## *Authors' response* to "Comment on: 'Homozygous knockout of the *piezo1* gene in the zebrafish is not associated with anemia'"

We welcome the Comment of Faucherre et al.[1] in response to our correspondence[2] concerning their report of hemolytic anemia in zebrafish embryos subjected to morpholino knockdown of piezo1.[3] We note, however, that in their Comment, Faucherre et al. neither dispute nor question our descriptions or analyses[2] of their paper on piezo1 morpholino knockdown.[3] Nor do they question any of our data showing the absence of detectable anemia in zebrafish red cells from piezo1$^{-/-}$ germline knockout embryos and adults,[2] a phenotype quite different from that reported in their morpholino knockdown studies.[3] The rationale of Faucherre et al. for contributing their Comment seems rather to be based on the following contentions.

1. Faucherre et al. state that an article by Rossi et al.[4] "has added a new angle to this debate" [concerning phenotypic differences (between morpholino knockdowns and germline knockouts in zebrafish)], suggesting we did not discuss it. However, we note that this very paper was, indeed, discussed and cited in our correspondence.[2]

2. Faucherre et al. state that this same new angle "may have led to an oversight by Shmukler et al.," but Faucherre et al. do not elaborate on the nature of the oversight. They note only that our correspondence "do[es] not explain why there appears to be such a large discrepancy between these [mouse and zebrafish] phenotypes". Indeed, neither we nor Faucherre et al. can further explain this difference in a brief exchange of correspondence. As was true for Rossi et al.[4] and for Kok et al.[5], the explanation requires directed experimentation for each individual gene for which knockdown and knockout phenotypes differ (a situation describing as many as 80% of zebrafish genes).[5]

3. Faucherre et al. further state that "the lack of any phenotype in the piezo1 KO zebrafish line has possibly led the authors to misinterpret [the data of] Cahalan et al." We cited[2] the data of Cahalan et al. as they were published.[6] We pointed out that the mild hemolytic anemia of the mouse with red blood cells deficient in PIEZO1 was fully compensated, and that the normal hematocrit and red cell number thus resembled the normal hematologic phenotype of piezo1$^{-/-}$ zebrafish. In their Comment, Faucherre et al. choose to emphasize the qualitative similarity between the mild hemolysis and red cell volume increase of the mouse and the apparently severe hemolysis and red cell volume increase in the morpholino knockdown zebrafish. Each interpretation, although divergent, is legitimate; neither is a "misinterpretation".

4. Faucherre et al. contend that "the piezo1 KO zebrafish line is a global KO of piezo1 and should first be compared to the mouse global KO of piezo1 not the erythrocyte-specific [conditional] KO line." We respond without qualification that phenotypic comparison of zebrafish morpholino knockdowns with the corresponding germline knockouts needs no justification.

Faucherre et al. further note in their Comment that the "zebrafish piezo1 KO line .... appears to all intents and purposes to have no observable phenotype"[in contrast to the embryonic lethal vascular phenotype in KO mice]. We agree, and note in addition that recently described patients with autosomal recessive lymphatic dysplasia secondary to homozygous loss-of-function mutations in human PIEZO1 are remarkable for normal vasculature and an asymptomatic, fully compensated, very mild hemolytic state of incomplete penetrance.[7,8]

The concern of Faucherre et al. about these contrasting phenotypes associated with PIEZO1 loss-of-function only serves to highlight their curious choice not to report the vascular phenotype of their piezo1 morpholino knockdown fish, despite their commendable use of the Lmo2:dsRed reporter line[3] (delineating vasculature as well as erythrocytes). Figure 3 from the original report by Faucherre et al. presents images of dsRed-expressing erythrocytes from morpholino-injected Lmo2:dsRed reporter line fish[3] (which of the two morpholinos used was not described but seems to be MO1, assuming conditions of their Online Supplementary Figure S5 applied also to Figures 3 and 4). The absence of comment about a vascular phenotype in the dsRed-expressing blood vessels of this reporter line suggests the likelihood of normal vasculature, as was indeed observed in germline piezo1$^{-/-}$ fish by Kok et al.[5] If so, the difference in vascular phenotype between the global knockout mouse and the PIEZO1-deficient fish would seem to be independent of whether the knockout mouse is compared to a morpholino knockdown fish or to a germline knockout fish.

*Boris E. Shmukler,[1] Nathan D. Lawson,[2] Barry H. Paw,[3] and Seth L. Alper[1]*

*[1]Division of Nephrology and Department of Medicine, Beth Israel Deaconess Medical Center, Boston; Department of Medicine, Harvard Medical School, Boston; [2]Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester; [3]Hematology Division, Department of Medicine, Brigham and Women's Hospital, Boston; Hematology-Oncology Division and Department of Medicine, Boston Children's Hospital; Departments of Medicine and Pediatrics, Harvard Medical School, Boston, MA, USA*

*Correspondence: salper@bidmc.harvard.edu*
*doi:10.3324/haematol.2015.137810*

*Key words: piezo 1, zebrafish, anemia, erythrocytes.*

*Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.*

### References

1. Faucherre A, Kissa K, Nargeot J, Mangoni ME, Jopling C. Comment on: Homozygous knockout of the piezol gene in the zebrafish is not associated with anemia. Hawmatologica 2016;101:(1):xxx
2. Shmukler BE, Huston NC, Thon JN, et al. Homozygous knockout of the piezo1 gene in the zebrafish is not associated with anemia. Haematologica. 2015 Aug 20. [Epub ahead of print]
3. Faucherre A, Kissa K, Nargeot J, Mangoni ME, Jopling C. Piezo1 plays a role in erythrocyte volume homeostasis. Haematologica. 2014;99(1):70-75.
4. Rossi A, Kontarakis Z, Gerri C, et al. Genetic compensation induced by deleterious mutations but not gene knockdowns. Nature. 2015; 524(7564):230-233.
5. Kok FO, Shin M, Ni CW, et al. Reverse genetic screening reveals poor correlation between morpholino-induced and mutant phenotypes in zebrafish. Dev Cell. 2015;32(1):97-108.
6. Cahalan SM, Lukacs V, Ranade SS, Chien S, Bandell M, Patapoutian A. Piezo1 links mechanical forces to red blood cell volume. eLife. 2015;4.
7. Fotiou E, Martin-Almedina S, Simpson MA, et al. Novel mutations in PIEZO1 cause an autosomal recessive generalized lymphatic dysplasia with non-immune hydrops fetalis. Nat Communi. 2015;6:8085.
8. Lukacs V, Mathur J, Mao R, et al. Impaired PIEZO1 function in patients with a novel autosomal recessive congenital lymphatic dysplasia. Nat Commun. 2015;6:8329.