Comment on: 'Homozygous knockout of the piezo1 gene in the zebrafish is not associated with anemia'

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We read with interest the recent letter by Shmuckler et al. 'Homozygous knockout of the piezo1 gene in the zebrafish is not associated with anemia' published in Haematologica.[1] In their letter the authors describe the characterization of a zebrafish piezo1 knockout (KO) line generated using zinc finger nucleases. Having ensured that the piezo1 mRNA had been truncated the authors then compared their piezo1 KO zebrafish with our previous results on piezo1 morphants (generated using antisense morpholinos to block mRNA translation or splicing) published as an article in Haematologica 'Piezo1 plays a role in erythrocyte volume homeostasis'.[2] In our article we reported that knockdown of piezo1 in zebrafish embryos resulted in fragile, overhydrated, spherocytic erythrocytes leading to hemolysis and anemia. In contrast, Shmuckler et al. report that they did not observe any of these defects in erythrocytes obtained from either embryonic or adult piezo1 KO zebrafish save for a slight reduction in volume as opposed to the increase in volume we observed in piezo1 morphants. The authors subsequently discuss why there is a discrepancy between our two reports and while they highlight the possibility that the piezo1 morpholinos are non-specific (although 2 different morpholinos that have the same effect specifically on erythrocytes would be highly unusual) they also acknowledge that the problem may also lie with their piezo1 KO line. Lastly, the authors compare their findings with a recent article published in eLife by Cahalan et al. entitled 'Piezo1 links mechanical forces to red blood cell volume' in which piezo1 was specifically knocked out in mouse erythrocytes.[3] They conclude that the findings of Cahalan et al. are consistent with their own observations that knocking out piezo1 has little effect on erythrocytes.

However, having carefully studied the letter by Shmuckler et al. we believe an oversight may have been made by the authors which has subsequently led to a possible misinterpretation of the data from Cahalan et al. First, we would like to offer some background information on the current morpholino/knockout controversy within the zebrafish community. At the beginning of 2015 Kok et al. published an article in Developmental Cell comparing morphant phenotypes with KO phenotypes in zebrafish.[4] The conclusion of this article was that around 80% of the published morphant phenotypes did not occur when the same genes were knocked out. They concluded that these discrepancies were most likely due to non-specific effects of morpholinos.[4] This was obviously very worrisome, with some members of the zebrafish community suggesting that all morpholino-based phenotypes must be verified by KO of the same gene. However, an article was published later this year in Nature by Rossi et al. which has added a new angle to this debate.[5] In their article the authors describe how knockdown of a particular gene using morpholinos leads to a specific vascular phenotype in zebrafish embryos. However, when they generated the KO line they did not observe this phenotype. By comparing the KO and morphant proteomes and transcriptomes they were able to determine that a compensatory response had been activated in the KO line that was absent from the morphants. In particular they found a number of genes were upregulated in the KO line which effectively rescued the phenotype observed in morphants. This argues that just because a KO zebrafish line fails to produce the same phenotype as a morphant it cannot be immediately concluded that the morphant is at fault.

This phenomenon may also have led to an oversight by Shmuckler et al. In their letter the authors conclude that the lack of any observable erythrocyte phenotype in their piezo1 KO zebrafish line is in agreement with the mouse erythrocyte specific piezo1 KO phenotype described by Cahalan et al. However, 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 KO line. Complete KO of piezo1 in mice, as Shmuckler et al. indicate, results in early embryonic lethality associated with defective vasculogenesis. This phenotype is very different from the zebrafish piezo1 KO line which appears to all intents and purposes to have no observable phenotype. The authors do not explain why there appears to be such a large discrepancy between these phenotypes. We suggest at least two possibilities, firstly, piezo1 could be completely dispensable in zebrafish or a compensatory mechanism has been triggered in the zebrafish piezo1 KO line, similar to the observations made by Rossi et al. Furthermore, we believe that the lack of any phenotype in the piezo1 KO zebrafish line has possibly led the authors to misinterpret the data from Cahalan et al. Cahalan et al. showed that erythrocyte-specific KO of piezo1 in mice leads to overhydrated, fragile erythrocytes which undergo intravascular hemolysis. This would appear to be more in line with our own observations of piezo1 morphant zebrafish. In fact Shmuckler et al. described that the only measurable phenotype in the zebrafish piezo1 KO line is a slight reduction in erythrocyte volume which appears to be at odds with the data from Cahalan et al. Although we agree the anemic phenotype we observe in piezo1 morphants is more pronounced than that of the mouse phenotype it would appear that the actual mechanism by which piezo1 regulates erythrocyte volume is conserved.

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