Genetic Aspects of Development*:

Vascular Anomalies & Overgrowth Syndromes

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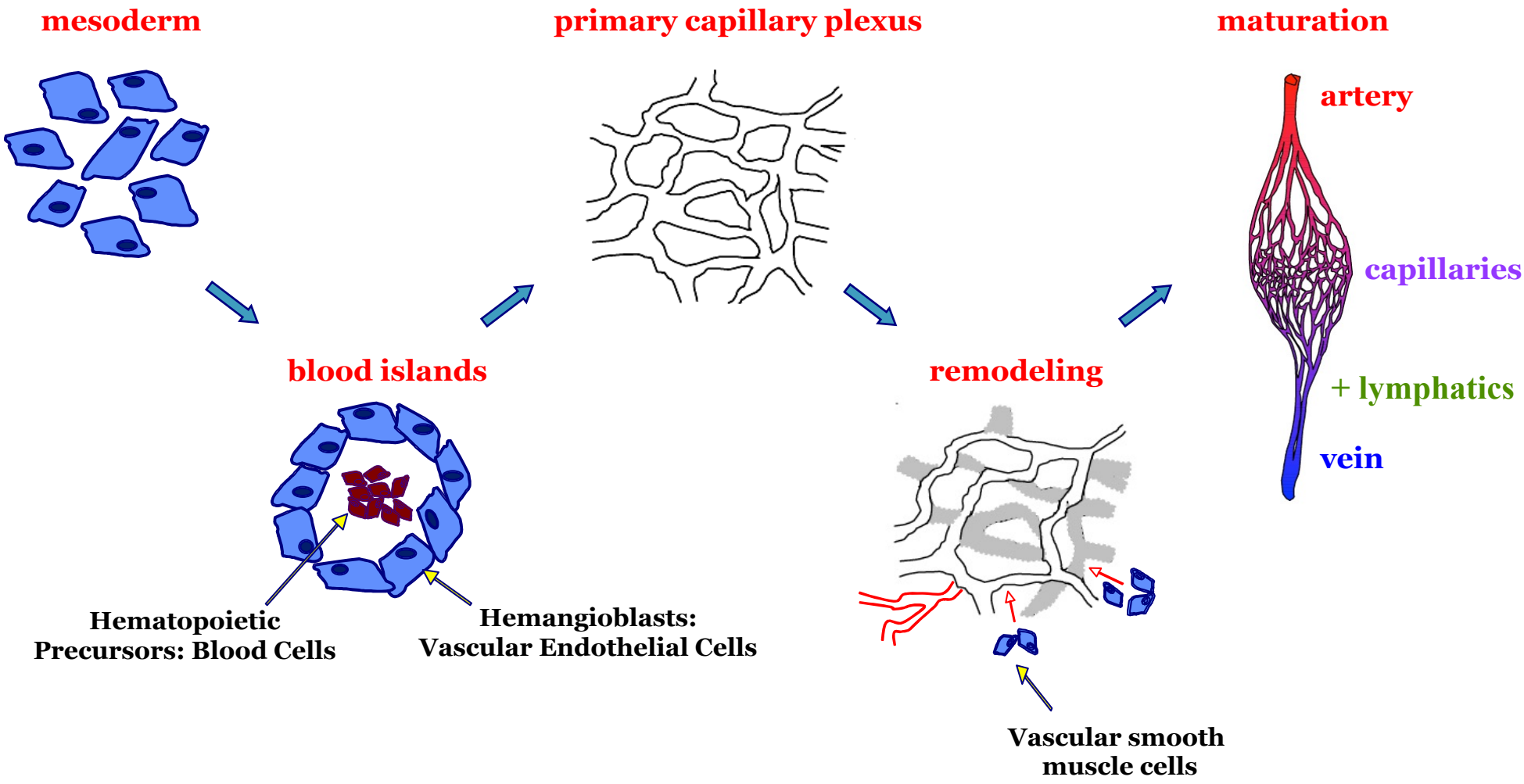
*** Thompson & Thompson: Genetics in Medicine, 8th ed., chapter 14**

Vasculogenesis and Angiogenesis

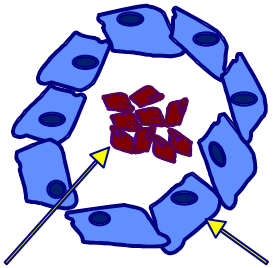


Vasculogenesis and Angiogenesis

mesoderm

bFGF, VEGFA,
NRP1,2

blood islands

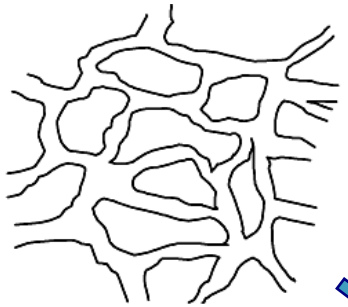


Hematopoietic Precursors: Blood Cells

Hemangioblasts: Vascular Endothelial Cells

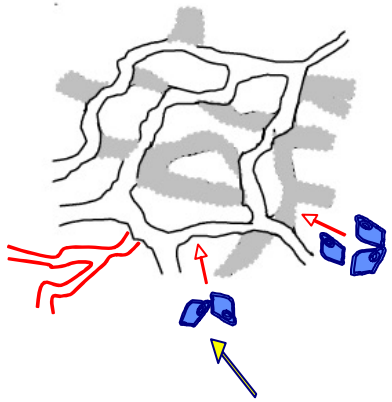
VEGFA-VEGFR2,
TGFB1, TGFBR2

primary capillary plexus



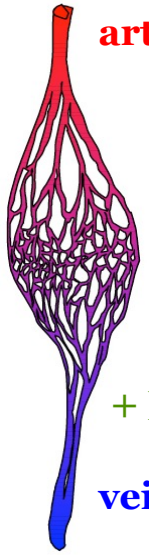
VEGFA,
VEGFR1,VEGFR2

remodeling



Vascular smooth muscle cells

maturation



artery

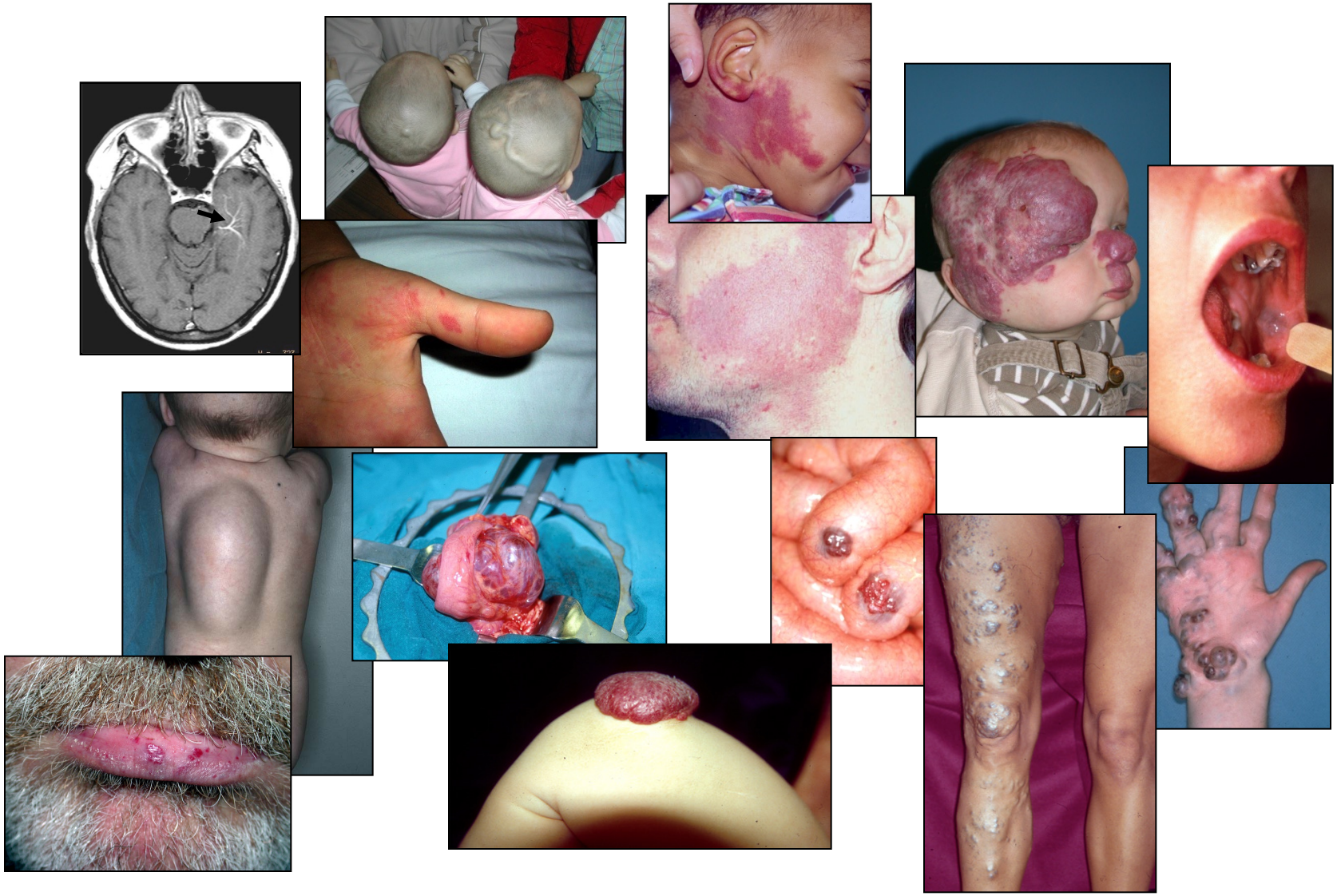
capillaries

+ lymphatics

vein

TIE2-ANGPT 1,2, VEGFR3-VEGFC, Ephrins-EPHs, CoupTFII, RASA1, SHP2, VE-CAD, ...

Developmental defects
➔ Vascular Anomalies



Diagnosis: clinical history, examination & tests

Vascular Anomalies: characteristics

- Large clinical variability
- Commonly sporadic; rare familial forms (*13 hereditary forms*)
- Localized lesions: Single (sporadic) or multiple (familial)
- Pure forms versus associations & syndromes

Classification of Vascular Anomalies

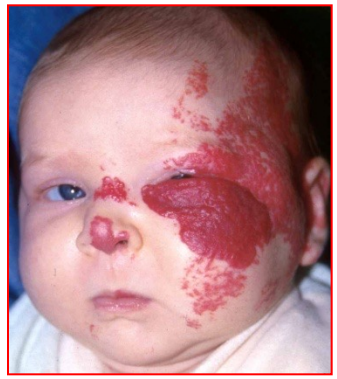
Tumors

Malformations

Mulliken JB and Glowacki, *Plast Reconstr Surg* 1982; Wassef et al, *Pediatrics* 2015; https://www.issva.org/UserFiles/file/ISSVA-Classification-2018.pdf

Classification of Vascular Anomalies

Tumors



Malformations

Hemangioma

- Infantile hemangioma (IH)
- Non-involuting congenital hemangioma (NICH)
- Rapidly-involuting congenital hemangioma (RICH)
- Partially-involuting congenital hemangioma (PICH)

Hemangioendothelioma

Angiosarcoma

Lymphangiosarcoma

Mulliken JB and Glowacki, *Plast Reconstr Surg* 1982; Wassef et al, *Pediatrics* 2015; https://www.issva.org/UserFiles/file/ISSVA-Classification-2018.pdf

Classification of Vascular Anomalies

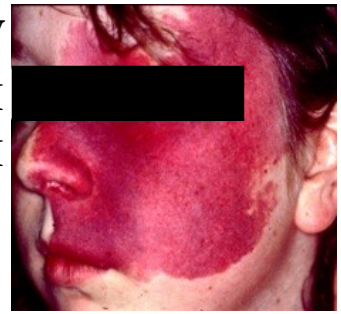
Tumors

Malformations

Capillary

CCM

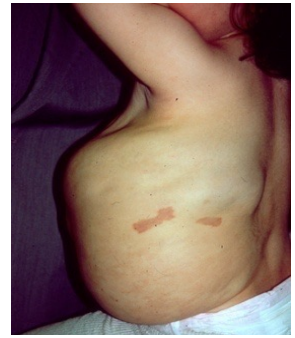
CM-AVM



Lymphatic

LE

LM

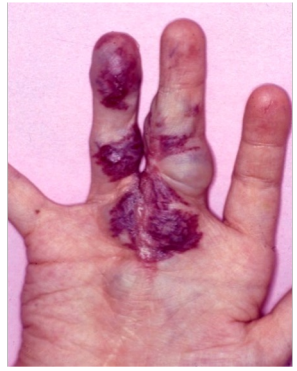


Venous

GVM

VMCM

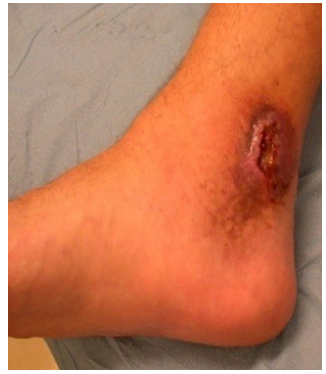
VM



Arterial

CM-AVM

HHT



Combined: AVM, CVM, CLVM, LVM, CLAVM ...

(Overgrowth) syndromes: Maffucci, KTS, PWS, MCLMR ...

Mulliken JB and Glowacki, *Plast Reconstr Surg* 1982; Wassef et al, *Pediatrics* 2015; https://www.issva.org/UserFiles/file/ISSVA-Classification-2018.pdf

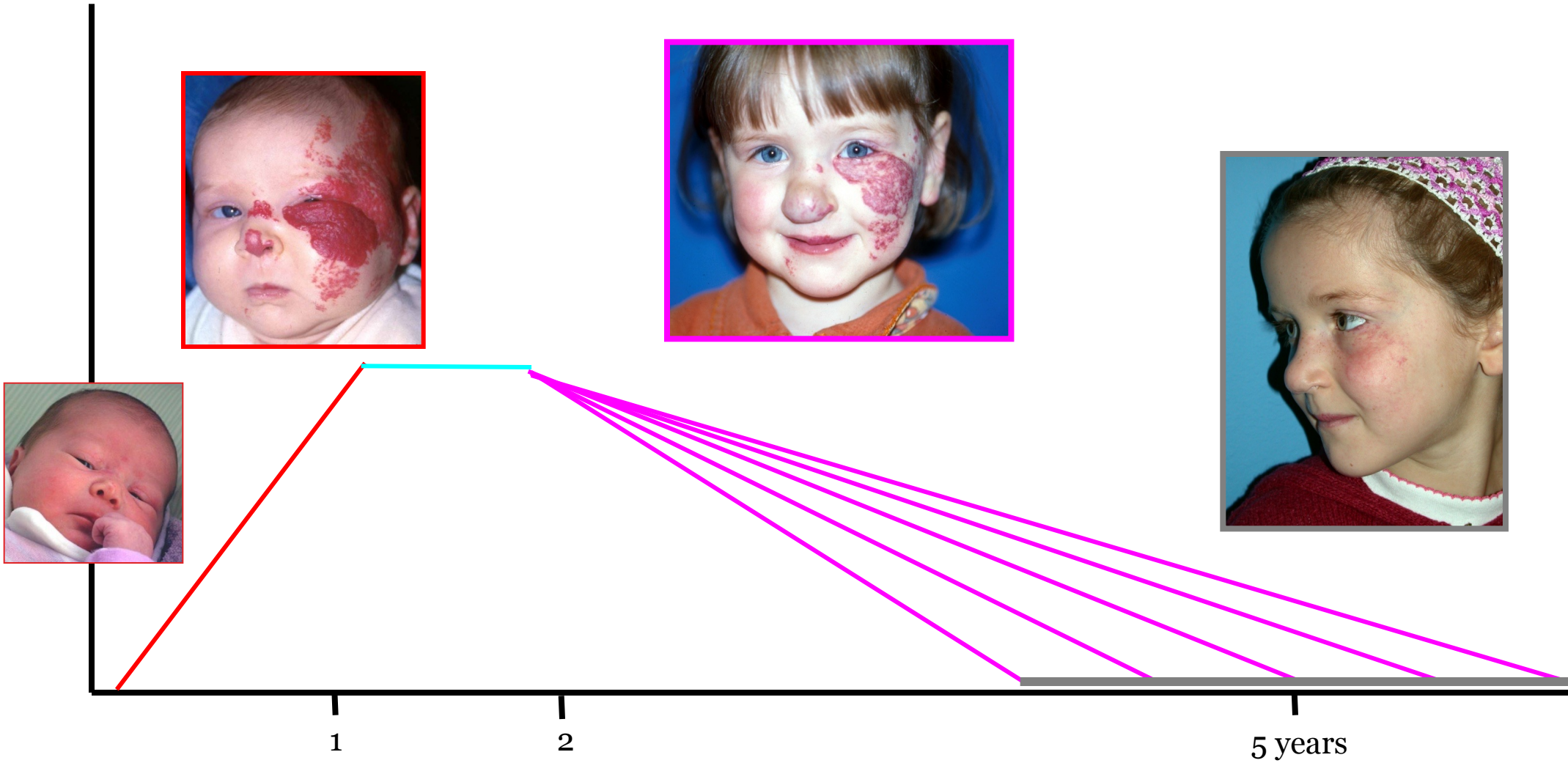
Genetic bases of vascular anomalies

1. Predisposing susceptibility genes (vs. causative)
2. Locus heterogeneity
3. Inherited with reduced penetrance
4. Somatic mutations
5. Clinical phenotypic variability

