International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-1, January 2016 Pages 32-36

Mutational Analysis Mapping on The Molecular Structure of The ACVRL1 Protein and Implications For Rendu-Osler-Weber (ROW)

George N. Goulielmos*#, Elias Eliopoulos*, Alexandros Karatzanis, Maria I. Zervou, Emmanuel Fountakis, Stylianos G. Velegrakis, Trias Thireou, Emmanuel P. Prokopakis

***Abstract*—About 80% of Rendu-Osler–Weber (ROW) patients carry mutations in endoglin (*ENG*) or activin receptor-like kinase1 (*ACVRL1*; *ALK1*) genes. In order to investigate the molecular mechanisms that govern the pathogenic effect of the mutations in *ACVRL1*, we have collected and analyzed the mutational effect of over 80 different predominant mutations, as well as their location, on the 3D molecular structures of N- and C-terminal domains of ACVRL1. We have used macromolecular modeling on the protein structural components of ACVLR1 and structural component interface analysis to locate position and interaction of point mutations. Specific mutations were identified using genomic DNA sequencing from blood leucocytes. Out of the 151 point mutations reported for the *ALK1* gene, the majority are located on the surface of the ARD and PK structural domains, with some on the interaction interface. New observed mutation Cys90Phe found in two Cretan ROW patients, located on loop F4 of ARD, introduces conformational steric hindrance and disruption of stability. We have mapped point mutations on the structural domains of ACVLR1, correlating location and severity of ROW. In addition, we report the identification and location of a novel missense mutation, Cys90Phe, which has not yet been described. It is identified in a Cretan ROW patient, and associated with severe clinical appearance according to the Curacao criteria.**

George N. Goulielmos, Laboratory of Molecular Medicine and Human Genetics, School of Medicine, University of Crete, Heraklion, Greece, Phone No. +302810394673,

Elias Eliopoulos, Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, 75, Iera Odos, 11855, Athens, Greece, Phone No. +2105294223,

Alexandros Karatzanis, Department of Otorhinolaryngology, University of Crete School of Medicine, Heraklion, Greece, Phone No. +302810 392348,

Maria I. Zervou, Laboratory of Molecular Medicine and Human Genetics, School of Medicine, University of Crete, Heraklion, Greece, Phone No. +302810394672,

Emmanuel Fountakis, Department of Otorhinolaryngology, University of Crete School of Medicine, Heraklion, Greece, Phone No. +302810392348,

Stylianos G. Velegrakis, Department of Otorhinolaryngology, University of Crete School of Medicine, Heraklion, Greece, Phone No. +302810392348

Trias Thireou, Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, 75, Iera Odos, 11855, Athens, Greece, Phone No. +2105294223,

Emmanuel P. Prokopakis, Department of Otorhinolaryngology, University of Crete School of Medicine, Heraklion, Greece, Phone No. +302810 392347,

*equal contribution

#corresponding author

***Index Terms*—ACVRL1, mutation, Rendu-Osler-Weber (ROW), 3-D model.**

I. Introduction

Hereditary hemorrhagic telangiectasia (HHT), which is also known as Osler–Weber–Rendu (ROW) syndrome, leads to abnormal blood vessel formation on the skin, mucous membranes, and often internal organs such as lungs, liver, and brain [1]. Five indistinguishable genetic types of ROW have been recognized and defined by associated gene loci so far, and three of these types of the disease have been linked to particular genes, namely activin receptor-like kinase 1 (*ACVRL1* or *ALK-1*), Endoglin (ENG) and *SMAD4* [2]. HHT1 (MIM #187300), with a high incidence of gastrointestinal bleeding, pulmonary and cerebral arteriovenous malformations, and HHT2 (MIM #600376), with a high incidence of hepatic arteriovenous malformations, which account for more than 80% of ROW, are caused by heterozygous mutations in *ENG* [3],[4] and activin *ACVRL1* [5] genes, respectively [6],[7]. Alterations in the sequence of both genes include point mutations, deletions, insertions, nonsense, and missense mutations, splice-site alterations, and intragenic rearrangements [8]. Additionally, an association of the ROW phenotype with colonic polyposis and mutations in the *SMAD4* gene, which is located in 18q21.1 and codes for SMAD4 (involved in the TGF-beta signaling pathway) has been demonstrated as well [9].

