# The identification of distinct haplotypes in VHL tumor-predisposition syndrome and congenital erythrocytosis

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Von-Hippel-Lindau (*VHL*) gene, tumor suppressor, is a negative regulator of hypoxia inducible factors (HIFs); HIFs are involved in many biological processes including unique energy metabolism of tumors (Warburg effect), erythropoiesis, iron metabolism, and vasculogenesis. Loss-of-function mutations in *VHL* gene cause VHL tumor-predisposition syndrome (VHL-TPDS) or congenital erythrocytosis phenotype (CEP) but not both. VHL-TPDS is caused by autosomal dominant mutations, leading to malignant tumors such as pheochromocytoma, renal cell carcinoma, paraganglioma, and hemangioblastoma after another somatic mutation in trans occurs in the *VHL* locus. First described congenital erythrocytosis due to an autosomal recessive mutation in *VHL* (R200W) was Chuvash erythrocytosis (CE), with high erythropoietin (EPO) levels caused by stabilization of HIFs` $\alpha$ subunits under normoxia. However, neither the patients with CE nor the patients with different *VHL* mutations causing erythrocytosis, ever developed any VHL tumors. Therefore, it is unknown why mutations in the same gene cause tumors versus erythrocytosis but never both.

Our data and other evidence indicate that this phenotypic difference between VHL-TPDS and congenital CEP is not due to the location of the mutations. Importantly, some individuals with compound heterozygosity for the Chuvash $VHL^{R200W}$ mutation and other missense VHL mutations known to be associated with VHL-TPDS would be expected to develop tumors, yet those individuals do not develop VHL tumors.

The novel *VHL* mutations generate increased levels of a previously unrecognized cryptic *VHL* transcript composed of a portion of intron 1 and exon 2 of *VHL.* The increased levels of this transcript were associated with either germline intronic mutations or synonymous germline mutations in exon 2, which facilitate alternative splicing that generates the aberrant transcripts of *VHL* gene. Surprisingly, the same changes were associated with CEP in some families but not in other families with VHL-TPDS.

We analyzed published haplotype of *VHL* gene in 4 samples with VHL-TPDS, 13 with CEP, 15 with CE (homozygote for Chuvash $VHL^{R200W}$), and 19 healthy controls. Genomic DNA was isolated from granulocytes collected from whole blood by density gradient method. We genotyped 7 single nucleotide polymorphisms (SNPs) including rs1056286, rs776517,rs779805, rs2600005, rs166538, rs458952, rs378630 located between 226 kb upstream and 100 kb downstream of *VHL* gene using Taqman SNP genotyping assays (Thermofisher). The 4 patients with VHL-TPDS had same genotype of five SNPs located between 3`kb of upstream to 8kb of downstream of *VHL* gene, which is different from patients with Chuvash $VHL^{R200W}$. However, other CEP patients did not have same haplotype.

We postulate that there are functional genetic variants including single nucleotide polymorphisms (SNPs) and genome structural variants (SVs), such as copy number variation, insertion, deletion, or duplication that predispose to the VHL-TPDS, while other variants also increase HIFs signaling and EPO production, leading to CEP but prevent tumor development. These two haplotypes

conferring two distinct phenotypes and their role in VHL-TPDS in tumorigenesis remain to be defined.