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Whole exome sequencing identified genetic variations in Chinese hemangioblastoma patients

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Abstract

Hemangioblastomas (HBs) are uncommon tumors characterized by the presence of inactivating alterations in the von Hippel-Lindau (*VHL*) gene in inherited cases and by infrequent somatic mutation in sporadic entities. We performed whole exome sequencing on 11 HB patients to further elucidate the genetics of HBs. A total of 270 somatic variations in 219 genes, of which there were 86 mutations in 67 genes, were found in sporadic HBs, and 184 mutations were found in 154 genes in familial HBs. C: G>T: A and T: A>C: G mutations are relatively common in most HB patients. Genes harboring the most significant mutations include *PCDH9*, *KLHL12*, *DCAF4L1*, and *VHL* in sporadic HBs, and *ZNF814*, *DLG2*, *RIMS1*, *PNN*, and *MUC7* in familial HBs. The frequency of CNV varied considerably within sporadic HBs but was relatively similar within familial HBs. Five genes, including *OTOGL*, *PLCB4*, *SCEL*, *THSD4* and *WWOX*, have CNVs in the six patients with sporadic HBs, and 3 genes, including *ABCA6*, *CWC27* and *LAMA2*, have CNVs in the five patients with familial HBs. We found new genetic mutations and CNVs that might be involved in HBs; these findings highlight the complexity of the tumorigenesis of HBs and pinpoint potential therapeutic targets for the treatment of HBs.

Keywords

Hemangioblastomas; von Hippel-Lindau gene; whole exome sequencing; copy number variation; single nucleotide variant

Introduction

Hemangioblastomas (HBs) are highly vascularized neoplasms; they account for only 1–2.5% of all tumors in the central nervous system (CNS) [Conway and others 2001; Hussein

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Competing interests

The authors have declared that no competing interests exist.

2007; Lonser and others 2003]. They often reside in a strikingly limited subset of CNS locations, including the cerebellum, brainstem, retina, and spinal cord. Due to their unique location and the existence of rich vascular networks in these areas, HBs can cause bleeding and rupture, or they can become symptomatic with/without an associated cyst. Therefore, CNS-HBs are a major cause of morbidity and mortality [Shehata and others 2008].

HBs are benign tumors with characteristic and well-described histopathological features, including rapid proliferation of vascular and stromal cells, which are regarded as the underlying neoplastic cells [Hussein 2007]. However, the ontogenesis of HBs remains a mystery for nearly a century since the discovery of HBs. Consequently, the World Health Organization has not classified HBs as neoplasms [Louis and others 2007]. Accordingly, exploration of the oncogenesis of HB will not only shed light on its etiology, pathology, and diagnosis, it will also provide useful insight into treating it.

HBs occur sporadically or in von Hippel-Lindau (*VHL*) disease. Although there are vast differences between the two subtypes of HBs in terms of genetics, age of onset, clinic recurrence, and other involved organs, they share identical histopathology. Germline mutations or aberrations in the *VHL* gene have been identified as the root cause of VHL disease. It has been estimated that only approximately 4% of *VHL* gene carriers are asymptomatic [Beitner and others 2011; Catapano and others 2005], with CNS-HBs occurring in up to 60–80% of VHL patients [Maher and others 1990; Richard and others 2000; Wanebo and others 2003]. It is also thought that there were 4–25% of seemingly sporadic HBs are VHL somatic mutations [Fukino and others 2000; Hes and others 2000; Neumann and others 1989]. These observations indicate that a dysfunctional or absent VHL protein (pVHL) could be involved in the tumorigenesis of HB, but it alone might not be enough to induce the lesions [Ma and others 2011; Nordstrom-O'Brien and others 2010]. Additional genetic variations that may contribute to the tumorigenesis of HB have not yet been identified [Nordstrom-O'Brien and others 2010; Sims 2001].

