MAYO CLINIC

PROGRESS: Beginning to Understand the Genetic Predisposition to PSC

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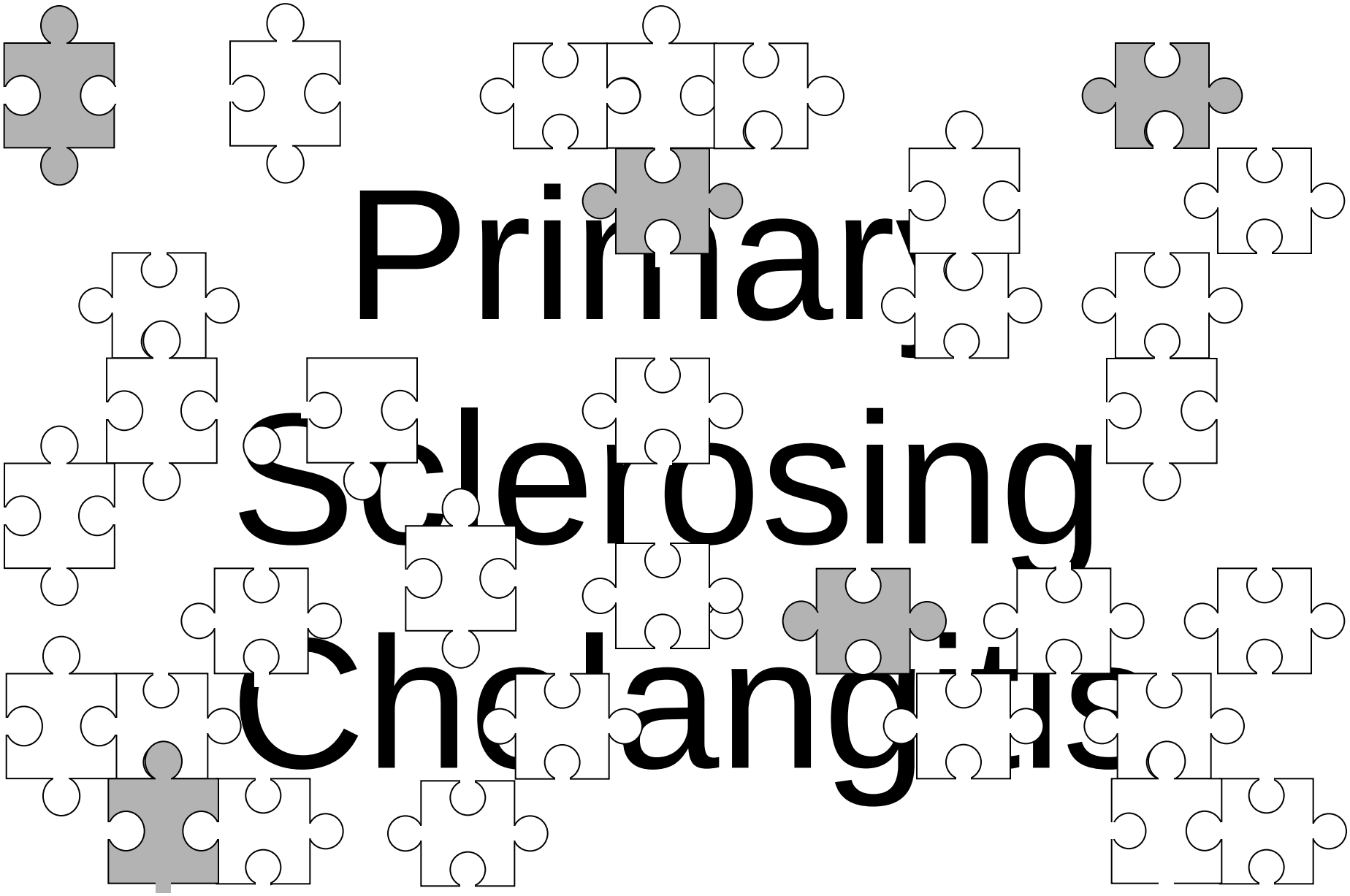
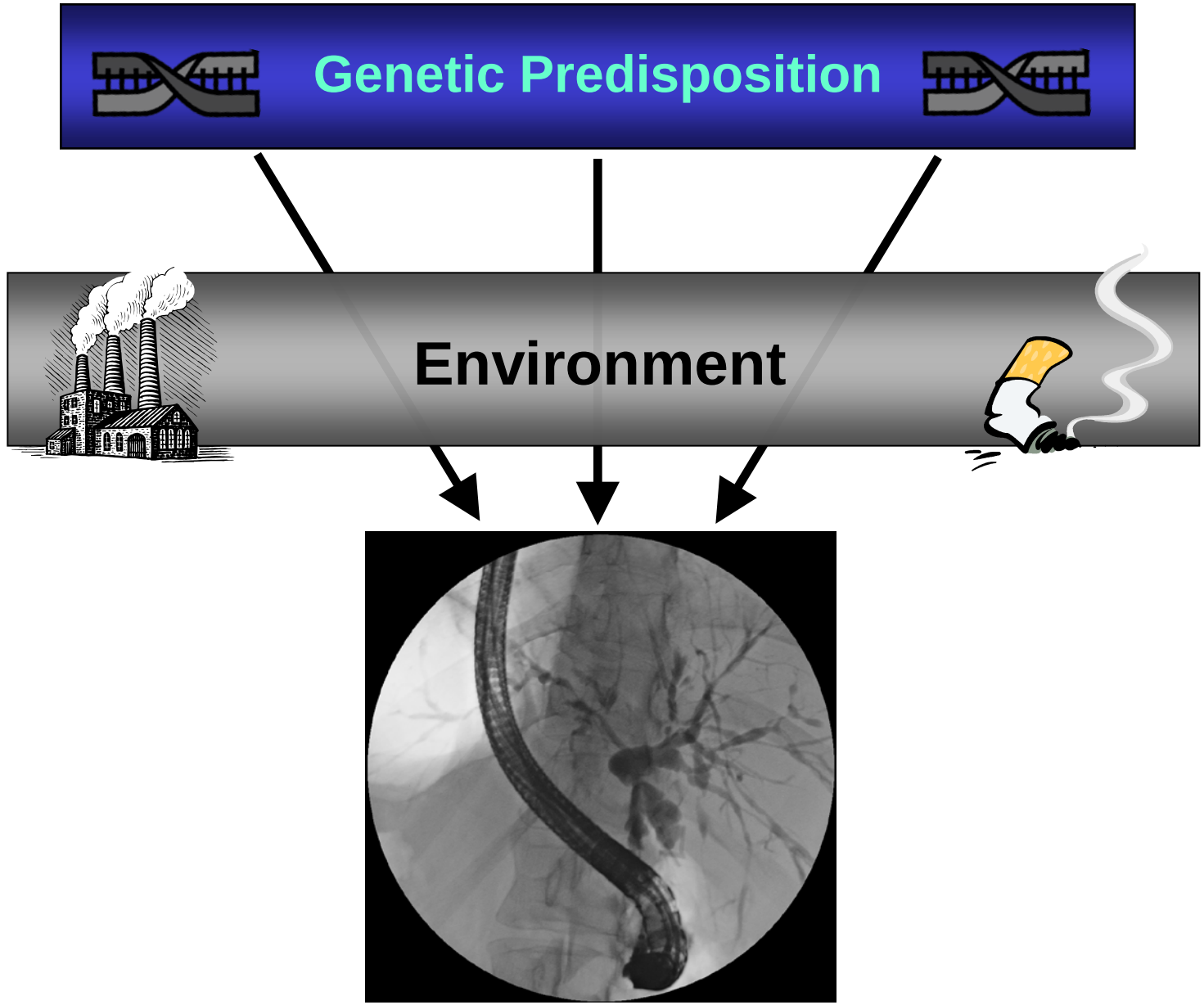

