

Cavernous Malformation Genetics

Gene Testing:

CCM1, CCM2, CCM3, CCM4, CCMn

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***Founding Director, Genetic Alliance BioBank**

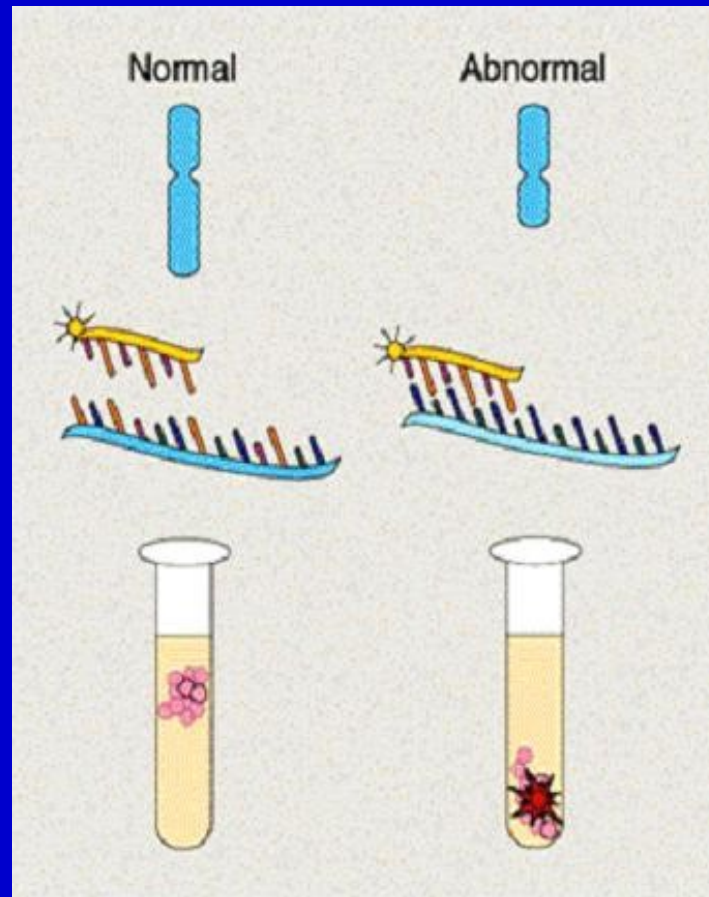
***Genetic Alliance, Washington, DC**

What are the questions that pertain to genetic testing?

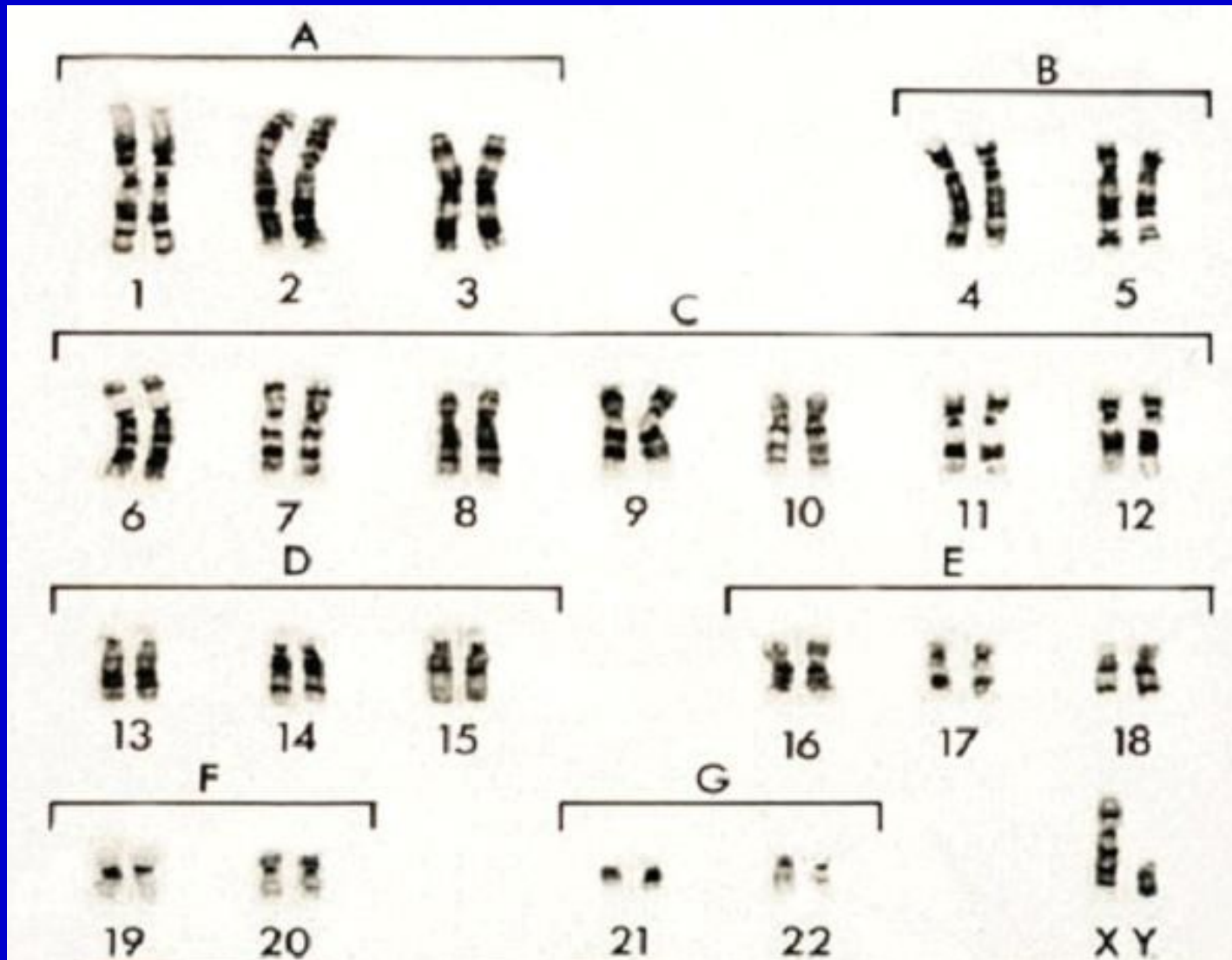
- What kind of genetic test is it?
- How would the genetic test be used?
- Would the genetic test help or hurt me?
- How is the genetic test applied in my situation?
- Where can I find a lab that does the test?
- What is involved in ordering this genetic test?
- How would a genetics consultation help?

