Enzymatic Activity of the K173Q Polymorphism of NPP1: Kinetic Analysis for Assessing Implications in Disease

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K173Q is a polymorphism of ecto-nucleotide pyrophosphatase/ phosphodiesterase 1 (NPP1), an enzyme which inhibits bone mineralization and ectopic calcification by hydrolyzing ATP to produce pyrophosphate (PPi). Inactivating variants of NPP1 result in deadly hypercalcification disorders. K173Q introduces a single-nucleotide polymorphism to the SMB2 domain and has been implicated in increased vascular calcification in patients of renal disease, suggesting its role as an inactivating variant. However, enzymatic activity of K173Q has not yet been verified. This study sought to (1) determine and compare the relative steady state kinetic efficiencies of the wild-type protein, NPP1-WT, and the variant NPP1-K173Q and (2) connect this kinetic analysis to the K173Q pathogenicity. NPP1-WT and NPP1-K173Q were purified through transient transfection in HEK293 cells, and HPLC was performed to quantify enzyme efficiency. NPP1-WT exhibited a kcat of 55±7 min-1 enzyme-1 compared to 59±10 min-1 enzyme-1 for NPP1-K173Q, suggesting the polymorphism is nearly equally as active as NPP1-WT (p=0.779). K173Q does not appear to inactivate the NPP1 enzyme as previously suggested, clarifying that non-catalysis interactions of an NPP1 variant may contribute to pathogenicity, which significantly broadens the implications of NPP1 in disease. Future studies should focus on elucidating mechanisms of K173Q-induced vascular calcification in renal disease to develop novel treatments for K173Q pathogenicity.