Furthermore, identified mutations span the entire *VHL* gene and occur in nearly every codon [Nordstrom-O'Brien and others 2010]. Dysfunctional or absent pVHL has organ-specific expression and age-dependent penetrance. Moreover, different types of *VHL* mutations could also influence tumorigenesis. For example, *VHL* deletions and protein truncating mutations appear to confer a higher risk of CNS-HBs than missense mutations [Cybulski and others 2002; Franke and others 2009; Maher and others 1996; Maranchie and others 2004]. Previous studies have indicated the importance of different pathways in the development of HB neoplasms as well as the existence of additional unknown genetic alterations that might drive disease progression [Dulaimi and others 2004]. In this study, we performed a whole exome sequencing to explore the possible genetic variations that may contribute to the tumorigenesis of HBs.

Materials and Methods

Study Participants

Eleven HB samples and the corresponding peripheral blood samples (including six sporadic HBs and five familial HBs, respectively) were selected randomly from the sample bank in

the Department of Neurosurgery, Huashan Hospital, Shanghai Medical College, Fudan University. These patients underwent resection of CNS-HBs between November 2008 and June 2015 at our hospital. All the specimens were ultimately diagnosed by experienced neuropathologists. The five patients with familial HBs were from three families. All of the familial HB patients had a family history of HBs, and *VHL* mutations were identified in genetic testing. All the sporadic HB patients had no family history of HB, showed no clinical signs of *VHL* HBs, and did not show any *VHL* mutation. This research was approved by the institutional review board of Huashan Hospital (KY2009-313). Informed consent was obtained from all patients.

Extraction of DNA

In this study, the sample detection included concentration, sample integrity and purification of the extracted DNA. Concentration was detected using a fluorometer or a Microplate Reader (Qubit Fluorometer, Invitrogen, Carlsbad, CA, USA). Sample integrity and purification were detected by Agarose Gel Electrophoresis (concentration of agarose gel: 1%, voltage: 150 V, electrophoresis time: 40 min). Library construction followed the standard commercial guidelines. Briefly, 1 µg–2µg of genomic DNA was randomly fragmented using Covaris AFA process to an average size of 250–300 bp. Fragments were end repaired, and an extra A base was added to the 3' end. Illumina adaptors were ligated to the fragments, and proper cycles of polymerase chain reaction (PCR) amplification were applied to each sample after ligation. DNA was quantified using either Agilent 2100 Bioanalyzer or the Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). We used the required amount of pre-capture library for each capture reaction. Agilent SureSelect, NimbleGen SeqCap EZ Hybridization and Wash kits (Roche, Basel, Switzerland) were used for whole-exome capture following the standard manufacturer's protocol.

Exome Capture and Sequencing and Bioinformatics Analysis

Genomic DNA was extracted from the tumor and blood of each of the 11 patients, and then it was fragmented randomly. After electrophoresis, DNA fragments of the desired length were gel purified. Adapter ligation and DNA cluster preparation were performed and the samples were subjected to Solexa sequencing [Mardis 2008; Wang and others 2008; Wheeler and others 2008]. WES was performed using the Complete Genomics (CG) platform, which utilizes two proprietary technologies, DNA nanoball (DNB) sequencing and combinatorial probe-anchor ligation (cPAL). The results showed a high concordance with the Illumina platform and a high sensitivity [Lam and others 2012; Porreca 2010]. To minimize the likelihood of systematic bias in the sampling, two paired-end libraries with an insertion size of 500 bp were prepared for all the samples and then they subjected to whole exome sequencing. This resulted in at least 30-fold haploid coverage for each sample. Raw image files were processed using the Illumina pipeline for base calling with default parameters, and the whole exome sequences of each individual patient were generated as 90-bp-paired-end reads.

We used the Burrows-Wheeler Aligner program [Li and Durbin 2009] to map the raw sequencing data for each individual to the human reference genome (build hg19). We then used Picard to sort and mark duplicated reads. Gene mutations or alterations in peripheral

blood were considered to be nonspecific genetic variations and were excluded from the final analyses. The SNVs were detected by VarScan [Koboldt and others 2009]. P-values were calculated by Fisher's exact test on the read counts, using reference and variant allele [Koboldt and others 2009]. The CNVs of the normal samples and the tumor samples were detected by in-house pipeline CNV-Detection (BGI-Tech, Shenzhen, Guangdong, China) based on the Broad Institute's Segseq algorithm [Chiang and others 2009]. Segseq can detect breakpoints at any read position. The segments were iteratively joined by eliminating the breakpoints. Statistical significance was calculated based on the number of reads in the tumor and normal samples in the entire segment. This enables a more accurate estimation of statistical significance because of the increased number of aligned reads [Chiang and others 2009]. We then used ANNOVAR to annotate the variant results [Wang and others 2010], based on which subsequent advanced analysis could be conducted. Quality control (QC) was performed at each stage of the analysis pipeline.