Three kinds of genetic tests

- Cytogenetic
- Molecular
- Metabolic



Karyotype



Clinical vs. Research Testing

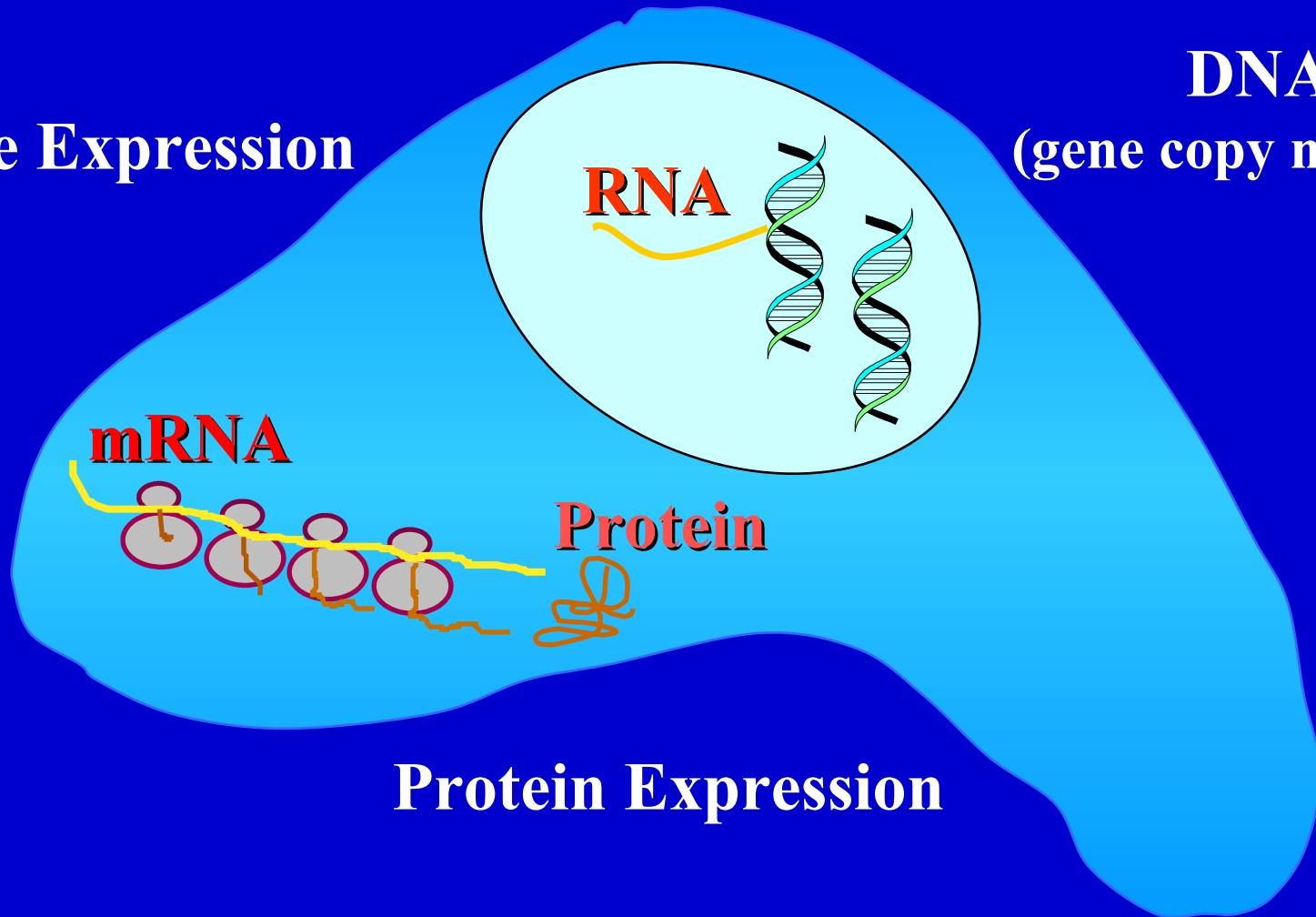
- **Clinical testing - Done for the purpose of prevention, diagnosis, or treatment as part of patient care. Results are reported to the provider.**
- **Research testing - Done for the purpose of understanding a condition better, or developing a clinical test. Results are usually not provided.**

Mutation Analysis

DNA** codes for **RNA** which codes for **Protein

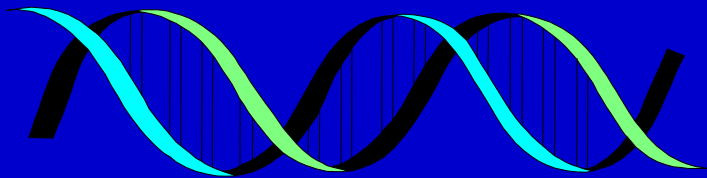
Gene Expression

DNA
(gene copy number)



Protein Expression

Genetic Code/English Language



=

Sentence

Exon

=

Word

A T C G

=

A to Z

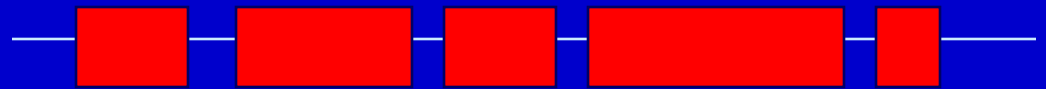
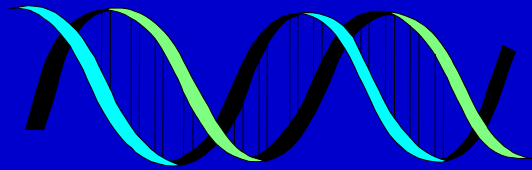
Nucleotide bases

26 Letters

Genetic Code Variations

Mutation vs. Polymorphism

Sentence = The cat chases the mouse.



Sentence = The cat chases the mouse.