Many different mutations are so far known and it is likely that there is predominance of either type in a specific population. *ENG* mutations are more frequently found in patients from Northern Europe and the Americas, while Mediterranean populations have the majority of *ACVRL1* mutations. To date, 397 *ENG* and 332 *ACVRL1* mutations and polymorphisms have been reported (HHT Mutation Database) and the majority of *ACVRL1* (~53%) are located in exons 3, 7, 8 and 10 [7]. The number is continuously increasing, indicating that any nucleotide may be mutated, except probably those encoding for the transmembrane region (exon 13) or cytoplasmic domain (exon 14) [10]. It has been proposed that disease severity is more pronounced in HHT1 (caused by mutations in *ENG* gene) compared to HHT2 (mutations in *ACVRL1* gene). Considering all these limitations, genetic testing of ROW is valuable, especially in cases with limited symptoms, not only to confirm the clinical diagnosis but also to identify asymptomatic carriers among a

ROW family [11]. Of note, the severity and age of onset vary considerably both between families and among members of the same family, making diagnosis very difficult [10].

The mechanisms that govern the pathogenic effect of the mutations in *ACVRL1* remain to be further elucidated. To this end, in this study we have located their position on the experimental 3D structures of the N- and C-terminal domains of ACVRL1 [12],[13]. Furthermore, we applied the mutations and constructed three-dimensional models of the mutants, as a first step before their further classification according to the clinical severity of ROW. In addition, herein we report the identification of a novel missense mutation, Cys90Phe, identified recently in a Cretan ROW patient.

II. Materials and Methods

A. *Construction of ACVRL1 domains' three-dimensional (3D) model*

The 3-D experimental molecular structures of the N- (PDB code 3FA0) and C– (PDB code 3MY0) terminal domains of ACVRL1 have been used to create molecular models and generate specific mutants using Schrödinger LLC PRIME [14]. The derived models of mutants were checked for folding and packing differences against the wild type protein domains, as well as for their interaction with the TGF-β family ligands BMP9 and BMP10 and LDN-193189, respectively, *in silico* using Schrödinger Piper [15] and Schrödinger LLC GLIDE docking software [16]. PYMOL (The PyMOL Molecular Graphics System, Version 1.7.0.4 Schrödinger, LLC) was used for structural representations. Both structural domains were analysed for aminoacid residue accessibility, interface and interaction with neighboring subunits using PISA [17].

B. *Genetic analysis*

Blood sample of ROW patients of Greek origin with the tentative diagnosis of HHT2 was referred to our laboratory for molecular genetic analysis. Mutational analysis and examination of medical records were carried out with informed consent and under research protocols according to the declaration of Helsinki. Whole blood was collected in EDTA-containing tubes and genomic DNA was isolated from blood leucocytes by using the commercial kit Qiamp DNA Blood Mini kit (QIAGEN Inc, CA, USA). The coding exons and exon-introns boundaries of the two genes, *ENG* and *ACVRL1*, were amplified by polymerase chain reaction (PCR) using genomic DNA as template and sequenced (Genetic Diagnostic Laboratory, Department of Genetics, School of Medicine, University of Pennsylvania, USA).

III. Results

A. *Mapping of ROW associated mutations*

We have mapped 151 ROW associated mutations on the ACVRL1 domains. 31 are located on the N-terminal Activin Receptor domain (ARD) and 120 on the C-terminal Kinase domain (PK) (Fig. 1 and 2). The two domains are linked through a transmembrane Leucine rich helical domain consisting of twenty two aminoacid residues (Fig. 1a). Moreover, we have classified all these mutations according to severity and position, based on the existing literature

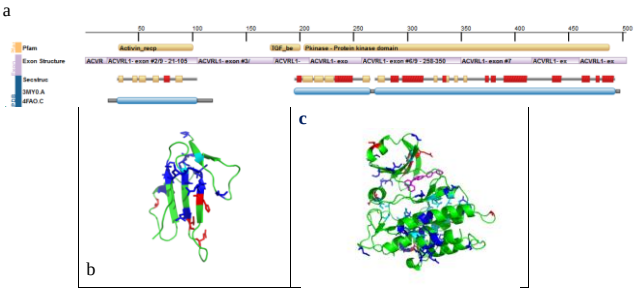

Figure 1. Representation of the ACVLR1 (ALK1) structure (gene and protein) (from Protein Data Bank). (a) Gene structure and protein domain secondary structure. (b) 3D structure of N-terminal (Activin Receptor) and C-terminal (Protein Kinase) domains of ALK1 with distribution of occurring mutations colored according to position and interaction (red for interface, blue for external and cyan for internal residues).