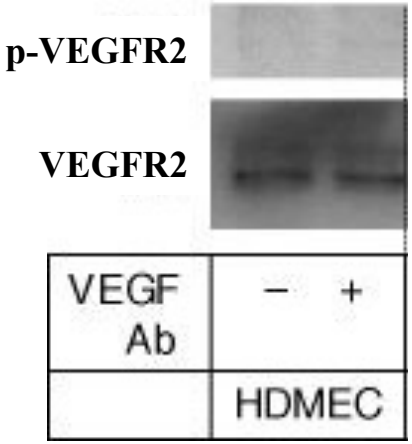
Genetic bases of vascular anomalies

1. Predisposing susceptibility genes (vs. causative)
   - Multigenic (vs. monogenic)
   - Polymorphism (vs. mutation)

Vascular Tumors: Infantile Hemangioma



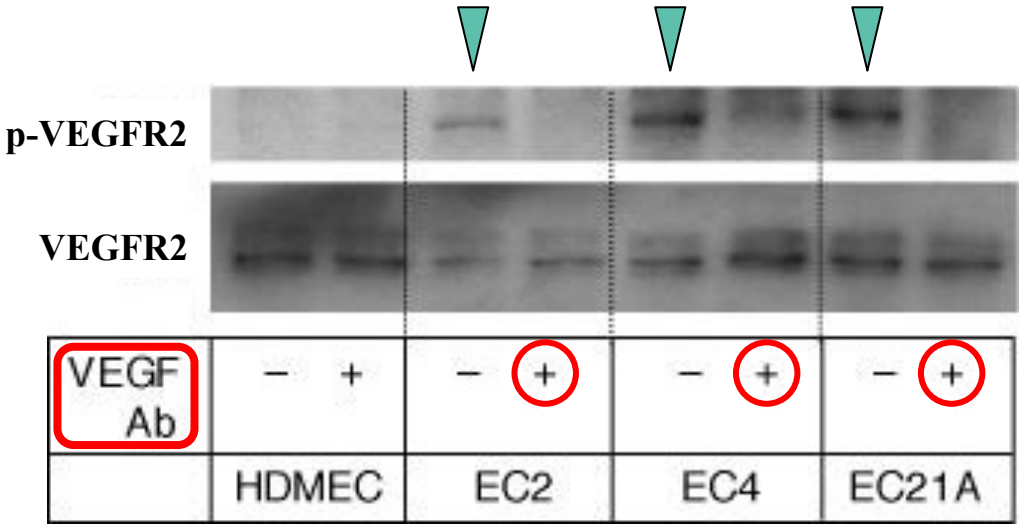
Infantile Hemangioma Cells



Jinnin *et al*, *Nat Med* 2008

Infantile Hemangioma Cells

VEGF-dependent increased VEGFR2 phosphorylation



Hemangioma cells

→ Chronic activation of VEGF signaling

> Phosphorylation

AKT, ERK1/2, RASA1, STAT4....

> Transcription

VEGF, GLUT1

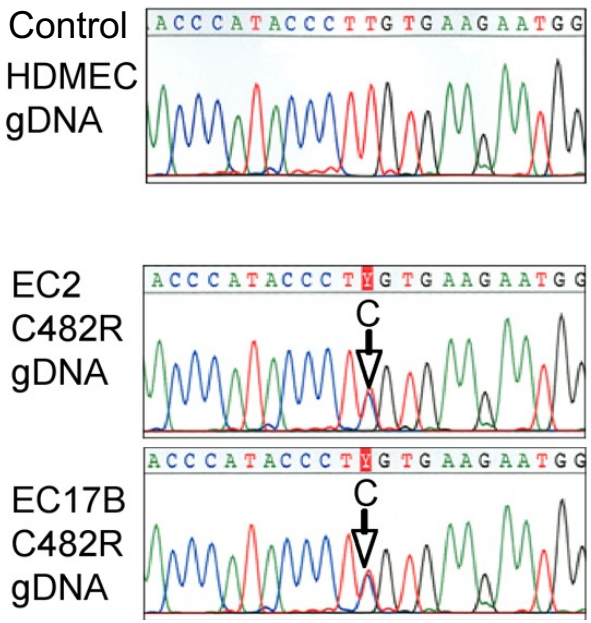
> Hem EC proliferation

Jinnin et al, Nat Med 2008

Hemangioma Etiopathogenesis

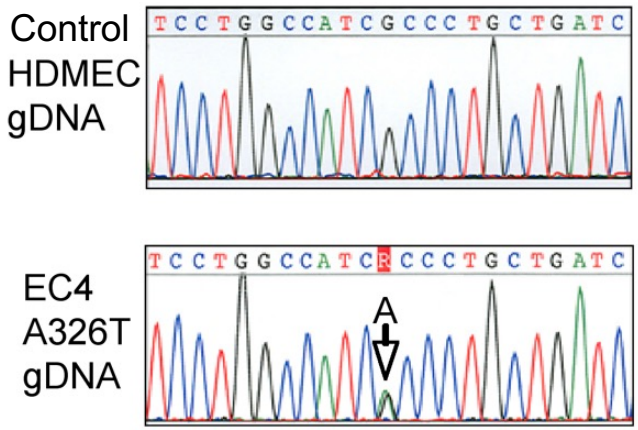
Screening of EC cell lines for 24 candidate genes that regulate
EC migration/proliferation/adhesion/hypoxia response

Two with *VEGFR2* variant



10/ 105 hemangioma patients, 12/295 controls
(~10%) (~4%)

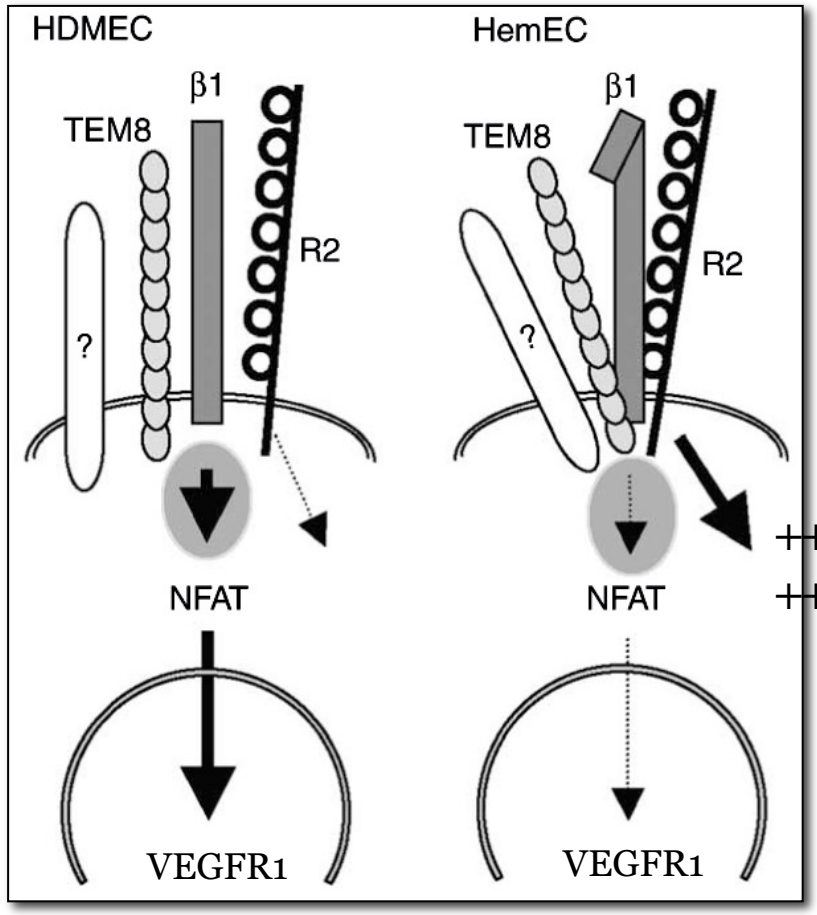
One *TEM8* variant



0/ 110 hemangioma patients, 0/295 controls

Jinnin *et al*, *Nat Med* 2008

Hemangioma Etiopathogenesis



- All Hem ECs showed pathway dysregulation → Changes not necessary
- Associated SNP also present in controls → Changes not sufficient

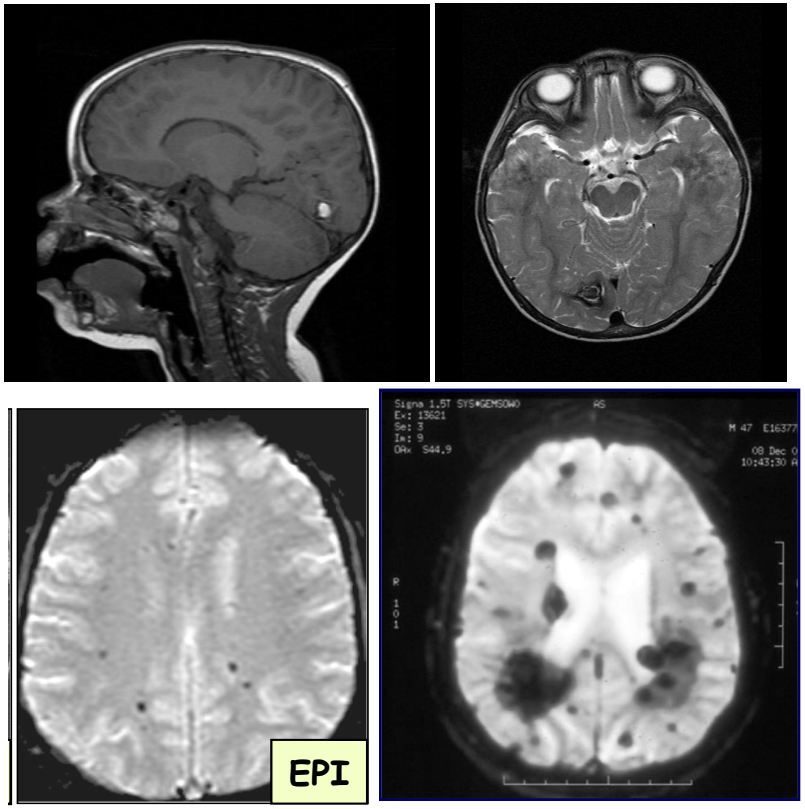
→ Particular *combinations* of predisposing germline changes cause disease

Jinnin *et al*, *Nat Med* 2008

Genetic bases of vascular anomalies

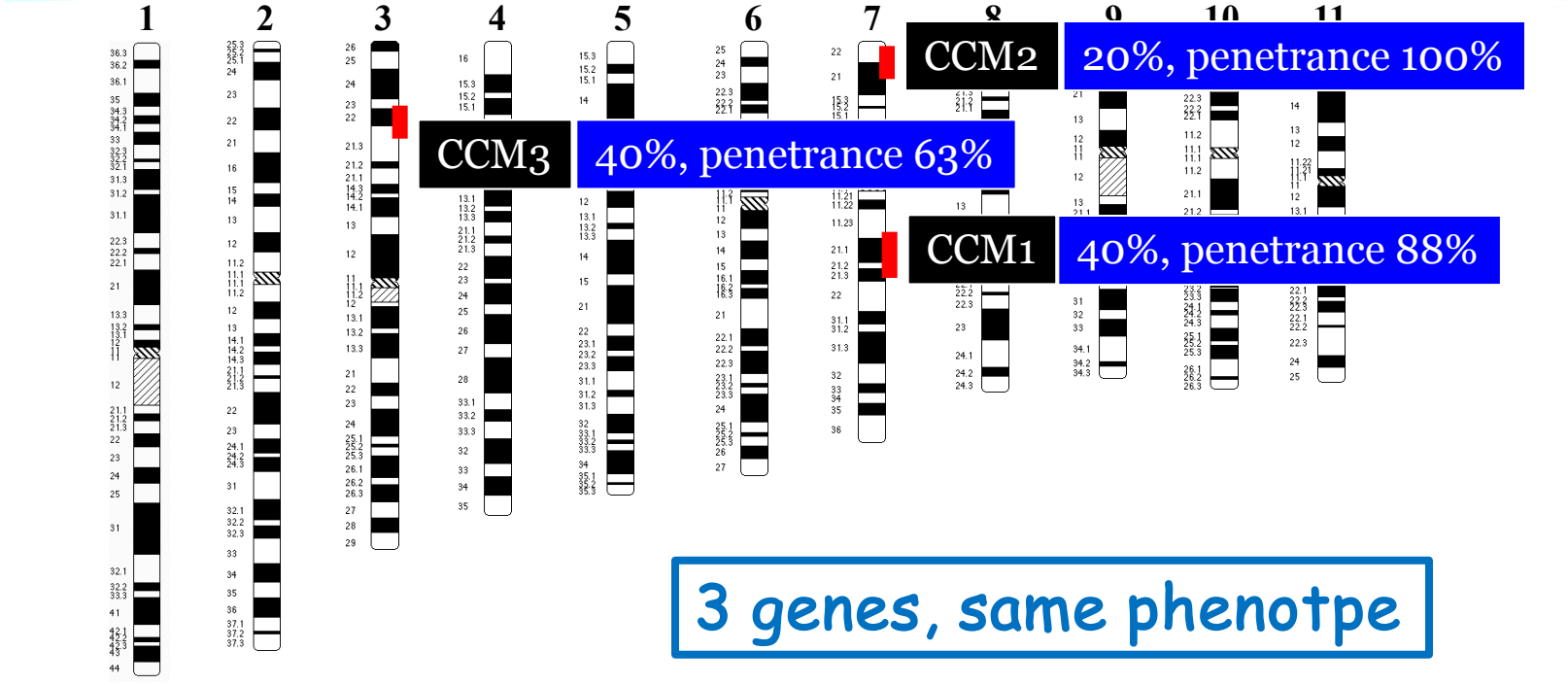
2. Locus heterogeneity
   - "One" phenotype, many causes
     - CCM
     - PLE

Cerebral Cavernous Malformation (CCM)

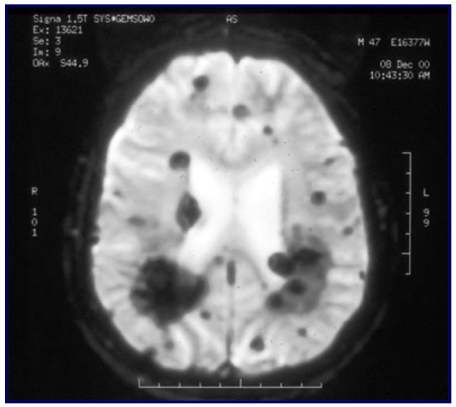


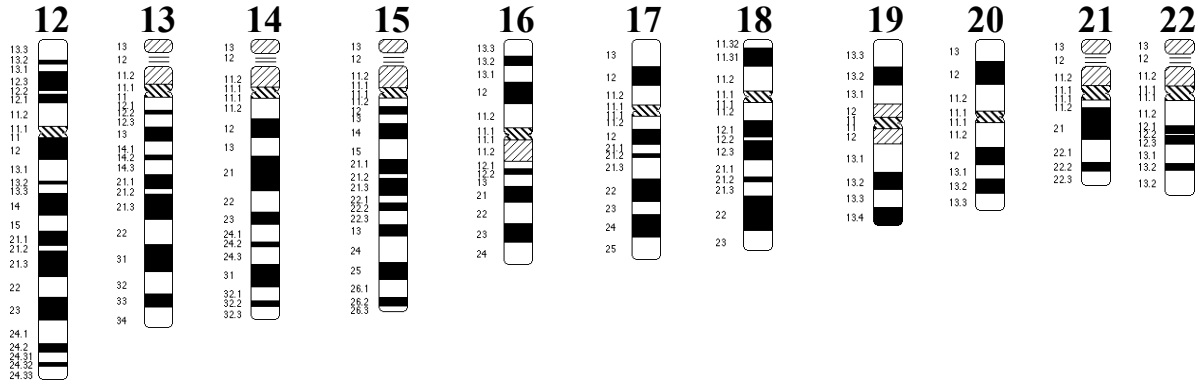
- Incidence: 0.1-0.5%
- Single or multiple lesions
- Epilepsy, headache, haemorrhage;
  Asymptomatic: 15-20%
- Autosomal dominant inheritance (>80%)
- Variable expressivity

