Smoking is a significant cause of global mortality. In recent years, advances have been made in identifying genetic loci associated with nicotine dependence. Among these, the most well-established associations implicate acetylcholine nicotinic receptor subunit genes located on chromosome 15q25. Recently, it has been posited that the relationship between these genes and nicotine dependence is conditioned by age of smoking initiation. In this study, four samples derived from case-control cohort studies nested within a multi-site U.S. cohort study, the Nurses’ Health Study (NHS), are examined to assess the association between key loci in the CHRNA5-CHRNA3-CHRNB4 acetylcholine nicotinic receptor subunit genes and nicotine dependence as measured by cigarettes per day. Evidence for the well-established nicotine dependence locus tagged by rs16969968, as well as other loci in the region, is evaluated. Additional analyses assess whether associations with cigarettes per day vary by age of smoking onset.