Congenital superior oblique muscle palsy as an isolated event is a common cause of vertical deviation of the eyes. The recent studies on the etiology indicated that congenital superior oblique muscle palsy could be attributed to a genetic abnormality. ARIX gene is involved in the development of oculomotor and trochlear nerve which control eye movement. In this study, we identified ARIX gene polymorphisms in patients with congenital superior oblique muscle palsy and investigated the relation between ARIX gene and congenital superior oblique muscle palsy. The three exons of the ARIX gene were sequenced by genomic DNA amplification with polymerase chain reaction and direct sequencing in 15 patients with superior oblique muscle palsy and 54 normal individuals. PCR products cloned into plasmids were also sequenced. A family with father and a daughter each having congenital superior oblique muscle palsy was also involved in this study. Four patients with congenital superior oblique muscle palsy carried heterozygous nucleotide changes in the ARIX gene. One patient with the absence of the superior oblique muscle had T7C in the 5′-UTR of the exon 1 and C-44A in the promoter region, both of which were located on the same strand. Another unrelated patient with congenital superior oblique muscle palsy had C76G in the 5′-UTR of the exon 1 and C-9A in the promoter region on the same strand. G153A in the 5′-UTR of the exon 1 was found in common in two affected members of a family with congenital superior oblique muscle palsy. The nucleotide change (G153A) in the 5′-UTR of the exon 1 co-segregated with congenital superior oblique muscle palsy in one family. Other four nucleotide changes in the exon 1 or the promoter region were found only in patients with congenital superior oblique muscle palsy. These nucleotide polymorphisms may be one of risk factors for the development of congenital superior oblique muscle palsy.