Results

Basic Characteristics of the Study Participants

The basic characteristics of the 11 study participants are presented in Table 1. Six of the participants had sporadic HB, and five had familial HB. Of the participants with familial HB, patient 21 and 23 came from the same family while patient 24 and 25 were from a different family. Four of the patients were female (three had sporadic HB and one had familial HB), and the HBs appeared in different locations.

Single Nucleotide Variation

We identified a total of 270 somatic single nucleotide variation (SNV) mutations in the 11 tumors, of which 67 (26.8%) might affect protein coding (61 missense, five nonsense and one splice site; Table 2). These 270 somatic mutations are in 219 genes, with four genes (*MAP4K3*, *NBPF20*, *PGM5*, and *ZNF814*) having two mutations and 47 mutations located in intergenic regions. We found that, for most of the patients in this study, C: G>T: A transitions were the most common mutation in individual HB samples; however, the T: A>C: G mutation were also very common in many HB patients (Fig. 1A). Some patients, such as patient 12 and patient 21, have more mutations in the coding regions (Fig. 1B). An examination based on HB type indicated that there were 86 mutations in the patients with sporadic HBs (mean 14.3), of which 51 represent novel mutations, and 184 mutations in the patients with familial HBs (mean 36.8), of which 139 represent novel mutations (Table 2). The 86 mutations in the sporadic HBs are in 67 genes with 19 mutations in the intergenic regions (Supplementary Fig. 1). The 184 mutations in the familial HBs are in 154 genes with two genes (*MAP4K3* and *ZNF814*) having two mutations each and 28 mutations in the intergenic regions (Supplementary Fig. 2).

Genes with the Most Significant Mutations

For sporadic HBs, the most significant mutation (c.2639C>G; $p=3.22\times 10^{-11}$) is found in the *PCDH9* gene. Other genes harboring strongly significant mutations for familial HBs include *KLHL12*, *DCAF4L1*, and *VHL* (all $p<5\times 10^{-8}$). For familial HBs, the most significant mutation (c.1010C>T; $p=6.18\times 10^{-40}$) is found in the *ZNF814* gene, which also harbors a

synonymous mutation ($p=1.01\times 10^{-22}$). Other genes harboring strongly significant mutations for familial HBs include *DLG2*, *RIMS1*, *PNN* and *MUC7* (all $p<5\times 10^{-19}$; for the complete list of significant mutations, please see online Supplementary Table 1 and Supplementary Table 2).

Supplementary Figs. 3 and 4 schematically illustrate two identified somatic mutation networks for sporadic HBs and familial HBs, respectively. In sporadic HBs, we identified several statistically significant networks that are involved in different functions, such as the spliceosomal complex ($P=3.77\times 10^{-5}$) and RNA splicing ($P=1.66\times 10^{-4}$). In the *VHL* HBs, we did not identify any network that was found to be statistically significant after correction for multiple comparisons using the false discovery rate.

Copy Number Variations

We detected a large number of copy number variations (CNVs) in all the HB samples across the chromosomes. Specifically, we identified a total of 26,072 CNVs in patients with sporadic HB (Supplementary Fig. 5) and 8,000 CNVs in patients with familial HBs (Supplementary Fig. 6). The frequency of CNVs varied considerably among patients with sporadic HBs: one patient (patient 13) had 13,310 CNVs and one patient (patient 15) had 4,167 CNVs, while patient 16 only had 707 CNVs across the genome. In contrast, the frequency of CNVs was generally lower and relatively similar among patients with familial HB: on average, there were 1,600 CNVs per patient; patient 21 had the smallest number of CNVs (1358) and patient 22 had the largest number of CNVs (1,901).

