The role of genetics in pulmonary arterial hypertension

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Abstract

Group 1 pulmonary hypertension or pulmonary arterial hypertension (PAH) is a rare disease characterized by proliferation and occlusion of small pulmonary arterioles, leading to progressive elevation of pulmonary artery pressure and pulmonary vascular resistance, and right ventricular failure. Historically it has been associated with a high mortality rate, although over the last decade, treatment has improved survival. PAH includes idiopathic PAH (IPAH), heritable PAH (HPAH), and PAH associated with certain medical conditions. The etiology of PAH is heterogeneous, and genetics play an important role in some cases. Mutations in *BMPR2*, encoding bone morphogenetic protein receptor type 2, a member of the transforming growth factor beta (TGF-β) superfamily of receptors, have been identified in 70% of cases of HPAH, as well as 10-40% of cases of IPAH. Other genetic causes of PAH include mutations in activin receptor-like type 1 (*ACVRL1*), endoglin (*ENG*), SMAD family member 9 (*SMAD9*), caveolin 1 (*CAV1*) and potassium two-pore-domain channel subfamily K member 3 (*KCNK3*). T-box 4 (*TBX4*) mutations have been identified in 10-30% of pediatric PAH patients, but rarely in adults with PAH. PAH in children is much more heterogeneous than in adults and can be associated with several genetic syndromes, congenital heart disease, pulmonary disease, and vascular disease. In addition to rare mutations as a monogenic cause of HPAH, common variants in cerebellin 2 (*CBLN2*) increase the risk of PAH by approximately twofold. A PAH panel of genes is available for clinical testing and should be considered for use in clinical management, especially for patients with a family history of PAH.

Introduction

Based on the World Health Organization (WHO) clinical classification, pulmonary hypertension falls into five groups: pulmonary arterial hypertension (PAH) (Group 1);

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pulmonary hypertension due to left heart disease (Group 2); pulmonary hypertension due to chronic lung disease and/or hypoxia (Group 3); chronic thromboembolic pulmonary hypertension (Group 4); and pulmonary hypertension due to unclear multifactorial mechanisms (Group 5).

Pathologically, PAH is characterized by excessive pulmonary vasoconstriction, abnormal vascular remodeling (including medial hypertrophy, intimal fibrosis, and adventitial proliferation) and obliteration of small pulmonary arterioles, leading to progressive elevation of pulmonary artery pressure, which in turn increases afterload of the right ventricle [1] (Figure 1). In response to vascular stress, the effects of genetic risk factors, such as *BMPR2* mutations, will interplay with those of other signaling molecules (kinases and growth factors). There is hypertrophy of smooth muscle cells, deposition of extracellular matrix, proliferation of endothelial cells, propagation of adventitial fibroblast cells and remodeling of small pulmonary arteries. Despite recent improvements in management, PAH remains a devastating disease. The pathogenesis of PAH is complex and incompletely understood, but includes both genetic and environmental factors that alter vascular structure and function [2]. Understanding the genetic etiology of PAH should facilitate better diagnosis and development of novel therapies in the future.

Genetics of PAH

Familial cases of PAH have been long recognized [3] and are usually inherited as autosomal dominant traits with incomplete penetrance; females are preferentially affected. HPAH accounts for about 6% of PAH [4]. BMPR2, encoded by the gene most commonly mutated in HPAH, is a member of the transforming growth factor-beta (TGF-β) receptor superfamily; this comprises a large series of cytokine growth factors that control a host of cellular functions, among them proliferation, migration, differentiation, apoptosis, and extracellular matrix secretion and deposition. Multiple studies have identified *BMPR2* mutations in ~70% of HPAH and ~10-40% of IPAH cases [5-8]. To date, 390 different *BMPR2* mutations have been identified (www.hgmd.cf.ac.uk). These are all loss-of-function mutations, and include nonsense, missense, splicing and frameshift mutations, as well as deletions or re-arrangements affecting one or more exons or the entire *BMPR2* gene [9]. It is suggested that nonsense or frameshift mutations cause truncation of the protein and lead to nonsense-mediated decay, while missense mutations cause retention of the mutant protein in the endoplasmic reticulum or plasma membrane [10]. *BMPR2* mutations occur in about 20% of adult idiopathic PAH cases (Table 1) [11-22]. Although there is some variability, the average penetrance of *BMPR2* mutations is only about 20%, and a "second hit" is proposed to be a modulator of disease progression [23-24]. Using lymphocyte-derived human cell lines to examine *BMPR2* expression, unaffected mutation carriers were shown to have higher levels of wild-type *BMPR2* transcripts than affected patients [25], suggesting that the penetrance of the disease is regulated by the level of expression of the normal *BMPR2* allele. These authors also suggested that there is an estrogen receptor-alpha binding site at the *BMPR2* promoter and that *BMPR2* expression is reduced in lymphocytes from female patients compared with male patients [26].