[5]-[8],[11],[12],[18]-[25] and the HTT mutation database (http://hhtmutation.org/ or http://arup.utah.edu/database/HHT/) dealing with the clinical consequences and manifestations of each of them (Fig. 2).

From this study, it is apparent that mutations on *ACVRL1* fall into three categories according to their location in the three-dimensional molecular domains:

• those located at the interacting interface that may alter interaction or recognition properties between the N-terminal domain (ARD) and the interacting TGF-β family ligands or at the cytoplasmic protein kinase domain active site,

• those located on the non-interacting surface but playing an important role in the molecule's folding process and finally,

• those located in the core of the protein domains that may alter charge distribution or introduce conformational instability.

Each mutation has been examined individually with respect to its location in the two molecular domains and the intra- and inter-molecular interactions formed. On the C-terminal domain (PK) 120 effective mutations are distributed mainly in the core of the domain [26] with some on the interacting surface [27]. On the N-terminal domain (ARD), 7 mutations and one insertion are on the interacting interface while most mutations (14) are located in the core of the domain contributing to conformational instability.

	Activin Receptor domain				Kinase domain			
	INTERACTION INTERFACE	BINDING SITE	SURFACE	INTERNAL	INTERACTION INTERFACE	BINDING SITE	SURFACE	INTERNAL
SEVERE	4	0	11	2	5	4	27	12
MILD	0	0	0	0	1	0	5	0
UNKNOWN	3	0	9	2	10	4	37	15

Figure 2. Distribution of observed mutations according to position and clinical significance (severity). Mutations found in the different structural domains of *ALK1* according to location and severity.

B. *Structural and functional implications of the new Cys90Phe mutation*

All mutations have been characterized according to severity and location on the ACVLR1 extracellular and cytoplasmic domains (Fig. 3a,b and 3c,d, respectively).

One family out of seventeen ROW families, which have been reported in Crete, has been analyzed by sequencing analysis so far. Two related cases (father and daughter) were found to be heterozygous for a germline mutation, that is a

single base substitution, c.269G>T, in exon 3 of the genomic

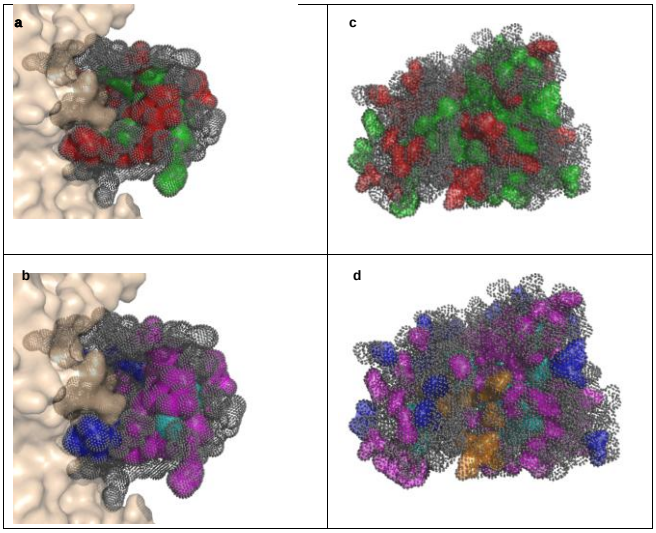

Figure 3. Distribution of observed mutations on the structural domains of ALK1: in the Activin Receptor (N-terminal) domain (PDB code 4FAO) according to clinical significance (**a**) and position in structure (**b**); in the Kinase (C-terminal) domain (PDB code 3MY0) according to clinical significance (**c**) and position (**d**). Mutations in red are those that create severe clinical effects, in yellow those with mild effects and in green those that have not been linked to clinical effects. Mutations in dark blue are located in the interaction interface, in orange in the ligand binding site, in magenta on the protein's surface while in light blue are mutations located in the internal of the protein domains.