We identified five genes, *OTOGL*, *PLCB4*, *SCEL*, *THSD4*, and *WWOX*, which has CNVs in the six patients with sporadic HB (Table 3). We identified three genes, *ABCA6*, *CWC27*, and *LAMA2*, with CNVs in the five patients with familial HBs (Table 4).

Discussion

In this study, we performed whole exome sequencing (WES) on six patients with sporadic HBs and five patients with familial HBs to explore the potential genetic variations that may contribute to the tumorigenesis of HBs. We identified many mutations, the majority of which represent novel mutations, in both sporadic HBs and familial HBs. We found a large number of CNVs in many patients with sporadic HBs, but fewer CNVs in patients with familial HBs. To the best of our knowledge, this is the first WES study to examine potential genetic variations for HBs.

Nowadays, WES has become a very powerful strategy in the discovery of rare causal variants of inherited diseases and in the identification of additional somatic genetic variations that may account for unexplained heritability [Cirulli and Goldstein 2010]. In this study, we screened and identified a number of potential new mutations in patients with HBs. For example, in patients with familial HBs, we found mutations in *ZNF814*, *DLG2*, *RIMS1*, *PNN*, and *MUC7*. Further studies are needed to examine whether and how these additional genetic mutations contribute to HB tumorigenesis and if they are associated with age-dependent penetrance of HBs with synergetic inactivation of *VHL*. In sporadic HBs, we found the most significant mutations in *PCDH9*, *KLHL12*, and *DCAF4L1* but not in *VHL*.

The exact roles of these genetic mutations need to be elucidated. Taken together, our findings provide additional insights into the genetic basis of HBs, and they enhance our understanding of the complexity of the clinical phenotypes of HBs.

CNVs are considered to be a significant source of genetic diversity, and they can influence phenotypic variability, such as disease susceptibility [Diskin and others 2009]. Many of the genes harboring the identified genetic variations are also implemented in different tumor-related pathways. For example, we found CNVs in WW domain-containing oxidoreductase (*WWOX*) in the six patients with sporadic HBs. *WWOX* is located at 16q23.3–24.1, an area with a high frequency of gene deletions or chromosomal alterations. *WWOX* is a tumor suppressor gene that regulates multiple cancer-related pathways [Aqeilan and Croce 2007; Salah and others 2010]. It exerts its cancer-suppressing function by suppressing the carcinogenic effects of oncogenes [Gaudio and others 2006], and/or by inducing cell apoptosis via activation of other tumor suppressor genes [Aqeilan and others 2004]. *WWOX* loses its expression in various tumors [Lewandowska and others 2009]. Previous studies have reported that the CNVs in *WWOX* are associated with increased risk of lung cancer and gliomas in a Chinese population [Yang and others 2013; Yu and others 2014]. We found that there were five common CNVs in *WWOX* (<http://dgv.tcag.ca/dgv/app/home?ref=GRCh37/hg19>), none of which were among the list of CNVs that we identified in patients with sporadic HBs. Therefore, the CNVs we found in this study represent novel CNVs in *WWOX* that might be involved in the function of *WWOX* with respect to its role in cancer suppression and HB-tumorigenesis for sporadic HBs. Importantly, because the CNVs we identified in *WWOX* did not occur in the matched peripheral blood DNA, the occurrence of CNVs in *WWOX* within sporadic HBs might be due to acquired environment influences. Future studies are needed to explore the potential mechanism of the identified CNVs in influencing the function of *WWOX*.

A recent study identified a reverse relationship between CNVs and mutations in 12 types of cancer such as glioblastoma multiforme and head and neck squamous cell carcinoma, and it found that tumors can roughly be divided into two classes based on somatic genetic variations: the M class predominantly has mutations while the C class predominantly has CNVs [Ciriello and others 2013]. Overall all, we found a large number of CNVs (mean=4345) but a relatively smaller number of mutations (mean= 14) in patients with sporadic HBs; in contrast, we found significantly fewer CNVs (mean=1,600) but relatively more mutations (mean=37) in familial HBs. However, we did not observe this inverse relationship in some of the individual patients. For example, we identified a large number of CNVs in patient 13 who had sporadic HB (n=13,310), but he had an average number of SNVs (n=14). Another sporadic HB patient (patient 16) had the least number of CNVs (n=707), but an average number of SNVs (n=13). These results highlight the genetic heterogeneity underlying the tumorigenesis of HB.