Additional genes related to TGF-β superfamily receptor signaling have been identified as rare causes of PAH; these include activin receptor-like type 1 (*ACVRL1*), endoglin (*ENG*), SMAD family member 8 (*SMAD8*; also known as *SMAD9)* and SMAD family member 4 (*SMAD4*) [27-34] (Figure 2). The TGF-β family is involved in regulation of multiple cellular functions and homeostasis, including proliferation, differentiation, apoptosis and endothelialmesenchymal transition (endo-MT) [35]. TGF-β plays an important role in the respiratory system [36]. The TGF-β/ACVRL1/ENG signaling pathway activates or phosphorylates Smad1/5/8 and Smad2/3 through receptors such as BMPR2, and induces growth factors such as fibroblast growth factor 2 (FGF2) and platelet-derived growth factor B (PDGFB) [37]. TGF-β regulates the synthesis of platelet-derived growth factors through the non-canonical pathway (C-terminal Src kinase or MAP kinase) in endothelial cells, which can stimulate the growth of vascular smooth muscle cells [38].

Hereditary hemorrhagic telangiectasia genes

Hereditary hemorrhagic telangiectasia (HHT) is a rare autosomal dominantly inherited disease characterized by telangiectases and arteriovenous malformations (AVMs), including pulmonary AVMs, with variable clinical presentations due to different locations of the lesion. Mutations in *ENG* or *ACVRL1* are identified in 80-85% of HHT patients, while *SMAD4* mutations are found in 1-2% of HHT [39, 40]. SMAD4 is a "co-SMAD" that forms a complex with receptor-activated SMADs (SMAD2/3 or SMAD1/5/8).

Growth differentiation factor 2 (*GDF2*, previously known as bone morphogenetic protein 9, *BMP9)* is a rare genetic cause for HHT [41]; one study identified three heterozygous missense mutations in *GDF2/BMP9* in 1.6% (3/191) of HHT patients [42], but heterozygous mutations in *GDF2/BMP9* have not been reported in HHT-PAH or isolated PAH. Recently, though, a homozygous *GDF2/BMP9* nonsense mutation c.C76T; p.Gln26Ter was identified in a child with isolated PAH [43]. The parents were heterozygous for the mutation but were phenotypically normal. It is estimated that 30% of HHT patients have pulmonary arteriovenous malformations, and a small percent (<1%) of HHT patients have PAH [44]. In this small proportion of patients with HHT-PAH, some have PAH that is clinically and histopathologically indistinguishable from other heritable forms of PAH, while others have PAH associated with pulmonary arteriovenous fistulas [45]. The underlying causative factor in these patients is, typically, mutations of *ACVRL1*. Up to 20% (16/83) of all detected mutations in *ACVRL1* are associated with the development of PAH, and, of these 16 mutations, 81% are repeatedly observed with PAH [46-47]. In rare instances (n=5), mutations of *ACVRL1* cause PAH without HHT [48-49].

Caveolin 1

Mutations in caveolin 1 (*CAV1*) are a rare cause of HPAH and IPAH [50]. CAV1 encodes a membrane protein which is enriched in cell surface caveolae and plays an important role in mediating TGF-β, G-protein and nitric oxide signaling in PAH. *CAV1* is expressed ubiquitously, but with high expression in adipocytes, endothelial cells, and fibroblasts [51]. Homozygous or heterozygous *CAV1* mutations have been identified in lipodystrophy or partial lipodystrophy patients [52-53]. Recently, heterozygous *CAV1* mutations were reported in isolated PAH or PAH associated with lipodystrophy [51, 54-55]. Mechanisms of

CAV1 mutation in PAH have been extensively studied. In mouse, *Cav1* is expressed in epithelial and endothelial cells of the septum which is located between the alveolar space and the blood capillaries in the lung [56]. In humans, *CAV1* was detected in the endothelium of pulmonary arteries [50]. BMPR2 localizes to caveoli and directly interacts with CAV1 in various cell types, including vascular smooth muscle cells [57-58]. Other studies suggest that BMPR2 signaling through SRC regulates CAV1 trafficking in PAH [59] (Figure 2).