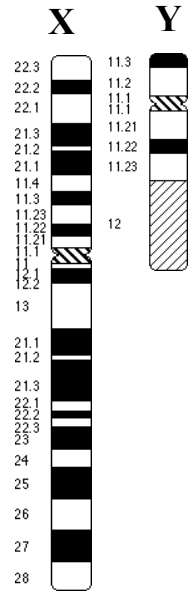
Genetic basis of CCM



3 genes, same phenotpe

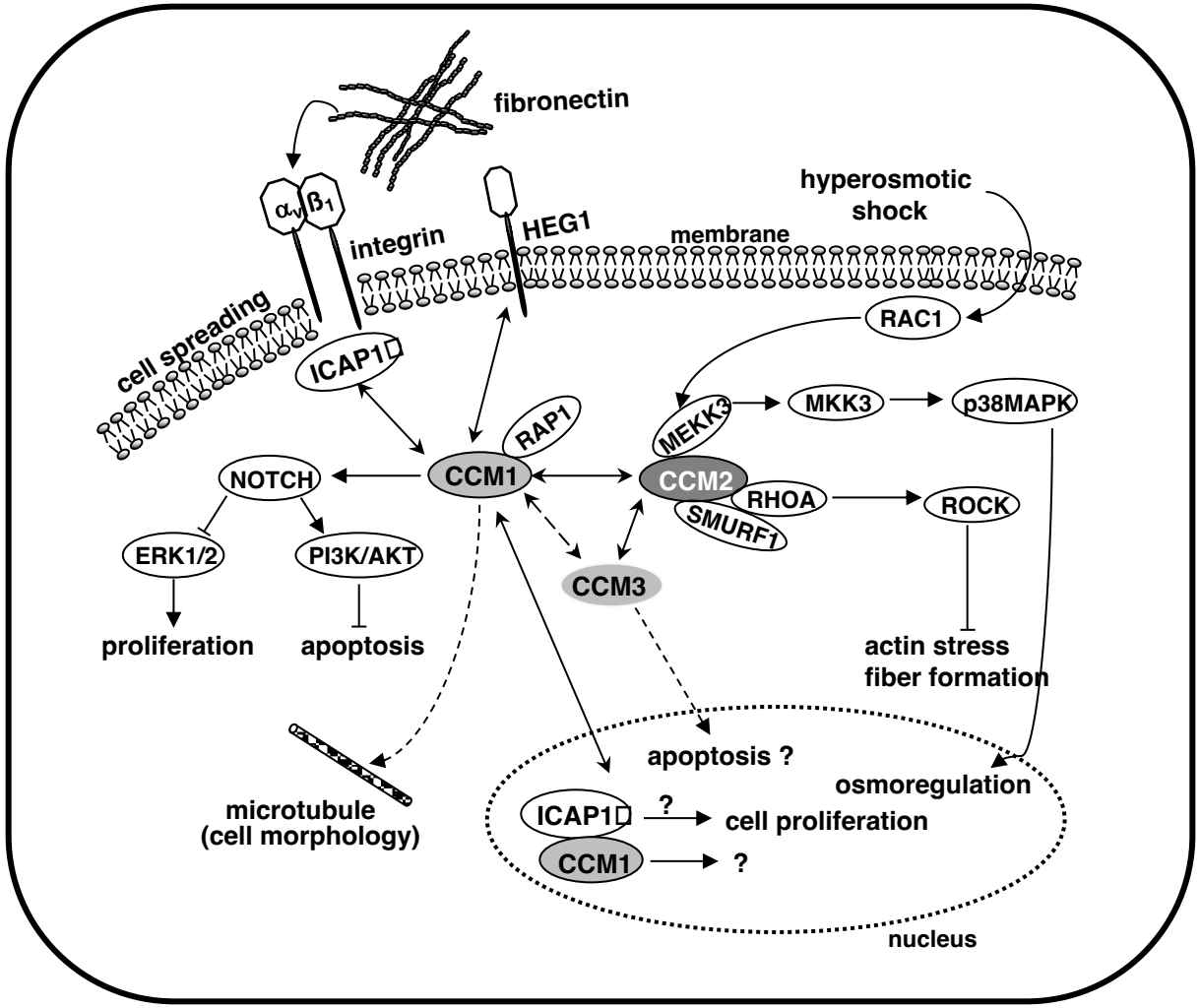






Dubovsky *et al.*, *Hum Mol Genet*, 1995; Craig *et al.*, *Hum Mol Genet*, 1998

Pathophysiology of CCM



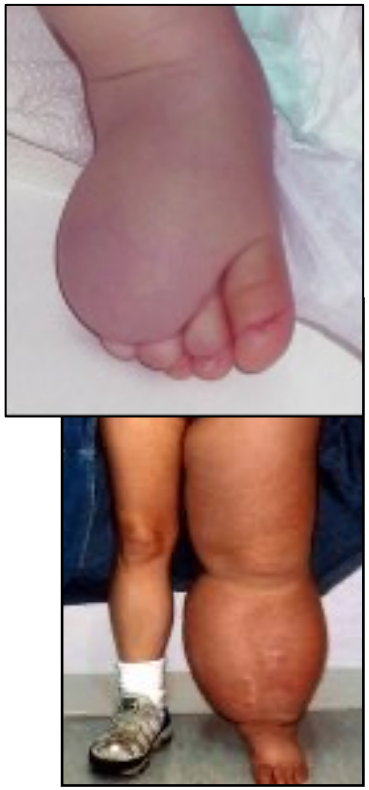
CCM1: KRIT1, CCM2: malcavernin/MGC4607, CCM3: PDCD10

Adapted from Revencu et al, *J Med Genet* 2006

Genetic bases of vascular anomalies

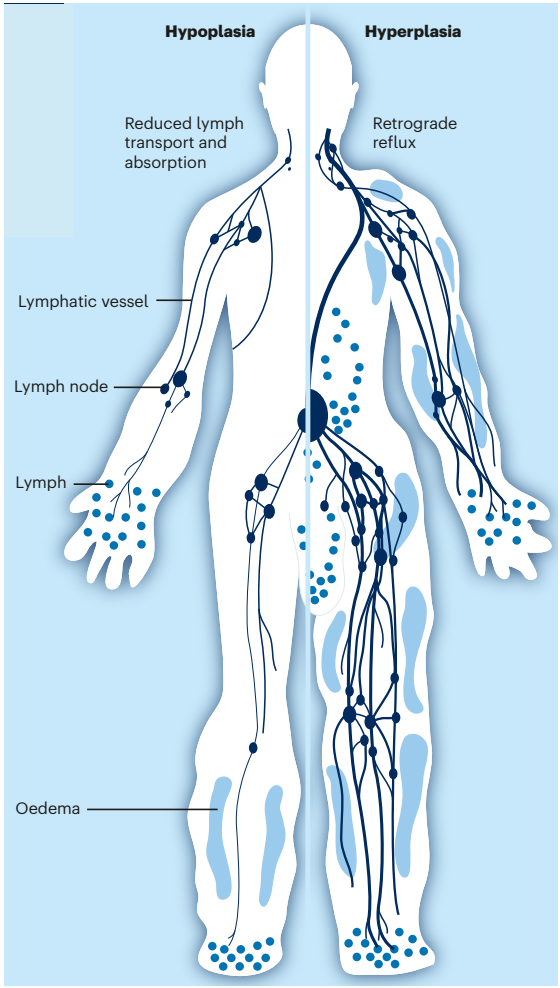
2. Locus heterogeneity
   - "One" phenotype, many causes
     - CCM
     - PLE

Primary lymphedema (PLE)



- Chronic accumulation of lymph within tissues
- Predisposition to infections
- Important dysfunction of extremities

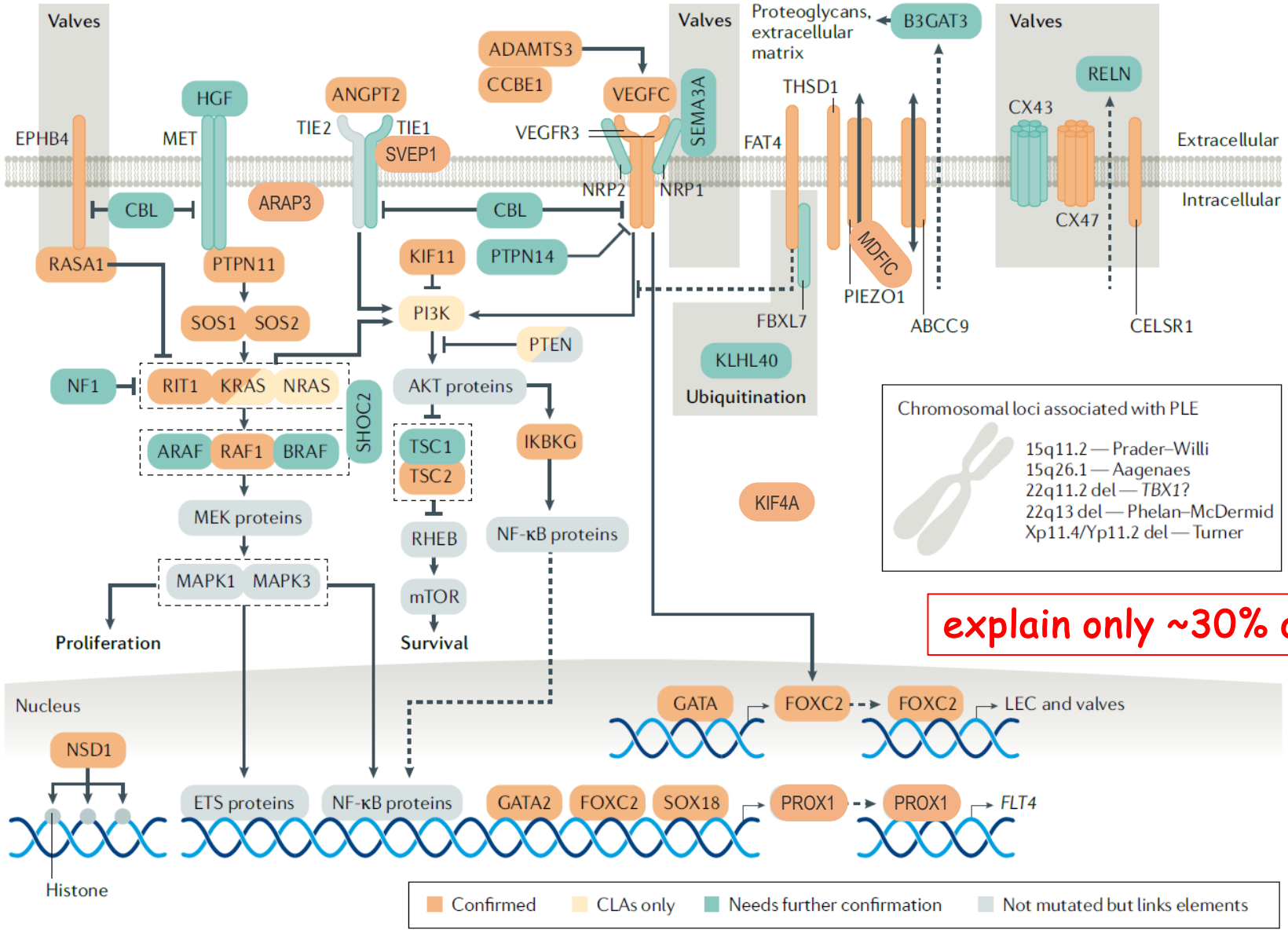
  - abnormal development and/or function of lymphatic vessels
  - genetic predisposition



Brouillard et al, *Nat Rev Dis Primer* 2021

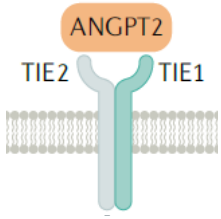
Primary Lymphedema: Highly heterogeneous

(32 clear mutated genes/loci and 18 awaiting confirmation)



Adapted from Brouillard et al, *Nat Rev Dis Primer* 2021

Different mechanisms for a same gene: e.g. *ANGPT2*



ANGPT2 mutations	T299M	N304K	C435S	R492Q	*Whole-gene deletion*	Cryptic splice site
Zygocity	Het	Het	Het	Het	Het	Hom
Inheritance	AD	AD	AD	AD	de novo	Recessive (lethal)
Secretion	normal	Reduced with partial reduction of WT	Not secreted, dominant-negative on WT	Not secreted, dominant-negative on WT	(not tested)	Not secreted
Global effect	GOF	LOF / DN	LOF / DN	LOF / DN	Haplo-insufficiency	Splicing/NMD

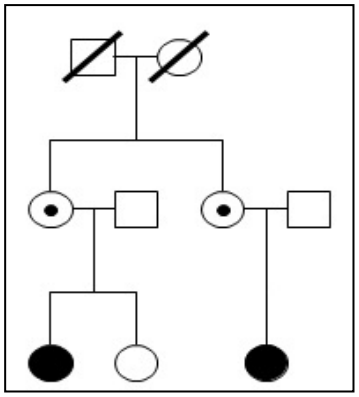
Leppänen & Brouillard et al, *Sci Transl Med* 2020; Smeland & Brouillard et al, *J Med Genet* 2021

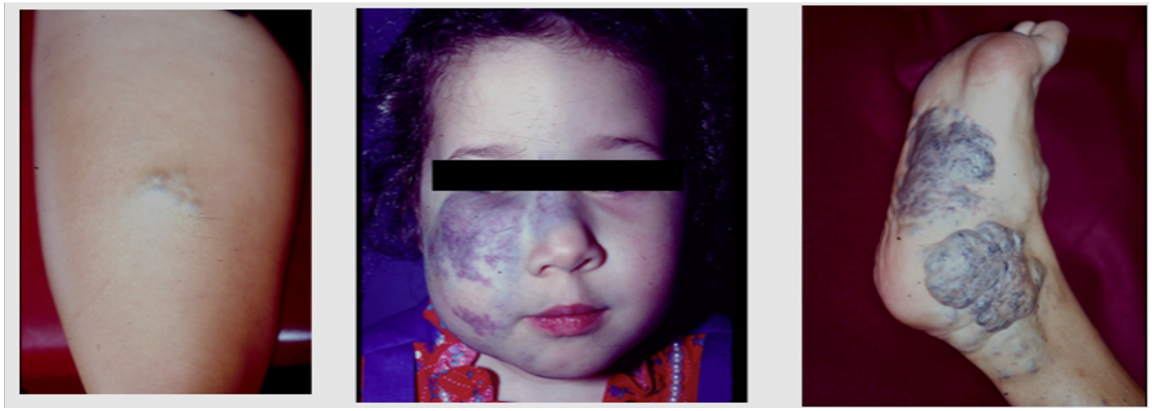
Genetic bases of vascular anomalies

3. Inherited with reduced penetrance
   - Second-hits

Glomuvenous Malformation (GVM)

- 5% of venous anomalies
- Caused by loss-of-function mutations in *glomulin*
- Autosomal dominant, with reduced penetrance & phenotypic heterogeneity

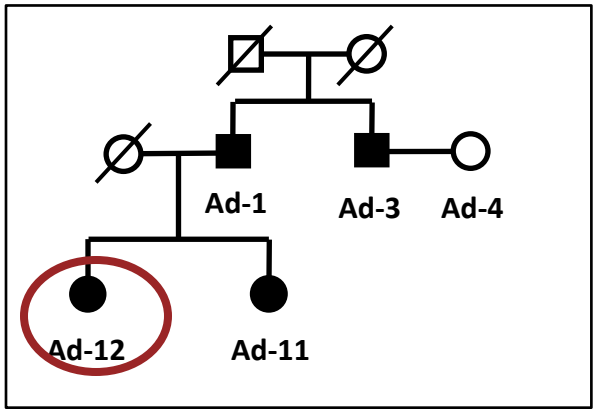


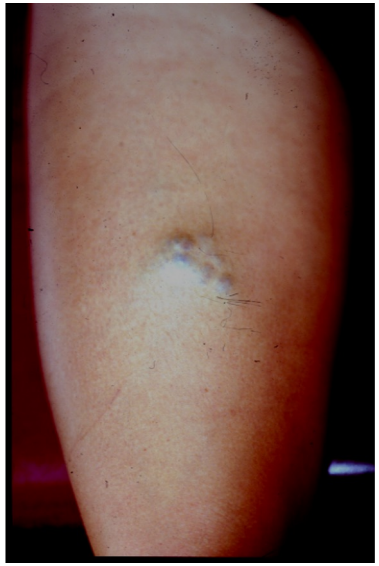


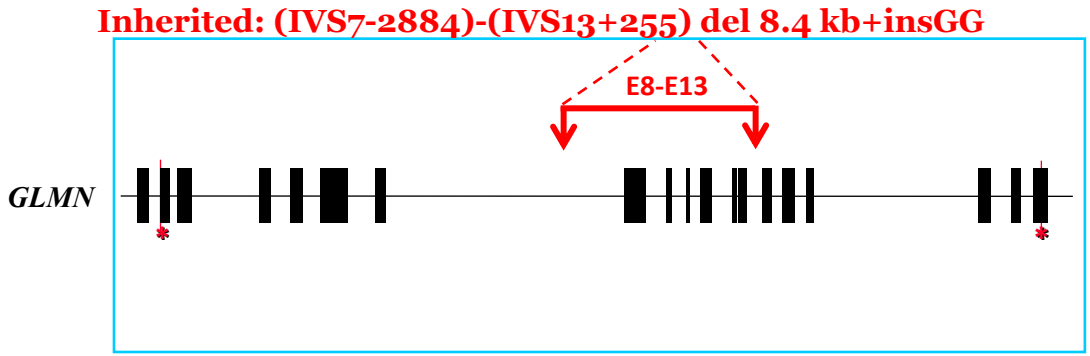
- Does lesion-formation require an additional somatic event? *(Knudson's hypothesis)*

