Amelogenesis imperfecta (AI) is a group of inherited disorders characterized by abnormal enamel formation. AI has traditionally been classified based on the phenotype and the inheritance pattern (X-linked, autosomal recessive (AR), or autosomal dominant (AD)). Despite progress in knowledge of ADAI and X-linked AI, surprisingly little is known about the molecular basis of ARAI. A major difficulty in identifying the molecular etiology of ARAI is that linkage analysis has decreased application to AR conditions (unless consanguinity is present) and a candidate gene approach is frequently required. Objectives: To identify genetic mutations responsible for ARAI. Methods: Five candidate genes were chosen based on either their known role in enamel development or gene expression microarray data on developing enamel. Ameloblastin (AMBN), Enamelin (ENAM), Matrix Metalloproteinase 20 (MMP20), Kallikrein 4 (KLK4), and a novel RIKEN clone being characterized in our laboratory were sequenced. DNA was obtained from clinically well characterized ARAI study participants affected with hypoplastic, hypocalcified or hypomaturation AI. The exons and exon/intron boundaries of the candidate genes were amplified by PCR. Amplicons were sequenced and compared with reported human gene sequences (NCBI). Results: Screening of 22 patients from 18 families with ARAI did not identify mutations in the coding region of these five genes in 20 patients. Two individuals with AR Hypomaturation AI were homozygous for a KLK4 mutation (Hart et al., J Med Genet 2004). Conclusion: The lack of coding region mutations in these families suggests that ARAI is genetically heterogeneous involving genes other than those studied. Alternatively, the candidate genes studied could be causative of ARAI due to mutations in the 5' regulatory region that was not sequenced in the present study. While a candidate gene approach can identify mutations in ARAI, alternative strategies to determine the molecular etiology of these conditions is needed. Supported by NIDCR grant DE12879.