Primary
Sclerosing
Cholangitis

PSC

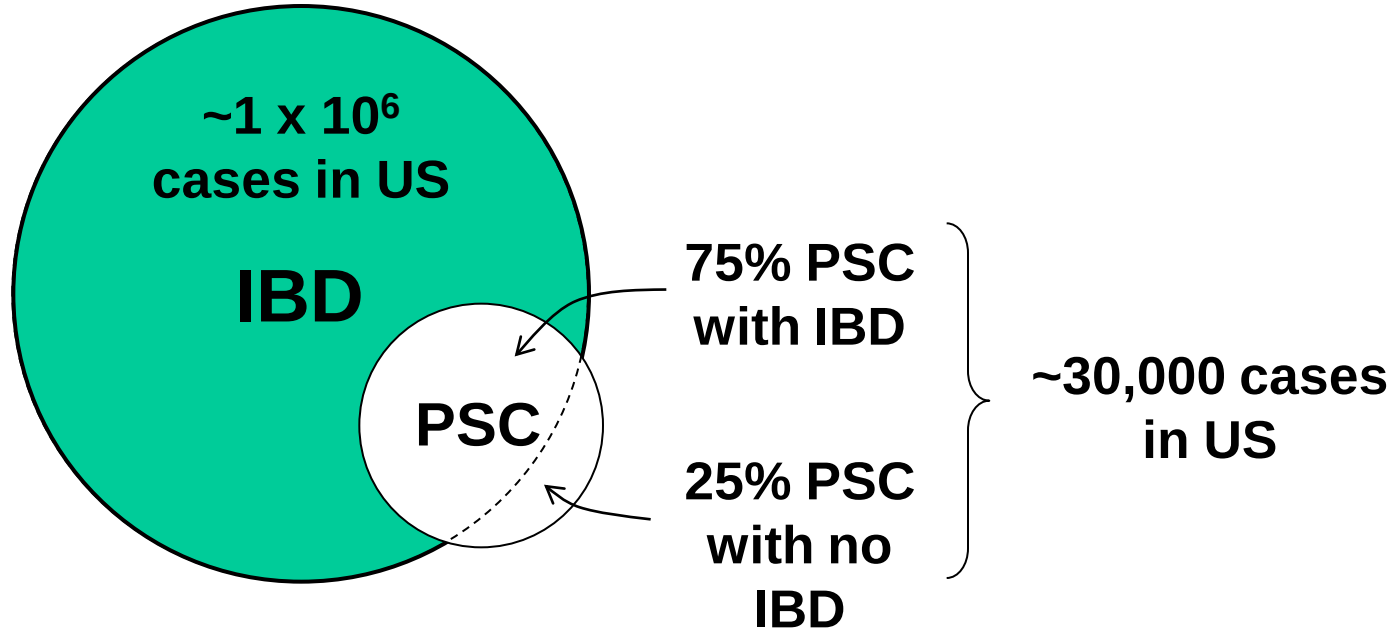
- In 1924, Delbet described the first case of PSC
- Early 1980's first PSC case-series reports in medical literature
  Drs. R. Wiesner, N. LaRusso, Mayo Clinic, USA
  Dr. R. Chapman, United Kingdom
- To date, etiology of PSC remains unknown
- No medical therapy available

What is the cause of PSC?

Proposed Pathogenesis of PSC?



