Genomic landscape of cerebral cavernous malformations: focusing on the *CCM1, CCM2, CCM3* genes

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Background

Cerebral cavernous malformation (CCM) is a common cerebral vascular malformation and presents with diverse clinical manifestations, including epilepsy, headache, and intracranial hemorrhage. **Familial CCM (fCCM),** with the hallmark of multiple lesions, has significant higher risks of rebleeding and epilepsy than the sporadic cases [1, 2]. Three causative genes for fCCM have been recognized: CCM1 (*KRIT1*), CCM2 (*MGC4607*) and CCM3 (*PDCD10*), all involved in signalling networks responsible for the maintenance of junctional integrity of vascular endothelial cells [3, 4]. The advancement of the next generation sequencing (NGS) has unraveled that most of the sporadic cases with multiple lesions are genetic cases with a de Novo mutation or a mutation inherited from an asymptomatic parent. NGS also unraveled the important roles of somatic mutations of CCM. However, there is still in lack of a comprehensive genetic study of CCMs in Taiwanese population.

Object

This study is aimed to investigate the prevalence of the pathogenic variants in the *CCM1~3* genes in the CCM patients of Taiwanese population and to establish the correlations of the genotypes and phenotypes.

Method

We prospectively recruited 63 unrelated CCM patients who has been followed at Taipei Veterans General Hospital for at least one year. The pipeline of NGS study is shown in **Figure 1**. There are two parts of genetic analysis in this study: (1) all probands were screened for **germline mutations** by **targeted sequencing** the coding regions of the three genes. The clinical significance of a variant was determined according to the according to the 2015 American College of Medical Genetics guideline [5]. (2) For those who received surgical resection due to symptomatic CCMs, **paired whole exome sequencing** of the CCM lesion and the blood DNA was performed to search **somatic mutations** of the lesion tissue.

