Variant analysis in Chinese families with hereditary hemorrhagic telangiectasia

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Abstract
Background: Hereditary hemorrhagic telangiectasia (HHT) is a vascular dysplasia disorder characterized by epistaxis, mucocutaneous telangiectasias and arteriovenous malformations in internal organs. Recurrent epistaxis is the primary complaint in 90%-96% of HHT patients and the other symptoms come with age. The aim of this study was to analyze HHT-associated gene variant spectrum in Chinese HHT patients and to assess whether genetic testing could contribute to the early diagnosis.

Methodology/Principal: Thirty one HHT families including 62 individuals were recruited. Variants in the coding regions of four genes involved in HHT were amplified and analyzed using Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA).

Results: Twenty unique variants, including 8 novel variants were found in 24 of the 31 (77.4%) kindred. Diagnosis is confirmed for 7 possible individuals from 6 kindred. Thirteen ACVRL1 variants were detected from 17 isolated HHT families. Variants in ACVRL1 from 8/17 (47.1%) families were located in exon8. Seven ENG variants were found in 7 unrelated families throughout the coding region.

Conclusion: We conclude that ACVRL1 gene variant is 2.4 times more prevalent than that in ENG in Chinese individuals with HHT, and exon8 of the ACVRL1 gene may be a hotspot region. Genetic testing could contribute to early diagnosis for HHT.

KEYWORDS
ACVRL1, ENG, epistaxis, hereditary hemorrhagic telangiectasia, variants

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1 | INTRODUCTION

Hereditary hemorrhagic telangiectasia, also known as Rendu-Osler-Weber syndrome, is a rare autosomal dominant genetic disorder, which affects 1 in 5–8,000 individuals (Faughnan et al., 2011; Govani & Shovlin, 2009; Lesca et al., 2007; Shovlin, 2010). Characteristic features of HHT include recurrent epistaxis, the presence of mucocutaneous telangiectasias, arteriovenous malformations (AVMs) in internal organs, and family history of HHT (Guttmacher, Marchuk, & White, 1995). The clinical diagnosis of HHT is based on the Curacao criteria (Shovlin et al., 2000), which propose that three or more of the four characteristic features described above define a definite diagnosis, where as two of these features suggest a “possible” diagnosis and one or none of these features indicate unlikely HHT. The penetrance for HHT is age-dependent. Epistaxis is the first and the primary manifestation in 90%–96% of HHT patients (Guttmacher et al., 1995). Thus, the diagnosis for children and sporadic patients with recurrent epistaxis only is hard to decide.

At least four genes, including Endoglin (ENG, OMIM: 131195; McAllister et al., 1994) resulting in HHT1 (OMIM: 187300), Activin A Receptor Type II-like 1 (ACVRL1, OMIM: 601284) resulting in HHT2 (OMIM: 600376) (Johnson et al., 1996), SMAD family member 4 (SMAD4, OMIM: 600993) resulting in HHT syndrome associated with juvenile polyposis (JP-HHT, OMIM: 175050) (Gallione et al., 2006) and Bone morphogenetic proteins 9 (BMP9, OMIM: 605120) resulting in a vascular anomaly syndrome (HHT5, OMIM: 615506) (Wooderchak-Donahue et al., 2013), are thought to be responsible for about 90% HHT patients diagnosed by the clinical features. The remaining ~10% of HHT patients have an unidentified genetic cause, which may be resulted from intronic variants in the known genes or caused by a novel gene (McDonald et al., 2011; Wooderchak-Donahue et al., 2018). The aforementioned genes were all part of the transforming growth factor (TGFβ) signal pathway and integral to angiogenesis. Pathogenic variants in any of these genes may disrupt the balance between pro- and antiangiogenic signals for normal vascular development, resulting in HHT.

