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Short report

# A germline 1;3 translocation disrupting the *VHL* gene: a novel genetic cause for von Hippel-Lindau

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## ABSTRACT

Von Hippel-Lindau (VHL) disease is an autosomal dominant hereditary tumour susceptibility disease caused by germline pathogenic variation of the *VHL* tumour suppressor gene. Affected individuals are at risk of developing multiple malignant and benign tumours in a number of organs.

In this report, a male patient in his 20s who presented to the Urologic Oncology Branch at the National Cancer Institute with a clinical diagnosis of VHL was found to have multiple cerebellar haemangioblastomas, bilateral epididymal cysts, multiple pancreatic cysts, and multiple, bilateral renal tumours and cysts. The patient had no family history of VHL and was negative for germline *VHL* mutation by standard genetic testing. Further genetic analysis demonstrated a germline balanced translocation between chromosomes 1 and 3, t(1;3)(p36.3;p25) with a breakpoint on chromosome 3 within the second intron of the *VHL* gene. This created a pathogenic germline alteration in *VHL* by a novel mechanism that was not detectable by standard genetic testing.

Karyotype analysis is not commonly performed in existing genetic screening protocols for patients with VHL. Based on this case, protocols should be updated to include karyotype analysis in patients who are clinically diagnosed with VHL but demonstrate no detectable mutation by existing genetic testing.

## INTRODUCTION

Von Hippel-Lindau (VHL) disease is an autosomal-dominant hereditary tumour susceptibility disease where patients are at increased risk of developing multiple malignant and benign tumours in a number of organs. Patients affected with VHL are at risk for the development retinal haemangiomas, central nervous system (CNS) haemangioblastomas, clear cell renal cell carcinomas (ccRCC) and renal cysts, pheochromocytomas and paragangliomas, pancreatic neuroendocrine tumours, endolymphatic sac tumours, pancreatic neuroendocrine tumours and epididymal and broad ligament cystadenomas.<sup>1</sup> The genetic cause of VHL was identified as germline mutation of the *VHL* tumour suppressor gene.<sup>2</sup>

An individual with a family history of VHL can be diagnosed if they present with a single VHL-associated tumour, such as a retinal or cerebellar haemangioblastoma or a renal cell carcinoma (RCC). To receive a positive diagnosis, individuals with no family history of VHL must present

with two or more retinal or cerebellar haemangioblastomas, or a single haemangioblastoma and a visceral tumour.<sup>3–5</sup> Mutational analysis of the *VHL* gene is important for diagnosis of VHL as it allows for presymptomatic identification of mutation-positive at-risk individuals. This enables optimal surveillance of these patients allowing for the early detection of tumours and timely surgical or therapeutic intervention.

Germline *VHL* mutations have been identified in over 900 families worldwide. Numerous mutation types have been observed including single nucleotide substitutions, small insertion–deletion mutations, splice site mutations and larger deletions resulting in partial or complete loss of the *VHL* gene and these data can be found in the UMD-VHL mutations database (<http://www.umd.be/VHL/>).<sup>6–10</sup> Current Clinical Laboratory Improvement Amendment (CLIA)-based genetic testing has a very high detection rate of germline *VHL* sequence alterations in patients with VHL, but to date, germline chromosomal translocation has not been reported in association with VHL.<sup>11 12</sup> Germline translocations involving chromosome 3 have been reported that occur away from the *VHL* locus, but these patients present with bilateral and multifocal ccRCC and show no evidence of susceptibility to other VHL-associated tumours.<sup>13–16</sup>

This report presents a patient with no family history of VHL, who had multiple VHL-associated phenotypic features consistent with a clinical diagnosis of VHL, but who had no discernable germline sequence alteration of the *VHL* gene detected by standard genetic testing. Further in-depth genomic analysis demonstrated the first example of a germline-balanced chromosomal translocation of chromosomes 1 and 3 in which the breakpoint occurred within the *VHL* gene, resulting in a novel genetic mechanism for VHL.