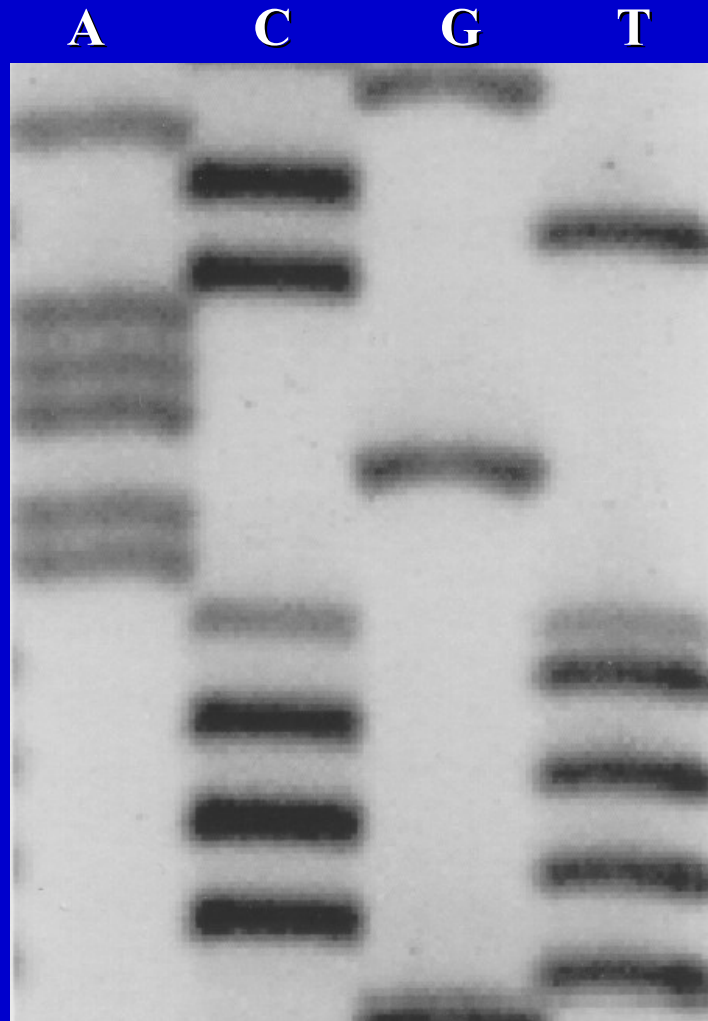


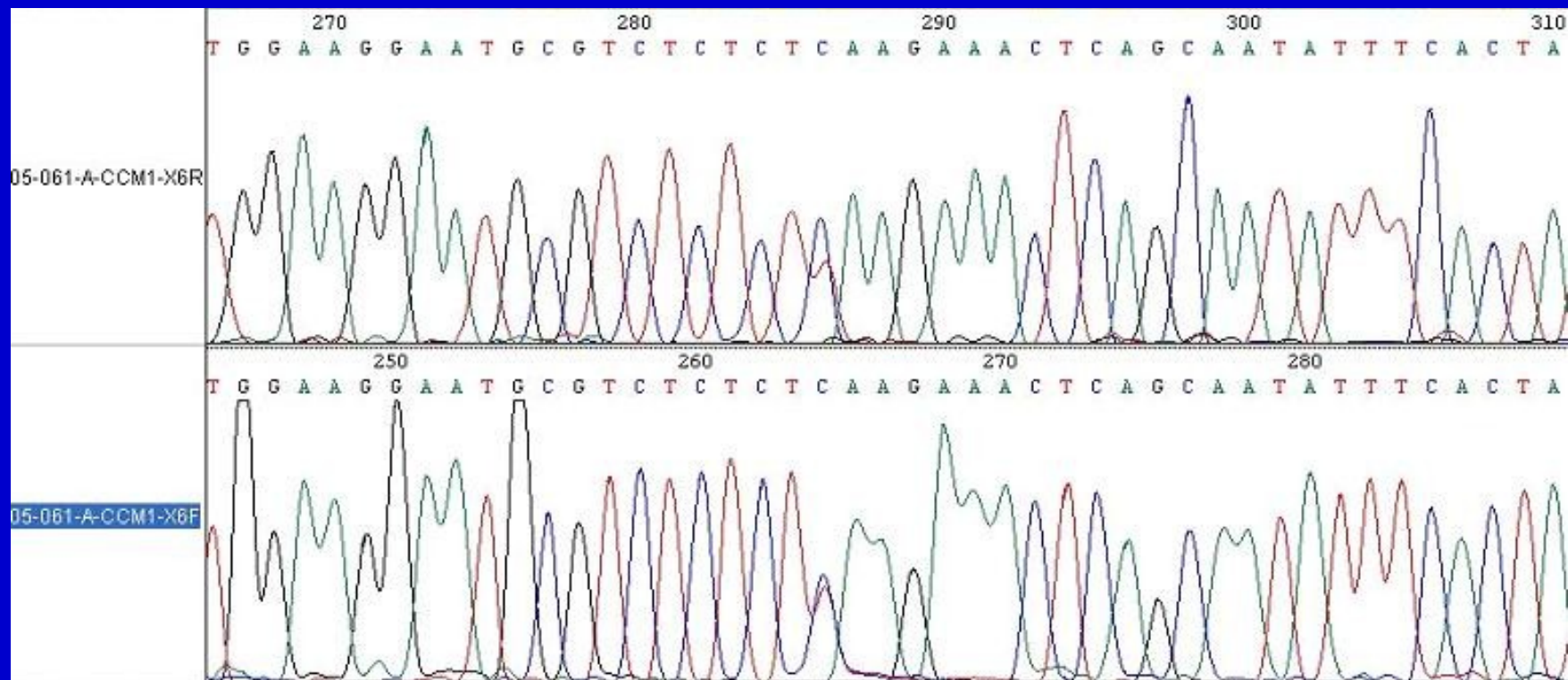
**POINT
MUTATION**

Sentence = The rat chases the mouse.

Sequence ladder for a portion of the *CCM1/KRIT1* gene showing the common Hispanic mutation: (C1363T) transition in exon 10 changes a GLN to a premature termination codon (Q>X).

	Typical	/	Mutant
	G		G
	A		A
	C		C
	T		T
	C		C
	A		A
	A		A
	A		A
	G		G
	A		A
	A		A
GLN/Stop	C		T
	T		T
	C		C
	T		T
	C		C
	T		T
	C		C
	T		T
	G		G





Genetic Code – frameshift mutation

Sentence = The cat chases the mouse.



MUTATION

Sentence = T hec atc atchas est hemou se

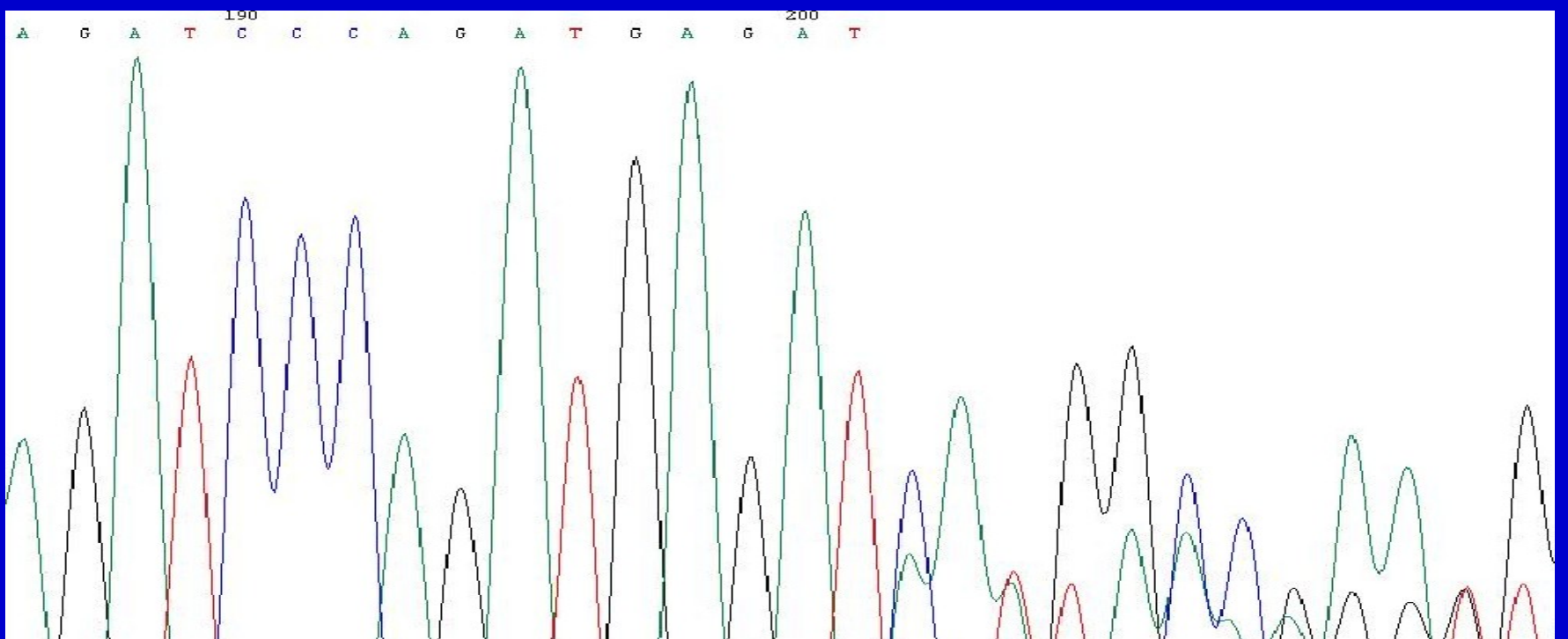
Sentence = The cat chases the mouse.