sequencing of *ACVRL1* gene (GENBANK Accession number 4557242), thus resulting in a missense mutation Cys90Phe of ACVRL1 domain. This is a novel mutation that has not been previously reported in the ROW (HHT) Mutation database (www.hhtmutation.org). Of note, no mutations of the *ENG* gene were detected. However, this mutation was not detected on two cases (daughters) of this family. Based on the clinical manifestations of the patients, the novel mutation is characterized as pathogenic. Considering that this mutation has not previously been detected in patients from other studies, this codon seems to not be frequently affected.

Cys90 is located on the ACVRL1 extracellular domain (res 30-104) that interacts with Activin (BM9 in the available crystal structure [12] through the amphipathic helix α1 (Fig. 4a). This helix is held in position by the disulphide bond Cys77-Cys89 and through β-strand β5 and the disulphide Cys90-Cys95 to the core of the protein domain (Fig. 4b). The mutation Cys90Phe not only disrupts the disulphide bridge but also introduces steric hindrance between strands β5 and β4 and loop F2 (Fig. 4c).

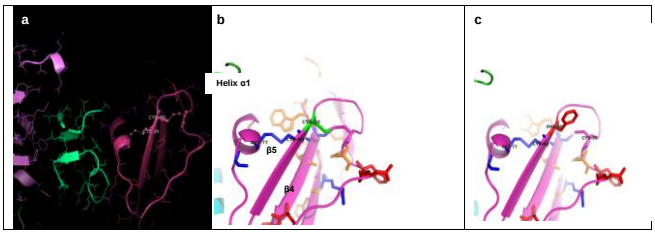

Figure 4. The Cys90Phe mutation on ACVLR1.
a. The location of the mutation on the ACVLR1 extracellular domain (pink) with respect to the activin (BM9) receptor (in green). **b**. The network of disulphide bridges in ACVLR1 that holds interaction helix α1 stable on the core β-sheet. **c**. Mutation Cys90Phe located in loop F4 introduces steric hindrance is the protein's conformation and disruption of stability due to the breaking of the Cys90-Cys77 disulphide bridge.

IV. Discussion

Osler–Weber–Rendu (ROW) syndrome or HHT, is an autosomal dominant genetic disorder, appearing a wide ethnic and geographic distribution and occurring in one in 5000 to 8000 people, depending on the geographic or ethnic region [28]-[30].

In recent years, our scientific interest has focused on a clinical type of ROW characterized by severe recurrent bleeding tendency. This preferentially involves the nose, with the most common symptom being recurrent massive epistaxis [1]. The diagnosis of ROW is made clinically on the basis of the Curacao criteria established in June 1999 by the Scientific Advisory Board of the ROW Foundation International Inc. The four diagnostic criteria include epistaxis, telangiectasias, occurring in more than 95% of patients, visceral lesions and family history (first-degree relative with ROW). Genetic linkage studies managed to map two variants of the disease, to mutations in at least two genes, *ENG* on chromosome 9q33-34 [4] and *ACVRL1* on 12q13 (13) [18], respectively. The corresponding products of both genes are specific endothelial transmembrane receptors for the transforming growth factor b (TGFb) superfamily of ligands that are involved in controlling cell proliferation, differentiation, migration, and adhesion in the vascular system [8],[31],[32]. ACVRL1 and ENG are single-pass transmembrane receptors [27]. TGF-superfamily consists of more than 30 ligands including bone morphogenic protein (BMP), growth and differentiation factors (GDF), activins, seven type I receptors and five type II receptors, as well as, several co-receptors and accessory proteins [33]. Biochemical studies have suggested that BMP9 is the plausible physiological ligand of ALK1 and, therefore, may represent a significant step in understanding HHT as it allows us to reconsider the molecular mechanisms behind the disease pathogenesis [34]. A kinetic and thermodynamic analysis shows that BMP9 displays a significant discrimination in type II receptor binding [12]. This data provides insights into ACVRL1 signaling that could serve as a basis for further angiogenic therapies [12].

