Fatty acid composition is an important indicator of beef quality. The objective of this study was to search the potential candidate region for fatty acid composition. We performed pool-based genome-wide association studies (GWAS) for oleic acid percentage (C18:1) in a Japanese Black cattle population from the Hyogo prefecture. GWAS analysis revealed two novel candidate regions on BTA9 and BTA14. The most significant single nucleotide polymorphisms (SNPs) in each region were genotyped in a population (n = 899) to verify their effect on C18:1. Statistical analysis revealed that both SNPs were significantly associated with C18:1 (p = .0080 and .0003), validating the quantitative trait loci (QTLs) detected in GWAS. We subsequently selected VNN1 and LYPLA1 genes as candidate genes from each region on BTA9 and BTA14, respectively. We sequenced full-length coding sequence (CDS) of these genes in eight individuals and identified a nonsynonymous SNP T66M on VNN1 gene as a putative candidate polymorphism. The polymorphism was also significantly associated with C18:1, but the p value (p = .0162) was higher than the most significant SNP on BTA9, suggesting that it would not be responsible for the QTL. Although further investigation will be needed to determine the responsible gene and polymorphism, our findings would contribute to development of selective markers for fatty acid composition in the Japanese Black cattle of Hyogo.