In/Del

Sentence = The chases the mouse.

The FRD cat chases the mouse.



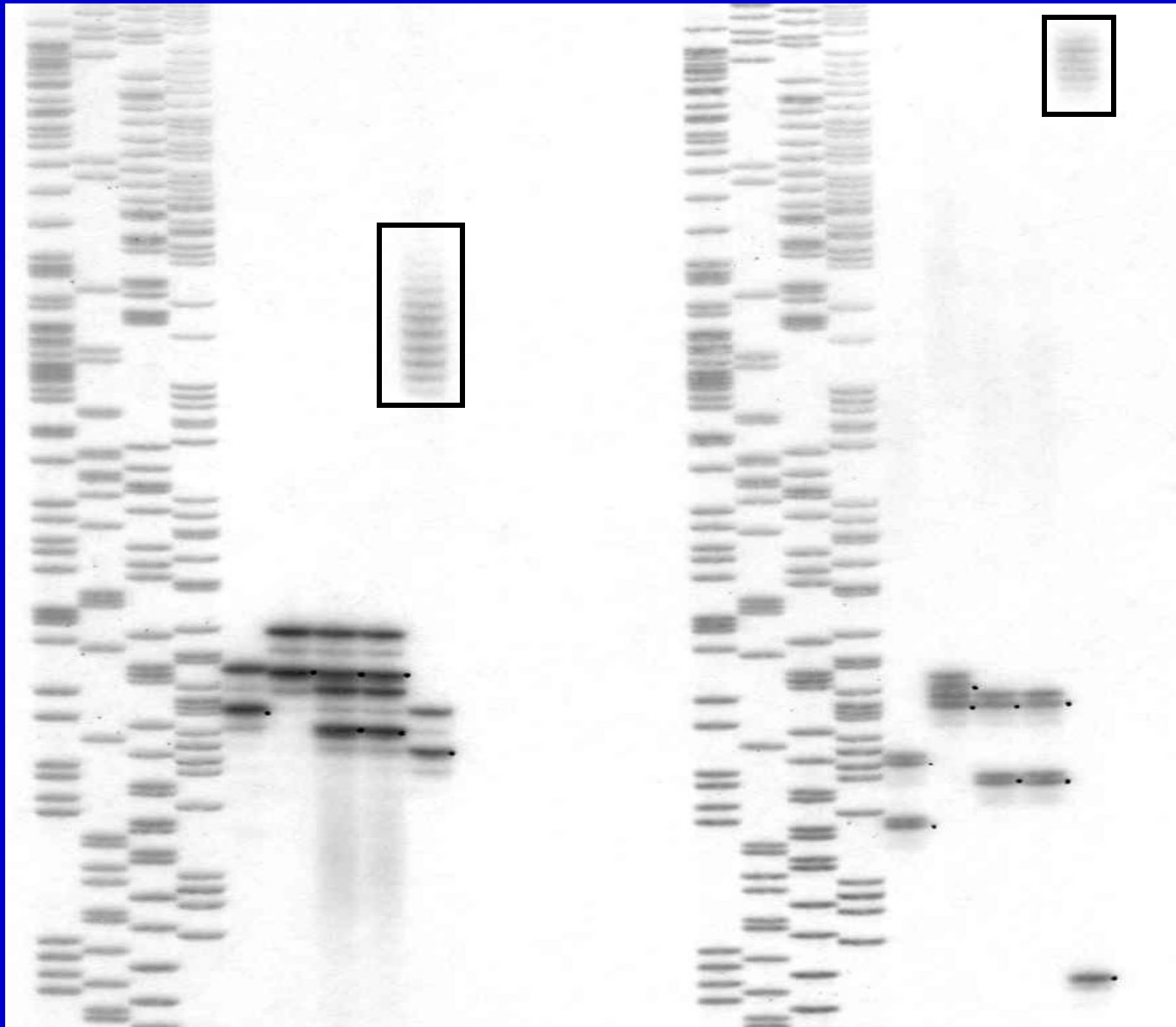
Sentence = The cat chases the mouse.



**DUPLICATION
MUTATION**

Sentence = The cat cat chases the mouse.

Tri Nucleotide Repeat Disorders



Other Types of Mutations:

Mutations in non coding regions of genes

Splice site mutations

Uses of Molecular Genetic Testing

- **Diagnostic**
- **Predictive**
- **Carrier**
- **Prenatal**
- **Newborn Screening**

CCM Timeline

- **Traits: 1966; MIM 1487** Known genetic traits, < 10 Mapped
- **Genes: 1973; HGM-I** < 100 Genes mapped, Several chr. with NO genes at all.
- **Traits: 1989; OMIM**, >1,000 Known genetic traits > 250 Disease genes mapped.
- **1994: Marshfield maps CCM1 locus to 7q, Human Genome Project (HGP)**
- **1995: CCM Consortium founded to coordinate research efforts**
 - Marshfield, Duke, Barrow, UMINN, NIH (all other interested groups invited but decline)
- **1998: CCM2 and CCM3 loci identified.**
- **1999: CCM1 identified as KRIT1**
- **HGP: Completed spring 2000** Really done 2001 Really, really done 2003 or perhaps never
- **2000: Clinical test for “Common Hispanic Mutation” available** (7 months)
- **2003: CCM2 gene identified**
- **2004: Clinical test for full CCM1 and CCM2 gene sequence available** (5 months)
- **2005: CCM3 gene identified as PDCD10, clinical test online 3 months later.**
- **2005: Evidence for CCM4 and beyond??**
- **2006: Angioma Alliance Joins Genetic Alliance BioBank**
 - Forms Angioma Alliance Tissue Bank
- **2006: Deletions found to cause a significant portion of CCMs**
- **2007: First Angioma Allianace UK meeting**