Previous studies have indicated that the disorder was caused predominantly by variants in either ENG (McAllister et al., 1994; McDonald et al., 1994; Shovlin et al., 1994) or ACVRL1 (Johnson et al., 1995; Vincent et al., 1995) genes. More than 500 variants have been reported in the two genes. Many of the variants were specific for each family, however, recurrent or founder variant has been reported in some populations, suggesting that the variant spectrum for HHT families may vary in different populations. Indeed, it has been shown that American, North European and Japanese families have fewer ACVRL1 variants than ENG variants (Komiyama, Ishiguro, Yamada, Morisaki, & Morisaki, 2014; McDonald et al., 2011). Presently there is only one report in the literature on the clinical and genetic characteristics of Chinese HHT patients (Chen et al., 2013). Thus, the aim of our study was to expand on this database on the variant spectrum of Chinese patients with HHT, and to assess whether genetic testing could set the diagnosis for Chinese patients with HHT.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

The study was approved by the Ethics Committee of Beijing TongRen Hospital and performed in accordance with the guidelines of the World Medical Association’s Declaration of Helsinki. Written informed consent was obtained from all subjects or from next of kin, and carers or guardians of minors/children.

2.2 | Cohort

A total of 62 individuals, including 36 females and 26 males, from 31 unrelated families with one or more members suffering from HHT were recruited from the outpatient clinic of Otolaryngology, Head and Neck Surgery Department at Beijing TongRen Hospital, who come from the different provinces in China. All the patients were of Han Chinese origin and aged between 4 years old to 73 years old; with a mean age of 42.9 ± 15.7 years. Clinical diagnosis of HHT was made according to the Curacao criteria (Shovlin et al., 2000). A cohort of 100 individuals without recurrent epistaxis, telangiectasias and the family history of HHT were also recruited as normal controls. Subjects were excluded if they or their first degree family members had any inherited vascular diseases.

2.3 | DNA extraction

DNA was extracted from the peripheral blood leukocytes using the DNA Isolation Kit (Roche, Indianapolis, USA).

2.3.1 | Single nucleotide variants and indel analysis

The protein coding sequences together with intron/exon boundaries of the four related genes (ENG, NM_000118.3; ACVRL1, NM_000020.2; SMAD4, NM_005359.5; BMP9, NM_016204.2) were amplified using polymerase chain reaction (PCR) for all DNA samples. The purified PCR products were directly sequenced using BigDye Terminator v.3.1 Cycle sequencing Kit (Applied Biosystems, Foster City, USA) and analyzed on ABI 3,730 DNA Analyzer (Applied Biosystems, Foster City, USA). PCR and sequencing primer pairs were designed using online Primer 3.0 software (Koressaar & Remm, 2007) (Table S1). The coding region and the flanking sequences (about 50 bases around the
coding region) of the four genes were captured. Nucleotide alterations were identified by sequence alignment with the NCBI Reference Sequence (Build137). When a novel missense variant was identified, the paralog and ortholog sequences were compared using the CLUSTAL O (1.2.4) Multiple Sequence Alignment Program (Bayrak-Toydemir, Mao, Lewin, & McDonald, 2004). The functional impact on the protein as an amino acid substitution was assessed using SIFT software (Bayrak-Toydemir et al., 2006).

2.4 | Deletion/duplication detection

Large deletions and duplications in the ACVRL1 and ENG genes of individuals who tested negative via PCR amplification and sequencing were detected using the SALSA MLPA kit (P093-B1 HHT/PPH1, MRC-Holland, the Netherlands), according to the manufacturer’s instructions. MLPA peak plots were analyzed using the Coffalyser. Net software (MRC-Holland) to normalize and calculate the dosage ratios. Limit dosage ratios of $\leq$0.7 and $\geq$1.35 were set for deletion and duplication, respectively.

Additionally, when a variant likely to be pathogenic was identified in a proband, the variant was screened in other family members to assess whether the variant was co-segregated with the patients and normal individuals. Furthermore, one hundred unrelated normal individuals were analyzed for each novel variant detected.

2.5 | Evaluation of variants

The classification for variants uses the joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Variants have been classified as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign and benign (Richards et al., 2015). The calculation and analysis for the probability of observed cosegregation was according to the method recommended by Jarvik & Browning (2016).