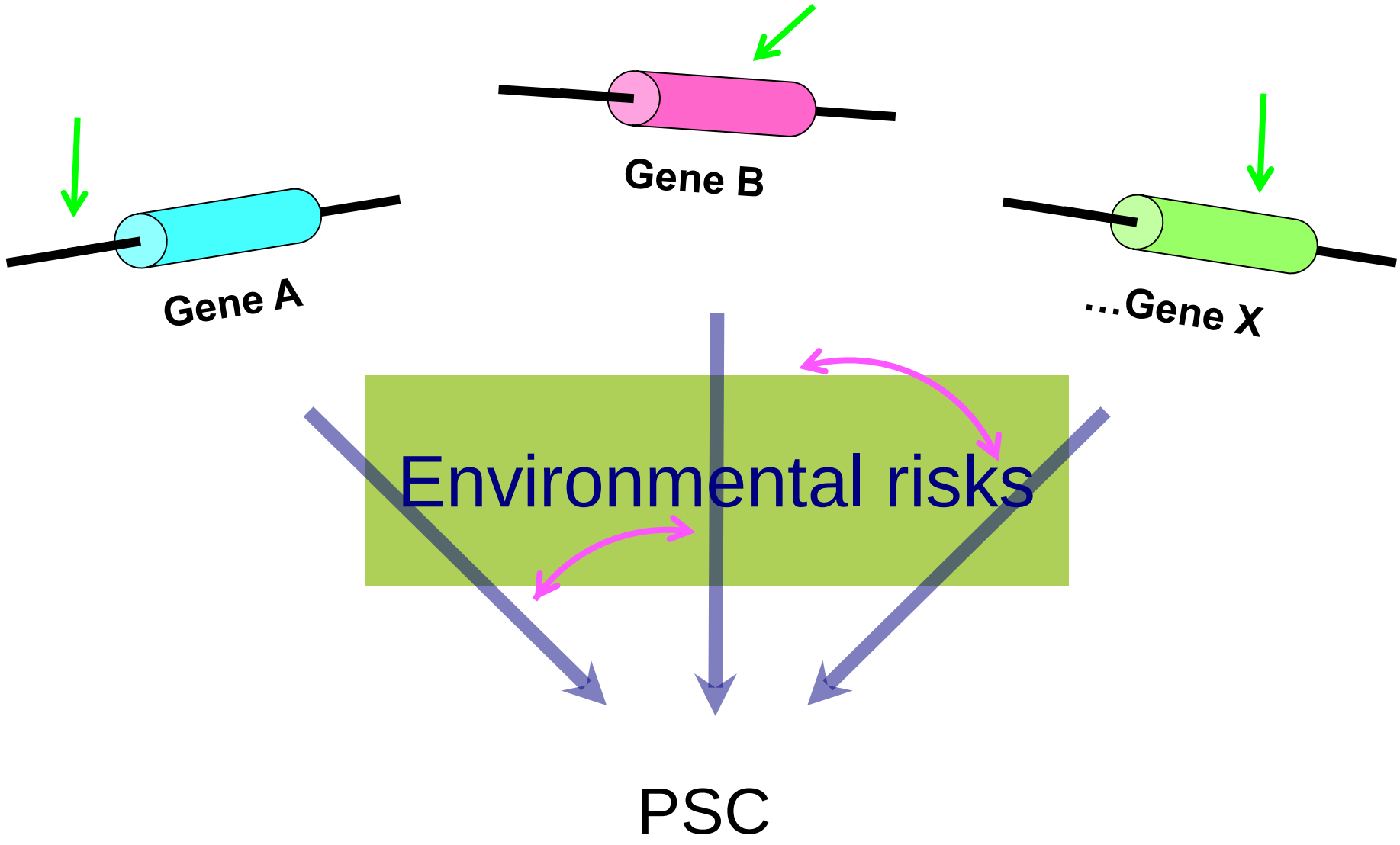
PSC and IBD


~1 x 10^6
cases in US
IBD
PSC
75% PSC
with IBD
25% PSC
with no
IBD
~30,000 cases
in US

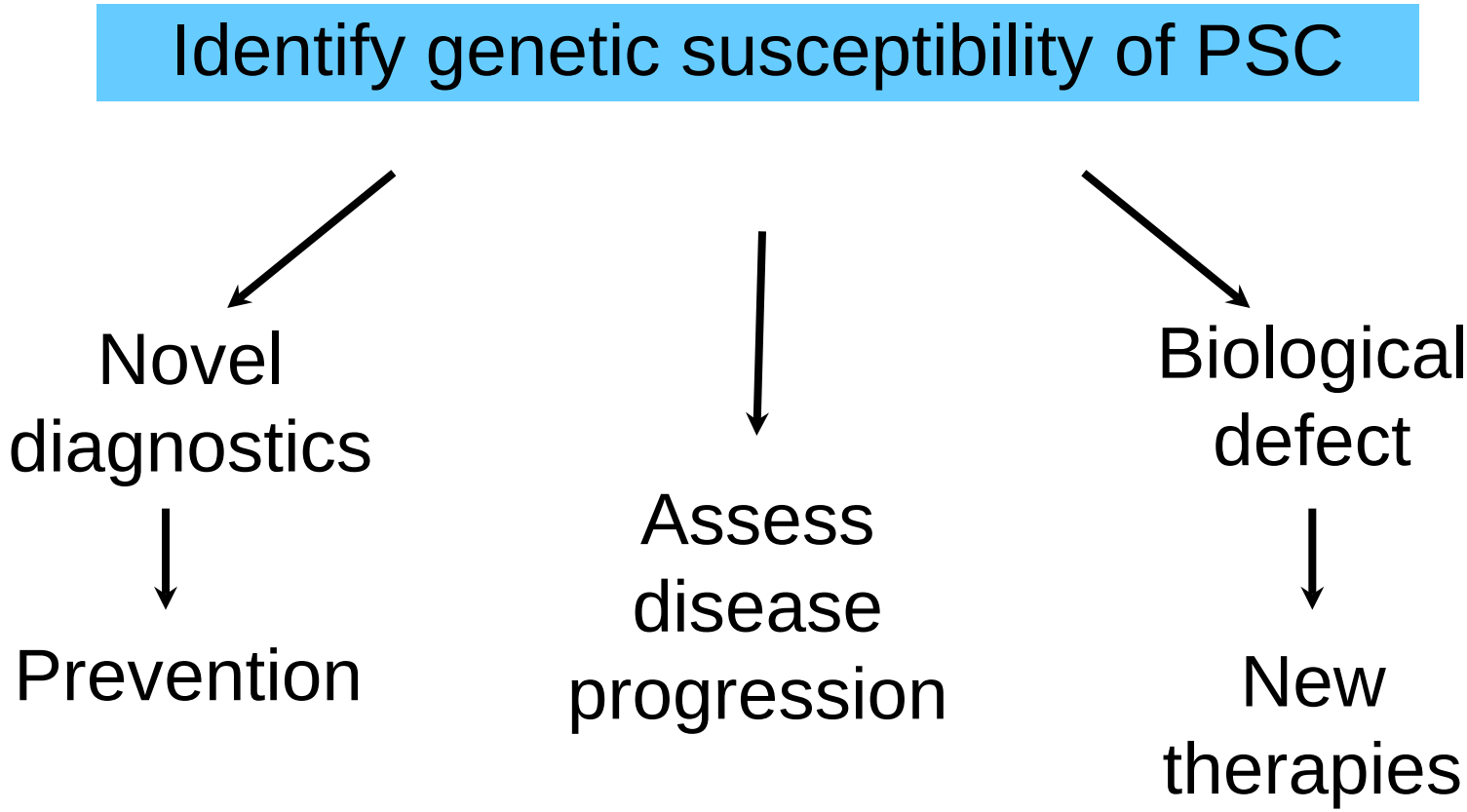
PSC is a Heterogeneous Disease

How can we find the causes of PSC?