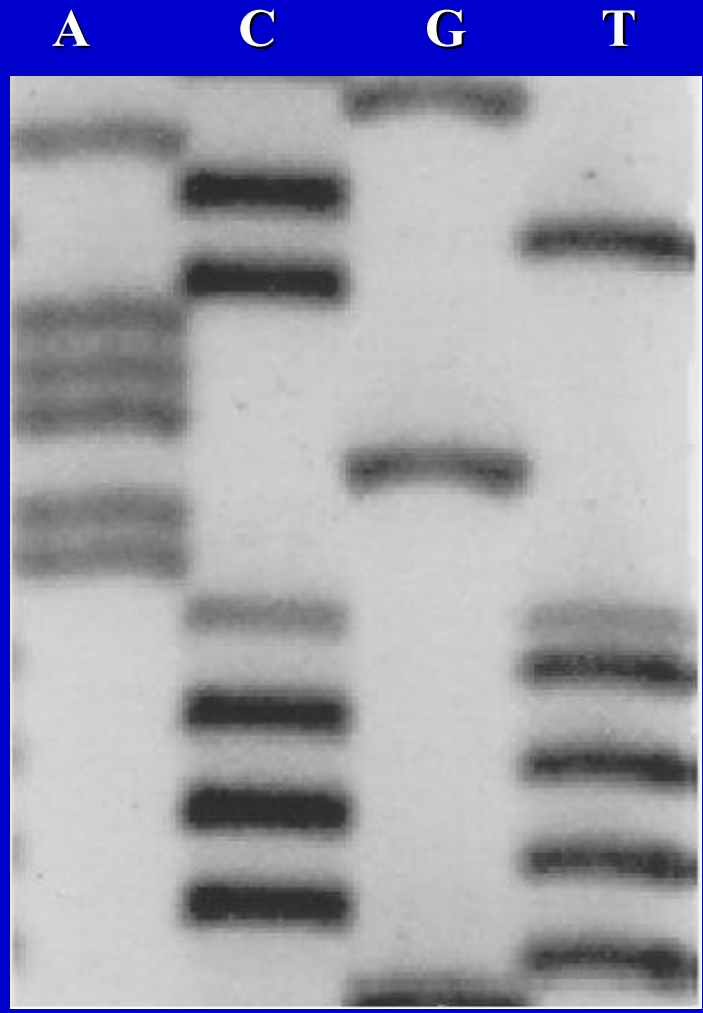
■ Molecular Genetics of Familial Cerebral Cavernous Malformation

■ <u>Name</u>	<u>Symbol</u>	<u>Locus</u>	<u>Protein Name</u>	<u>% CCMs</u>
■ CCM1	<i>KRIT1</i>	7q21	KRev Interaction Trapped 1	~40 (~70 Hispanic)
■ CCM2	<i>MCG4607</i>	7p13	Malcavernin	~15
■ CCM2	<i>MCG4607</i>	7p13	Malcavernin	~25 (deletions)
■ CCM3	<i>PDCD10</i>	3q26.1	Programmed Cell Death 10	~7
■ CCM4	?????	3q26.3-27.2	?????????	??
■ CCMn				~15

- **CCM1 targeted mutation analysis:**
- **A single mutation in the exon 10 of the CCM1 gene (1363C>T) referred to as the "common Hispanic mutation" had been identified in about 70% of families of Hispanic heritage. No other common mutation has been detected in families of other ethnic derivation.**
- **Non-Mexican individuals with a positive family history and/or multiple CCMs have an approximately 40% chance of carrying a CCM1 mutation.**
- **Clinical Penetrance: Have a mutation but no clinical symptoms: 62-88%**
- **Patients can also have a lesion but experience no clinical symptoms : 3-5%**

Sequence ladder for a portion of the *CCM1/KRIT1* gene showing the common Hispanic mutation: (C1363T) transition in exon 10 changes a GLN to a premature termination codon (Q>X).

	Typical	/	Mutant
	G		G
	A		A
	C		C
	T		T
	C		C
	A		A
	A		A
	A		A
	G		G
	A		A
	A		A
GLN/Stop	C		T
	T		T
	C		C
	T		T
	C		C
	T		T
	C		C
	T		T
	G		G



- **Sequence analysis: CCM2**
- **Between 13% and 20% of individuals with familial CCM have mutations in the CCM2 gene.**
- **No CCM2 mutations were seen in 31 individuals with no family history of CCM.**
- **.**

CCM del exon2-10

- This deletion of exons 2 to 10 of the CCM1/KRIT gene accounts for nearly 15% of CCM cases (perhaps up to 30% in CCM1/2/3 mutation negative patients)
- Other CCM gene deletions may account of another 10%.

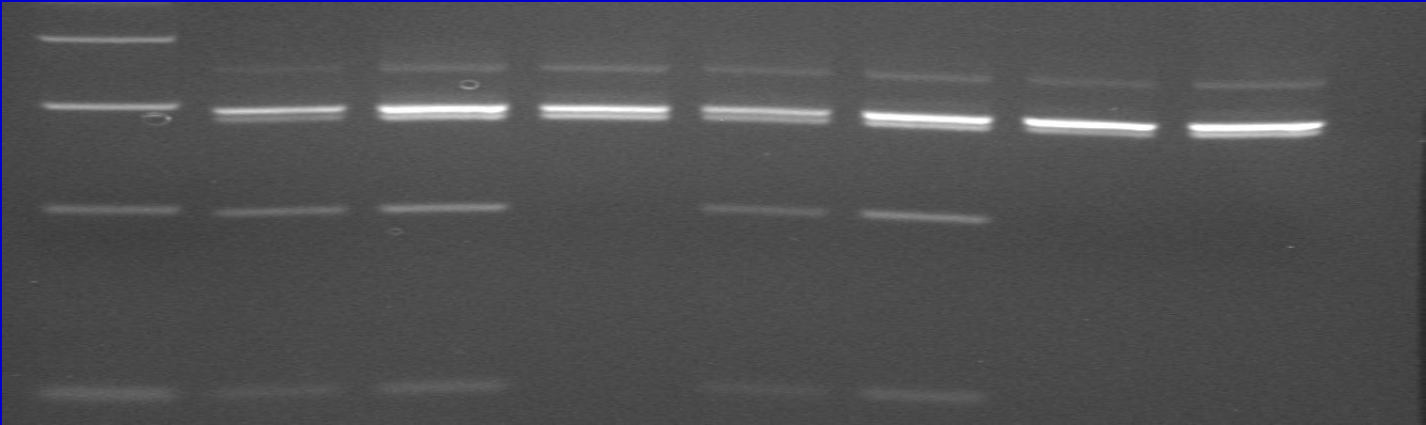
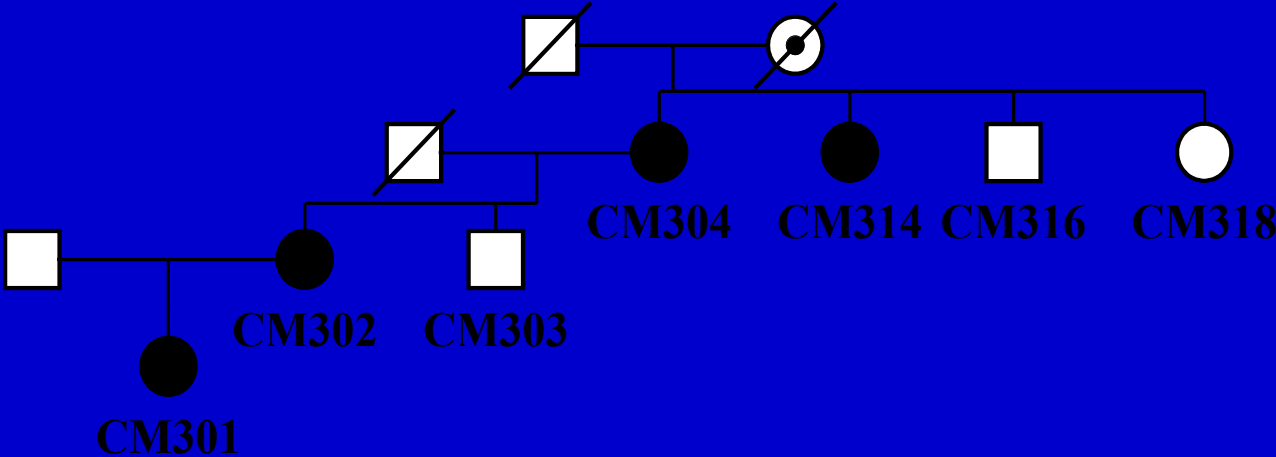
- **Sequence analysis: CCM3**
- **Although initial estimates suggested that 40% of individuals with familial CCM might be linked to the CCM3 locus recent data indicate that this number is probably too high.**
- **In families with CCM who had no evidence of a mutation in CCM1 or CCM2 by sequence analysis, Bergametti et al (2005) found a CCM3 sequence alteration in 8 of 20 and Marchuk (2006) found a CCM3 mutation in only 10% of CCM1/2 negative families studied.**