PAH due to *KCNK3* Mutations

Potassium two-pore-domain channel, subfamily K member 3 (*KCNK3)*, also known as *TASK-1*, encodes an outwardly rectifying potassium channel that is sensitive to changes in extracellular pH. Genetic and electrophysiological studies suggest that *KCNK3* mutation is a rare genetic cause of PAH [60]. K^+ channels are the largest and most diverse family of ion channels. According to their structure and composition, K^+ channels are classified into three main groups: voltage gated (6 transmembrane domains or 6TM), inward rectifiers (2TM) and two-pore-domain (4TM) channels [61-62]. Two-pore-domain channels (K_{2P}) are the most recently discovered family of potassium channels. KCNK3, also called TASK-1 or $K_{(2P)}3.1$, belongs to this family of K^+ channels, characterized by 4 transmembrane domains and 2 pore domains per subunit. Abundant expression of *Kcnk3* is described in rat, rabbit and human pulmonary artery smooth muscle cells. The function of this channel is to conduct leak K^+ current, maintain the resting membrane potential and regulate vascular tone of the pulmonary artery. Activation of the K^+ channel causes K^+ efflux, membrane hyperpolarization and vasodilatation [63-66]. Besides *KCNK3*, single nucleotide polymorphisms in potassium voltage-gated channel subfamily A member 5 (*KCNA5*), another gene in the potassium channel family, have been reported in PAH patients. It is suggested that *KCNA5* is a genetic risk factor for the disease and KCNA5 has roles in determining pulmonary vascular tone, oxygen sensing, cell proliferation, and apoptosis [67-68].

Pediatric PAH

The prevalence of PAH in children is estimated to be ~2.2 cases per million, an order of magnitude lower than the estimated prevalence of 15-50 cases per million in adults [69]. Pediatric PAH includes IPAH, HPAH and PAH associated with congenital heart disease and with abnormal lung development.

The genetics of PAH in children has not been extensively studied, but the data suggest that PAH in children may have a different genetic etiology from that in adults. The pediatric studies have been limited in sample size, and additional larger studies need to be performed. One study identified no *BMPR2* mutations in 13 children with IPAH [70]. In a cohort of 57 pediatric PAH patients, 18 *BMPR2* and 7 *ACVRL1* mutation carriers were identified [71]. A study of PH associated with congenital heart disease (CHD) demonstrated that 22% of children had *ACVRL1*, *ENG* or *BMPR2* mutations [8]. In a mixed cohort of 40 childhood PAH cases, including 29 IPAH/HPAH and 11 PAH-CHD, six *BMPR2*, two *ACVRL1* and two *ENG* mutations were identified [72]. The differences in genetic results in different pediatric PAH studies are probably due to a combination of small sample sizes, different genetic backgrounds and selection criteria, including whether the PAH was associated with

other diseases. *BMPR2* mutation-positive children appeared less likely to respond to acute vasodilators than mutation-negative children [17].

TBX4 is a transcription factor in the T-box gene family, expressed in the atrium of the heart, the limbs, and the mesenchyme of the lung and trachea. Jointly with TBX5, TBX4 has been shown to interact with FGF10 during lung growth and branching [73]. Heterozygous loss of function mutations in *TBX4* have been previously reported to cause small patella syndrome (SPS; MIM# 147891) [74], and heterozygous microdeletions of 17q23.2 encompassing *TBX4* were identified in eight patients with developmental delay, limb abnormalities, CHD, and one with pulmonary hypertension [75-76]. One missense and two frameshift variants in *TBX4* were identified in 6/20 (30%) pediatric PAH patients [21], and two missense variants in *TBX4* were identified in 2/49 adult PAH patients (4.1%), suggesting a higher frequency of *TBX4* mutations in pediatric PAH. Although it had not initially been clinically noted, some of the patients and the asymptomatic parents carrying *TBX4* mutations were later assessed for skeletal signs of small patella syndrome (SPS); in all five available probands and two carrier parents there was radiological evidence of skeletal malformations [21]. In a cohort of 165 adult-onset PAH, only one possible mutation (non-frameshift insertion c. 310_312 ins AAG, p.L103_V104 ins E) in *TBX4* was identified [22]. Rarely, *TBX4* mutations have been associated with abnormal lung development, such as acinar dysplasia of the lungs [77]. Table 2 provides a summary of pediatric PAH genetic studies [16-17, 21, 70-72, 78-80].