We identified several mutations/CNVs in genes that are involved in angiogenesis, such as vascular endothelial growth factor A (*VEGFA*) and *VEGFC*. *VEGFA*, located at 6p12, is a member of the cysteine knot family of growth factors, and it is produced by most cells in the body [Ferrara 2004]. It is well known for its essential role in the growth of blood vessels and for its involvement in angiogenesis in brain tumors [Jain and others 2007]. A recent study

also reported that *VEGFA* was over-expressed in HB tissues in comparison to normal surrounding tissues from the same patients [Kruizinga and others 2016]. We also observed many mutations/CNVs in the intergrin (ITG) gene family, such as *ITGA8*, *ITGA1*, *ITGB5*, and *ITGB8*. *ITGB8*, located on the cytogenetic band 7p21.1 of the human chromosome 1, can prevent neuronal apoptosis that is induced by deprivation of oxygen-glucose, and inhibition of *ITGB8* leads to down regulation of *VEGF* [Zhang and others 2012]. *ITGB8* is also involved in the management of neurovascular physiology. Its removal in perivascular cells leads to the inability to regulate the active phases of blood vessel growth, leading to problems with neurovascular homeostasis [Salajegheh 2016]. Suppression of *ITGB8* can promote tumor growth and angiogenesis [Fang and others 2011]. Many of the other genes that we identified in the ITG family also play important functions regarding angiogenesis or the pathogenesis of various types of tumors [Salajegheh 2016]. These findings suggest potential pharmacological targets for the prevention and/or treatment of HBs.

Our study has some limitations. First, the sample size is rather limited. This prevented us from differentiating driver mutations from passenger mutations [Tokheim and others 2016]. More studies with larger sample sizes are needed to validate our findings. Second, the patients in this study were from a single hospital in China, and it is uncertain as to whether our findings can be generalized to patients of different races/ethnicities. Third, although we identified many genetic variations that might contribute to the tumorigenesis of HBs, in this exploratory analysis, we did not examine the underlying mechanisms. Future studies are also needed to investigate how multiple genetic variants interact with each other in the development of HBs. Finally, in this study, we used VarScan to detect the SNVs. Previous research indicated that this caller had a sensitivity >70% in whole-exome sequencing [Kroigard and others 2016], and had medium specificities [Cai and others 2016]. It exhibited advantages in comparison with other callers in the detection of somatic SNVs with high frequencies. However, because we did not perform analytical validation of the identified somatic variants, we cannot rule out the possibility of false positive findings. Various factors, such as sample preparation, exome enrichment laboratory procedure and random/systematic sequencing and alignment errors, can lead to false positive findings. We are conducting an ongoing study which aims at including more patients with HBs to validate the findings from this study.

In summary, we performed WES in six patients with sporadic HBs and five patients with familial HBs. We found many SNVs and CNVs, many of which were reported to play essential roles in angiogenesis or the pathogenesis of cancers. Our findings highlight the complexity of the pathogenesis of HBs, even in patients with familial HBs. Future studies exploring the underlying mechanisms will further illustrate how multiple genetic variations lead to the initiation and progression of HB, and they can help pinpoint therapeutic targets for the treatment of HBs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

ANNOVAR	Annotate Variation
CNS	central nervous system
CNV	copy number variation
HB	hemangioblastoma
SNV	single nucleotide variant
WES	Whole exome sequencing

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Fig. 1. Distribution of single nucleotide variants in patients with HBs

A: Distribution of all single nucleotide variants.

B: Distribution of single nucleotide variants in coding regions.

Patients 11–16 were those with sporadic HBs, and patient 21–25 were those with familial HBs.

HB, hemangioblastoma; SNV, single nucleotide variant

Table 1

Basic characteristics of the HB patients.