■ **Molecular genetic testing: Research**



- **If no mutation is detected in the CCM1, CCM2, or CCM3 genes, research testing may be performed to identify families that demonstrate linkage to the putative CCM4 locus.**

Inheritance of the Common Hispanic *CCM1/KRIT1* Mutation.



CCM1 Clinical DNA testing

Summary of Results

<u>GENE/EXON#</u>	<u>MUTATION</u>	<u>CODING CHANGE</u>	<u>OCCURS</u>	
CCM1 X1	None			
CCM1 X2	152-15delAAGT	Frame shift	2	
CCM1 X3	268C>T	Arg90STOP	2	
	363_369delTAAATAC	Leu157STOP	5	
CCM1 X4	Exon4+1G>A	Splice site	1	
CCM1 X5	None			
CCM1 X6	842_delA	Frame shift	2	
	x6+1G>A	Splice site	2	
CCM1 X7	873_874delTC	Frame shift	2	
CCM1 X8	None			
CCM1 X9	Exon9-1G>C	Splice site	1	
	1197_1201delCAAA	Frame shift	9	AKA: 1201_1205delCAAA
	208delA	Asn403Leu	1	
CCM1 X10	1267 C>T	Arg>STOP	3	
	1288_1289insG	Frame shift	2	
	1360_1363delTCTC	Leu453fs	1	
	1360_1361delTC	Frame shift	1	
	1362_1363delTC	Frame shift	1	
	1363C>T	Gln>STOP	75+	Common Hispanic
CCM1 X11	1423delA	Frameshift	1	
	1512T>A	Pro504Pro	1	
	1513C>T	Gln505STOP	1	
CCM1 X12	x12+4_7delAGTA	Frameshift	1	
CCM1 X13	None			
CCM1 X14	1,839 T>G	Gly>Gly	1	
	1935delT	Frame shift	2	
	1879C>T	Gln627STOP	2	
CCM1 X15	2042delT	Met706STOP	3	
CCM1 X16	None			

CCM2/CCM3 Clinical DNA testing Summary of Results

<u>GENE/EXON#</u>	<u>MUTATION</u>	<u>CODING CHANGE</u>	<u>OCCURS</u>	
CCM2 X1	1A>G	Met1Gly	1	
CCM2 X1b	Nonr			
CCM2 X2	del3bp 55C>T	delK65 Arg19STOP	2 4	loss of codon
CCM2 X3	None			
CCM2 X4	314delT	Leu105STOP	1	
CCM2 X5	None			
CCM2 X6	Nonr			
CCM2 X7	None			
CCM2 X8	Exon8+1G>A	Splice site	4	
CCM2 X9	None			
CCM2 X10	1091A>G 1250_1251delAG	His364Arg Frameshift	1 1	

<u>GENE/EXON#</u>	<u>MUTATION</u>	<u>CODING CHANGE</u>	<u>OCCURS</u>	
CCM3 X4	None			
CCM 3 X5	None			
CCM 3 X6	205delA 214G>C	Val88STOP Val>Leu	2 2	
CCM 3 X7	322C>T 363_364delC	Arg108STOP Frame shift	2 2	
CCM 3 X8	Exon8+1 G>A	Splice Site	3	
CCM 3 X9	None			
CCM 3 X10	None			

**headaches, seizures, focal
neurological deficit,
cerebral hemorrhages**



MRI (gradient-echo)



multiple CCM lesions

solitary CCM lesion



**family history -
(MRI proven)**

**family history +
(MRI proven)**

**family history -
(MRI proven)**



**genetic predisposition
(CCM 1, 2, 3 gene screening)
high risk of familial transmission**

**unknown etiology
low risk of familial
transmission**

Genetic Testing is Context Specific

- **Why are we testing THIS patient at THIS time?**
- **Be aware of the potential ramifications.**