Other genes are likely to play an important role in childhood PAH. Two novel missense mutations in *BMPR1B* were identified in pediatric IPAH [81]. These two variants are located in highly conserved BMPR1B protein regions. Functional studies showed that BMPR1B mutant protein increased phosphorylation of SMAD8. BMPR1B (also known as ALK6) is one of the BMP type I receptors which interact with BMP type II receptors and mediates BMP signaling. BMPR1B expression was significantly increased in pulmonary artery smooth muscle cells (PASMC) isolated from BMPR2-negative PAH patients, while BMPR1B transcripts were hardly detectable in control PASMC [82].

Two novel missense mutations (G840E and T900P) in *NOTCH3* were identified in two PAH patients. These variants are located in highly conserved NOTCH3 protein regions. Functional studies indicated that these mutations increased cell proliferation and viability [83], suggesting a possible gain of function. As the receptor for JAGGED1, NOTCH3 is involved in vascular homeostasis and PAH [84]. Evidence suggests that TGF-β1 regulates Notch3, indicating that Notch3 is in the TGF-β signaling network [86].

Genetics and environmental factors interact in PAH

The genetics of PAH across multiple genes are characterized by female predominance and incomplete penetrance of dominantly inherited mutations. The age of onset can be modified by a complex interplay of multiple mutations. One *BMPR2* mutation carrier has been shown to have a loss of Chromosome 13 in the pulmonary vasculature, associated with loss of *SMAD9* [23]. Some patients have been found to carry multiple variants in PAH risk genes [86]. Differences in *BMPR2* expression or isoform ratios may provide an explanation for the reduced penetrance among *BMPR2* mutation carriers [87]. The onset of PAH can be

modified by association with other diseases or exposures. Heterozygous *BMPR2* sequence variants have been identified in a small subset of patients with PAH associated with exposure to fenfluramine [13, 88], CHD [16], and veno-occlusive disease [89-90], raising the possibility that these factors represent triggers in the setting of inherited susceptibility. In contrast, *BMPR2* mutations have not been identified in modestly sized series of PAH associated with the scleroderma-spectrum disease, human immunodeficiency virus [91-92], Down syndrome [93], neurofibromatosis 1 [94], Gaucher disease [95] or autoimmune polyendocrine syndrome [96]. Additionally, the onset of disease can be modified by age [97], sex [26], epigenetic modifications [98], lifestyle [99] and environmental factors [100].

HPAH and IPAH generally have similar clinical courses, although there are some subtle but important differences. *BMPR2* mutation carriers are younger at diagnosis and have worse hemodynamic parameters [17]. Both children and adults with PAH and *BMPR2* mutations are less likely to respond to vasodilators and are unlikely to benefit from treatment with calcium channel blockade [17, 101]. Symptomatic HPAH patients carrying *ACVRL1* mutations, most without HHT, have an earlier age of onset and more rapid disease progression than HPAH patients with *BMPR2* mutations, despite responsiveness to vasodilators at the time of diagnosis [102]. In contrast, many (but not all) *TBX4*-related cases of PAH follow a more benign course compared with *BMPR2* mutation carriers [22].

Commons Variants Increase the Risk of PAH

The cause of most IPAH is currently unknown, but common variants have been hypothesized to contribute to disease risk. A large international genome-wide association study (GWAS) identified common variants in cerebellin 2 (*CBLN2*) that were associated with HPAH and IPAH [103].

Genetic Testing for PAH

Clinical genetic testing is now available for *BMPR2* or for panels of PAH-associated genes, including *BMPR2*, *ACVRL1*, *ENG*, *SMAD4*, *SMAD9*, *CAV1*, *KCNA5*, *KCNK3*, *GDF2 (BMP9)* and *NOTCH3*. Genetic testing is appropriate for patients with HPAH, IPAH, and pediatric PAH, and is largely used to stratify risk for relatives of PAH patients. Genetic testing can be done by targeted sequencing together with some form of gene dosage analysis such as MLPA (multiplex ligation-dependent probe amplification). Genetic counseling should be offered for predictive testing to review the complexities associated with genes with moderate penetrance and without currently available prevention [104]. For at-risk family members, patient education to ensure awareness of disease symptoms and disease surveillance by echocardiogram every 3 years should enable early diagnosis and treatment which may improve outcomes.