3 | RESULTS

Overall, 62 individuals from 31 HHT families were recruited. Among them, five individuals were sporadic with no family history of HHT and the other 57 individuals came from 26 families with other affected members (Figure S1).

Table 1 shows the characteristics of all the participants in the study. Epistaxis was the most frequent clinical feature in our cohort and all the individuals had the manifestation. Overall, 32 patients were diagnosed as definite HHT patients and 11 as possible HHT patients, with the HHT onset age ranging from 3 to 50 years old. Four subjects were classified as “carriers” based on the presence of pathogenic gene variant and the missing symptoms, which may be explained by their rather young age (the age were described in Table 1).

A total of 20 variants were identified in 24 of 31 kindreds (sequences shown in Figure S2), with 24 definite HHT cases from 19 kindreds, 9 possible HHT individuals from 8 kindreds and 4 carriers from 4 kindreds, which were responsible for 77.4% (24/31) of all HHT families. No variant was detected in 7 families (7/31, 22.6%), including 5 families with 8 definite HHT patients and 2 families with 2 possible HHT patients (Table 1).

All the 20 variants were single nucleotide variants (SNVs) or small indels located in ACVRL1 and ENG gene. We didn’t find any pathogenic variant in the SMAD4 or BMP9 gene. No gross alteration was found in the MLPA analysis for ACVRL1 and ENG.

A total of 13 variants in ACVRL1 gene were detected from 17 isolated HHT families (17/24 families, 70.8%). The ACVRL1 variant of c.200G>A in exon3, the c.1120C>T and c.1232G>A in exon8 were recurrent in unrelated families. Overall, variants found in 8/17 (47.1%) of all families with ACVRL1 variants were located in exon8 (including 5 unique variants) (Figure 1). The distribution of other ACVRL1 variants was illustrated on Figure 1. Similarly, seven variants in ENG gene were identified in 7 HHT families (7/24 families, 29.2%). The distribution of the seven ENG variants was showed on Figure 1.

A total of eight novel variants, which have not been reported previously, were found in this study. Four of these were detected in the ENG gene (c.593del, c.840del and c.1878+7C>T and c.841A>G), and the other four novel variants were located in the ACVRL1 gene (c.576del, c.1207C>G, and c.552_559delinsTCTGCTCAGGTGCAGTCT and c.1042G>A). We further analyzed the pathogenic potential for the different type of novel variants in the following section.