Patient ID	Age	Sex	Type of HB	Features of HB (location and VHL-related presentation)	Family history of HB
11	60	F	Sporadic	Left cerebellum HB	No
12	26	F	Sporadic	Fourth ventricle HB	No
13	56	M	Sporadic	Cerebellum HB	No
14	56	M	Sporadic	Left CPA HB	No
15	59	M	Sporadic	Right cerebellum HB	No
16	47	F	Sporadic	Left cerebellum HB	No
21	47	F	Familial HB	Posterior cranial fossa HBs (bilateral renal cell carcinoma)	Yes, mother
22	32	M	Familial HB	T6-7 HB (5 yrs after cerebellum HB operation, multiple cysts in the left kidney and pancreas)	Yes, father and sister
23	25	M	Familial HB	Multiple HBs in medulla oblongata and cerebellum sample from the cerebellum	Yes, mother
24	39	M	Familial HB	Cerebellum HB	Yes, mother and sister
25	25	M	Familial HB	Multiple cranial HBs in sample from the cerebellum	Yes, mother and cousin

CPA, ; VHL, ; F, female; M, Male; HB, hemangioblastoma

Table 2

Somatic single nucleotide variants in patients with HBs.

Patients	Missense	Nonsense	Splice site	Other	Total mutation	Novel mutation
11	0	0	0	9	9	1
12	11	2	1	19	33	29
13	4	0	0	10	14	9
14	1	0	0	10	11	10
15	0	0	0	6	6	0
16	0	0	0	13	13	2
Total	16	2	1	67	86	51
21	27	2	0	65	94	81
22	2	0	0	13	15	5
23	8	1	0	23	32	29
24	7	0	0	9	16	12
25	1	0	0	26	27	12
Total	45	3	0	136	184	139

Patients 11–16 were those with sporadic HBs, and patient 21–25 were those with familial HBs.

HB, hemangioblastomas.

Table 3

The five genes having CNV in all of the six patients with sporadic HBs.

Gene	Chr	Start	End	Copy Ratio	Type	Patient
<i>OTOGL</i>	12	80707425	80714079	1.70	amplification	11
<i>OTOGL</i>	12	80764407	80765658	1.99	amplification	11
<i>OTOGL</i>	12	80661063	80665359	0.66	deletion	12
<i>OTOGL</i>	12	80730572	80732809	0.53	deletion	12
<i>OTOGL</i>	12	80496991	80605596	1.71	amplification	13
<i>OTOGL</i>	12	80660659	80663966	1.26	amplification	13
<i>OTOGL</i>	12	80750095	80831570	1.26	amplification	14
<i>OTOGL</i>	12	80672958	80703998	0.60	deletion	15
<i>OTOGL</i>	12	80707447	80714034	0.63	deletion	15
<i>OTOGL</i>	12	80714535	80717256	0.45	deletion	15
<i>OTOGL</i>	12	80752478	80761196	0.59	deletion	15
<i>OTOGL</i>	12	80762203	80770863	0.71	deletion	15
<i>OTOGL</i>	12	80722584	80726771	1.31	amplification	16
<i>PLCB4</i>	20	9255315	9317733	1.38	amplification	11
<i>PLCB4</i>	20	9353750	9364815	1.45	amplification	11
<i>PLCB4</i>	20	9215047	9317649	0.69	deletion	12
<i>PLCB4</i>	20	9449377	9453533	0.61	deletion	12
<i>PLCB4</i>	20	8862743	9287807	1.89	amplification	13
<i>PLCB4</i>	20	9287952	9364795	1.28	amplification	13
<i>PLCB4</i>	20	9382481	9388309	1.48	amplification	13
<i>PLCB4</i>	20	9343176	9351983	1.36	amplification	14
<i>PLCB4</i>	20	9346283	9364729	0.74	deletion	15
<i>PLCB4</i>	20	9299560	9318813	1.32	amplification	16
<i>SCEL</i>	13	78137954	78143397	1.35	amplification	11
<i>SCEL</i>	13	78201874	78210222	0.45	deletion	12
<i>SCEL</i>	13	77904169	78124018	2.00	amplification	13
<i>SCEL</i>	13	78133951	78142864	1.28	amplification	13