Conclusion

The genetics of pulmonary hypertension are complex due to the genetic heterogeneity, incomplete penetrance and sexual dimorphism in adults. There are likely to be additional genes as well as genetic and environmental modifiers for PAH still to be identified. Furthermore, the genes involved may differ between children and adults with PAH. Larger

genetic studies, including epidemiological data and hormonal levels will be necessary to tease apart these complexities.

Acknowledgements

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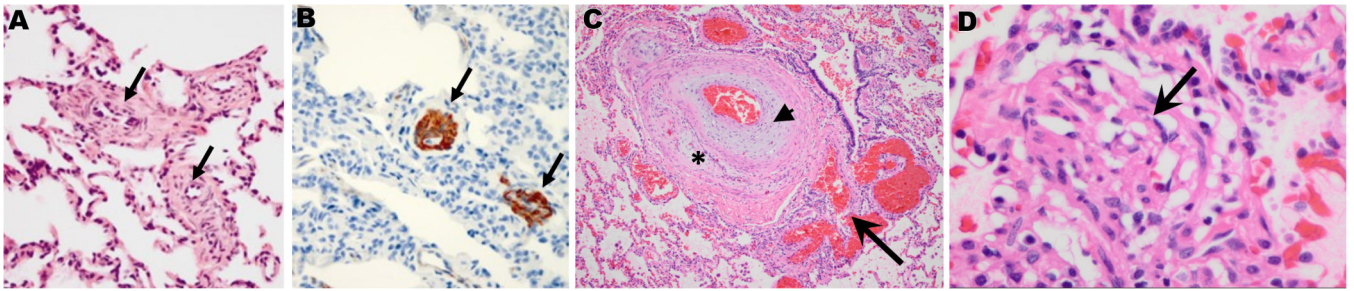
Figure 1.
Typical pathological features of PAH. **A, B**: Lung biopsy from a PAH patient with a *CAV1* mutation (grade I and II). H&E staining shows medial thickening of small pulmonary arteries (Panel A, arrows), and pulmonary vascular smooth muscle cell proliferation -- confirmed by immunohistochemical staining of alpha smooth muscle actin (Panel B, arrows) [51]. **C, D**: Lung tissue from a PAH patient with a *KCNK3* mutation. Panel C shows intimal proliferation, fibrosis (arrow head) and recanalization (asterisk), with an adjacent angiomatoid lesion (arrow) typical of pulmonary arterial hypertension (grade III). Grade IV disease includes plexiform lesions characterized by endothelial and intimal proliferation (Panel D, arrow) [61].
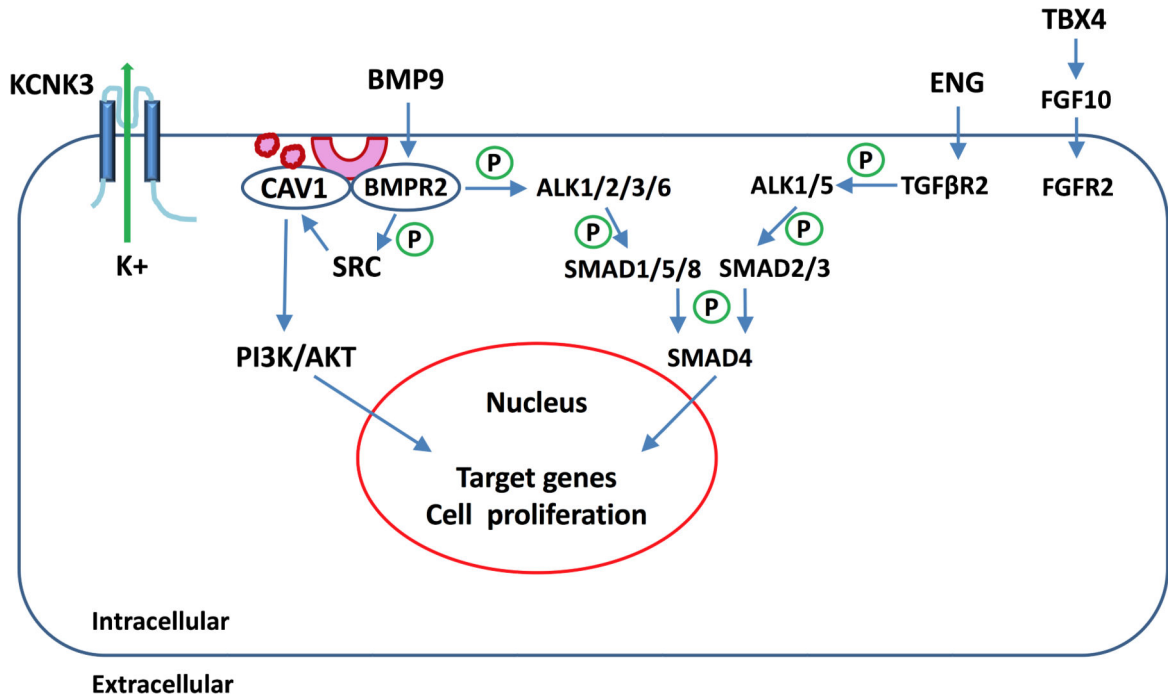
Figure 2.
PAH genes and signaling pathways. BMP ligands, such as BMP9, bind to BMPR2. Upon ligand binding, BMPR2 phosphorylates BMPR1, including ALK1, ALK2, ALK3 or ALK6 (ACVRL1, ACVR2, BMPR1A or BMPR1B respectively). The ligand-receptor complex phosphorylates SMAD1/5/8 and subsequently phosphorylates SMAD4. The phosphorylated SMADs translocate to the nucleus and regulate expression of target genes. TGF-β ligands, such as ENG, bind TGFβR2 to phosphorylate TGFβRI, including ALK1 (ACVRL1) and ALK5 (TGFBR1). The complex phosphorylates SMAD2/3 which phosphorylates SMAD4 and translocates to the nucleus. CAV1 interacts with BMPR2 to prevent vascular proliferation. BMPR2 phosphorylates SRC, which regulates cell proliferation or regulates CAV1 to activate PI3K/AKT activity. KCNK3 encodes a 2-pore potassium channel and regulates membrane potential as well as vascular tone. TBX4 regulates fibroblast growth factor 10 (FGF10), which binds with fibroblast growth factor receptor 2 (FGFR2) to regulate development in lung.

