Atrial fibrillation (AF) is the most common cardiac arrhythmia in the human population, with an estimated incidence of 1-2% in young adults but increasing to more than 10% in 80+ years patients. Albeit AF is a frequent electrophysiological disorder, to date, the genetic bases of AF remain rather elusive. Point mutations in a large variety of ion channels have been described in familial cases of AF, yet explaining a minority of cases. Recently, genome wide association studies (GWAS) have unraveled risk SNPs highly associated with AF, among which the most significant are located in 4q25 locus. Surprisingly these risk alleles are located in a gene desert, being the closest gene PITX2. Experimental evidences of Pitx2 loss-of-function in mice revealed that this homeodomain (HD)-containing transcription factors plays a pivotal role in atrial electrophysiology. Therefore these data, underscore PITX2 as a candidate gene for AF. In this context, we have recruited 31 AF patients from the Regional Hospital for genetic analyses of both the risk alleles and PITX2 ORF re-sequencing. Among those patients, we have found two point mutations in the HD of PITX2 and three other mutations in the 5' untranslated region. A 65 years male patient with recurrent AF displayed two distinct HD-mutations, G947A (Q103H) and G1008A (E124Q), which both resulted in a change within a highly conserved amino acid position. Curiously, no 4q25 risk variants were present in this subject. Both PITX2 HD mutations were further followed for functional studies. We generated plasmid constructs with mutated version of each nucleotide variant (MD4 and MD5, respectively) as well as a dominant negative control construct, in which the PITX2 HD was lacking (DN). Functional analyses demonstrated PITX2 MD4 and PITX2 MD5 decreased Nppa-luciferase transactivation by 50% and 40%, respectively, in a similar range as PITX2 DN (50%). Co-transactivation with other cardiac-enriched transcription factors, such as Gata4 and Nkx2.5, was similarly impaired, further supporting the pivotal role of these mutations for correct PITX2 function. We are currently evaluating the functional consequence of these mutations in a cardiomyocyte context. Preliminary data suggest that distinct AF-related genes are similarly impaired. In summary, we have identified novel PITX2 mutations in an AF patient, which functionally impairs the transactivating capacity of PITX2.