Boon et al, *Hum Mol Genet* 1994; Brouillard et al, *Am J Hum Genet* 2002

First somatic 2nd hit identified in a vascular malformation

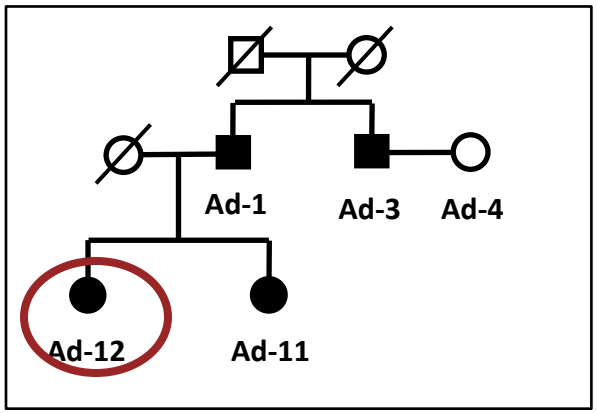


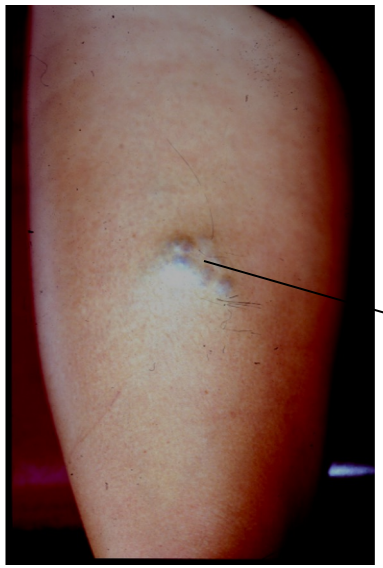




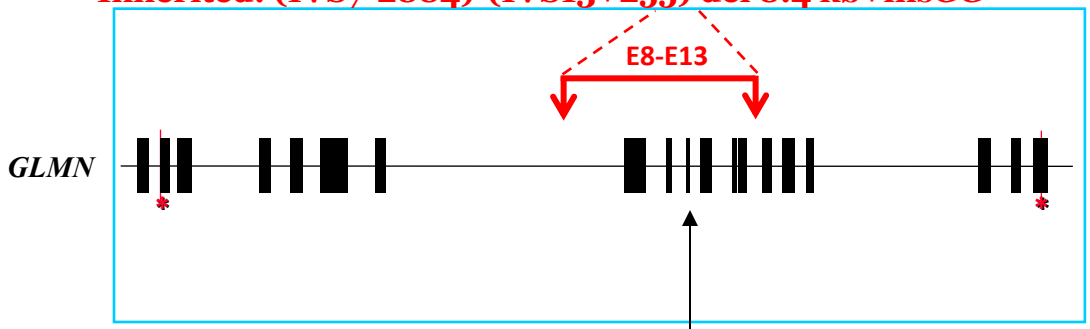
Brouillard et al. *Am. J Hum Genet* 2002

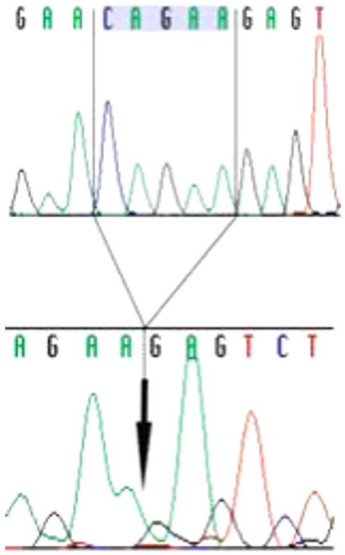
First somatic 2nd hit identified in a vascular malformation





Inherited: (IVS7-2884)-(IVS13+255) del 8.4 kb+insGG





Somatic: 980delCAGAA (Exon 10)

Brouillard et al. *Am. J Hum Genet* 2002

Complete local loss of glomulin

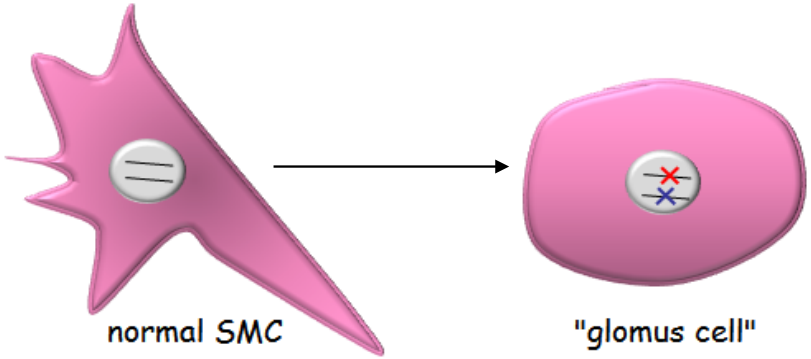
In all cells of the patient

wt

inherited mutation

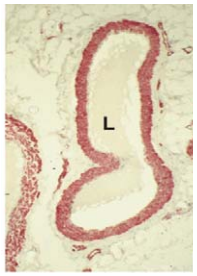
2nd somatic mutation

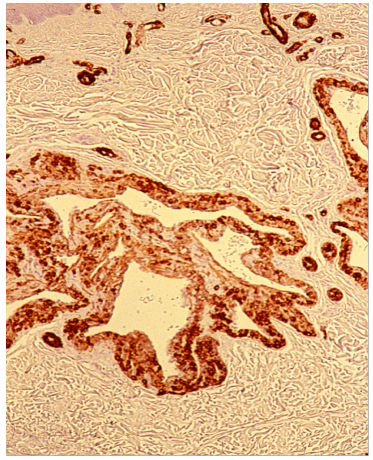
inherited mutation

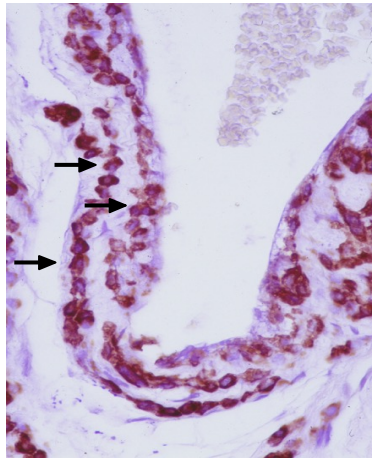


GVM lesion

Normal vein



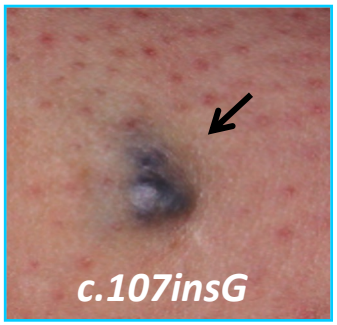




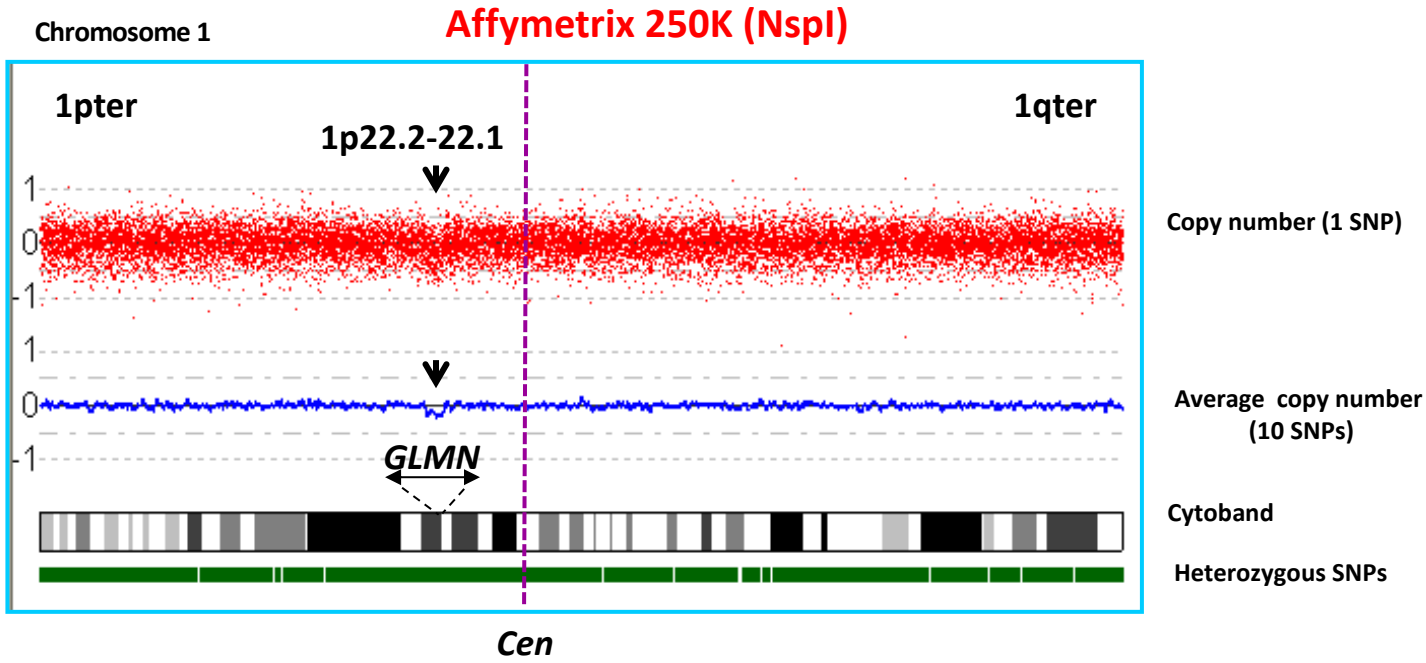
SMC α actin

Local loss of wild-type glomulin expression: genomic deletion?

GVM71_12



Loss of wt *RNA*

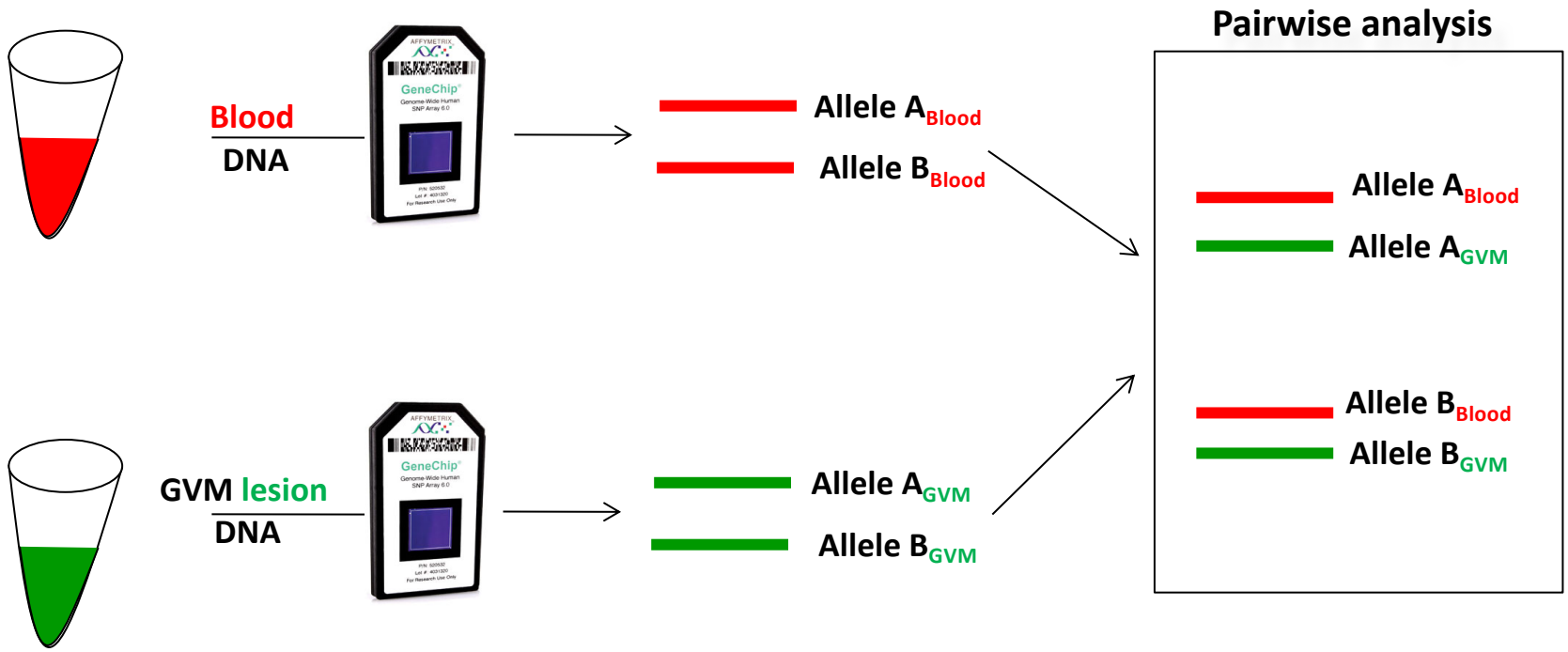


Other tissues showed no chromosomal alteration by SNP-chips

Amyere et al, *Am J Hum Genet* 2013

Somatic 2nd hits in GVM

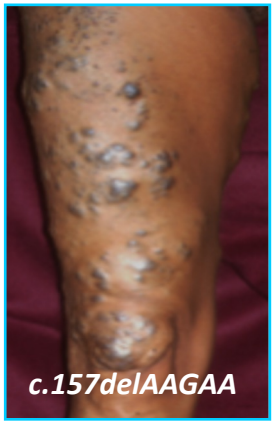
Pairwise comparison of allele-specific copy number



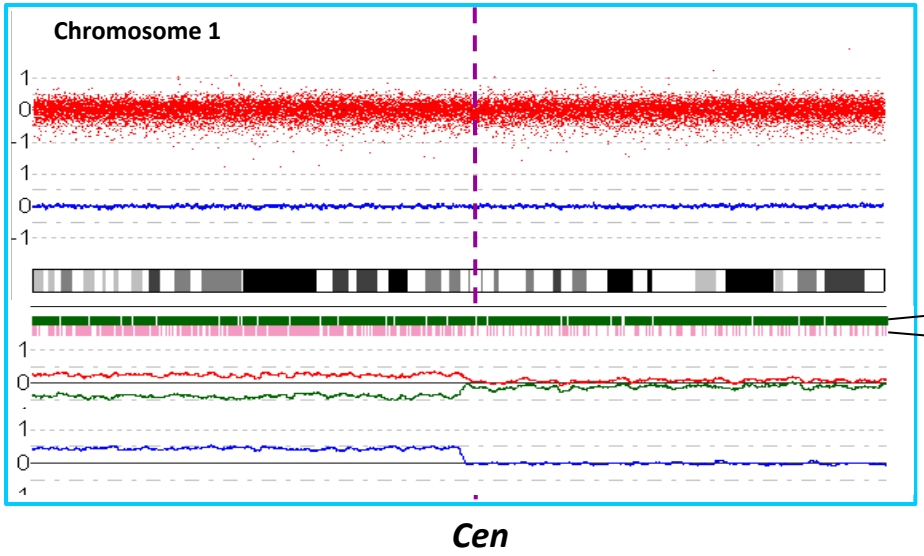
Amyere et al, *Am J Hum Genet* 2013

Somatic 2nd hits in GVM

GVM22



Loss of WT allele RNA in GVM tissue



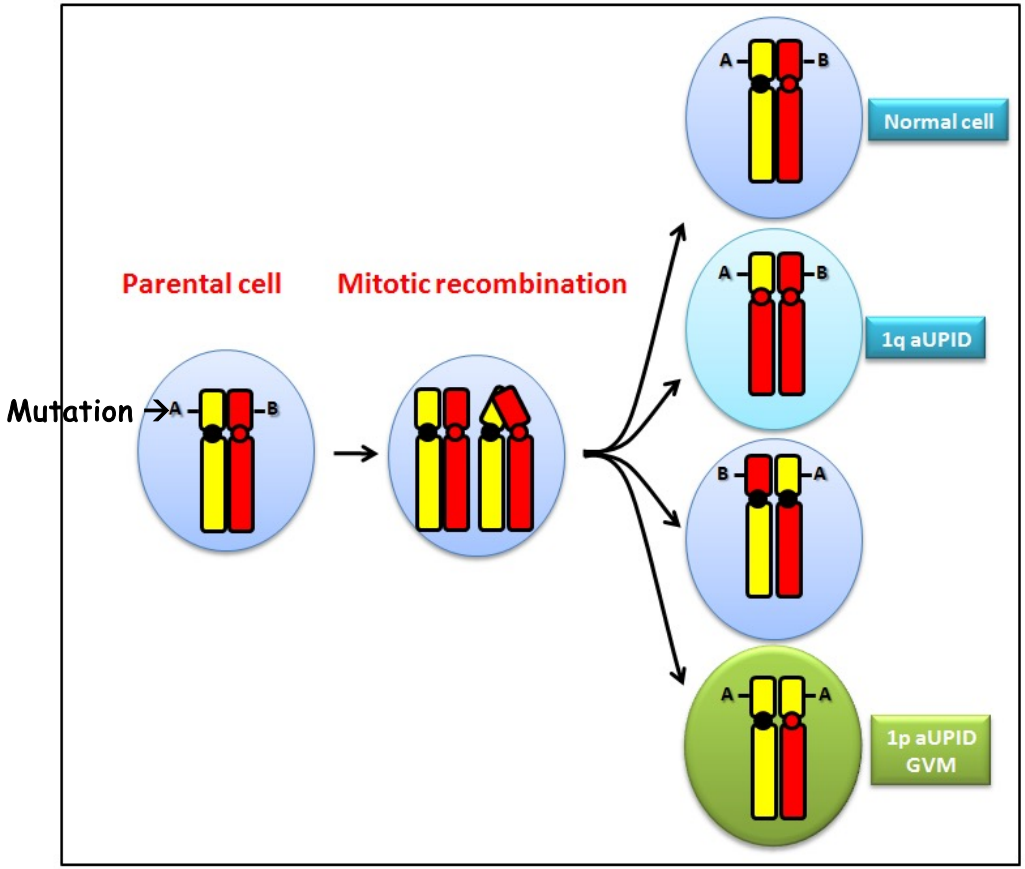
Similar observation in 11 other GVM lesions, not in controls

Amyere et al, *Am J Hum Genet* 2013

aUPID: A novel mechanism for glomulin loss

Allelic imbalance (LOH) without copy number change in tissue:

acquired UniParental IsoDisomy



Amyere et al, *Am J Hum Genet* 2013

Autosomal dominant with incomplete penetrance: "recessive" at the level of the cell!