Activin receptor-like kinase 1 (ALK1) or ACVRL1, an endothelial cell-specific type I receptor of the TGF-β superfamily, is an important regulator of normal blood vessel development and is primarily implicated in the development of ROW. It regulates pathological tumor angiogenesis and represents an important therapeutic target [12]. Considering the multiple genetic, pharmacological and histopathological evidence that supports a critical role for ACVRL1 signaling in the regulation of blood vessel formation, either in tumors or in normal tissues [27],[33],[34], the growing interest in ACVRL1 as a therapeutic target that complements existing anti-angiogenesis treatments seems justified. Indeed, a soluble ACVRL1 ligand trap (Acceleron Pharma), and an anti-ACVRL1 antibody (Pfizer) are presently in clinical trials for treatment of advanced solid tumors [35]. Thus, ACVRL1 has been considered to be an important therapeutic target and several agents, directed to ACVRL1, are currently in clinical trials as anti-angiogenic cancer therapeutics. Considering the biological and clinical importance of the ACVRL1 signaling pathway, we sought to construct a 3-D model of various domains of ACVRL1 protein and localize known mutations

as well as a new one on it. The protein is rendered nonfunctional through missfolding or aggregation, caused by specific mutations directly associated with position on the 3D structure or functionality. These mutations were found in the extracellular, transmembrane and kinase domains of ACVRL1 including missense mutations as well as nonsense mutations, small deletions or insertions.

The majority of ROW causing mutations affect aminoacid residues integral to the core structure while the minority may have an effect in BMP9 binding. In addition, it has been suggested that mutations in exon 5 lead to a more severe phenotype [6].

Presently, we are in a process of identifying this mutation of the *ACVRL1* gene of the members of 17 families from Eastern Crete, in an attempt to confirm the new Cys90Phe mutation and not only to validate its role regarding the geographic distribution of the disease in Crete, but to disclose the correlation of the severity of clinical appearance as well.

Molecular diagnosis of the disease causing mutation(s) in either one of the two ROW associated genes confirms the clinical diagnosis and, as a consequence, may be of benefit for patients and their close relatives [26]. Although a confirmed molecular diagnosis may provide an opportunity for early detection of the disease and appropriate management, it has been commented that ROW patients should be examined for mutations only after detailed clinical examination [22]. Screening for mutations in *ACVRL1* and *ENG* is justified especially in order to identify younger family members at risk of carrying a mutation. Further studies are under way to correlate mutations to amino acid residue functionality and clinical significance. Thus, correlation of position/type of mutation with clinical significance may elucidate our understanding of the contribution of particular mutations to the clinical pathology and severity of the disease in ROW patients and contribute greatly in terms of genetic counseling and prediction of ROW symptoms.

As far as treatment is concerned, the most promising intervention seems to be surgical closure of the nasal cavities [36], while other treatment modalities have been used so far with very results of success; namely, laser photocoagulation [37], and intranasal submucosal bevazicumab [38]. Given that ROW cannot be cured until now, the treatment of ROW patients remains symptomatic or has preventive character.

REFERENCES

[1] S. Dupuis-Girod, S. Bailly, H. Plauchu, Hereditary hemorrhagic telangiectasia: from molecular biology to patient care, J. Thrombosis and Haemostasis 8 (2010) 1447-1456.

[2] S.A. Abdalla, M. Letarte, Hereditary haemorrhagic telangiectasia: current views on genetics and mechanisms of disease, J. Med. Genet. 43 (2006) 97-110.

[3] K.A. McAllister, K.M. Grogg, D.W.Johnson, C.J. Gallione, M.A. Baldwin, C.E. Jackson, et al., Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1, Nat. Genet. 8 (1994) 345-351.

[4] M.T. McDonald, K.A. Papenberg, S. Ghosh, A.A. Glatfelter, B.B. Biesecker, E.A. Helmbold, et al., A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33-34, Nat. Genet. 6 (1994) 197-204.

[5] D.W. Johnson, J.N. Berg, M.A. Baldwin, C.J. Gallione, I. Marondel, S.J. Yoon, et al., Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2, Nat. Genet. 13 (1996) 189-195.