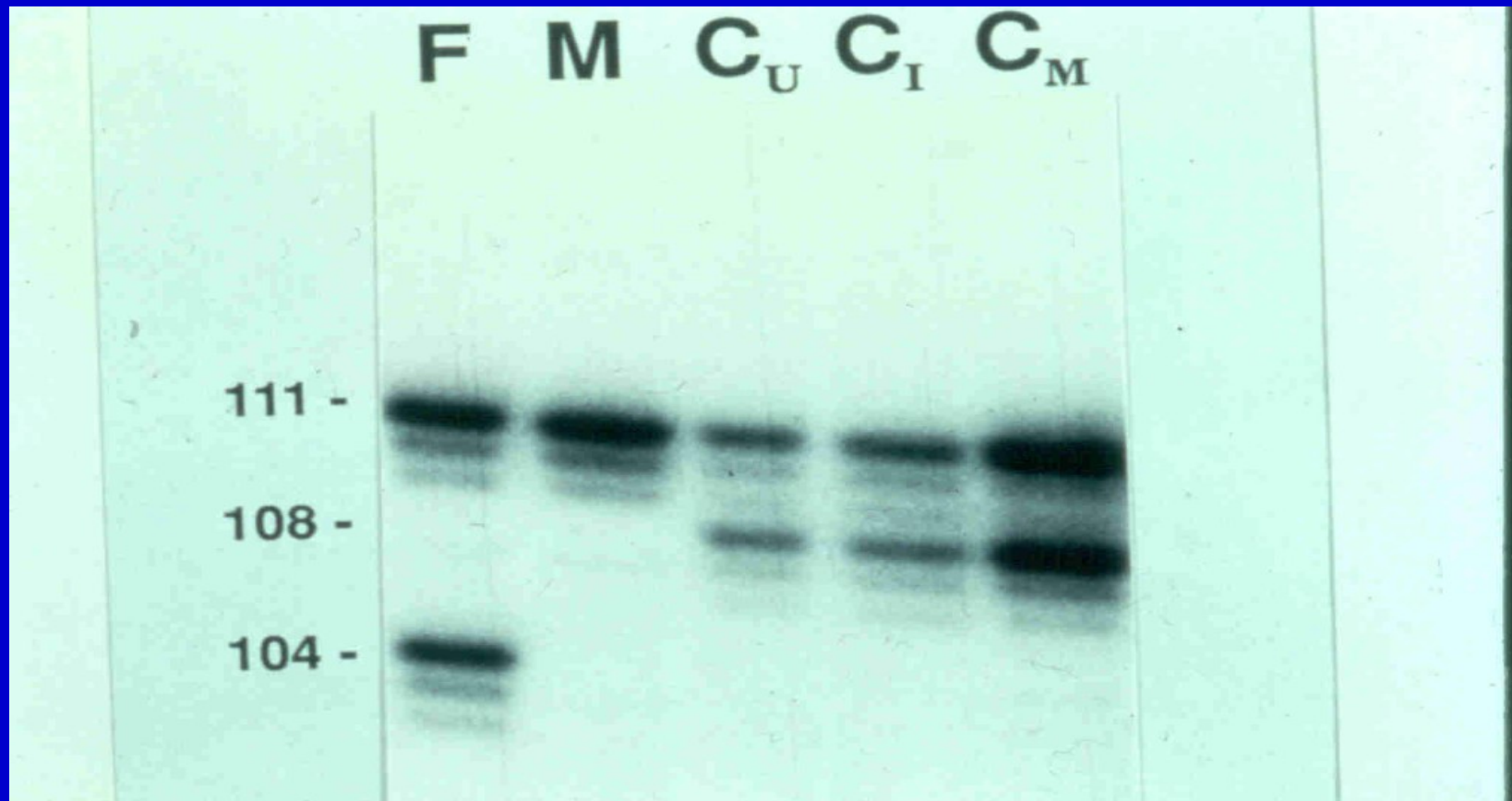
Would genetic testing help me?

- Reduces morbidity and mortality through close surveillance of at risk individuals
- Eliminates need for extra surveillance in individuals with no increased risk

Would genetic testing hurt me?

- **Psychological impact of knowing you have a life-threatening condition (or that you have escaped it and others that you care for have not)**
- **Possible insurance discrimination associated with high risk**
- **Family discord because other members who never agreed to be tested are now known to have this familial risk**

A Family Surprise



Genetic Testing Info



<http://www.genetests.org/>



Focused on Disease review

Medical Genetics

Database

www.genetests.org



- **Disease specific information**
- **Expert-authored**
- **Peer-reviewed**
- **Relevant Links**

**Interpreting genetic
testing will
require vigilance!**

What if a genetic test is not available?

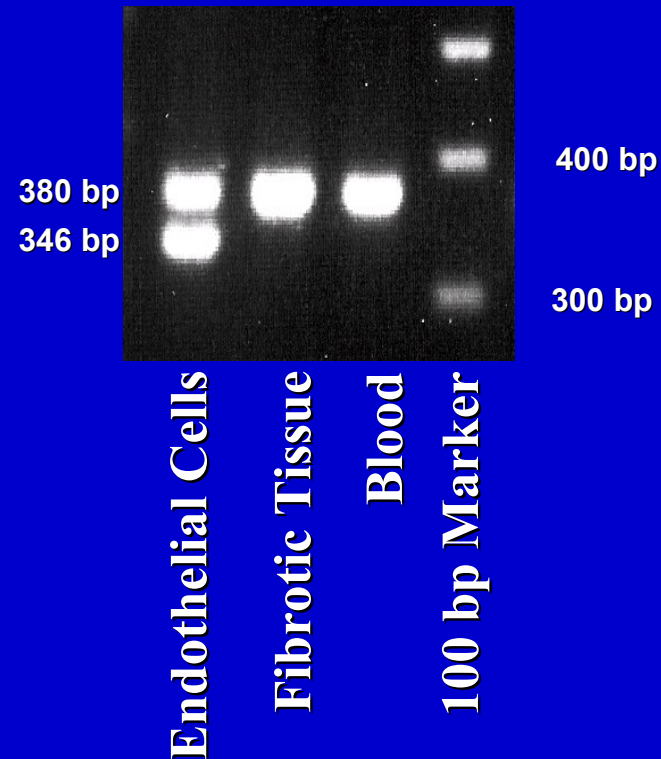
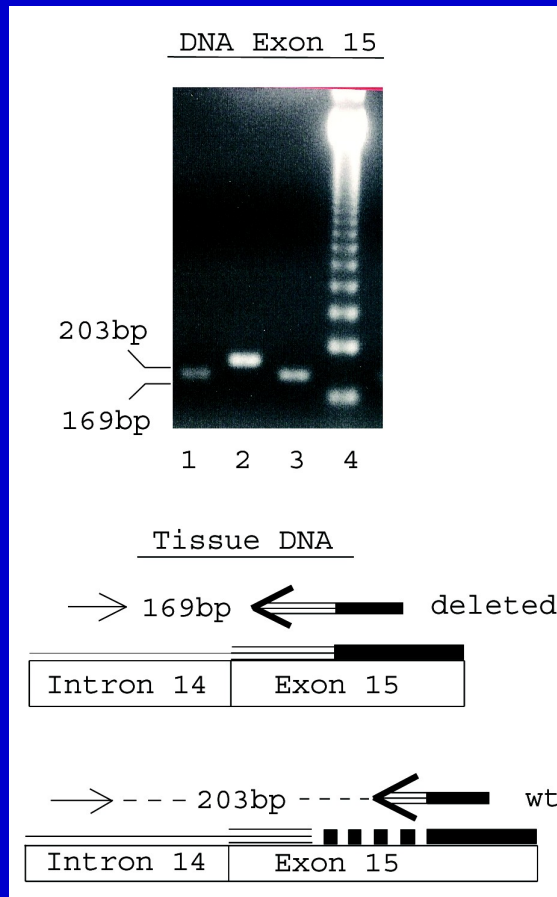
**Most people are seeking information
and coping strategies as much as
they are test results**

THINK RESEARCH

Informed Decision Making



Biallelic Somatic and Germ Line CCM1 Truncating Mutations in a Cerebral Cavernous Malformation Lesion



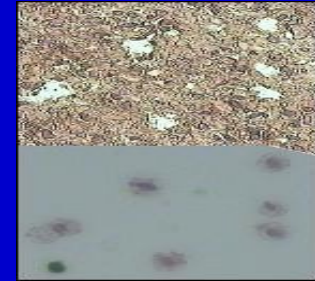
Gault et al. 2006

Typical vs. Malformation vascular tissue

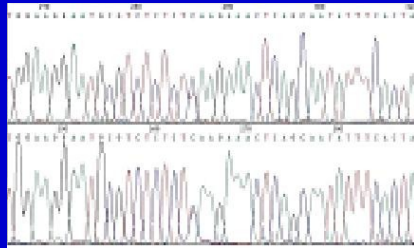
Malformation



Normal vasc.