PSC is a Complex Disease



Rationale for Studying Genetic Predisposition to PSC



PROGRESS

(PSC Resource Of Genetic Risk Environment & Synergy Studies)

Established in 2005

- To better understand the cause(s) and pathogenesis of PSC

- To improve prediction and therapy of PSC

PROGRESS

(PSC Resource Of Genetic Risk Environment & Synergy Studies)

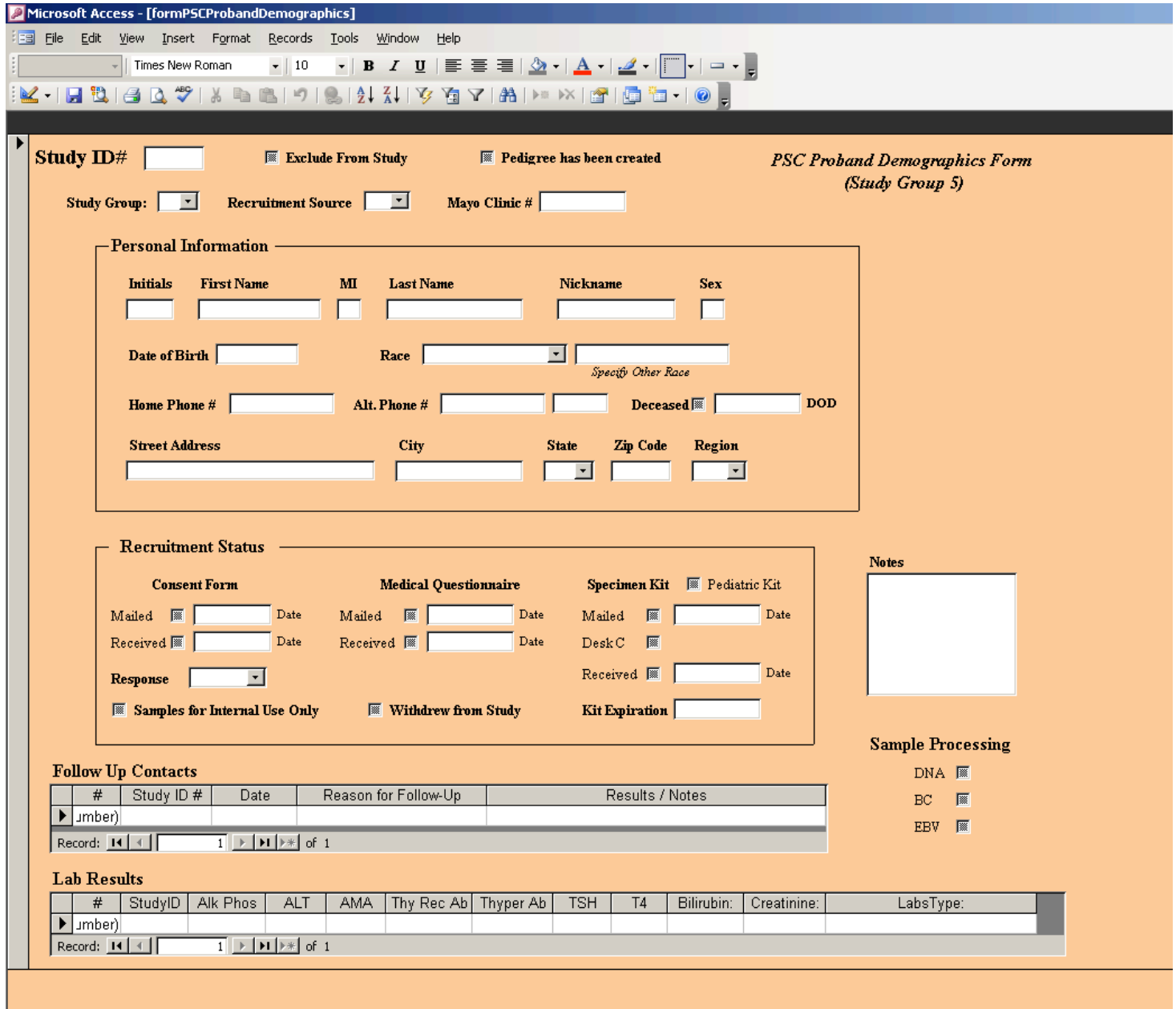
- Whole blood collection
  - biochemical testing
  - DNA isolation
  - cell-line creation
- Questionnaire data
- Family information (draw pedigrees)