[6] G. Lesca, N. Burnichon, G. Raux, M. Tosi, S. Pinson , M.J.G. Marion, et al., Distribution of ENG and ACVRL1 (ALK1) mutations in French HHT patients, Human Mutation 27 (2006) 598.

[7] C. Olivieri, F. Pagella, L. Semino, L. Lanzarini, C. Valacca, A. Pilotto, et al., Analysis of ENG and ACVRL1 genes in 137 HHT Italian families identifies 76 different mutations (24 novel). Comparison with other European studies, J. Hum. Genet. 52 (2007) 820-829.

[8] A.D. Bossler, J. Richards, C. George, L. Godmilow, A. Ganguly, Novel mutations in ENG and ACVRL1 identified in a series of 200 individuals undergoing clinical genetic testing for hereditary hemorrhagic telangiectasia (HHT): correlation of genotype with phenotype, Hum. Mutation 27 (2006) 667-675.

[9] C.J. Gallione, J.A. Richards, T.G. Letteboer, D. Rushlow , N.L. Prigoda, T.P. Leedom, et al., SMAD4 mutations found in unselected HHT patients, J. Med. Genet. 43 (2006) 793-797.

[10] L.A. Fernandez, F. Sanz-Rodriguez, F.J. Blanco, C. Bernabeu, L.M. Botella, Hereditary hemorrhagic telangiectasia, a vascular dysplasia affecting the TGF-beta signaling pathway, Clin. Med. Res. 4 (2006) 66-78.

[11] M.J. Kim, S.T. Kim, H.D. Lee, K.Y. Lee, J. Seo, J.B. Lee, et al., Clinical and genetic analyses of three Korean families with hereditary hemorrhagic telangiectasia, BMC Med. Genet. 12 (2011) 130.

[12] S.A. Townson, E. Martinez-Hackert , C. Greppi, P. Lowden, D. Sako, J. Liu, et al., Specificity and structure of a high affinity activin receptor-like kinase 1 (ALK1) signaling complex J. Biol. Chem. 287 (2012) 27313-27325.

[13] A. Chaikuad, I. Alfano, G. Kerr, C.E. Sanvitale, J.H. Boergermann, J.T. Triffitt, et al., Structure of the bone morphogenetic protein receptor ALK2 and implications for fibrodysplasia ossificans progressive, J. Biol. Chem. 287 (2012) 36990-36998.

[14] M.P. Jacobson, R.A. Friesner, Z. Xiang, B. Honig, On the role of the crystal environment in determining protein side-chain conformations, J. Mol. Biol. 320 (2002) 597-608.

[15] D. Kozakov, R. Brenke, S.R. Comeau, Vajda S, PIPER: an FFT-based protein docking program with pairwise potentials, Proteins 65 (2006) 392-406.

[16] R.A. Friesner, J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, et al., Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy, J. Medicinal Chem. 47 (2004) 1739-1749.

[17] E. Krissinel, K. Henrick, Inference of macromolecular assemblies from crystalline state, J. Mol. Biol. 372 (2007) 774-797.

[18] J.N. Berg, C.J. Gallione, T.T. Stenzel, D.W. Johnson, W.P. Allen, C.E. Schwartz, et al., The activin receptor-like kinase 1 gene: genomic structure and mutations in hereditary hemorrhagic telangiectasia type 2, Am. J. Hum. Genet. 61 (1997) 60-67.

[19] R.C. Trembath, J.R. Thomson, R.D. Machado, N.V. Morgan, C. Atkinson, I. Winship, et al., Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia, New England J. Med. 345 (2001) 325-334.

[20] R.E. Harrison, J.A. Flanagan, M. Sankelo, S.A. Abdalla, J. Rowell, R.D. Machado, et al., Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia, J. Med. Genet. 40 (2003) 865-871.

[21] T.G. Letteboer, R.A. Zewald, E.J. Kamping, G. de Haas, J.J. Mager, R.J. Snijder, et al., Hereditary hemorrhagic telangiectasia: ENG and ALK-1 mutations in Dutch patients, Hum. Genet. 116 (2005) 8-16.

