No mutations in the serotonin related \textit{TPH1} and \textit{HTR1B} genes in patients with monogenic sclerosing bone disorders

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\textbf{Introduction}

\textbf{Craniotubular hyperostosis:}
- Group of rare monogenic sclerosing bone disorders
- \uparrow bone mass: skull and tubular bones mainly affected
- Autosomal dominant disorders
  \rightarrow Mutations in the first \beta-propeller domain of LRP5
- Autosomal recessive disorders
  \rightarrow Mutations in \textit{SOST} or \textit{LRP4}

\textit{LRP5} \rightarrow regulates bone formation
- \textbf{Direct effect}: canonical Wnt signaling together with \textit{LRP4} and \textit{SOST}
- \textbf{Indirect effect}: regulating serotonin synthesis in the gut

\textbf{Serotonin dependent regulation of bone mass:}
- \textit{TPH1}: tryptophan hydroxylase 1
  \- Rate limiting enzyme for the serotonin synthesis
  \- \textit{Lrp5\textsuperscript{-/-}} mouse
    \- \downarrow bone mass and bone formation
    \- \uparrow tph1 expression
  \- \textit{Tph1\textsuperscript{-/-}} mouse:
    \- \downarrow bone mass and bone formation
    \- \downarrow serotonin level

- \textit{HTR1B}: 5-hydroxytryptamine receptor 1B
  \- Serotonin receptor on the osteoblast
  \- \textit{Htr1b\textsuperscript{-/-}} mouse:
    \- \downarrow bone mass and bone formation
    \- \downarrow serotonin level

\textbf{Material & Methods}

\textbf{Patients:}
- 53 patients diagnosed with some form of craniotubular hyperostosis
- No mutations in the known causative genes: \textit{LRP5}, \textit{LRP4} and \textit{SOST}

\textbf{Methods:}
- Direct sequencing of all coding exons and intron/exon boundaries of \textit{HTR1B} and \textit{TPH1}
  \- \textit{HTR1B}: 4 amplicons
  \- \textit{TPH1}: 11 amplicons

\textbf{Results}

\textbf{TPH1:}
- 5 variants found in our patient cohort:
  \- 4 known polymorphisms
  \- 1 unknown heterozygous variation: \textit{IVS1-36C>T}
    \- French woman with sclerosteosis
    \- Prediction programs (Spliceport and Netgene2)
      \rightarrow no effect on splicing

\textbf{HTR1B:}
- 9 known polymorphisms reported in the patient cohort
  \- 4 coding SNPs \rightarrow 2 rare non-synonymous SNPs (heterozygous)
    \- \textit{rs130060}
    \- \textit{rs150030508} Both SNPs are found in a Columbian boy diagnosed with sclerosteosis
      \rightarrow Compound heterozygous

\textbf{Sclerosteosis}
- Autosomal recessive disorder
- \uparrow bone mass of the skull and tubular bones
- Syndactyly

- \textit{Rs130060} (p.Phe124Cys\textsuperscript{-/-})
  \- Prediction programs: benign (Polyphen, polyphen2, mutPred, Sift)
  \- Previously reported homozygous and heterozygous
    \rightarrow not likely to be disease causing

- \textit{Rs150030508} (p.Ile225Thr\textsuperscript{-/-})
  \- Prediction programs: possibly damaging (Polyphen, polyphen2, mutPred, Sift)
  \- Sclerosteosis \rightarrow autosomal recessive
    \textit{Htr1b\textsuperscript{-/-}} \& \textit{htr1b\textsuperscript{-/-}} mice \rightarrow \uparrow bone mass, no syndactyly
    \rightarrow not likely to be the disease causing variant

\textbf{Discussion & Conclusion}

A few years ago, Yadav and colleagues suggested that \textit{LRP5} regulates bone formation not only via the canonical Wnt signaling but also via the regulation of the serotonin production in the gut (Yadav et al, 2008, Cell). In order to increase the knowledge on the involvement of serotonin in the regulation of bone formation, we screened two key proteins, \textit{TPH1} and \textit{HTR1B}, for mutations in the coding regions of the genes in patients diagnosed with several forms of sclerosing bone disorders. Unfortunately, we were not able to identify disease causing mutations in these genes. Therefore, we could not increase the insights of the role of serotonin in the regulation of bone formation by osteoblasts.