PROGRESS

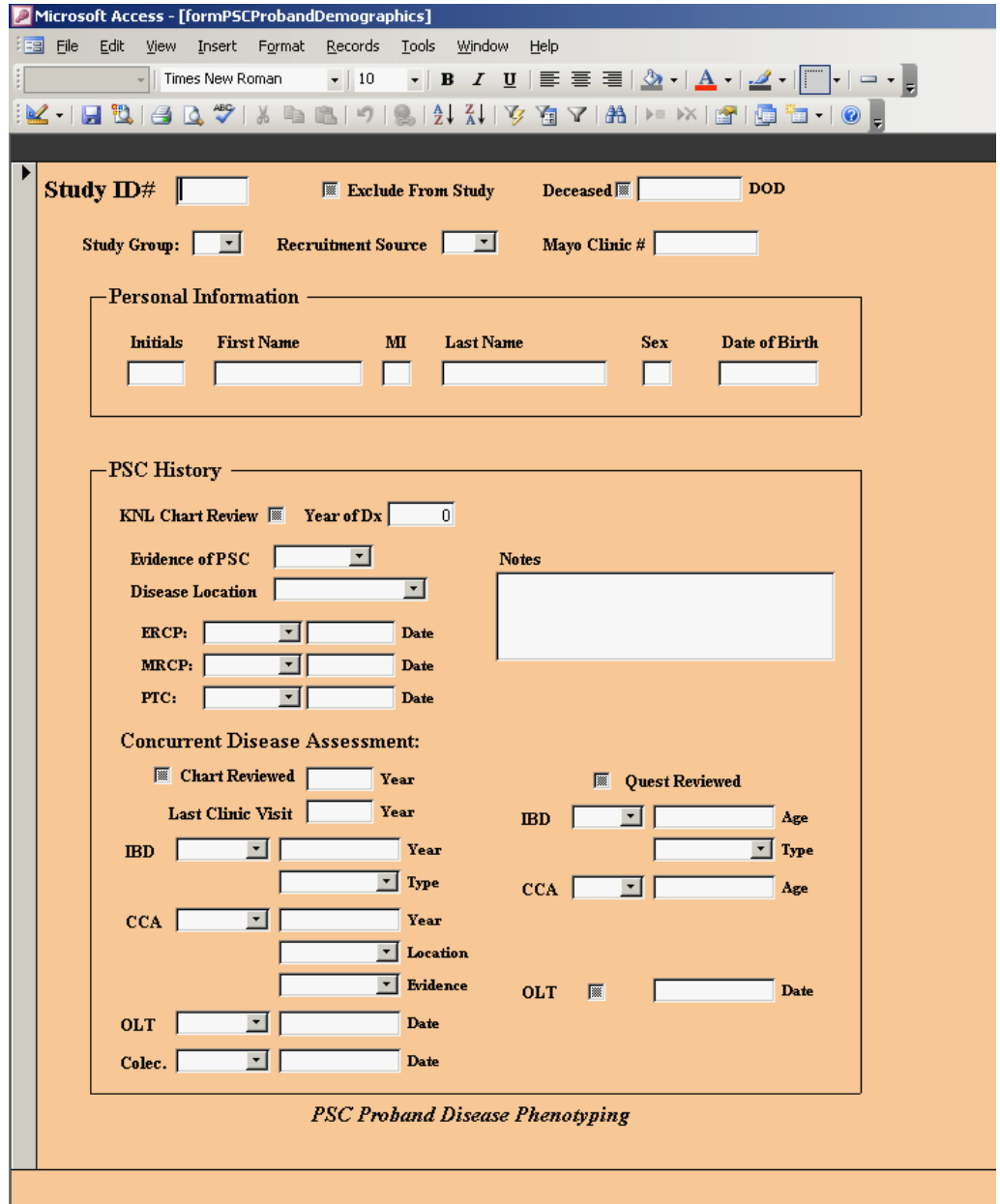
Study Requirements

- Read and sign a consent form
- Complete a questionnaire and a family information form
- Provide a sample of your blood
- Recipients of liver transplant are not excluded
- No need to visit Mayo Clinic to participate

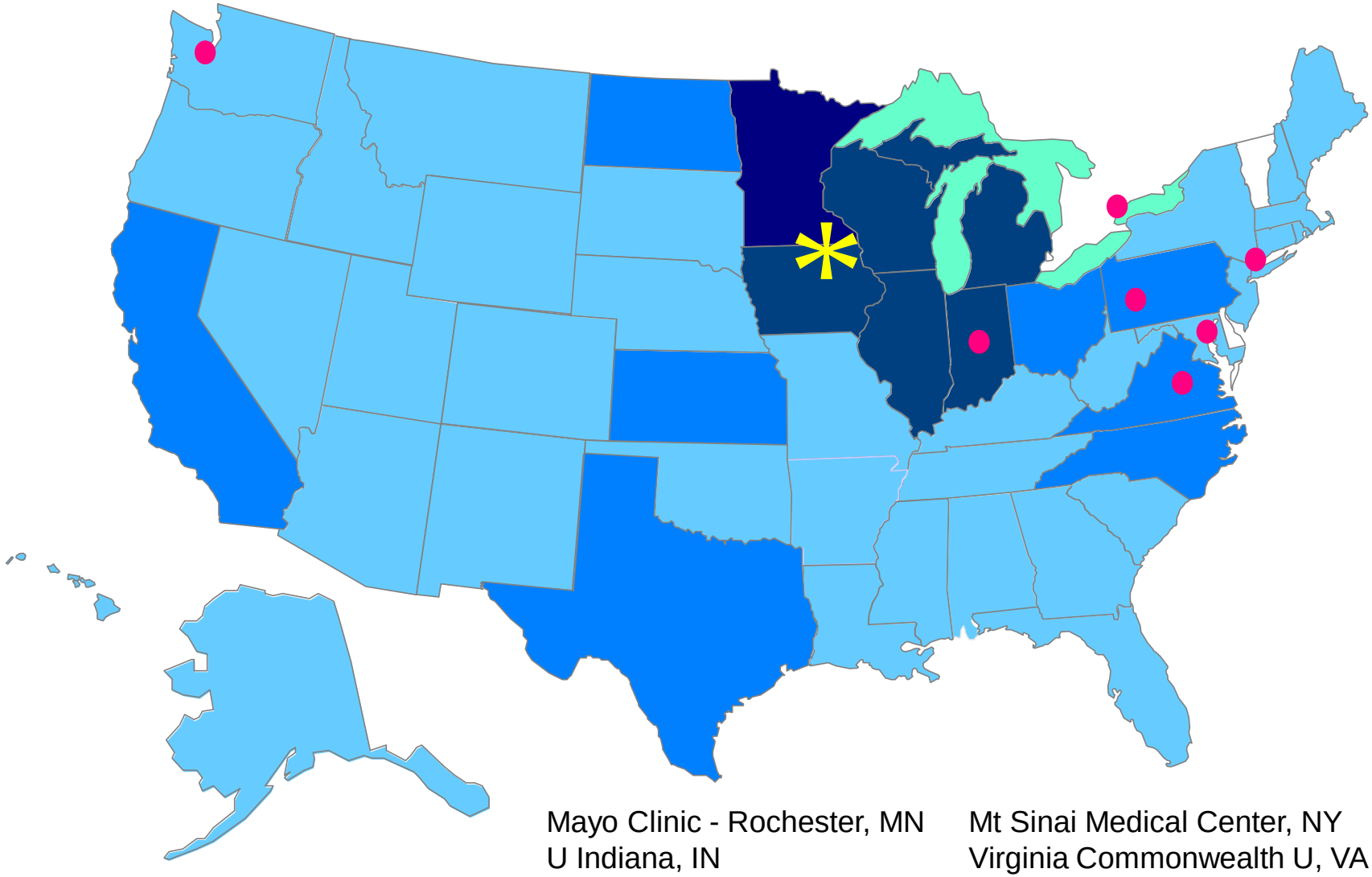
PROGRESS - Database Enrollment



PROGRESS - Database Phenotypes



PROGRESS Enrollment by State



Mayo Clinic - Rochester, MN
U Indiana, IN
Virginia Mason Clinic, WA
U Pittsburgh, PA

