Hydroxyprolinemia is an inborn error of amino acid degradation that is considered a non-disease. Known for more than 50 years, its genetic cause and prevalence have remained unclear. In MS/MS newborn screening, the mass spectrum of hydroxyproline cannot be differentiated from isoleucine and leucine causing false positive newborn screening test results for maple syrup urine disease (MSUD). We studied two siblings with hydroxyprolinemia via exome sequencing and confirmed the candidate gene in five further individuals with hydroxyprolinemia, who were all characterized biochemically and clinically. The prevalence was calculated based on the number of individuals with hydroxyprolinemia detected via MS/MS newborn screening at our centre from 2003 to 2014. In six cases, we identified homozygous or compound heterozygous mutations in PRODH2 as the underlying genetic cause of hydroxyprolinemia. One individual was heterozygous for a deletion in PRODH2 and had an intermittent biochemical phenotype with partial normalization of hydroxyproline concentrations. In one further individual with persistent hydroxyprolinemia no mutation in PRODH2 was found, raising the possibility of another defect of hydroxyproline degradation yet to be
identified as the underlying cause of hydroxyprolinemia. Plasma hydroxyproline concentrations were clearly elevated in all individuals with biallelic mutations in PRODH2. All studied individuals remained asymptomatic, giving further evidence that hydroxyprolinemia is a benign condition. The estimated prevalence of hydroxyprolinemia in Germany is about one in 47,300 newborns. Our results establish mutations in PRODH2 as a cause of human hydroxyprolinemia via impaired dehydrogenation of hydroxyproline to delta1-pyroline-3-hydroxy-5-carboxylic acid, and we suggest PRODH2 be renamed HYPD. Hydroxyprolinemia is an autosomal-recessively inherited benign condition. It is a frequent cause of false positive screening results for MSUD, the prevalence being about 2.5 times higher than that of MSUD.