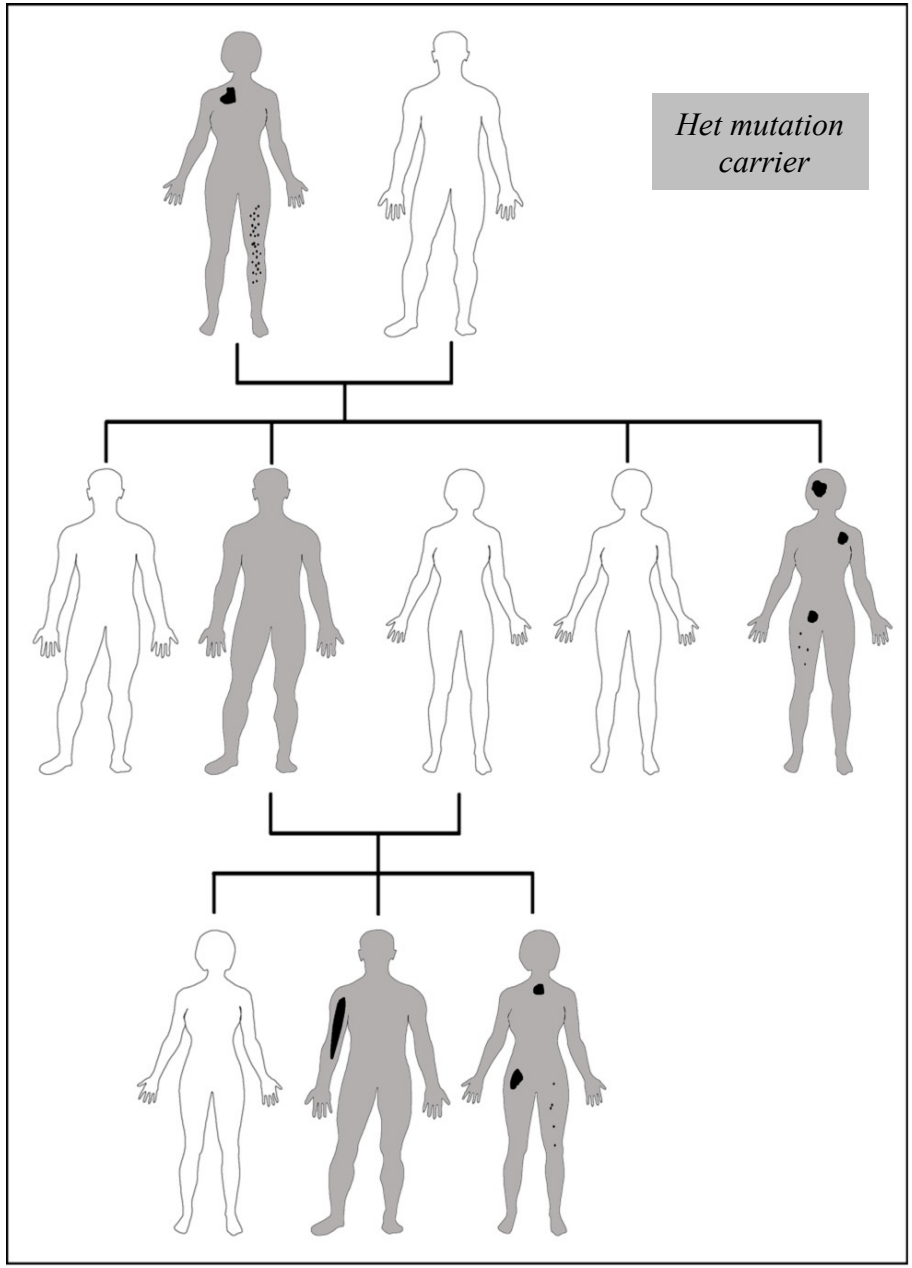
Since then, second hit mutations shown in

Glomuvenous malformation

Cutaneomucosal Venous Malformation (VMCM)

Cerebral Cavernous Malformation (CCM)

.....



Genetic bases of vascular anomalies

3. Inherited with reduced penetrance
   - Second-hits
   - Mosaicism/ tissue heterogeneity

Mosaicism in CM-AVM 1 (*RASA1*)

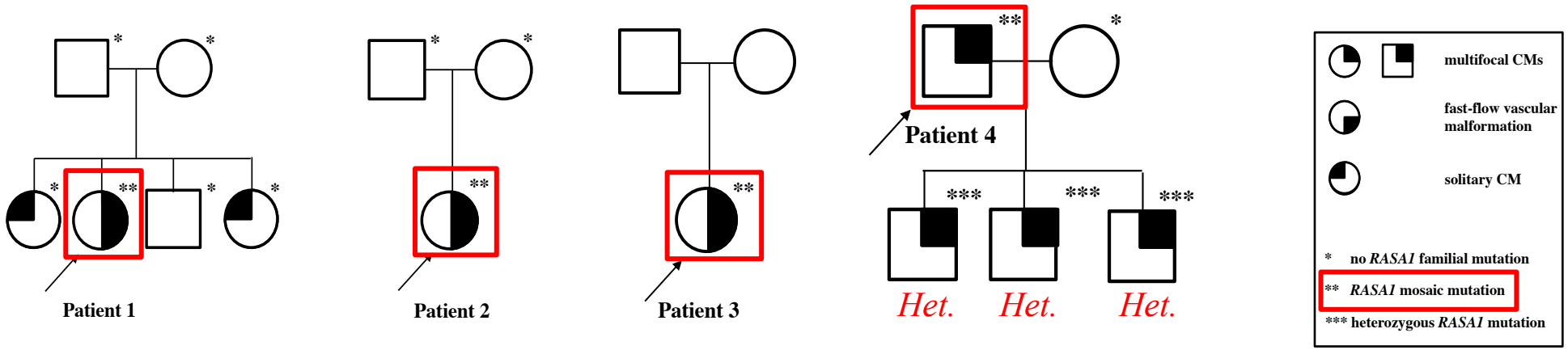


Table 1 Clinical and genetic data

	Tissue	*RASA1* variant	Allele frequency (read count) with AmpliSeq panel	Allele frequency (read count) with Sophia Genetics panel	Sanger sequencing	CMs	Fast-flow vascular malformations
Patient 1	Blood	*c.1879A>T*; p.(Lys627*)	35.7% (10/28)	25.3% (164/649)	+	3 CMs	Parieto-occipital AVF/spinal AVM from T1 to T8
Patient 2	Blood	*c.2035C>T*; p.(Arg679*)	2.7% (3/111)	3.1% (63/2011)	NP	4 CMs, Bier spots on hands and telangiectatic lesions on upper thorax, lower lip and tongue	Facial AVM
	AVM	*c.(2035C>T(;)c.1507C>T)*; p.(Arg679*(;) Gln503*)	13.6% (465/3407)+8% (171/2126)	NP	NP		
Patient 3	Blood	*c.1192C>T*; p.(Lys398*)	NP	8.5% (101/1189)	+	More than 20 CMs	Soft tissue and fatty hypertrophy with multiple AV microfistulas in the right foot
Patient 4	Blood	*c.2707C>T*; p.(Arg903*)	NP	6.1% (59/964)	NP	More than 10 CMs	–
	Saliva		NP	4.6% (36/783)	NP		
	CM		6.9% (21/305)	NP	NP		

The symbol – denotes absent and + denotes mutation seen by Sanger sequencing.
AV, arteriovenous; AVF, arteriovenous fistula; AVM, arteriovenous malformation;CM, capillary malformation; NP, not performed.

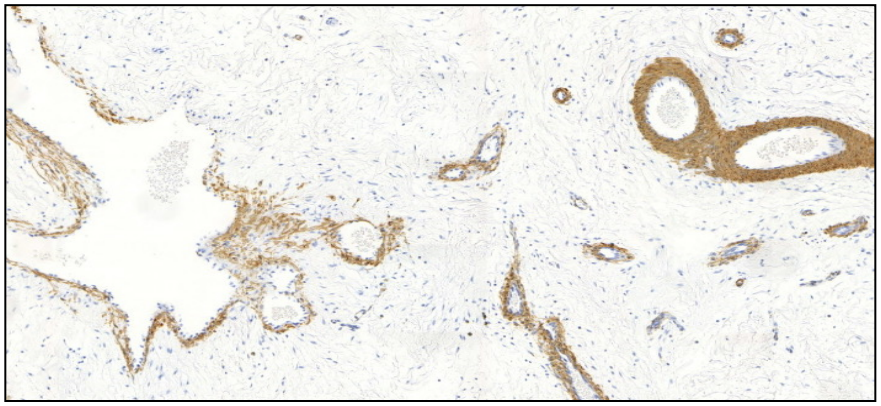
→ **Mosaic became germline**

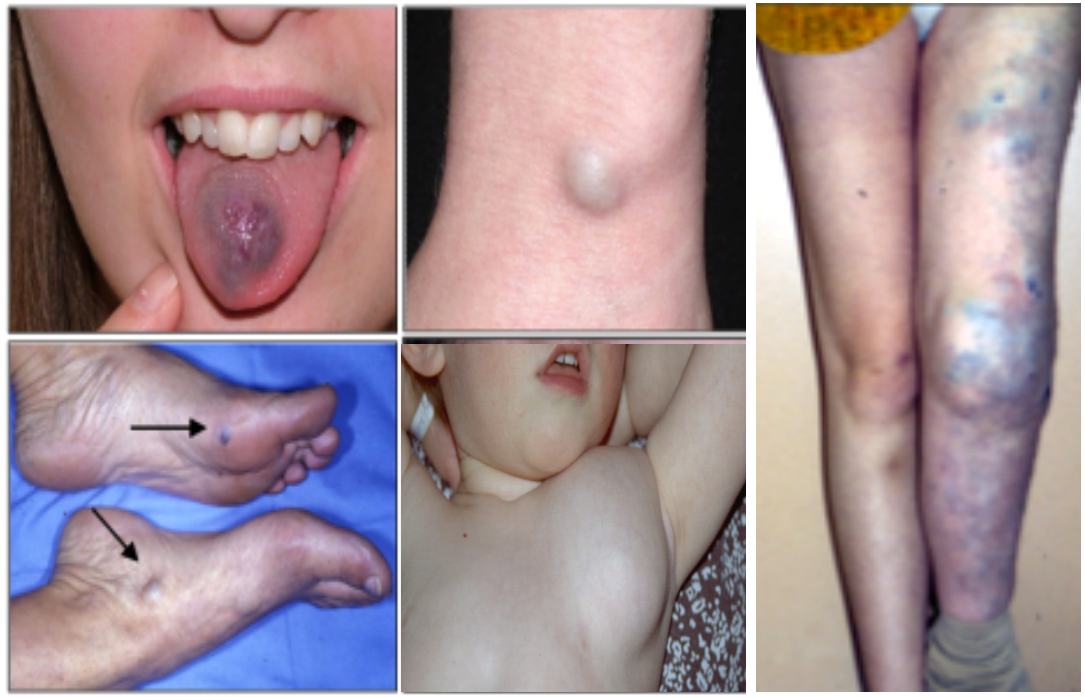
Revencu et al, *J Med Genet* 2019

Genetic bases of vascular anomalies

4. Somatic mutations
   - VMs & BRBN

Venous Malformations: mostly sporadic





- Enlarged venous channels
- Single EC layer, with patchy vSMC
- Typically sporadic (>98%)

- Familial forms (autosomal dominant) caused by TIE2/*TEK* mutations

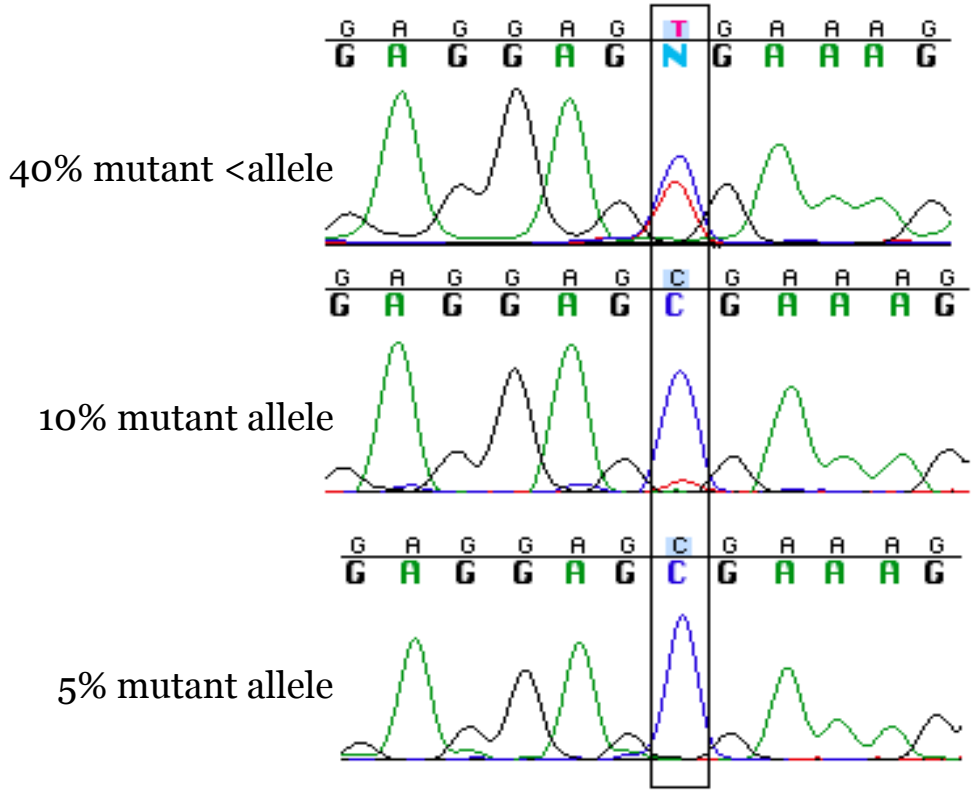
➔ Somatic mutations in the same gene in the sporadic cases?

Vikkula et al, *Cell* 1996

Sporadic VM tissues were negative

Tissular heterogeneity may hide mutation

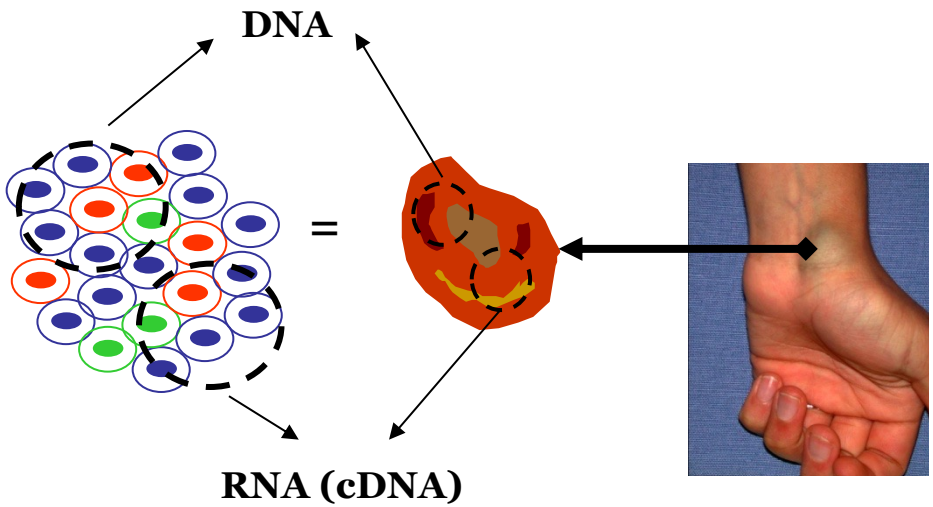
Sanger sequencing of gDNA (cumulative signal)



Mutations only in ECs?