Mt Sinai Medical Center, NY
Virginia Commonwealth U, VA
Johns Hopkins U, MD
U Toronto, ON, Canada

PROGRESS: Recruitment by Medical Center

	Consent	DNA	Questionnaire
Mayo Clinic	807	651	661
U. Indiana	106	105	95
U. Toronto, CA	51	50	25
U. Pittsburgh	42	40	33
V.M. Clinic	40	40	34
V.C.U.	18	16	10
Mt. Sinai, NY	33	29	22
Johns Hopkins	2	0	1
Total collaboration[a]	292	280	220
Total (all centers)	**1,099**	**931 (1,281*)**	**881**

[a]includes collection of 300 PSC DNAs from G.H and P. D. and 50 PSC DNAs from Poland.

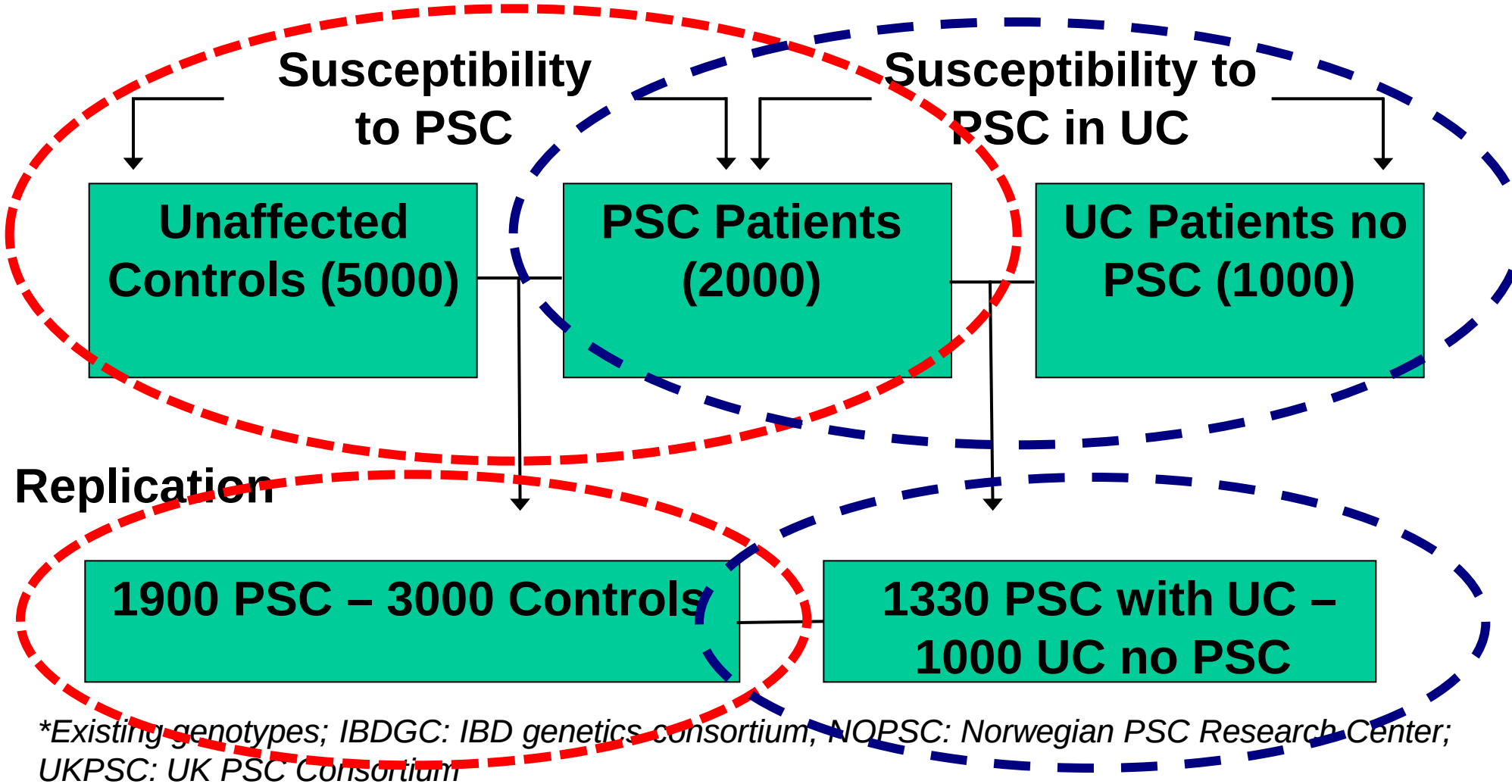
PROGRESS NIDDK Grant - Specific Aims

Aim 1: To expand PROGRESS, by:

- Continuing recruitment of PSC patients at Mayo Clinic
- Initiating referral of patients to PROGRESS by our external collaborators
- Fostering existing relationships with international PSC and IBD study groups

PROGRESS NIDDK Grant - Specific Aims

Aim 2: Genomic Wide Association Studies (GWAS)



