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

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BACKGROUND

Von-Hippel-Lindau (*VHL*) gene is a negative regulator of hypoxia inducible factors (HIFs); HIFs are involved in unique energy metabolism of tumors (Warburg effect), erythropoiesis, iron metabolism, and vasculogenesis. Loss-of-function mutations in the *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` α subunits under normoxia. However, neither the patients with CE nor the patients with CEP having other *VHL* mutations than CE, ever developed any VHL tumors.

Our data and other evidence indicate that this phenotypic difference between VHL-TPDS and 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 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 generates the aberrant transcripts of VHL. Surprisingly, the same changes were associated with CEP in some families but not in other families with VHL-TPDS.

Therefore, it is unknown why mutations in the same gene cause tumors versus erythrocytosis but never both.

METHOD

- Genomic DNA was isolated from granulocytes from 4 patients with VHL associated tumors including , 13 patients with VHL associated erythrocytosis, 15 patients with VHLR200W (CE), and 19 healthy controls (Table 1).
- 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 (Figure 1) using Taqman SNP genotyping assays (Thermofisher)
- Genotype calling was analyzed by QuantStudio™ Design and Analysis Software v2.3 (Thermofisher).

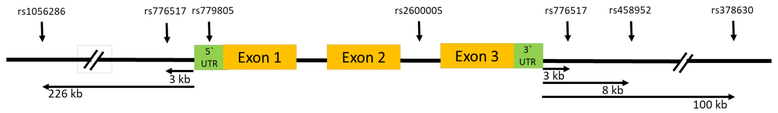

Figure 1. SNPs` location for haplotyping of *VHL* gene.

RESULTS

- 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 (red box in figure2 and table).
- 15 CE patients had same genotype of 6 SNPs located between 226 kb of upstream to 8 kb of downstream which is different from patients with Chuvash VHLR200W. However, other CEP patients did not have same haplotype (red box in figure2 and table).
- However, there were no district haplotype for CEP and healthy controls.

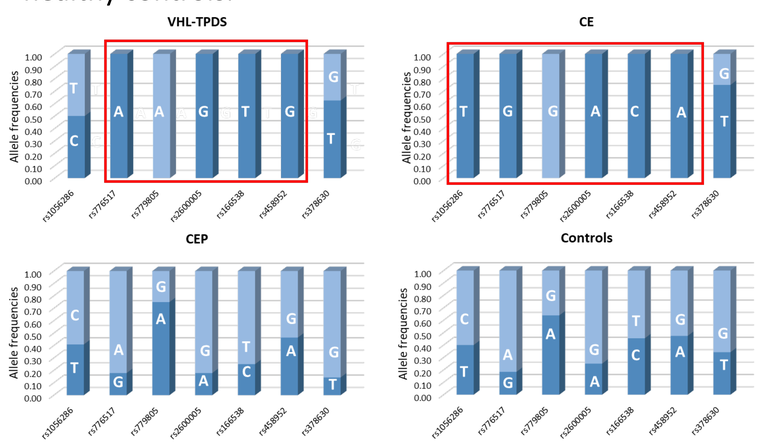

Figure 2. Allele frequencies of 7 SNPs in each group.

RESULTS (CONTINUE)

SNP ID	Location	Allele	VHL-TPDS	CE	CEP	Controls
rs1056286	226kb upstream of *VHL*	T	0.40	1.00	0.32	0.40
		C	0.60	0.00	0.46	0.60
rs776517	3 kb upstream of *VHL*	G	0.00	1.00	0.18	0.18
		A	1.00	0.00	0.82	0.82
rs779805	5`-UTR of *VHL*	A	1.00	0.00	0.75	0.64
		G	0.00	1.00	0.25	0.36
rs2600005	Intron 2 *VHL*	A	0.00	1.00	0.18	0.25
		G	1.00	0.00	0.82	0.75
rs166538	3 kb downstream of *VHL*	C	0.00	1.00	0.25	0.45
		T	1.00	0.00	0.75	0.55
rs458952	8 kb downstream of *VHL*	A	0.00	1.00	0.46	0.47
		G	1.00	0.00	0.54	0.53
rs378630	100kb downstream of *VHL*	T	0.60	0.75	0.14	0.34
		G	0.40	0.25	0.86	0.66

Table1. Allele frequencies of 7 SNPs in each group.

CONCLUSIONS

- We found that a distinct haplotype of *VHL* gene in 4 VHL-TPDS patients, suggesting that the region from 3kb of upstream from 5`UTR and 8kb of downstream from 3` UTR might gave genetic signatures which cause tumorigenesis but not erythrocytosis.
- CE patients regardless of their European or Asian origin have different haplotype in the same location of *VHL* genes compared to VHL-TPDS.
- Distinct genetic signature in the *VHL* gene was not found in CEP patients.

FUTURE STUDIES

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 the distinct function(s) with two distinct phenotypes and their role in VHL-TPDS in tumorigenesis remain to be defined.

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