Overcoming tissue heterogeneity:

cDNA-based screens



→ *TIE2 expression* primarily from ECs

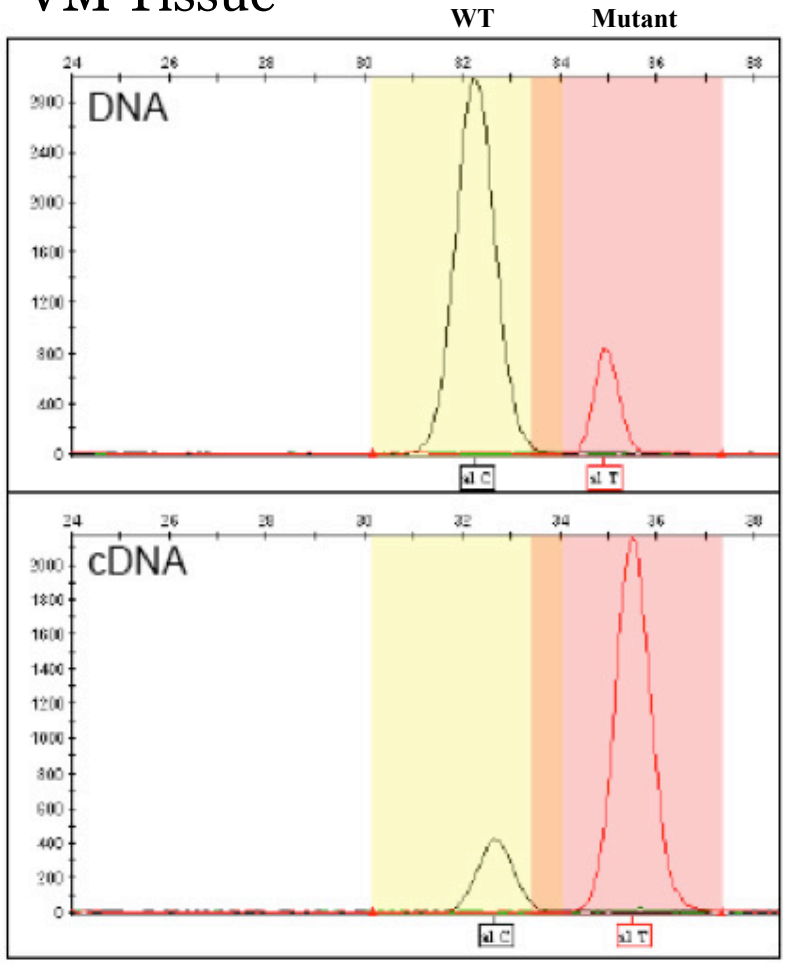
= abnormal EC

= normal EC

= other cells

RNA not always available …

VM Tissue



(Semi quantitative minisequencing): SNaPshot

Limaye et al., *Nat Genet*. 2009

Overcoming tissue heterogeneity:

Deep (Next Generation) Sequencing

gDNA sequencing

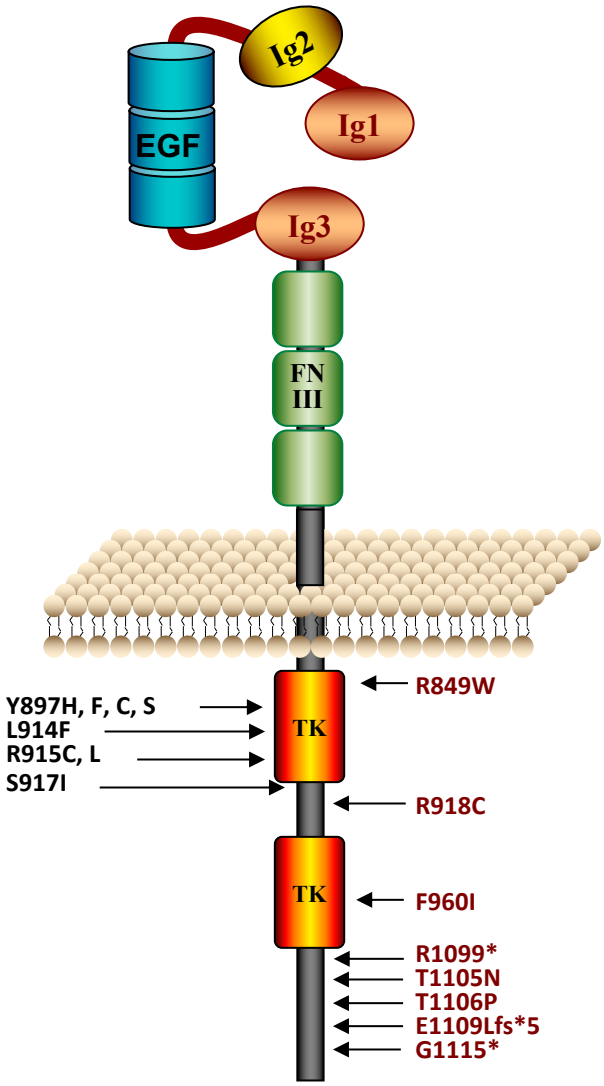
9 mut (T): 170 wt (C)

(~5%)

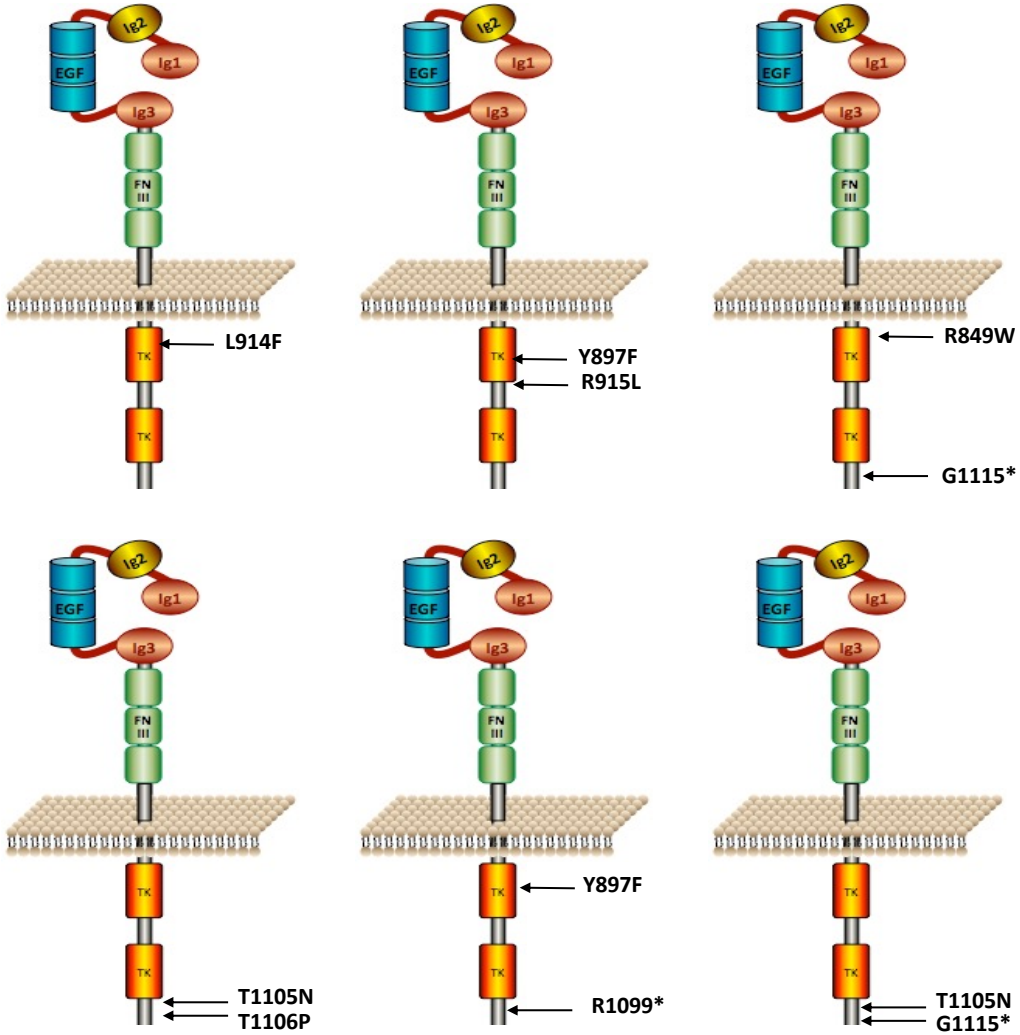
→ Should include negatives to distinguish low-freq alleles from background/noise!

Somatic TIE2/*TEK* mutations cause >60% of VMs

(1-10% of mutant alleles detected)

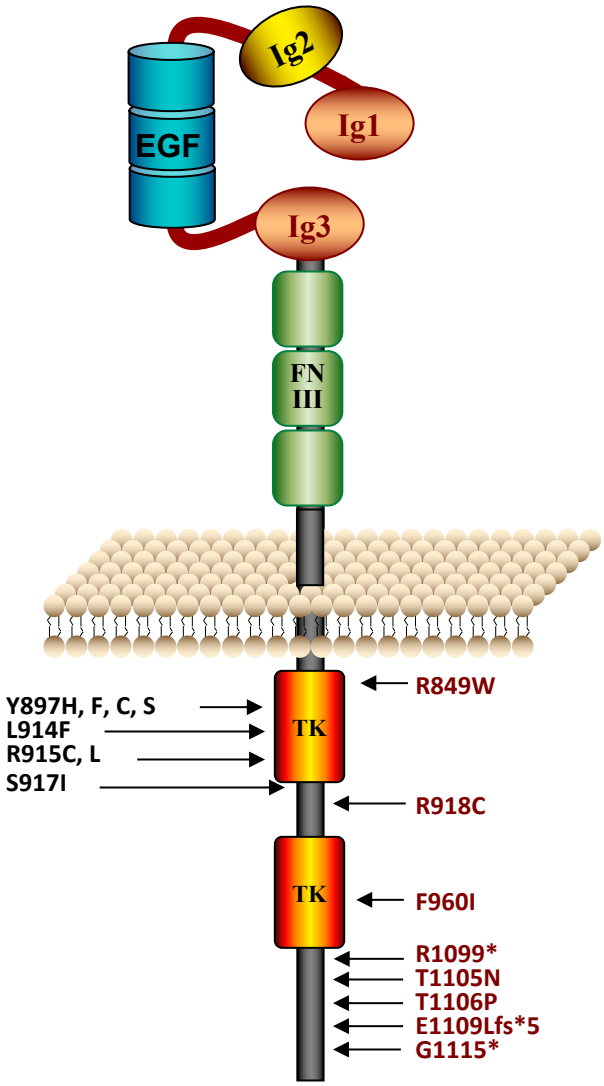


74%

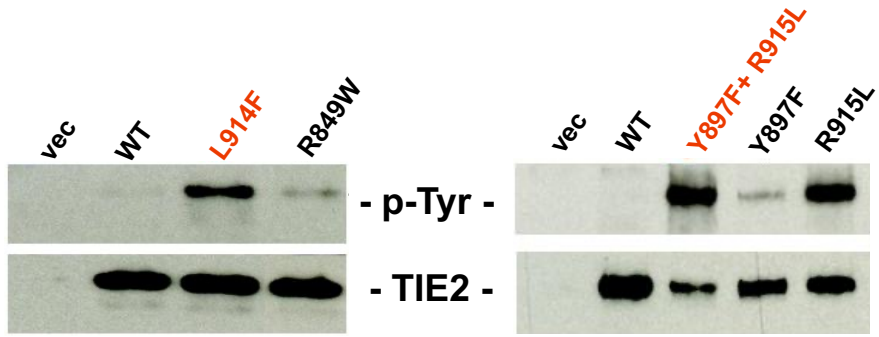


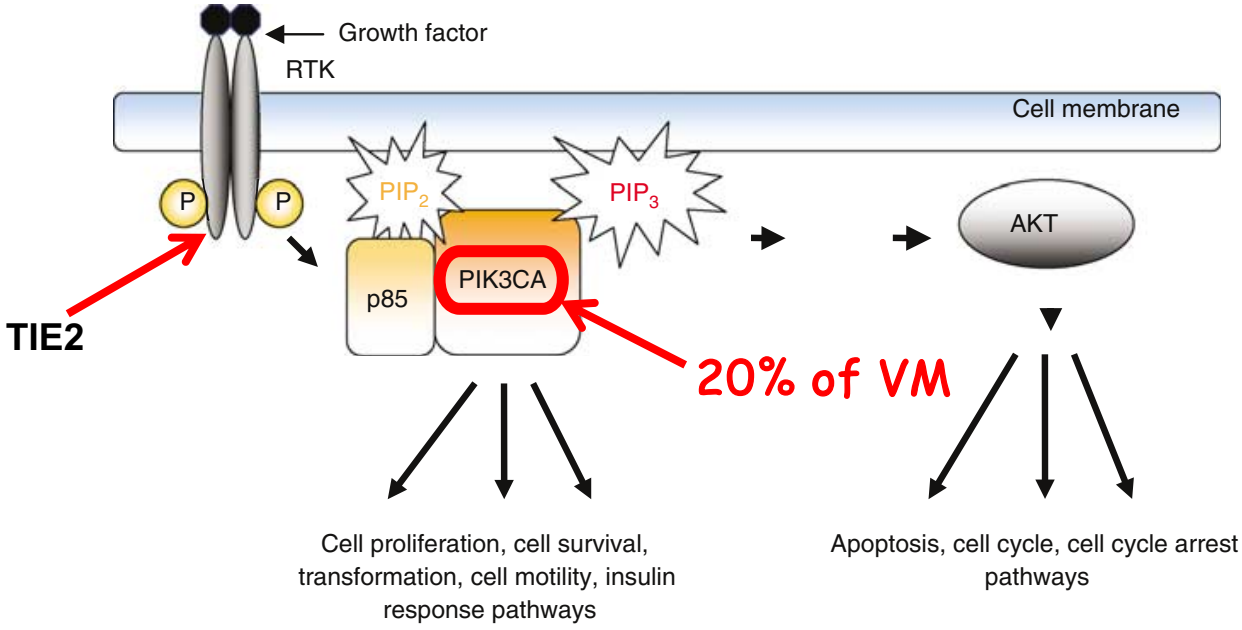
Limaye et al, *Nat Genet* 2009; Soblet et al, *Mol Syndromol* 2013

Somatic TIE2/*TEK* mutations cause >60% of VMs



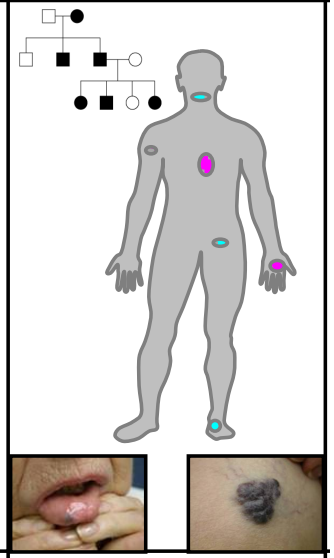
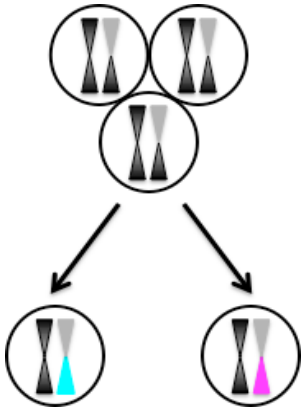
Hyper-phosphorylation of mutants





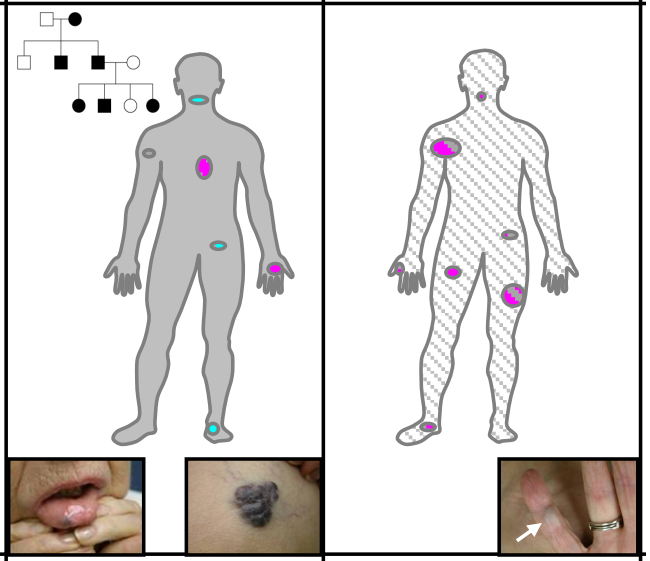
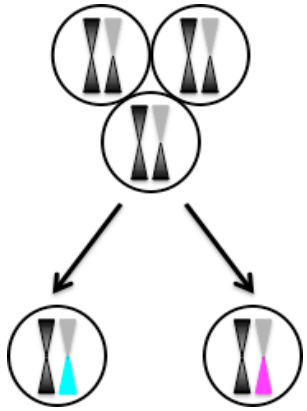
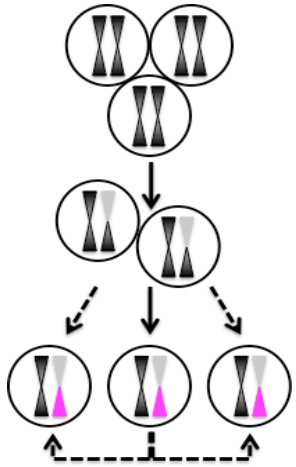
Limaye et al, *Nat Genet* 2009; Soblet et al, *Mol Syndromol* 2013, Uebelhoer et al, 2013, Natynki et al, *Hum Mol Genet* 2015 Limaye et al, *Am J Hum Genet 2015*

TIE2 Mutations : Variable Phenotypes

Disease	VMCM
Phenotype	
Frequent mutations	R849W (germline) – Y1108* (somatic)
Mutational mechanisms	