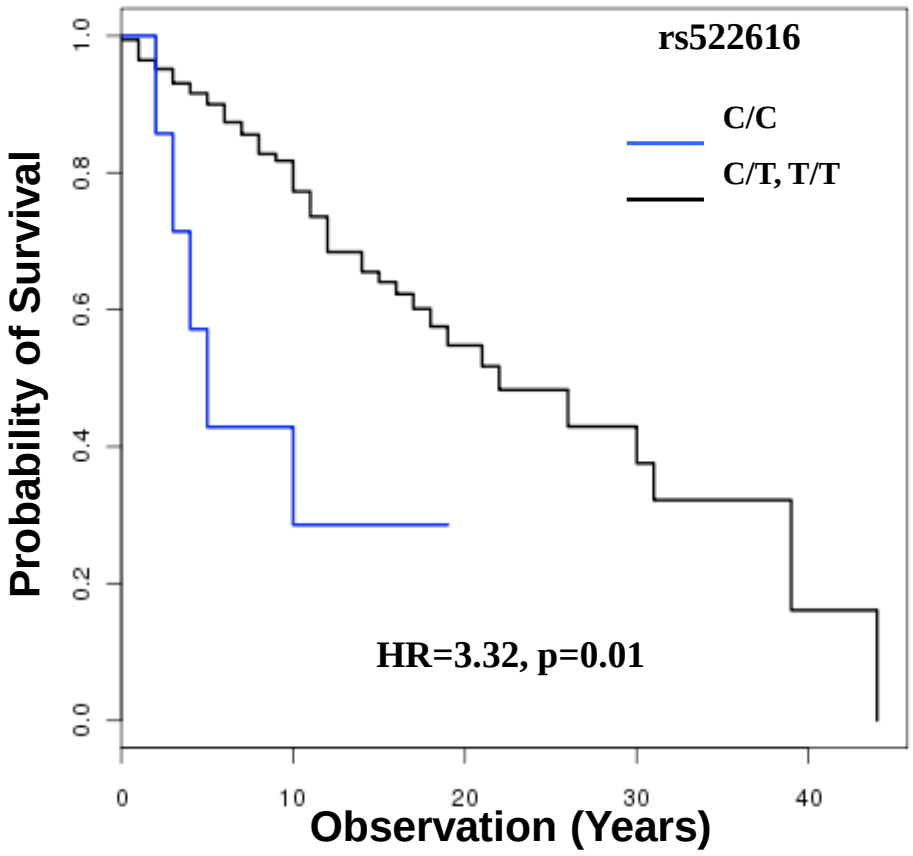
**Existing genotypes; IBDGC: IBD genetics consortium; NOPSC: Norwegian PSC Research Center; UKPSC: UK PSC Consortium*

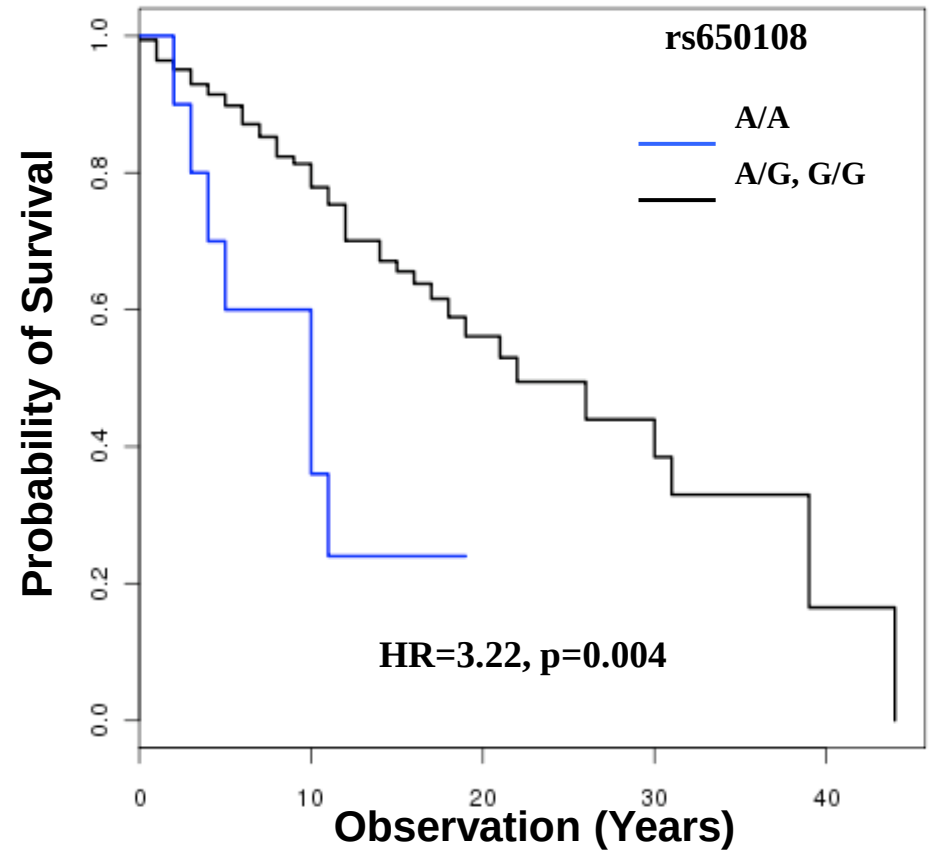
PROGRESS NIDDK Grant - Specific Aims

Aim 3

To determine environmental risk factors for PSC by performing a study of 1000 patients and 1000 controls utilizing the self-administered questionnaire data collected by PROGRESS

Outcome in PSC-UC Patients Homozygous for MMP3 rs522616 and rs650108 Genetic Variants