## MATERIALS AND METHODS

### Patient material procurement and consent

This patient was seen at the Urologic Oncology Branch (UOB) of the National Cancer Institute (NCI), National Institutes of Health (NIH) for clinical assessment.

### CLIA evaluation of VHL mutation and patient karyotype

Patient blood DNA was evaluated for *VHL* gene mutation or deletion/duplication by GeneDx



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(GeneDx, Gaithersburg, Maryland, USA), a CLIA (Clinical Laboratory Improvement Amendments) approved facility, and the CLIA karyotype analysis was performed by Quest Diagnostics (Quest Diagnostics, Chantilly, Virginia, USA).

### Germline nucleic acid extraction

Germline DNA from the patient's blood, saliva and skin samples were extracted using a Promega Maxwell 16 Blood DNA Purification Kit or Tissue DNA Purification Kit following manufacturer's protocol (Promega, Wisconsin, USA). DNA concentration and purity were evaluated using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA).

### Whole-genome sequencing (WGS)

The DNA from the patient was pooled with 11 other WGS samples and sequenced on HiSeq4000 using Illumina TruSeq Nano DNA Library Prep and paired-end sequencing for three runs. The HiSeq Real Time Analysis software (RTA 1.18) was used for processing image files, and the Illumina bcl2fastq v1.8.4 was used to demultiplex and convert binary base calls and qualities to fastq format. The samples had 513 to 948 million pass filter reads with base call quality above 77% of bases having Q30 and above. Adapters and low-quality bases in raw reads were trimmed using Cutadapt v1.18 before alignment with the reference genome (Human—hg19) using BWA v0.7.10. The average mapping rate of all samples was approximately 98% and the average sequencing genome coverage was 30×. The samples had 89%–93% non-duplicate reads and the GC content of mapped reads ranged from 39% to 40%.

### WGS analysis

Germline variants were called using GATK's Haplotype-Caller in joint genotyping mode. Variants were then filtered for quality with the following criteria: QD (quality by depth) <2.0, FS (Fisher strand) >60.0, MQ (mapping quality) <40.0, MQRankSum <−12.5, ReadPosRankSum <−8.0 for SNPs; QD <2.0, FS >200.0, ReadPosRankSum <−20.0 for INDELS (insertions or deletions of bases in the DNA). To prioritise cancer-related germline variants, we used the Cancer Predisposition Sequencing Reporter (version 0.5.1) to analyse the 218 manually curated cancer predisposition genes (Panel 0) for known or predicted pathogenic variants. Structural variants were called using Manta in paired germline mode and annotated using AnnotSV. Alignments around candidate translocation events were further reviewed using IGV (Integrative Genomics Viewer).

### PCR and Sanger DNA sequencing

PCR primers were designed on either side of the two potential breakpoint regions on the derivative versions of chromosomes 1 and 3 identified by WGS. The primer pairs used to amplify and sequence the translocation breakpoints were the following: chr1F (GAGTCATACATCAACCTCTAG)/chr3R (TGAGAATGAGACACTTTGAAAC), and chr3F (CTCAGCTAGGCAGT-TACTCT)/chr1R (CAAGGATTCTTTTCAGCCTTC). DNA sequencing was performed by PCR using a Qiagen Taq PCR Core Kit (Qiagen, Maryland, USA) according to the manufacturer's specifications, followed by bidirectional sequencing using the Big Dye Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems, California, USA) according to the manufacturer's specifications and run on an ABI 3130xl or 3730 Genetic Analyzer (Applied Biosystems). The three *VHL* coding exons were also amplified and sequenced using conventional methods. Sanger sequencing

was conducted at the CCR Genomics Core at the NCI, NIH, Bethesda, Maryland, USA. Forward and reverse sequences were evaluated using Sequencher 5.0.1 (Genecodes, Michigan, USA).