[22] L. Argyriou, S. Twelkemeyer, I. Panchulidze, L.E. Wehner, U. Teske, W. Engel, et al., Novel mutations in the ENG and ACVRL1 genes causing hereditary hemorrhagic teleangiectasia, Int. J. Mol. Med. 17 (2006) 655-659.

[23] P. Giordano, A. Nigro, G.C. Del Vecchio, C. Sabba, D. De Mattia, HHT in childhood: screening for special patients, Cur. Pharm. Design 12 (2006) 1221-1225.

[24] N.L. Prigoda, S. Savas, S.A. Abdalla, B. Piovesan, D. Rushlow, K. Vandezande, et al., Hereditary haemorrhagic telangiectasia: mutation detection, test sensitivity and novel mutations, J. Med. Genet. 43 (2006) 722-728.

[25] L.E. Wehner, B.J. Folz, L. Argyriou, S. Twelkemeyer, U. Teske, U.W. Geisthoff, et al., Mutation analysis in hereditary haemorrhagic telangiectasia in Germany reveals 11 novel ENG and 12 novel ACVRL1/ALK1 mutations, Clin. Genet. 69 (2006) 239-245.

[26] P. Bayrak-Toydemir, R. Mao, S. Lewin, J. McDonald, Hereditary hemorrhagic telangiectasia: an overview of diagnosis and management in the molecular era for clinicians. Genet. in Med. 6 (2004) 175-191.

[27] F. Lebrin, M. Deckers, P. Bertolino, P. Ten Dijke, TGF-beta receptor function in the endothelium, Cardiovascular Res. 65 (2005) 599-608.

[28] M. Dakeishi, T. Shioya, Y. Wada, T. Shindo, K. Otaka, M. Manabe, et al., Genetic epidemiology of hereditary hemorrhagic telangiectasia in a local community in the northern part of Japan, Hum. Mutation 19 (2002) 140-148.
[29] A.D. Kjeldsen, P. Vase, A. Green, Hereditary haemorrhagic telangiectasia: a population-based study of prevalence and mortality in Danish patients, J. Internal Med. 245 (1999) 31-39.
[30] F.S. Govani, C.L. Shovlin, Hereditary haemorrhagic telangiectasia: a clinical and scientific review, Eur. J. Hum. Genet. 17 (2009) 860-871.
[31] H.M. Arthur, J. Ure, A.J. Smith, G. Renforth, D.I. Wilson, E. Torsney, et al, Endoglin, an ancillary TGFbeta receptor, is required for extraembryonic angiogenesis and plays a key role in heart development, Dev. Biol. 217 (2000) 42-53.
[32] S.P. Oh, T. Seki, K.A. Goss, G. Renforth, D.I. Wilson, E. Torsney, et al., Activin receptor-like kinase 1 modulates transforming growth factor-beta 1 signaling in the regulation of angiogenesis, Proc. Natl. Acad. Sci. USA 97 (2000) 2626-2631.
[33] J. Massague, TGF-beta signal transduction, Annual Rev. Biochem. 67 (1998) 753-791.
[34] N. Ricard, M. Bidart, C. Mallet, G. Lesca, S. Giraud, R. Prudent, et al., Functional analysis of the BMP9 response of ALK1 mutants from HHT2 patients: a diagnostic tool for novel ACVRL1 mutations, Blood 116 (2010) 1604-1612.
[35] S.I. Cunha, K. Pietras, ALK1 as an emerging target for antiangiogenic therapy of cancer, Blood 117 (2011) 6999-7006.
[36] V.J. Lund, D.J. Howard, Closure of the nasal cavities in the treatment of refractory hereditary haemorrhagic telangiectasia, J. Laryngology Otology 111 (1997) 30-33.
[37] G.A. Velegrakis, E.P. Prokopakis, C.E. Papadakis, E.S. Helidonis, Nd:YAG laser treatment of recurrent epistaxis in heredity hemorrhagic telangiectasia, J. of Otolaryngology 26 (1997) 384-386.
[38] D. Ris, M. Burian, A. Wolf, V. Kranebitter, A. Kaider, C. Arnoldner, Intranasal submucosal bevacizumab for epistaxis in hereditary hemorrhagic telangiectasia: A double-blind, randomized, placebo-controlled trial, Head Neck 37 (2015) 783-787.