Genomic DNA

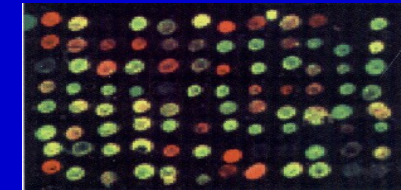
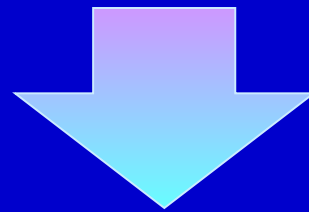


Gene Sequence
Do genomic and tissue mutations match?

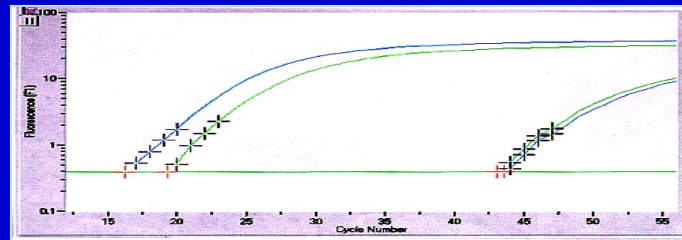
Laser Capture Microscopy
2-10,000 cells



Vascular Malformation Candidate
Genes or Gene Sequences

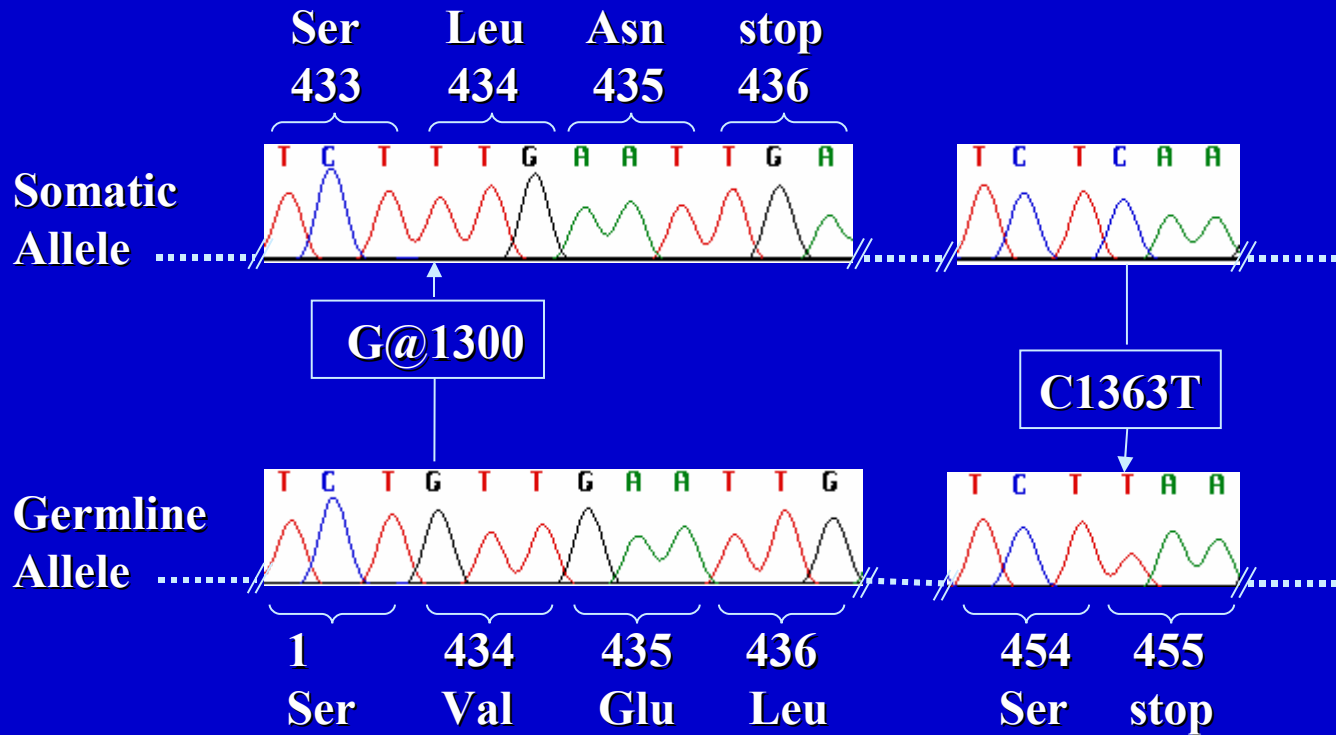


Microarray studies



Family studies, therapy, etc.

Krit1 RNA analysis

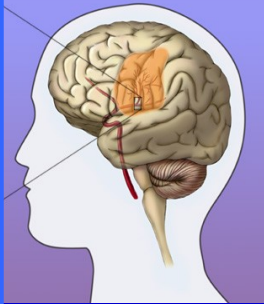


Somatic Loss of Heterozygosity Screens on single cells

Blood/Lesion

D7S492
CCM1 1363
D7S657

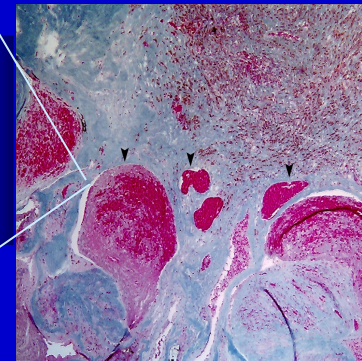
3	3
C	T
5	6



CCM Endothelial

D7S492
CCM1 1363
D7S657

3	3
C	T
5	6



Genotype / Phenotype correlations

The average number of affected individuals is significantly lower in CCM3 than in CCM1 /CCM2 families. No one has found any large CCM3 families.

Significantly higher proportion of CCM3 patients with an age of onset < 15

Significant difference in the average number of lesions detected via MRI imaging (CCM2 < CCM1)

Future Directions

- ♦ **Define the molecular anomaly in the ~10% of cases without any detected mutation.**
 - ♦ **Additional CCM gene(s) located in close vicinity of CCM1, CCM2 and /or CCM3**
 - ♦ **More deletions in any of the 3 CCM genes undetected by sequencing.**
 - ♦ **Somatic mosaicism of a de novo mutation undetected in the peripheral blood**
- ♦ **Approaches to identify those possible gene(s): candidate genes, translocations, search for more deletions ...**
- ♦ **Genetic background influences**