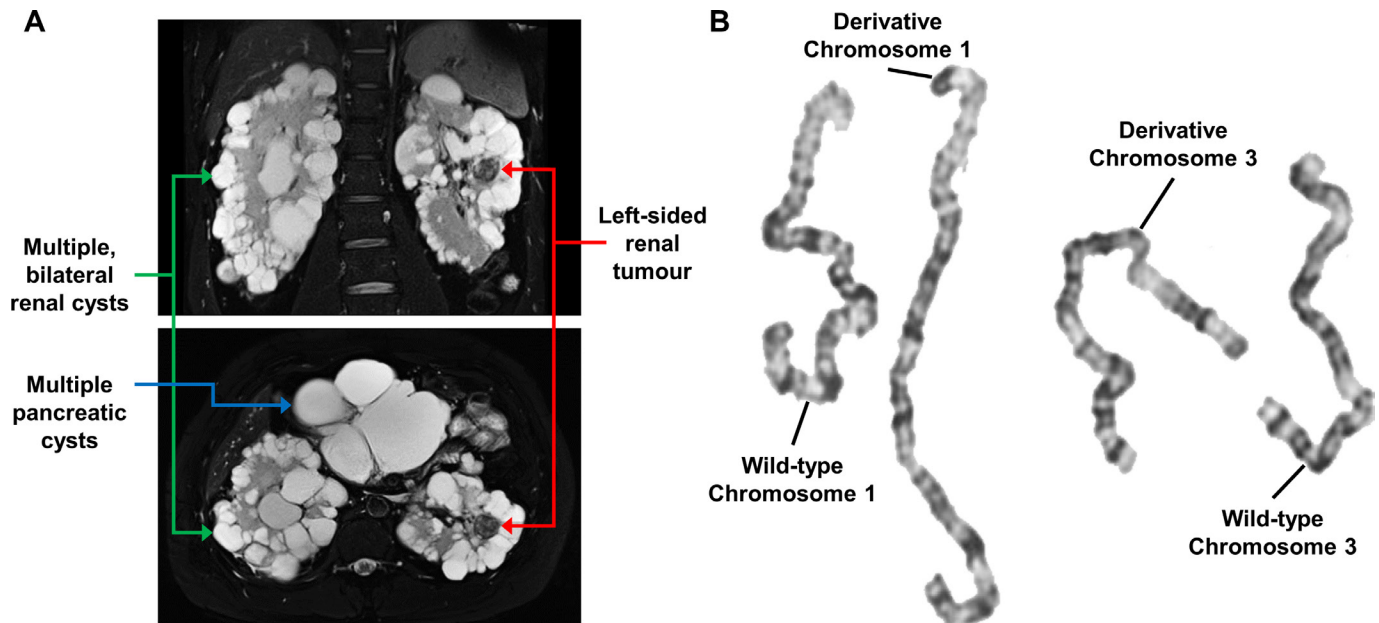
### RESULTS

A male patient in his 20s initially presented to the UOB at the NCI with several clinical phenotypic manifestations consistent with a diagnosis of VHL. At an outside institution, the patient had previously had multiple resections of CNS haemangioblastomas, a left robotic partial nephrectomy for multifocal ccRCC (five tumours ranging from 1.3 cm to 4.6 cm in largest dimension) and a right ablation of a central renal tumour. Previous germline mutation testing had been negative for *VHL* mutation and the patient reported no family history of kidney cancer or any other clinical manifestations consistent with VHL.

Further evaluation and imaging at the UOB revealed that the patient had bilateral epididymal cysts, numerous pancreatic cysts and numerous bilateral renal cysts with two solid lesions in the left kidney (2.3 cm and 1.7 cm) and one solid lesion in the right kidney (2.1 cm) (figure 1A). Currently, the adrenal glands appear unaffected. These clinical manifestations were consistent with a diagnosis of VHL. Germline genetic analysis was repeated using DNA derived from peripheral blood leucocytes to evaluate for point mutations, insertions, deletions or duplications in *VHL* and was negative. To assess the possibility of mosaicism, germline genetic testing was additionally performed on DNA derived from saliva containing buccal epithelial cells and from fibroblasts acquired via a skin punch biopsy. No alterations of the *VHL* gene were detected.

