta 2/C829F}$ (data not shown) or *Disp1*$^{\Delta 2/C829F}$; *Shh*$^{+/-}$ embryos (Fig. 2D,N); the tubular nasal process was shorter and the premaxillary bone was more extensive. In a proportion of the latter, truncated fused mandibles lack incisors (Fig. 2N); however, mandibular fusion was not observed in *Disp1*$^{\Delta 2C/C829F}$; *Shh*$^{Cre/+}$ embryos. The slightly weaker facial phenotype seen at term with each of the conditional removal combinations was evident at E10.5 when the distance between the *Fgf8*-expressing frontal-nasal processes is compared by whole-mount in situ hybridization (Fig. 2F-J). Variable weak midline *Shh* expression was observed rostral to the optic stalk in *Disp1*$^{\Delta 2/\Delta 2C}$; *Shh*$^{Cre/+}$ embryos at E9.5 (Fig. 2U-W). As expected, this resulted in *Ptch1* expression in adjacent nascent facial structures (Fig. 2P-R). Small, weak domains of *Shh* and *Ptch1* expression were observed close to the midline, localized to the region of the optic stalk in *Disp1*$^{\Delta 2C/C829F}$; *Shh*$^{Cre/+}$ embryos (not readily visible in Fig. 2T,Y). Only *Disp1*$^{\Delta 2/C829F}$; *Shh*$^{+/-}$ embryos, the strongest genetic combination, completely lacked *Shh* and *Ptch1* expression rostral to the diencephalon (Fig. 2S,X).

The authors apologise to readers for any inconvenience caused by the requirement to replicate these data and thank Mary Duah and Jill McMahon for replicating the experiments, Joe Vaughan and Celia Shneider for help with data acquisition, Renate Hellmiss for generating the figures and the journal *Development* for encouraging the reanalysis.

Publisher's note

Re: **Tian, H., Jeong, J., Harfe, B. D., Tabin, C. J. and McMahon, A. P.** (2005a). Mouse Disp1 is required in sonic hedgehog-expressing cells for paracrine activity of the cholesterol-modified ligand. *Development* **132**, 133-142.

In 2005, the McMahon laboratory reported that a re-examination of two papers published by their group in *Development* (Tian et al., 2004; Tian et al., 2005a) had revealed a duplication of Dr Tian's data in these papers. Following their analysis, the authors announced, with regret, that they must retract Tian et al. (2004), and this retraction was published by *Development* in November 2005, along with their apology to the editors and readership of the journal (Tian et al., 2005b). With respect to the second paper (Tian et al., 2005a), the authors' review, overseen by the Committee on Professional Conduct (CPC) for the Faculty of Arts and Sciences at Harvard University, found that the principal conclusions of the paper were supported by appropriate documentation but that the documentation for Fig. 2 was inadequate, requiring a replication of those data. The replicated data have been reviewed by *Development* and are published in this Corrigendum. *Development* and its publishers take very seriously issues relating to the authenticity of data. We acknowledge the dedication and openness of the McMahon laboratory in this matter, and the contributions of additional members of the laboratory, not on the paper's authorship list, in repeating these extensive experiments and data analyses for the Corrigendum.

References

Tian, H., Tenzen, T. and McMahon, A. P. (2004). Dose dependency of *Disp1* and genetic interaction between *Disp1* and other hedgehog signaling components in the mouse. *Development* **131**, 4021-4033.

Tian, H., Jeong, J., Harfe, B. D., Tabin, C. J. and McMahon, A. P. (2005a). Mouse Disp1 is required in sonic hedgehog-expressing cells for paracrine activity of the cholesterol-modified ligand. *Development* **132**, 133-142.

Tian, H., Tenzen, T. and McMahon, A. P. (2005b). Retraction: Dose dependency of *Disp1* and genetic interaction between *Disp1* and other hedgehog signaling components in the mouse. *Development* **132**, 5615.