ALPL is the gene coding the liver/bone/kidney alkaline phosphatase, ALPL, also known as tissue non-specific phosphatase (TNAP). This enzyme removes phosphate groups from various substrates and its function is essential for bone and tooth mineralization. In humans, mutations of ALPL are known to cause hypophosphatasia, a genetic disorder characterized by defective bone and/or tooth mineralization. Severe forms of the disease are autosomal recessive and can be fatal as resulting in an unmineralized skeleton. Milder forms are transmitted in either a dominant or a recessive way. So far, 254 mutations in ALPL leading to hypophosphatasia have been described in humans, 193 of which being missense mutations. Since a few years molecular evolutionary analysis of various proteins proved to be an efficient method, which allows to highlight residues and motifs that are important for the protein function and, as a consequence, to validate and/or predict sensitive positions for genetic disease. We performed such analysis using mammalian ALPL. We obtained the ALPL sequences of 42 species representative of the main mammalian lineages, and performed the analysis in order to determine sensitive positions defined as being unchanged during 220 Millions years of mammalian evolution. These positions were indicated on the human sequence, as well as the amino acids substituted by residues belonging to the same group. The residues affected by missense mutations leading to hypophosphatasia in humans were then compared to our results. Our evolutionary analysis shows that a large number of positions in the ALPL sequence are under selective pressure (399 positions out of 524), i.e., indicating their importance for the right function of the enzyme. Most (91%) of human missense mutations identified as responsible for hypophosphatasia are located on these sensitive positions. The few mutations (9%) that were not predicted by our model are discussed at the light of their involvement in the 3D structure of the protein. Many clusters of conserved positions correspond to protein regions of known function (active sites, homodimeric interface,…). For other regions the role of conserved positions is unknown, but certainly important for either the structure and/or biological function of ALPL. Furthermore, our analysis enables to predict that any novel substitution occurring on an evolutionary-conserved position will lead to hypophosphatasia. By contrast with time-consuming and expansive functional tests, our evolutionary analysis allows to either validate or invalidate at low cost any ALPL mutation, which would be suspected to be responsible for hypophosphatasia.