Germline translocations involving chromosome 3 have been reported and shown to result in an increased risk of kidney cancer, but to date no other *VHL*-associated clinical manifestations have been seen.<sup>13–16</sup> To investigate whether a translocation could cause these clinical features, a karyotype analysis was performed. The patient was shown to have a germline translocation between chromosomes 1 and 3, 46, XY, t(1;3)(p36.3;p25). The predicted breakpoint at chromosome 3p25 was in the same region as the *VHL* gene, potentially directly disrupting the *VHL* gene (figure 1B).

Paired-end WGS was performed on the DNA derived from peripheral blood leucocytes and several paired reads were found with one pair mate on chromosome 1 and the other within intron 2 of the *VHL* gene on chromosome 3. To confirm the breakpoints, primers were designed to amplify and Sanger sequence the breakpoint regions in both derivative chromosomes. The breakpoint on the derivative chromosome 3 had a 2 bp sequence present in both the wild-type sequences for chromosomes 1 and 3 with the last unique base of chromosome 1p at 716702 bp (hg19) and the first unique base of chromosome 3p at 10 191 126 bp (hg19) (figure 2). The breakpoint on the derivative chromosome 1 had an insertion of 10 bp which was a duplication of an upstream region of chromosome 3, with the last unique base of chromosome 3p at 10,191,119 bp (hg19) and the first unique base of chromosome 1p at 716698 bp (hg19) (figure 2). This results in a few base pairs being lost from wild-type sequence of chromosome 3 but no loss of sequence compared with wild-type chromosome 1. The break on chromosome 3p was confirmed to be within the second intron of the *VHL* gene creating a germline alteration that would result in either a truncated *VHL* protein or no protein being produced by this allele. However, the break on chromosome 1p did not occur within a gene, and the nearest telomeric gene, *LOC100288069*, was ~2.6 kb away and the nearest centromeric gene, *FAM87B*, was ~36 kb away. Neither



**Figure 1** Imaging and karyotype analysis of patient with von Hippel-Lindau (VHL) disease. (A) Coronal (upper) and axial (lower) MRI images of the abdomen of the patient with VHL showing multiple bilateral renal cysts (green arrows), one of the left-sided solid lesions (red arrows) and multiple pancreatic cysts (blue arrow). (B) Karyotype analysis of the germline DNA of the patient with VHL demonstrating a translocation between chromosomes 1 and 3.

of these genes were mutated in any of the 426 sporadic ccRCCs analysed by the Cancer Genome Atlas suggesting that alterations in either of these genes is unlikely to influence the risk of kidney cancer in this patient.

## DISCUSSION

The identification of patients with a clinical diagnosis of a disease, such as VHL, but no alteration in the known gene after standard genetic testing raises two questions. Are the current genetic tests unable to detect all the possible genetic abnormalities in the known gene or is there an alternative gene in which germline mutation produces the same clinical phenotype? An example of the first scenario would be a mutation within the intronic sequence that is not evaluated or detected by existing genetic tests and results in an aberrant splice event that inactivates the gene. This has previously been observed in patients with VHL from two separate studies in which a germline mutation was identified within the intron 1 of the *VHL* gene that created a cryptic exon.<sup>17 18</sup> This cryptic exon (termed E1') dysregulated *VHL* splicing and resulted in loss of protein expression and the germline mutation would not have been detected by standard genetic testing.<sup>17 18</sup> Whereas, an example of the second scenario would be mutation of the *TCEB1* gene which is another component of the VHL E3 ligase complex and has been reported as a potential mimic for VHL loss in sporadic ccRCC.<sup>19 20</sup> This report identifies a germline translocation that disrupts the *VHL* gene as a novel mechanism of gene loss in VHL that would not be detected by standard genetic testing.