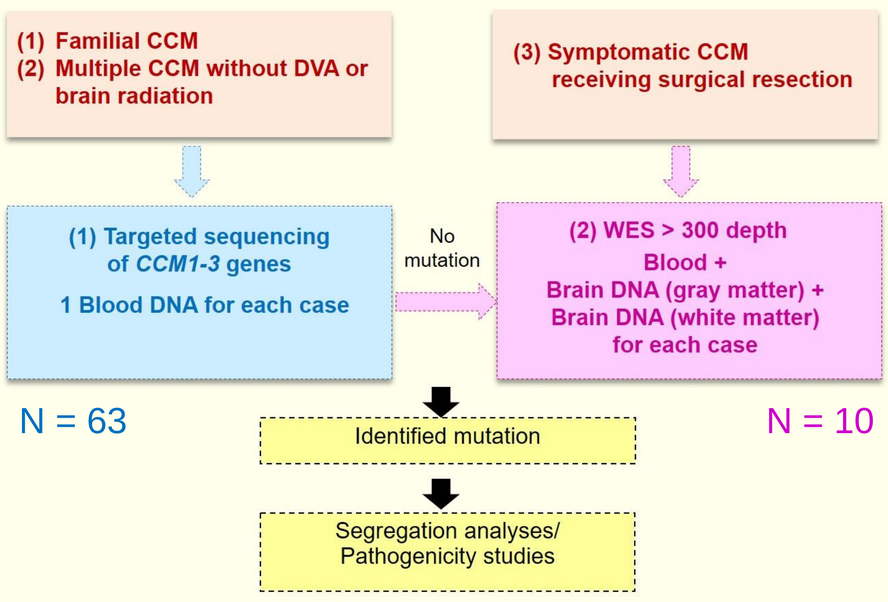
Results

Nine germline pathogenic/likely pathogenic mutations were identified, taking 14.1% of the cohort: E331X, Y11X, S615TfsX, G366DfsX, K570X, A257LfsX in *CCM1*; and T37fsX, A119PfsX, I57AfsX in *CCM2*. All are **novel variants** expected to cause loss of function of the proteins, supporting that loss of function of these genes are essential for the development of a CCM lesion. All these nine patients have **multiple CCMs** and six (66.7%) are familial cases. **Somatic mutations** in *CCM1* or *CCM2* were detected in four patients (6.3%). The novel *CCM2*:G59RfsX variant were detected in three unrelated probands, probably a hot spot mutation for CCM. One patient has two somatic mutations, CCM1:A555V and H483MfsX, providing evidence of the "double hits" theory of the pathogenesis of CCM. In contrast to those with a germline mutation, none of the four patients carrying somatic mutations has a positive family history and only one had multiple lesions. A pathogenic *CCM3* variant was not detected in the cohort, indicating it may be quite rare in our population. Taking together, 13 pathogenic variants in CCM1/CCM2 have been identified with the hit rate **20.3%**. The demographic features of the patients carrying above genetic variations are listed in **Table 1**.

Conclusion

Genetic diagnosis of a germline mutation will contribute to accurate risk assessment for the CCM patients, helpful in genetic counseling and individualized precision medicine. This is particularly critical for those with multiple lesions or a family history. Accumulating data of the somatic mutations would address a comprehensive genomic landscape of CCM, which will increase our understanding of the pathogenesis of CCM.

Figure 1. Next generation sequencing pipeline



CCM= cerebral cavernous malformation, DVA= a developmental venous anomaly, WES=whole exome sequencing

Table 1. Demographic features of the patients carrying CCM1 /CCM2 variants

No.	DNA No.	Genetic diagnosis	Type	Sex	Age at diagnosis	Age of seizure onset	Rebleeding	fCCM	Multiple lesions	Location^	T	Core features#	Epilepsy severity$
1	CM029	*KRIT1:p.E331X	Germline	M	27	27	Y	Y	Y	1, 2, 5, 6, 7	Y	1+2	2
2	CM109	*KRIT1:p.Tyr11X	Germline	F	51			Y	Y	2, 3, 7	Y	5	3
3	CM103	*KRIT1:p.Ser615ThrfsX	Germline	M	16			Y	Y	1		2+3	3
4	CM055	*KRIT1:p.Gly366AspfsX	Germline	F	23	7		Y	Y	1, 2, 4, 7, 8	Y	1+2+3	1
5	CM133	*KRIT1:p.Lys570X	Germline	F	55		Y	Y	Y	1+6		2+3+5	3
6	CM147	*KRIT1:p.Ala257LeufsX	Germline	M	54	46	Y		Y	1, 2, 7	Y	1+3	1
7	CM101	*CCM2:p.Ala119ProfsX	Germline	F	46	23	Y		Y	3		1+2+3	2
8	CM117	*CCM2:p.Ile157AlafsX	Germline	M	44				Y	8		2+3	3
9	CM017	*CCM2:p.Tyr37fsX	Germline	F	59		Y	Y	Y	1, 2, 3, 4, 6	Y	2+3	3
							5 (55.6%)	6 (67%)	9 (100%)				
10	CM073	*KRIT1:[p.Ala555Val; p.His483MetfsX]	Somatic	M	27	26	Y			1		1+2+3	1
11	CM005	*CCM2:Glu59ArgfsX	Somatic	F	36	34				2	Y	1+2	1
12	CM009	*CCM2:Glu59ArgfsX	Somatic	M	22	19				2	Y	1	1
13	CM019	*CCM2:Glu59ArgfsX	Somatic	F	33	31				2	Y	1+2+3	1
							1 (25%)	0	0				

*Novel variant, fCCM=familial CCM, T=temporal lobe
^Location in brain MRI: 1=Frontal, 2=Temporal, 3=Parietal, 4=Occipital, 5=Thalamus, 6=Brainstem, 7=cerebellar, 8=Basal ganglia
#Core features: 1.Epilepsy, 2. Headache, 3. Intracranial haemorrhage, 4.asymotinatic, 5.loss of consciousness
$Epilepsy severity: 1=DRE, 2=NDRE, 3=NE

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