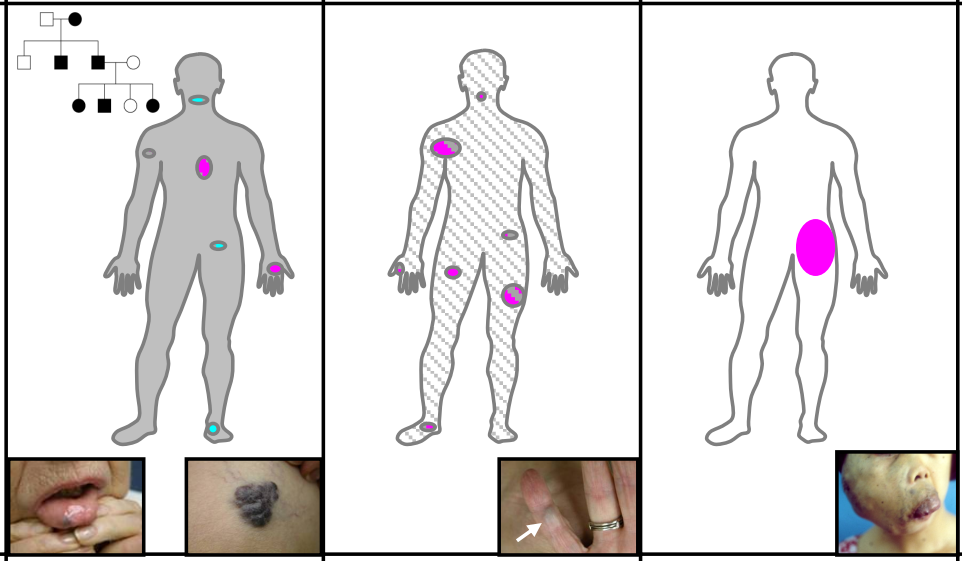
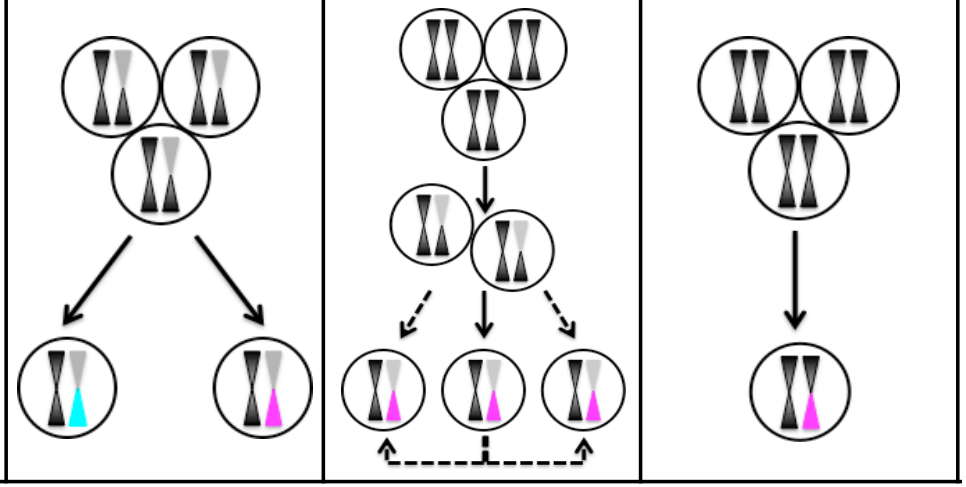
Nätynki et al, *Hum Mol Genet* 2015; Soblet et al, *J Invest Dermatol* 2017

TIE2 Mutations : Variable Phenotypes

Disease	VMCM	MSVM
Phenotype		
Frequent mutations	R849W (germline) – Y1108* (somatic)	R915C (mosaic) – Y897C (somatic)
Mutational mechanisms		

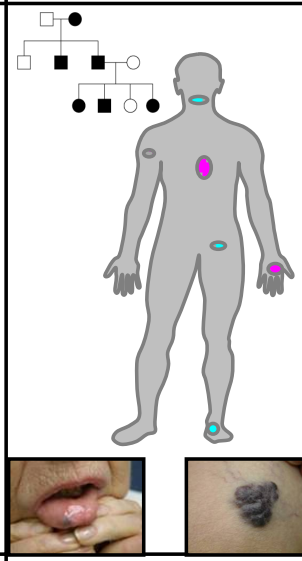
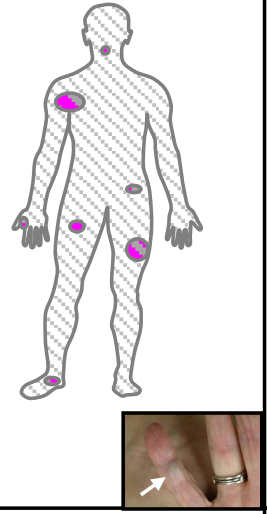
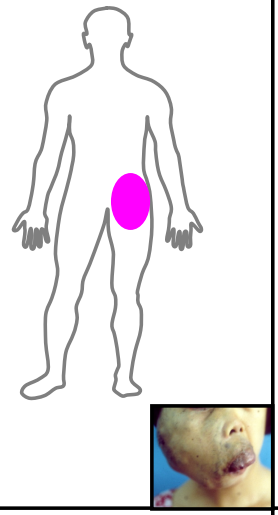
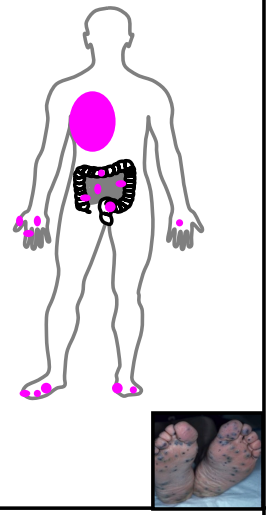
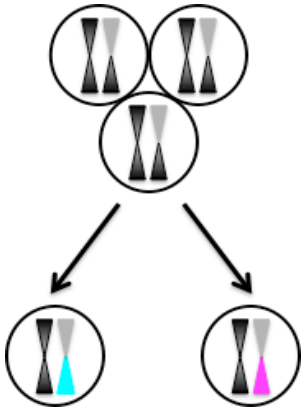
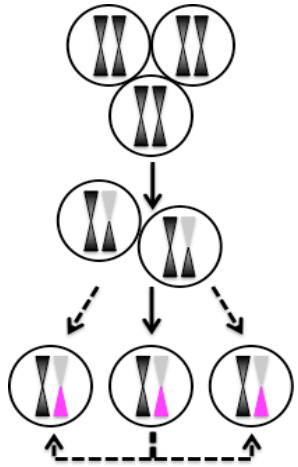
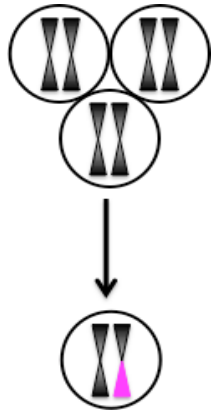
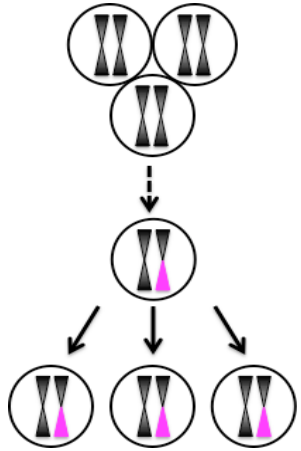
Nätynki et al, *Hum Mol Genet* 2015; Soblet et al, *J Invest Dermatol* 2017

TIE2 Mutations : Variable Phenotypes

Disease	VMCM	MSVM	VM
Phenotype			
Frequent mutations	R849W (germline) – Y1108* (somatic)	R915C (mosaic) – Y897C (somatic)	L914F (somatic)
Mutational mechanisms			

Nätynki et al, *Hum Mol Genet* 2015; Soblet et al, *J Invest Dermatol* 2017

TIE2 Mutations : Variable Phenotypes

Disease	VMCM	MSVM	VM	BRBN
Phenotype				
Frequent mutations	R849W (germline) – Y1108* (somatic)	R915C (mosaic) – Y897C (somatic)	L914F (somatic)	T1105N – T1106N (somatic, niche)
Mutational mechanisms				

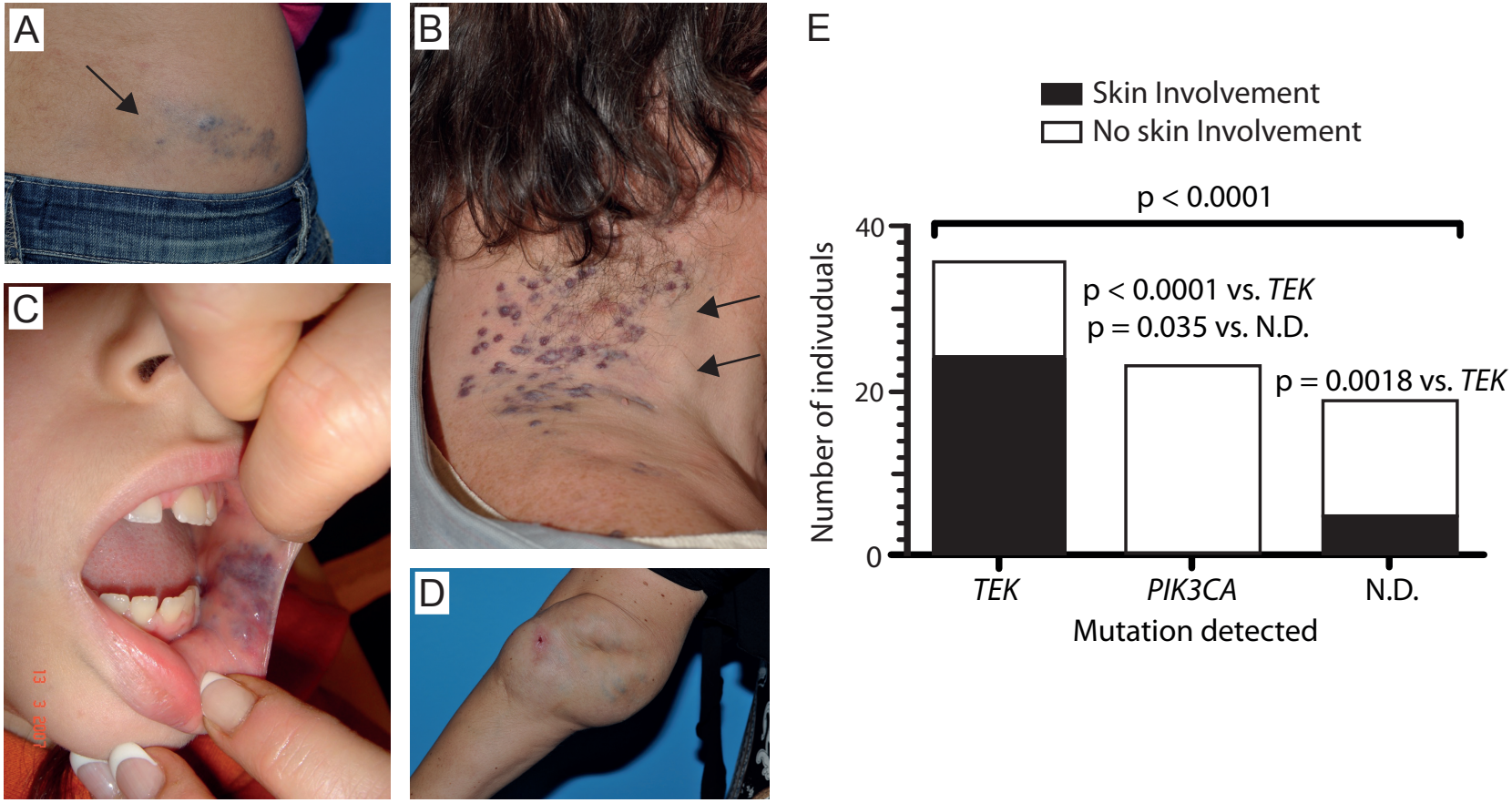
Nätynki et al, *Hum Mol Genet* 2015; Soblet et al, *J Invest Dermatol* 2017

Genetic bases of vascular anomalies

5. Clinical phenotypic variability
   - One gene, several clinical presentations
   - Spatio-temporal distribution of mutations/stochastic effect

Somatic *PIK3CA* mutations cause 20% of VM

(60% due to mutations in TEK)



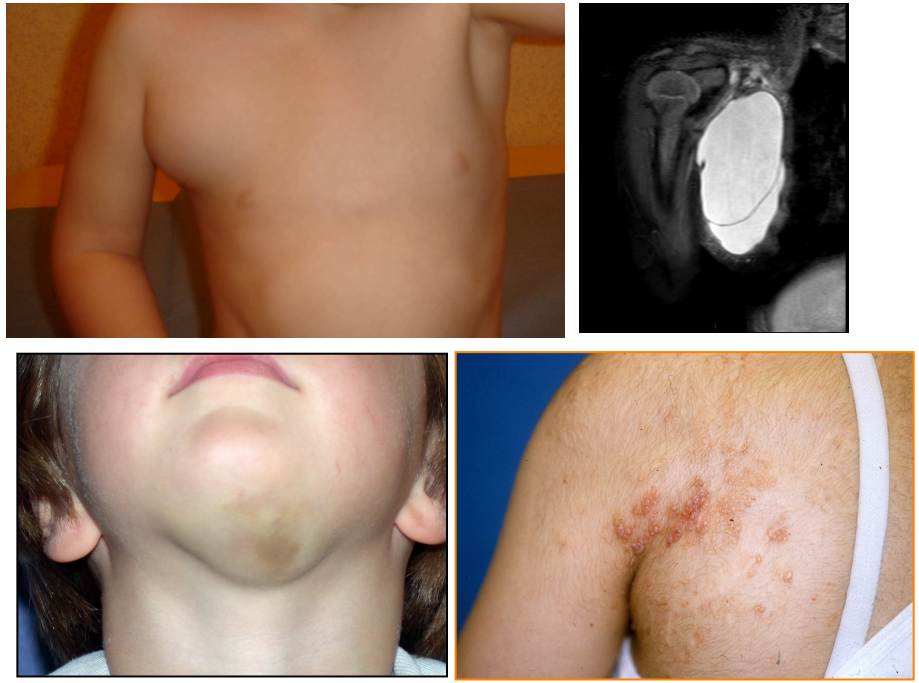
VM: Venous malformation

Limaye *et al*, *Am J Hum Genet* 2015

Somatic *PIK3CA* mutations also cause 75% of LM

(Lymphatic malformations)

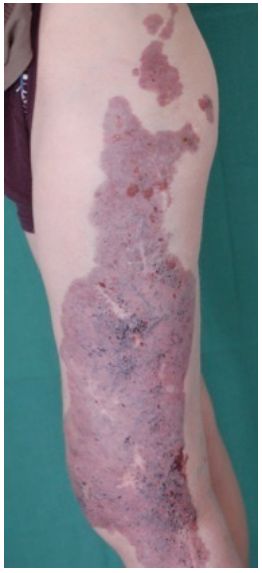
- No-flow malformations
- Filled with lymph
- Macro- or micro-cystic
- Present in utero
- Grow with the individual



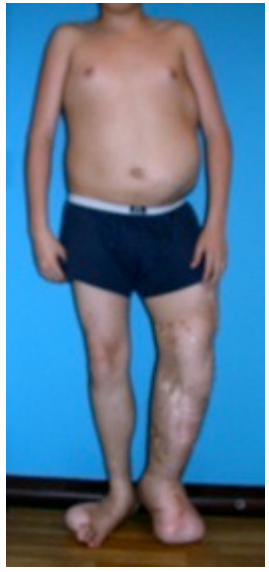
Boscolo et al, 2015; Osborne et al, 2015; Luks et al, 2015; Brouillard et al, *Orphanet J Rare Dis* 2021

Somatic *PIK3CA* mutations also cause PROS

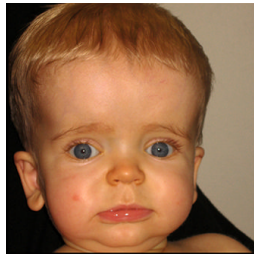
(PROS=PIK3CA-Related Overgrowth Spectrum)



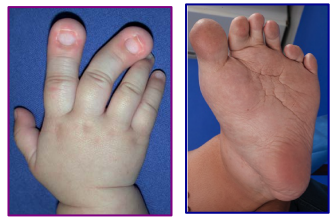
KT:
Klippel **T**renaunay
CLVM with overgrowth



CLOVES:
Congenital **L**ipomatous **O**vergrowth, **V**ascular malformation, **E**pidermal nevi, **S**coliosis