Four of the novel variants were out-of-frame indels, which may be pathogenic as haploinsufficiency of ENG or ACVRL1 was an underlying cause of HHT (Pece-Bybarca, Cymerman, Vera, Marchuk, & Letarte, 1999). These variants have never been found in public databases (dbSNPs, 1000Genome and ESP) neither the previous investigations. Patient’s phenotype is highly specific for HHT. Variant of c.552_559delinsTCTGCTCAGGTGCAGTCT in ACVRL1 was found in a sporadic individual. In regards to c.593del, c.840del in ENG and the c.576del in ACVRL1, variants testing for the family members found cosegregation with disease in more than one family member. Variant of c.593del was found in a four generation family (family ID: F3, Figure S1). Both the proband and her son, who were clinically diagnosed as definite HHT, were heterozygous with the variant. Samples of the other family members were not obtained. The second out-of-frame variant (c.840del) was detected in a four generation family with three patients. The
| Family ID | Individual ID | Gender | Age | Epistaxis MT | AVMs | Family history | Diagnosis | Gene | Exon | Nucleotide change | Amino acid change | Classification |
|-----------|---------------|--------|-----|--------------|------|----------------|-----------|------|------|------------------|-----------------|----------------|
| F1        | F1_III:1      | Male   | 55  | Yes          | No   | GIT            | Yes       | HHT  | ACVRL1 | EXON8  | c.1231C>T      | p.Arg411Trp     | Pathogenic     |
| F1        | F1_III:2      | Male   | 47  | Yes          | Na   | Na             | Yes       | Possible HHT | ACVRL1 | EXON8  | c.1231C>T      | p.Arg411Trp     | Pathogenic     |
| F1        | F1_III:3      | Female | 40  | Yes          | Na   | Na             | Yes       | Possible HHT | ACVRL1 | EXON8  | c.1231C>T      | p.Arg411Trp     | Pathogenic     |
| F1        | F1_IV:1       | Male   | 28  | /             | No   | No             | Yes       | Carrier | ACVRL1 | EXON8  | c.1231C>T      | p.Arg411Trp     | Pathogenic     |
| F2        | F2_II:1       | Female | 58  | Yes          | Yes  | PAVMs          | Yes       | HHT   | ACVRL1 | EXON3  | c.200G>A       | p.Arg67Gln      | Pathogenic     |
| F2        | F2_III:1      | Male   | 31  | /             | No   | No             | Yes       | Normal | /      | /     | /                |                 |                |
| F2        | F2_III:2      | Female | 72  | Yes          | Yes  | HAVMs          | Yes       | HHT   | ENG    | EXON5  | c.593del       | p.Pro198Argfs*24 | Pathogenic     |
| F3        | F3_II:1       | Male   | 48  | Yes          | Na   | Yes            | Yes       | HHT   | ENG    | EXON5  | c.593del       | p.Pro198Argfs*24 | Pathogenic     |
| F3        | F3_III:1      | Male   | 32  | Yes          | Yes  | HAVMs          | Yes       | HHT   | ACVRL1 | INTRON4 c.526–3C>G | /              | VUS             |
| F5        | F5_I:1        | Male   | 73  | Yes          | Na   | HAVMs, PAVMs   | Yes       | HHT   | ENG    | EXON7  | c.841A>G       | p.Ile281Val    | VUS             |
| F6        | F6_II:2       | Male   | 42  | Yes          | No   | No             | Yes       | Possible HHT | ACVRL1 | EXON10 | c.1436G>C      | p.Arg479Pro     | VUS             |
| F6        | F6_III:1      | Male   | 44  | /             | No   | No             | Yes       | Normal | /      | /     | /                |                 |                |
| F7        | F7_II:1       | Female | 61  | Yes          | Yes  | GIT            | Yes       | HHT   | /      | /     | /                |                 |                |
| F7        | F7_III:1      | Male   | 50  | Yes          | Yes  | Na             | Yes       | HHT   | /      | /     | /                |                 |                |
| F7        | F7_IV:2       | Female | 22  | Yes          | Yes  | Na             | Yes       | HHT   | /      | /     | /                |                 |                |
| F7        | F7_III:2      | Male   | 30  | /             | No   | No             | Yes       | Normal | /      | /     | /                |                 |                |
| F7        | F7_II:2       | Male   | 58  | /             | No   | No             | Yes       | Normal | /      | /     | /                |                 |                |
| F7        | F7_III:1      | Male   | 32  | /             | No   | No             | Yes       | Normal | /      | /     | /                |                 |                |
| F7        | F7_III:2      | Female | 37  | /             | No   | No             | Yes       | Normal | /      | /     | /                |                 |                |
| F7        | F7_II:3       | Female | 58  | /             | No   | No             | Yes       | Normal | /      | /     | /                |                 |                |
| F8        | F8_III:1      | Female | 35  | 3             | Yes  | Yes            | Yes       | HHT   | ENG    | EXON7  | c.840del       | p.Ile281Serfs*78 | Pathogenic     |
| F8        | F8_IV:1       | Male   | 10  | Yes          | No   | Na             | Yes       | Possible HHT | ENG    | EXON7  | c.840del       | p.Ile281Serfs*78 | Pathogenic     |
| F8        | F8_III:1      | Male   | 60  | Yes          | Yes  | HAVMs, GIT     | Yes       | HHT   | ENG    | EXON7  | c.840del       | p.Ile281Serfs*78 | Pathogenic     |
| F9        | F9_III:2      | Male   | 41  | Yes          | Yes  | Na             | Yes       | HHT   | ENG    | EXON14 | c.1878+7C>T    | /                | Likely pathological |
| F9        | F9_III:1      | Female | 43  | /             | No   | No             | Yes       | Normal | /      | /     | /                |                 |                |
| F9        | F9_III:4      | Male   | 40  | Yes          | Yes  | Na             | Yes       | HHT   | ENG    | EXON14 | c.1878+7C>T    | /                | Likely pathological |