Table 1

Results of genetic studies in adult PAH.

Reference	Number of patients	BMPR2	ACVRL1	ENG	TBX4
Deng Z, 2000 [11]	35	7 (20%)			
Humbert M, 2002 [13]	33	3 (9.1%)			
Morisaki H, 2004 [14]	34	13 (38.2%)			
Koehler R, 2004 [15]	99	11 (11.1%)			
Roberts KE, 2004 [16]	40	3 (7.5%)			
Rosenzweig EB, 2008 [17]	69	15 (21.7%)			
Sztrymf B, 2008 [12]	223	68 (30.5%)			
Wang H, 2010 [18]	76	14 (18.4%)			
Pfarr N, 2011 [19]	231	49 (21.2%)	0 (0%)	0 (0%)	
Kabata H, 2013 [20]	49	18 (36.7%)			
Kerstjens-Frederikse WS, 2013 [21]	49				2
Navas P, 2016 [22]	165	19 (11.5%)			4
Mutation frequency		220 (20.9%)	0 (0%)	0 (0%)	6 (2.8%)

Table 2

Results of genetic studies in pediatric PAH.

Reference	Number of patients	BMPR2	ACVRL1	ENG	TBX4
Grünig E, 2004 [70]	13	0 (0%)			
Roberts KE, 2004 [16]	66	3 (4.5%)			
Harrison RE et al., 2005 [80]	18	2 (11.1%)	1 (5.6%)	1 (5.6%)	
Rosenzweig EB, 2008 [17]	78	8 (10.3%)			
Fujiwara M, 2008 [78]	21	4 (19.0%)	5 (23.8%)		
Chida A, 2012 [71]	57	18 (31.6%)	7 (12.3%)		
Pfarr N, 2013 [72]	40	6 (15.0%)	2 (5.0%)	2 (5.0%)	
Kerstjens-Frederikse WS, 2013 [21]	20				6 (30.0%)
Levy M, 2016 [79]	66	5 (7.5%)	4 (6.1%)	0 (0%)	3 (4.5%)
Mutation frequency		46 (12.8%)	19 (9.4%)	3 (2.4%)	9 (10.5%)