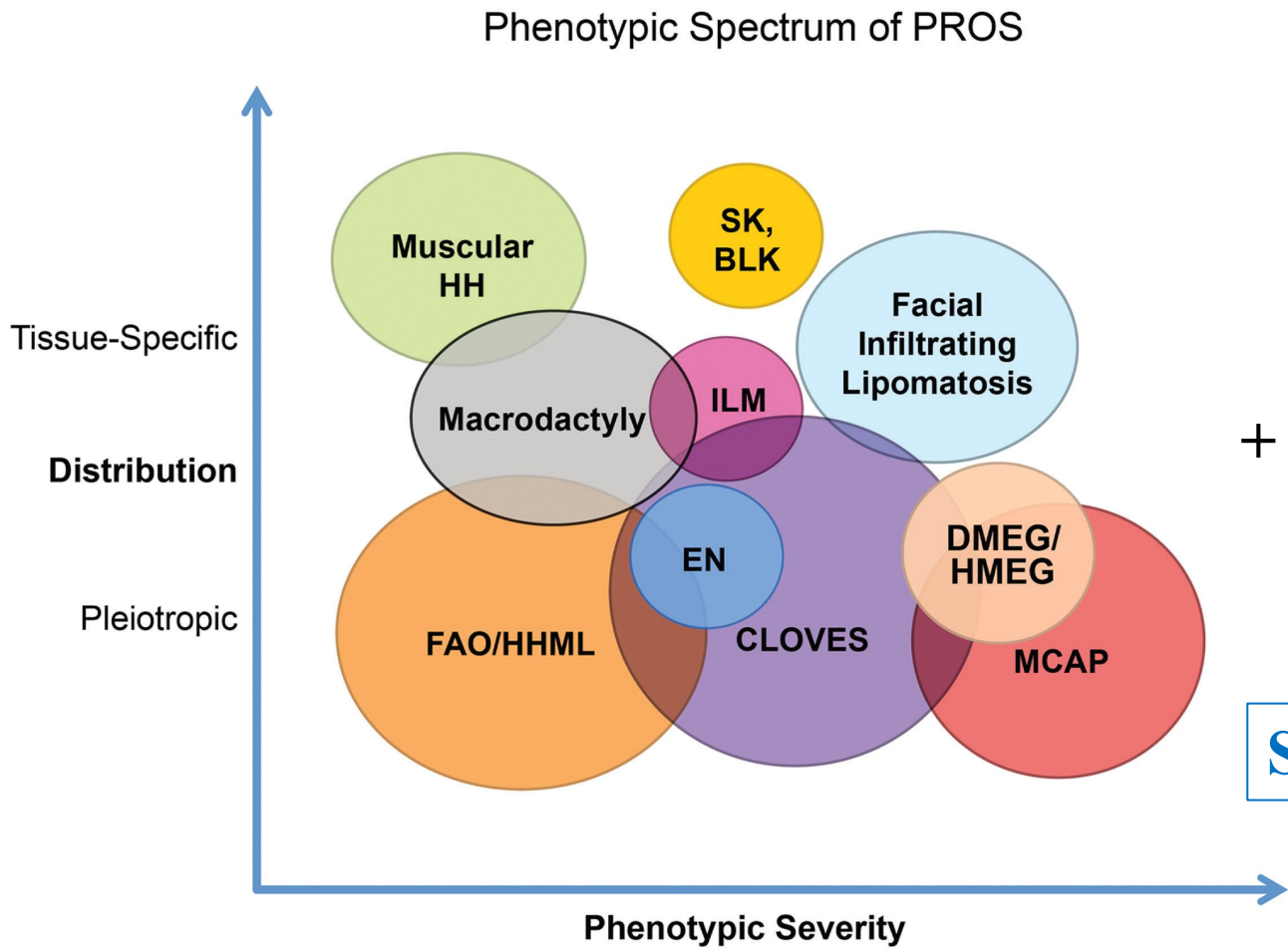
MCAP:
Megalencephaly **Cap**illary Malformation



Macrodactyly

Keppler-Noreuil et al, *Am J Med Genet* 2014; Brouillard et al, *Orphanet J Rare Dis* 2021

Somatic *PIK3CA* mutations cause a spectrum of phenotypes



+ CANCERS

Same mutations

Spatio-temporal occurrence of the mutation(s)

Keppler-Noreuil et al, *Am J Med Genet* 2014

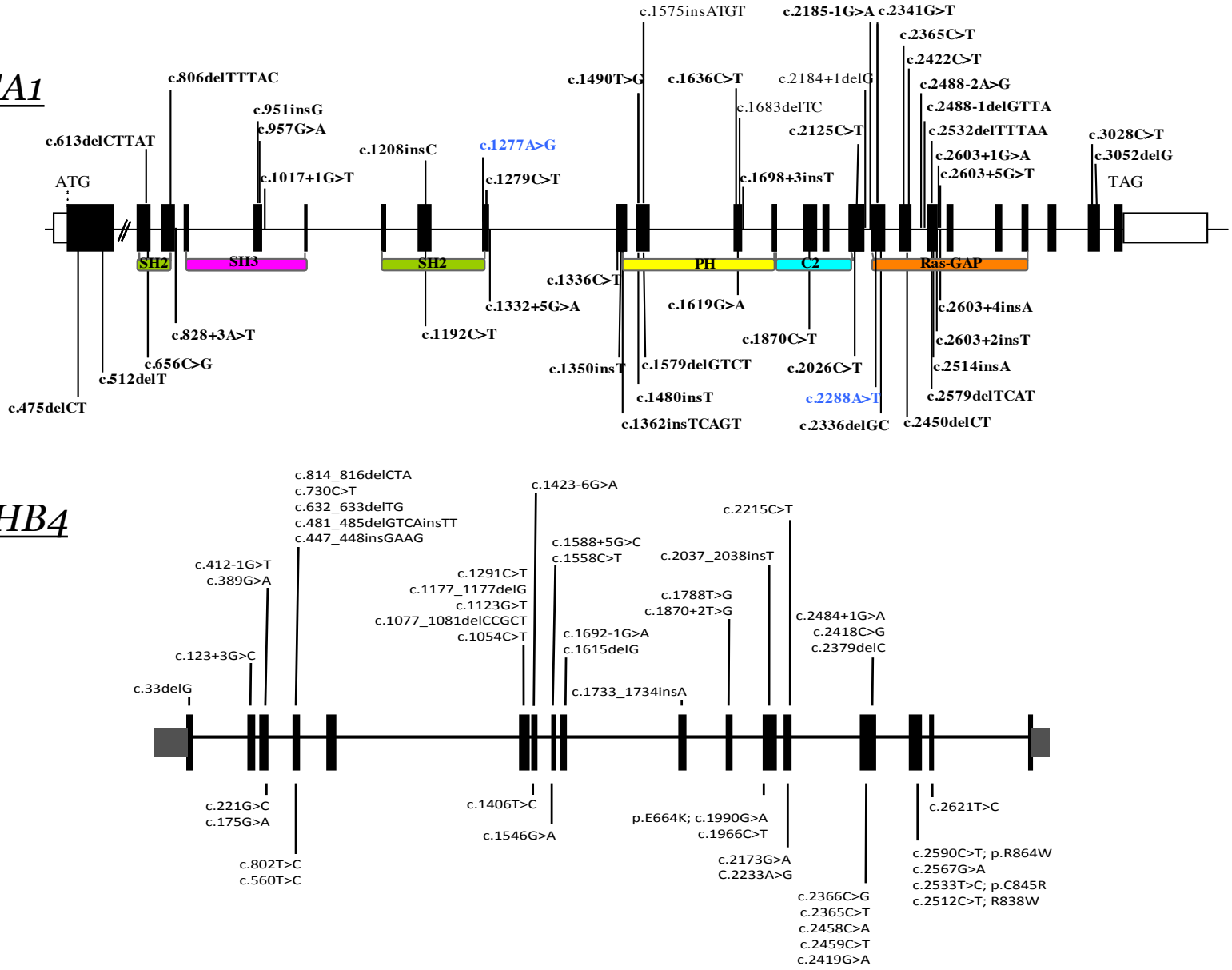
Genetic bases of vascular anomalies

5. Clinical phenotypic variability
   - One gene, several clinical presentations

   >> Spatio-temporal distribution of mutations/stochastic effect

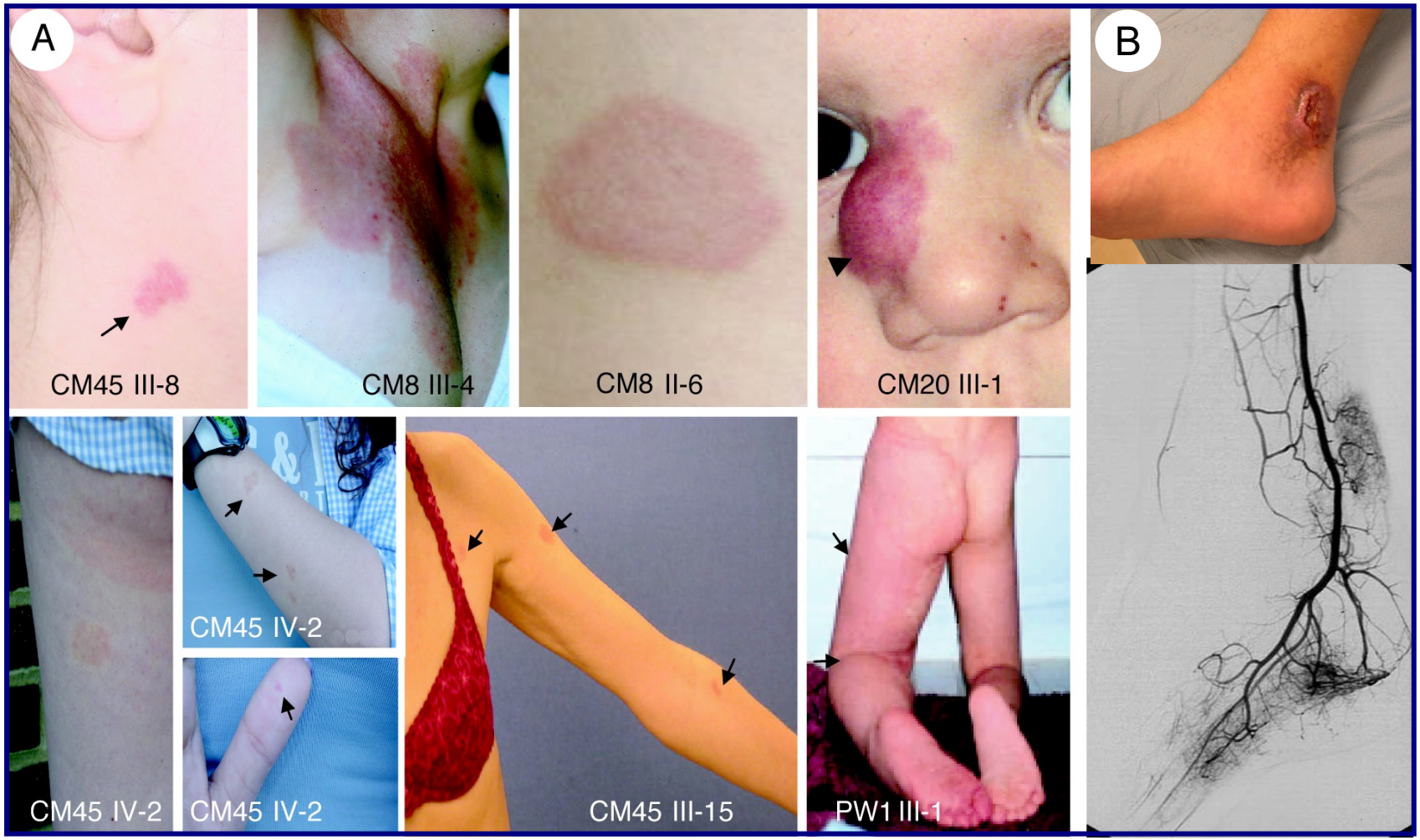
   - Two genes, several similar clinical presentations

CM-AVM: Capillary Malformation-Arteriovenous Malformation)
RASA1 and *EPHB4* Mutations



Revencu *et al*, *Hum Mutat* 2008; Amyere et al, *Circulation* 2017

Phenotypic variability in CM-AVM



Yet, more telangiectasia for CM-AVM2 (confounded with HHT)

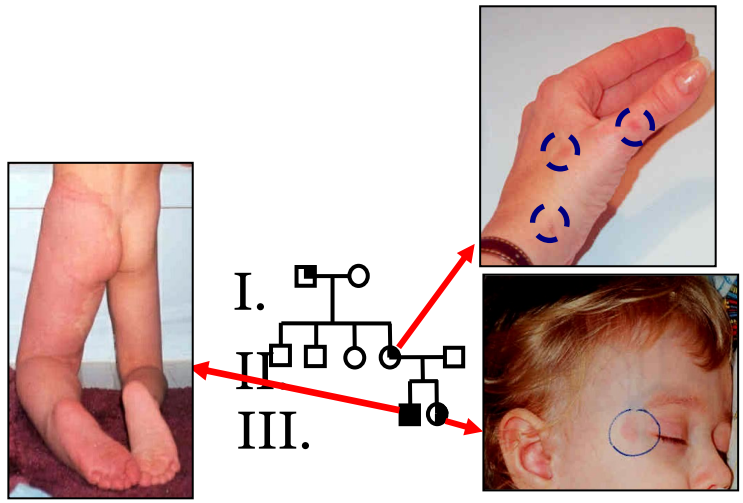
Eerola *et al, Am J Hum Genet* 2003; Revencu *et al, Inborn errors of development* 2nd Ed, 2008

RASA1 Phenotypes

314 individuals with RASA1 mutations (from 132 families)

306: multifocal CM (97%)
101: Accompanying fast-flow lesions
26 Parkes Weber syndrome **(8.5%)**
32 Intra-CNS AVM/AVF **(10%)**
43 Extra-CNS AVM/AVF **(13%)**

>> Large inter- and intra-familial variability



Revencu *et al*, *Hum Mutat* 2013

Genetic bases of vascular anomalies

1. Predisposing susceptibility genes (vs. causative)
   - Multigenic (vs. monogenic)
   - Polymorphism (vs. mutation)

2. Locus heterogeneity
   - "One" phenotype, many causes (CCM & PLE)

3. Inherited with reduced penetrance
   - Second-hits
   - Mosaicism/ tissue heterogeneity

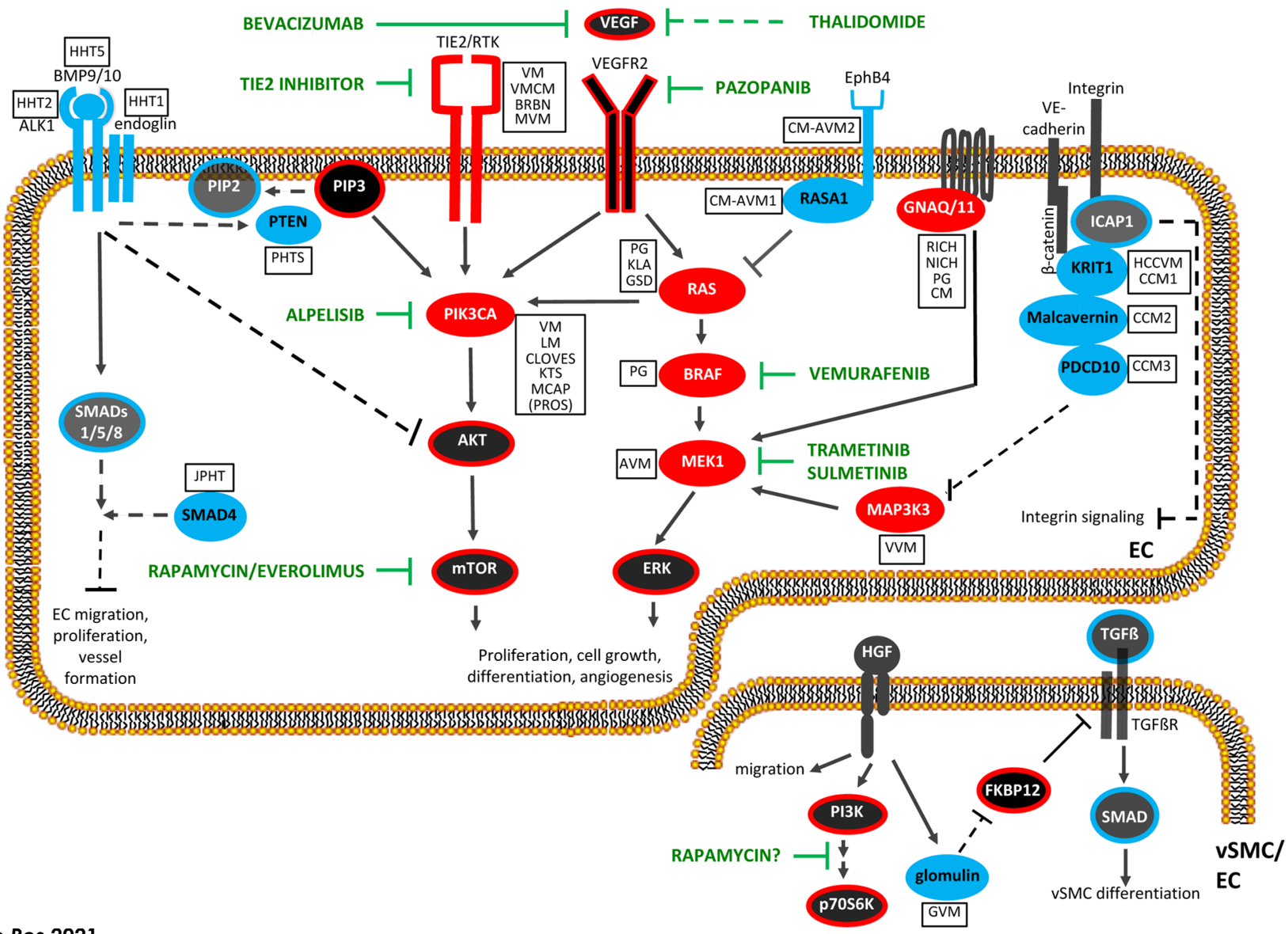
4. Somatic changes (VM & BRBN)

5. Clinical phenotypic variability
   - One gene, several clinical presentations
   - Spatio-temporal distribution of mutations/stochastic effect
   - Two genes, several similar clinical presentations

- Treatments for vascular anomalies ?

Vascular Anomalies : Targets for Treatment

Repurposing of Inhibitors?



Queisser et al, *Circ Res* 2021