(Continues)
| Family ID | Individual ID | Gender | Age | Onset age | Epist axis | MT | AVMs | Family history | Diagnosis | Gene | Exon | Nucleotide change | Amino acid change | Classification |
|----------|---------------|--------|-----|-----------|------------|-----|------|---------------|-----------|------|------|------------------|-----------------|---------------|
| F9 F9_II:1 | Female | 67 | 20 | Yes | Yes | GIT | Yes | HHT | ENG | EXON14 | c.1878+7C>T | / | Likely pathogenic |
| F9 F9_II:1 | Female | 39 | / | No | No | Na | No | Normal | / | / | / | / | / |
| F11 F11_II:1 | Male | 50 | 10 | Yes | Yes | Na | Yes | HHT | ENG | EXON4 | c.496del | p.Gln166Argfs*56 | Pathogenic |
| F12 F12_III:1 | Male | 53 | 20 | Yes | Yes | HAVMs | Yes | HHT | ACVRL1 | EXON8 | c.1232G>A | p.Arg411Gln | Pathogenic |
| F13 F13_II:1 | Female | 37 | 5 | Yes | Na | HAVMs | Yes | HHT | ENG | EXON4 | c.496del | p.Gln166Argfs*56 | Pathogenic |
| F14 F14_II:1 | Female | 45 | 17 | Yes | Yes | Na | Yes | HHT | ACVRL1 | EXON7 | c.853C>T | p.Leu285Phe | VUS |
| F15 F15_II:1 | Female | 50 | 15 | Yes | Yes | Na | Yes | HHT | ACVRL1 | EXON8 | c.1120C>T | p.Arg374Trp | Pathogenic |
| F16 F16_II:1 | Male | 23 | / | No | No | Na | Yes | Carrier | ACVRL1 | EXON8 | c.1120C>T | p.Arg374Trp | Pathogenic |
| F18 F18_II:1 | Female | 33 | 3 | Yes | Na | Na | Yes | Possible HHT | / | / | / | / | / |
| F18 F18_III:1 | Female | 11 | / | No | No | Na | Yes | Normal | / | / | / | / | / |
| F19 F19_II:1 | Male | 57 | 50 | Yes | Yes | Na | Yes | HHT | ACVRL1 | EXON7 | c.1042G>A | p.Asp348Asn | VUS |
| F20 F20_III:1 | Male | 36 | 28 | Yes | Yes | Na | Yes | HHT | ACVRL1 | EXON8 | c.1207C>G | p.Leu403Val | VUS |
| F21 F21_III:1 | Female | 58 | 15 | Yes | Yes | Na | Yes | HHT | ACVRL1 | EXON8 | c.1135G>A | p.Glu379Lys | Pathogenic |
| F21 F21_III:1 | Female | 50 | 14 | Yes | Yes | Na | Yes | HHT | ACVRL1 | EXON8 | c.1123G>A | p.Glu379Lys | Pathogenic |
| F22 F22_III:1 | Female | 53 | 45 | Yes | Yes | HAVMs | Yes | HHT | / | / | / | / | / |
| F23 F23_III:1 | Female | 41 | 3 | Yes | Yes | Na | Yes | HHT | / | / | / | / | / |
| F24 F24_III:1 | Female | 42 | 14 | Yes | Yes | HAVMs | Yes | HHT | ENG | EXON6 | c.772del | p.Tyr258Thrfs*101 | Pathogenic |
| F25 F25_II:1 | Male | 45 | 13 | Yes | Yes | Na | Yes | HHT | ACVRL1 | EXON8 | c.1232G>A | p.Arg411Gln | Pathogenic |
| F26 F26_II:1 | Female | 14 | / | No | No | Na | Yes | Carrier | ACVRL1 | EXON8 | c.1232G>A | p.Arg411Gln | Pathogenic |
| F27 F27_II:1 | Female | 59 | 20 | Yes | No | Na | Yes | Possible HHT | ACVRL1 | EXON5 | c.576del | p.Leu193Trpfs*65 | Pathogenic |
| F27 F27_II:1 | Female | 36 | / | No | No | Na | No | Normal | / | / | / | / | / |
| F27 F27_III:2 | Female | 33 | / | No | No | Na | No | Normal | / | / | / | / | / |
The proband, her father and her son were heterozygous with variant of c.840del (family ID: F8, Figure S1). The proband and her father were clinically diagnosed as HHT with more than three features. And the proband’s son, a possible HHT individual, was confirmed by the genetic testing. The novel c.576del was found in a four generation family (family ID: F27, Figure S1). In this family, a HHT patient and two normal individuals were recruited. The HHT patient was heterozygous with the c.576del variant and the two normal individuals were wildtype. And the genotype for all obtained members in F27 was co-segregated with the manifestation of the HHT. All the above findings indicated that these novel variants were likely to be pathogenic.

