107/A

Modeling and in silico analysis of the impact of eight missense mutations on human growth hormone activity using a novel approach

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Human growth hormone (hGH) plays a determinant role in growth and development of the human body during early stages of life. Harmful missense mutations of GH1 gene can give rise to a deficient hGH which is a recognized cause of short stature. It is crucial to understand the underlying molecular mechanisms to assign pathogenicity to missense mutations. However, most reported mutations of GH1 lack annotations. Not all missense mutations are deleterious; some are neutral; hence in vitro assays to initially characterize them would be a waste of resources while computational/theoretical approaches provide relatively accurate information readily with lesser cost. Here we performed a comprehensive analysis on eight missense mutations of GH1 (D112G, C53F, C53S, R77C, R183H, R16C, R16L, C189Y) to examine their impact on hGH activity using in silico analysis tools.

In this study, we developed a "successive relaxations" approach, starting from a zero-speculation initial model and incrementally adding more information in successive steps. X-ray structures were used to build homology models of hGH mutants using MODELLER and refined by molecular dynamics refinement protocol. UCSF Chimera was used for pre-processing, visualization and analysis. Our approach was validated by modeling an experimentally known mutant (G120R). L9P, M14S, V110I inert mutants were used as reference controls.

Comparing each mutant with the wild type and putative controls, we suggested a decrease in receptor activation by hGH_{D112G}. The effectiveness of signal transduction was affected due to changes in the interaction with the receptors by hGH_{C53F} due to its decreased stability; $hGH_{R77C,}$ by modifying the orientation of its receptor interaction; hGH_{R16L} and $hGH_{C189Y,}$ by weakening the interaction. hGH_{C53S} likely resulted in a bio-inactive complex. Effect of hGH_{R183H} and hGH_{R16C} on hGH bioactivity was predicted to be small and similar to wild type hGH. This is the first in-depth modeling study of hGH mutations that has included controls to evaluate the results, that looks at the potential effects of mutations in bound/unbound confirmations including the interactions with the receptor and within receptor subunits. Our approach produced plausible explanations in good agreement with experiment in all cases where data was available, thus supporting the applicability of this approach to understanding mutations of hGH.

Keywords: missense mutations, in silico analysis tools, human growth hormone

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