Review of: "Pseudoautosomal gene SHOX exhibits sex-biased random monoallelic expression and contributes to sex difference in height"

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Pseudoautosomal gene SHOX exhibits sex-biased random monoallelic expression and contributes to sex difference in height

Hattori et al.

Summary

The authors used a combination of approaches (expression microarray and qPCR for transcription analysis, and bisulfite sequencing for methylation analysis) and various materials (adult and child cartilage, cultured chondrocytes, clones of single fibroblasts) to study the expression profile of the pseudoautosomal *SHOX* gene in human males and females. They show sex-biased expression of *SHOX* and other PAR1 genes with lower expression in females compared to males. Furthermore, they show that in female fibroblasts, *SHOX* has random clonal monoallelic expression which is independent from X chromosome inactivation (XCI). The authors suggest that gender differences in *SHOX* epigenetic regulation and expression may be responsible for growth differences of human males and females.

Comments

- This is an interesting study with novel findings regarding *SHOX* random XCI-independent monoallelic expression in female fibroblasts. Though, as the monoallelic expression was random and found only in cultured adult fibroblasts, this provides limited information about teh regulation of *SHOX* and other PAR1 genes during the actual development.
- From another hand, if *SHOX*, indeed, undergoes such a complex regulation pattern in females, this somehow contradicts with the pronounced short stature phenotype due to *SHOX* haploinsufficiency in Turner syndrome patients. Based on the findings of this study, I would expect a milder phenotypic effect of *SHOX* haploinsufficiency. Could the authors comment on this, please?
- The authors mention that their results regarding lower expression of PAR1 genes in females compared to males is in line with the findings by Tukiainen et al. (2017). Indeed, this applies for the majority of PAR1 genes except for *SHOX* and *ASMT* for which very limited expression information was obtained. For example, Tukiainen et al. (2017) Figure 2a shows that out of the 29 tissues studied, information for

SHOX expression was available for just 5 tissues. I suggest the authors to reword their text accordingly.

- In the context of their findings, the authors should reference earlier studies suggesting that the Y chromosome may carry gene(s) for growth, though with no convincing evidence. For example, Kirsch et al. 2004.
- I think that the authors present a biased interpretation of reference 2 (Naqvi et al. 2019) and give too much credit to *SHOX* regarding human height. Height is a quantitative trait influenced by multiple genes.
- In Discussion page 8 the authors write "..the lack of *SHOX* orthologs in rodents and several other species...". This is true about murine rodents but I am not sure about 'several other species". For example, *SHOX* is present in all domestic species. Also, there is an autosomal paralog *SHOX2*. Rodent *Shox2*, for example, likely compensates the function of the missing X-linked gene being highly expressed in the developing limb. Thus, the authors' statement is misleading.
- Intriguingly, human *SHOX* and *SHOX2* are both highly expressed in fat cells and have low expression in other adult tissues. Thus, maybe the choice if tissues in this study was not the best? The authors should at least discuss this, as well as the possible role of *SHOX* and other PAR1 genes during development as presented by Bellott et al. 2014