The fifth novel variant (c.1878+7C>T,) was a substitution in the 3’UTR region of the ENG. It was found in a four generation family (family ID: F9, Figure S1). In this family, 6 DNA samples from 4 patients and 2 normal individuals were obtained and tested. All 4 patients were heterozygous with this variant of c.1878+7C>T, and the 2 normal individuals were wild type for this site. The variant was rare and not found in the previous reports and the public databases (dbSNPs, 1000Genome and ESP), thus, we assumed the variant only entered the pedigree once. The probability of observed cosegregation was calculated. The untyped relative (II:2) who must had passed the variant was assumed to be heterozygous. Considering definitely affected individuals (II:3, III:3, III:5 and IV:3), we observed four meioses, so the affected individuals contributed a factor of (1/2)^4 to the value of probability of observed cosegregation. The normal individual (III:1) from the family contributes a factor of (1−(1/2)) = 1/2. Thus, for family F9, the probability of observed cosegregation was 1/32, which is a strong evidence for the pathogenicity of c.1878+7C>T in the ACVRL1 (Jarvik & Browning, 2016).

The other three novel missense variants (c.841A>G in ENG, c.1207C>G and c.1042G>A in ACVRL1) were absent in the 100 normal individuals. Variants of c.1207C>G and c.1042G>A were not found in the public databases (dbSNPs, 1000Genome and ESP) and the previous studies. This variant of c.841A>G was reported in the EXAC (The Exome Aggregation Consortium) database (Cymerman, Vera, Karabegovic, Abdalla, & Letarte, 2003). The Minor allele frequency of this variant was 1.7e-5 in the dbSNPs database. SIFT was applied to predict whether the missense variants affect protein function. It was predicted to be “damaging”. Alignment for amino acid sequences from different species found that the novel missense variants were located in the conserved region (Figures S3 and S4).

**4 | DISCUSSION**

HHT presents clinically as a variety of symptoms including recurrent epistaxis, mucocutaneous telangiectasias, and visceral AVMs in lung, liver, gastrointestinal tract, brain or
spinal cord. In the present study, most of the patients diagnosed as definite HHT patients demonstrated epistaxis, mucocutaneous telangiectasia and family history. Nose bleeds were observed in all definite individuals, but also the 11 possible HHT individuals.

Our study has demonstrated that 77.4% of the kindred (24/31, 24 families including 24 definite HHT cases, 9 possible HHT cases, 4 carriers) had \textit{ACVRL1} or \textit{ENG} variant, which is in accordance with the findings of previous reports (Bayrak-Toydemir et al., 2006; Chen et al., 2013; Heimdal et al., 2016). However, the variant carrier percent in HHT from the ARUP institute is higher than that of our study, as the non-coding region variants are screened, which may contribute to about 1% of HHT patients (Wooderchak-Donahue et al., 2018). What’s more, none variant was found in \textit{SMAD4} and \textit{BMP9} gene, which may account for about 1%–2% of HHT patients. In the present study, a likely or clearly pathogenic variant was detected in 7 possible HHT cases and 4 individuals without any symptoms except for positive family history, which enabled us to set the diagnosis of HHT for the recurrent epistaxis patients and find the high risk individuals early. These findings suggest that the Curacao Criteria should be revised to take into consideration the results of genetic testing, which could confirm the recurrent nose bleeds and find the high risk individuals early (Torrying, Brusgaard, Ousager, Andersen, & Kjeldsen, 2014).