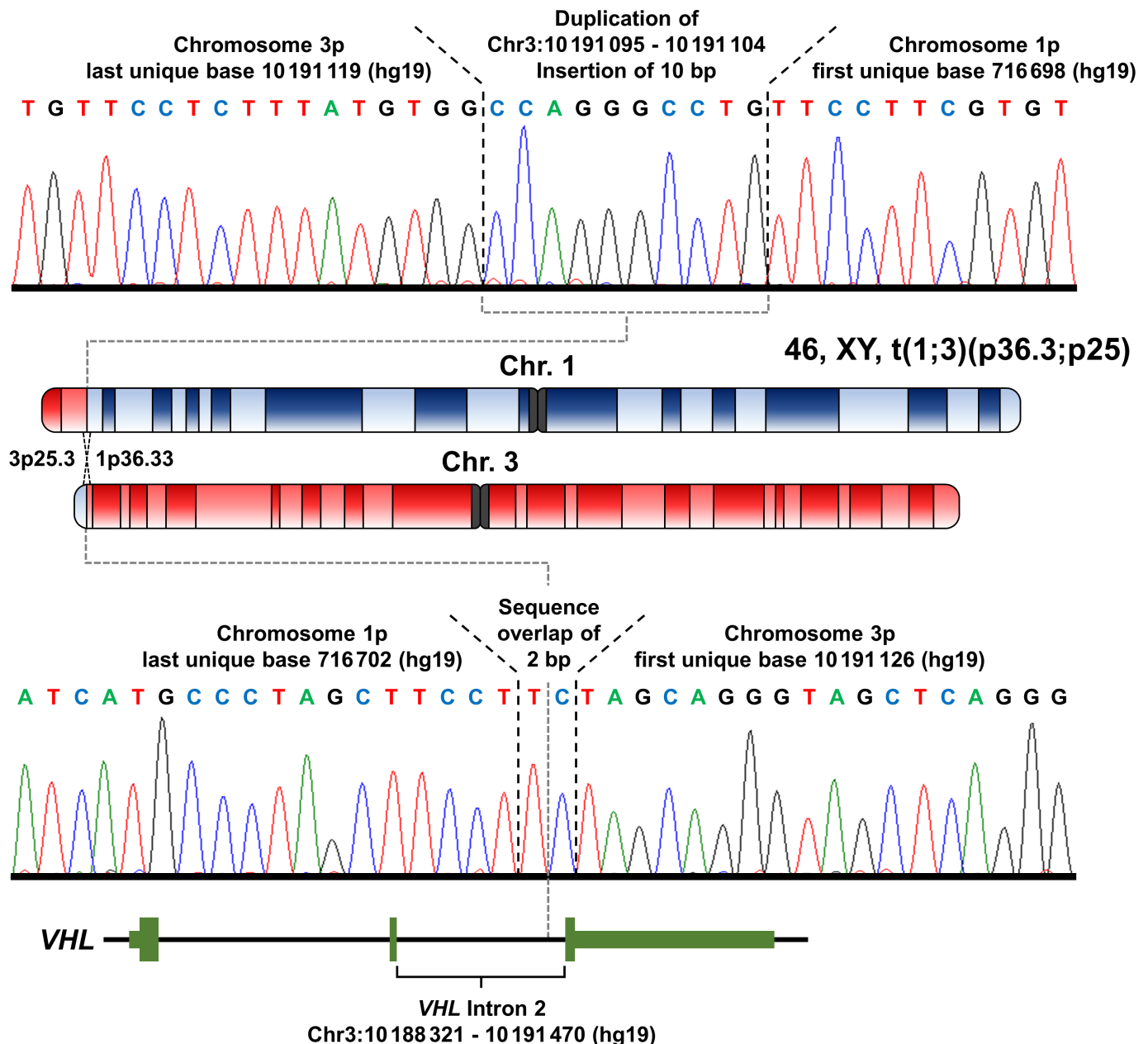
The patient reported here demonstrated a relatively typical presentation of the clinical features of VHL with no additional phenotypic features. The break on chromosome 3p obviously disrupted the *VHL* gene, but the break at the end of chromosome 1p did not occur within a gene. A significant number of germline *VHL* deletions have been reported in patients with VHL and the position of the breakpoints for these deletions frequently occur within DNA repeats, specifically *Alu* repeat regions.<sup>21</sup> In