Gene	Chr	Start	End	Copy Ratio	Type	Patient
<i>SCEL</i>	13	78130942	78133692	0.60	deletion	14
<i>SCEL</i>	13	78178371	78217054	1.32	amplification	14
<i>SCEL</i>	13	78176890	78191481	0.73	deletion	15
<i>SCEL</i>	13	78196903	78208368	0.50	deletion	15
<i>SCEL</i>	13	78042366	78125039	1.38	amplification	16
<i>THSD4</i>	15	71548883	71581017	0.69	deletion	11
<i>THSD4</i>	15	71704021	71731836	0.67	deletion	11
<i>THSD4</i>	15	71903510	71939503	0.55	deletion	12
<i>THSD4</i>	15	71411565	71446614	1.68	amplification	13
<i>THSD4</i>	15	71550735	71630571	1.57	amplification	13
<i>THSD4</i>	15	71634234	71700371	1.58	amplification	13
<i>THSD4</i>	15	71704946	71834240	1.90	amplification	13
<i>THSD4</i>	15	71834244	71950228	1.35	amplification	13
<i>THSD4</i>	15	71953052	72020695	1.68	amplification	13
<i>THSD4</i>	15	72037749	72041318	0.68	deletion	14
<i>THSD4</i>	15	71603850	71633595	0.66	deletion	15
<i>THSD4</i>	15	72039196	72050246	0.73	deletion	16
<i>WWOX</i>	16	78285500	78323808	1.40	amplification	11
<i>WWOX</i>	16	78267224	78289780	0.56	deletion	12
<i>WWOX</i>	16	78083567	78142310	1.40	amplification	13
<i>WWOX</i>	16	78151058	78387973	1.54	amplification	13
<i>WWOX</i>	16	78466792	78789897	1.64	amplification	13
<i>WWOX</i>	16	78792488	78859135	1.68	amplification	13
<i>WWOX</i>	16	78867130	79245296	1.65	amplification	13
<i>WWOX</i>	16	79246081	79340769	1.76	amplification	13
<i>WWOX</i>	16	78128470	78136040	0.48	deletion	14
<i>WWOX</i>	16	78466467	78482962	1.27	amplification	15
<i>WWOX</i>	16	78642591	78714289	1.41	amplification	16
<i>WWOX</i>	16	78873601	79034196	1.26	amplification	16

CNV, copy number variation

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Table 4

The three genes having CNV in all of the five patients with familial HBs.

Gene	Chr	Start	End	Copy Ratio	Type	Sample
<i>ABCA6</i>	17	67091701	67101720	1.32	amplification	21
<i>ABCA6</i>	17	67087558	67101675	1.28	amplification	22
<i>ABCA6</i>	17	67110783	67124511	1.30	amplification	22
<i>ABCA6</i>	17	67060848	67077218	0.72	deletion	23
<i>ABCA6</i>	17	67114246	67120997	1.28	amplification	24
<i>ABCA6</i>	17	67098740	67101581	0.60	deletion	25
<i>CWC27</i>	5	64081466	64084760	1.63	amplification	21
<i>CWC27</i>	5	64079888	64110852	1.38	amplification	22
<i>CWC27</i>	5	64064936	64070739	0.65	deletion	23
<i>CWC27</i>	5	64291614	64313873	0.63	deletion	24
<i>CWC27</i>	5	64096124	64100055	0.71	deletion	25
<i>LAMA2</i>	6	129419549	129467874	1.44	amplification	21
<i>LAMA2</i>	6	129475563	129478619	1.61	amplification	21
<i>LAMA2</i>	6	129591600	129601349	1.53	amplification	22
<i>LAMA2</i>	6	129774271	129777347	1.68	amplification	22
<i>LAMA2</i>	6	129475586	129478619	0.62	deletion	23
<i>LAMA2</i>	6	129794354	129799655	1.47	amplification	24
<i>LAMA2</i>	6	129377950	129389401	0.67	deletion	25

CNV, copy number variation