Previous studies have indicated that there is a considerable variation in the \textit{ENG}/\textit{ACVRL1} variant ratio in different populations. While one study in French HHT patients has demonstrated the \textit{ENG}/\textit{ACVRL1} variant ratio range from 0.37 to 0.51 (Lescsa et al., 2007), a \textit{ENG}/\textit{ACVRL1} variant ratio of 0.72 (13/18) has been found in Canadian patients (Abdalla et al., 2005), a ratio of 1.22 in American (USA) patients (Bayrak-Toydemir et al., 2006) and a ratio of 2.0 in Danish patients (Brusgaard et al., 2004). In the current study, 17 families (17/24, 70.8%) had variants in \textit{ACVRL1} gene and 7 families (7/24, 29.2%) in the \textit{ENG}, providing an \textit{ENG}/\textit{ACVRL1} variant ratio of 0.41 (7/17), which is comparable to the findings in the French patients. In comparison, another study of patients from 14 Chinese families has indicated a ratio is 0.25 (2/8) (Chen et al., 2013). It is possible that the wide variation noted in the \textit{ENG}/\textit{ACVRL1} variant ratios in different populations may be a consequence of differences in patient numbers and study methods employed in the different studies. However despite these differences, the findings from the two studies of Chinese HHT patients indicate that \textit{ACVRL1} variants, which were 2.4–4 times greater than \textit{ENG} variants, are the predominant cause of HHT in the Chinese patients.

In \textit{ACVRL1}, the c.1120C>T (p.Arg374Trp) and c.1232G>A (p.Arg411Gln) variants on exon8 were seen twice and three times in apparently unrelated families. These two variants have been reported in several families in previous studies (Abdalla, Cymerman, Johnson, Deber, & Letarte, 2003; Bayrak-Toydemir et al., 2006; Berg et al., 1997; Harrison et al., 2003; Johnson et al., 1996; Kjeldsen et al., 2001; Lescsa et al., 2004; Trembath et al., 2001), suggesting that these codons may be the hotspot or founder region of the \textit{ACVRL1} gene, which may need further studies in a larger sample size to proved. In this study, variants in 8/17(47.1%) families were located in exon8 of \textit{ACVRL1} gene. Indeed, a study by Chen and colleagues (Chen et al., 2013) investigating 14 Chinese HHT families also reported 8 unique \textit{ACVRL1} variants, of which 3/8 (37.5%) variants (c.1121G>A, c.1124A>G and c.1195T>C) were located in exon8. These
findings suggest that exon8 of the ACVRL1 gene may be a hotspot region, which may be useful in the effective genetic testing for HHT. In contrast, seven of the ENG variants were widely distributed throughout the gene, none of which was observed in multiple families.

In this study, a total of eight novel variants were found and the pathogenicity was evaluated. Four of the novel out-of-frame indels were proved to be pathogenic for HHT. The variant of c.1878+7C>T in ENG was found in a four-generation family. The cosegregation with HHT in this family was a strong evidence for the pathogenicity of c.1878+7C>T. Besides that, the variant was rare and absent in the public databases. These results strongly supported that it was to be “likely pathogenic”, although all variants in the two databases. These results strongly supported that it was to be strong evidence for the pathogenicity of c.1878+7C>T. About 47.1% of the ACVRL1 variants are located in exon8, despite a wide distribution throughout the gene. These findings suggest that exon8 of the ACVRL1 gene may be a hotspot region for HHT in Chinese patients. And c.1120C>T (p.Arg374Trp) and c.1232G>A (p.Arg411Gln) in ACVRL1 may be the commonest variants. Further studies with a larger sample size and functional analysis for the variants are needed to confirm this.

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CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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