our patient, the translocation breakpoint in the second intron of *VHL* on chromosome 3 occurred within an *MLT1H* long terminal repeat region (chr3:10 191 097-10 191 334) and ~30 bp upstream of an *AluJo* repeat (chr3:10 190 785-10 191 096), but the breakpoint on chromosome 1 was not near any DNA repeat region.

The patient had no family history of VHL suggesting that the germline translocation was a de novo event, but knowledge of this novel alteration will allow for screening of any subsequent offspring of the patient and confirmation of this de novo status. The patient can be managed by the current protocols for patients with VHL and counselled on the likelihood of any offspring inheriting VHL. In this specific case, the patient should also be counselled that a higher rate of miscarriage is associated with inheritance of a balanced translocation from either parent.<sup>22</sup>

Previous reports have identified patients with RCC with germline translocations involving chromosome 3 that occur away from the *VHL* locus and in these cases a three-hit model of carcinogenesis has been proposed.<sup>13-16 23 24</sup> The first hit is the translocation and the second hit is loss of the derivative chromosome containing 3p and the *VHL* gene. The cells would then require a third hit to the remaining wild-type version of *VHL*.<sup>23 24</sup> These patients tend to present with later onset of disease, due to the increased number of genetic events required, and only demonstrate bilateral and multifocal ccRCC with no evidence of the other VHL-associated tumours.<sup>13-16 23 24</sup> The patient in this study would not require the loss of the derivative chromosome as the translocation through the gene is analogous to deletion of the *VHL* gene and the normal two-hit hypothesis would apply. Thus, the patient shows the expected early age of onset for ccRCC and a full spectrum of additional VHL-associated tumours.

The discovery of this novel mechanism for germline *VHL* gene inactivation highlights the importance of continual refinement of mutational screening protocols. The existing screening protocols for patients with VHL do not include a karyotype analysis,



**Figure 2** Breakpoint mapping of germline translocation between chromosomes 1 and 3. Whole-genome sequencing identified the potential breakpoints on the derivative chromosomes resulting from the t(1;3)(p36.3;p25) translocation, and PCR primers were designed to amplify both breakpoint regions. Sanger sequencing of the resulting PCR products demonstrated the exact breakpoints on both derivative chromosomes. The breakpoint on the derivative chromosome 1 had an inserted 10bp duplication of upstream chromosome 3 sequence, with the last unique base of chromosome 3p at 10 191 119 bp (hg19) and the first unique base of chromosome 1p at 716 698 bp (hg19). The breakpoint on the derivative chromosome 3 had a 2 bp sequence overlap with the last unique base of chromosome 1p at 716 702 bp and the first unique base of chromosome 3p at 10 191 126 bp (hg19). The breakpoint on chromosome 3p occurred within intron 2 of the von Hippel-Lindau (*VHL*) gene.

but this should now be considered in patients who are clinically diagnosed with VHL but demonstrate no detectable germline mutation by current genetic testing protocols.

**Contributors** CJR, CDV and WML planned and coordinated the study. CJR, CDV, ML, XC, YZ, BT, MT, LSS, MWB and WML were involved in sequence data collection and interpretation. CJR, CDV, MWB and WML involved in clinical data collection. CJR, CDV, ML, BT, MT, LSS, MWB and WML wrote and edited the manuscript.

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