Juran et al., Liver International 2011

Immunochip Experiment

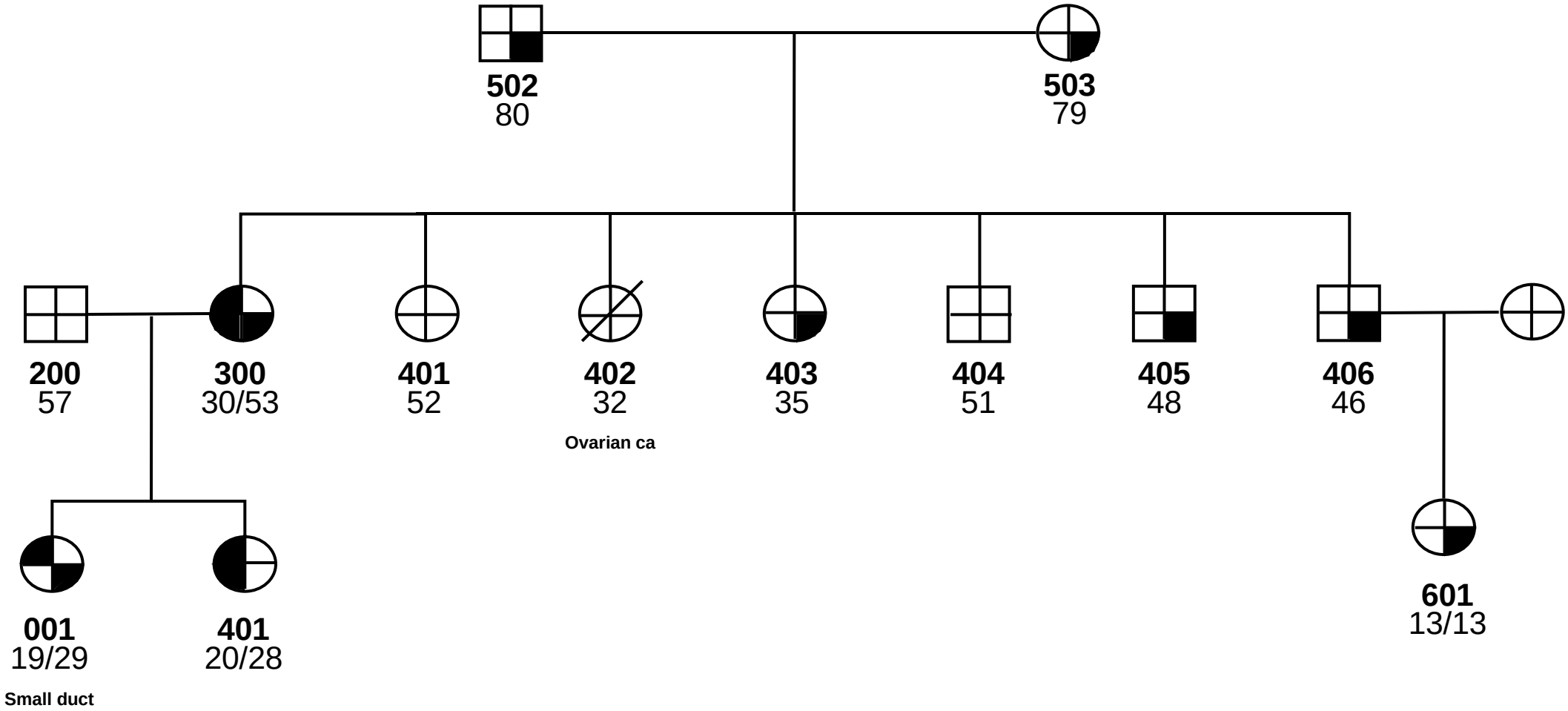
- 196,524 Single Nucleotide Polymorphisms (SNPs)
- 186 genetic loci with known autoimmune diseases associations

International PSC Immunochip Study

Origin	Cases	Controls
Belgium	163	1,425
Canada	323	0
Finland	308	504
France	45	0
Germany	852	5,435
Netherlands	255	3,421
Norway	504	1,412
Poland	43	541
Spain	27	284
Sweden	282	2,665
UK	1,121	8,970
USA	533	681
	4,456	25,338

Exome Sequencing of a Family

Pedigree #5139



Primary Sclerosing Cholangitis (PSC)

Orthotopic Liver Transplantation (OLT)

Inflammatory Bowel Disease (IBD)

Gallstone Disease (GSD)

Hypothesis and AIM

- We hypothesized that families with multiple members affected by PSC might carry rare genetic polymorphisms.

- We aimed to perform exome sequencing and analysis in this multiply-affected PSC family as a pilot to inform future large-scale efforts.

A Novel Genetic Variant of ABCB4 Gene in Pedigree #5139

SNP	gene_name1	daughter1	father	mother	daughter2
rs31653(A/C)	ABCB4	hom	hom		
rs31668(C/A)	ABCB4	hom	hom	hom	hom
rs2230028(T/C)	ABCB4			het	
(G/A) R595X	ABCB4	het		het	het
rs2109505(T/A)	ABCB4	het		het	het
rs1202283(G/A)	ABCB4	het	hom		het
rs2302387(G/A)	ABCB4	het		het	het

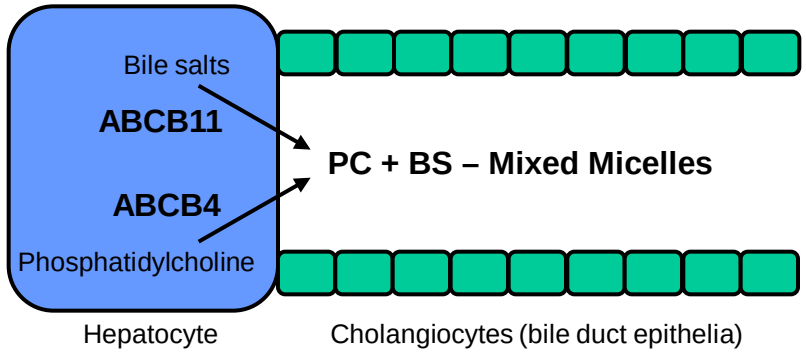
ABCB4 and Liver Disease

Defects in ABCB4 are known to cause a wide range of heritable cholestatic syndromes and contribute to cholelithiasis

- Progressive Familial Intrahepatic Cholestasis 3
- Intrahepatic Cholestasis of Pregnancy
- Low Phospholipid Associated Cholelithiasis
- PSC

Physiopathology of ABCB4 Deficiency

A.



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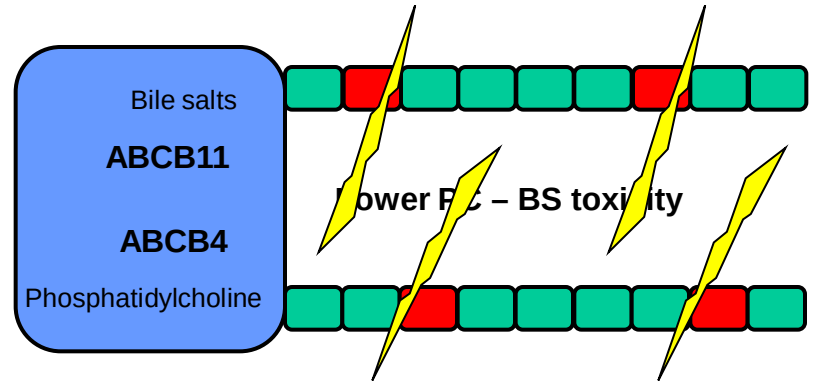
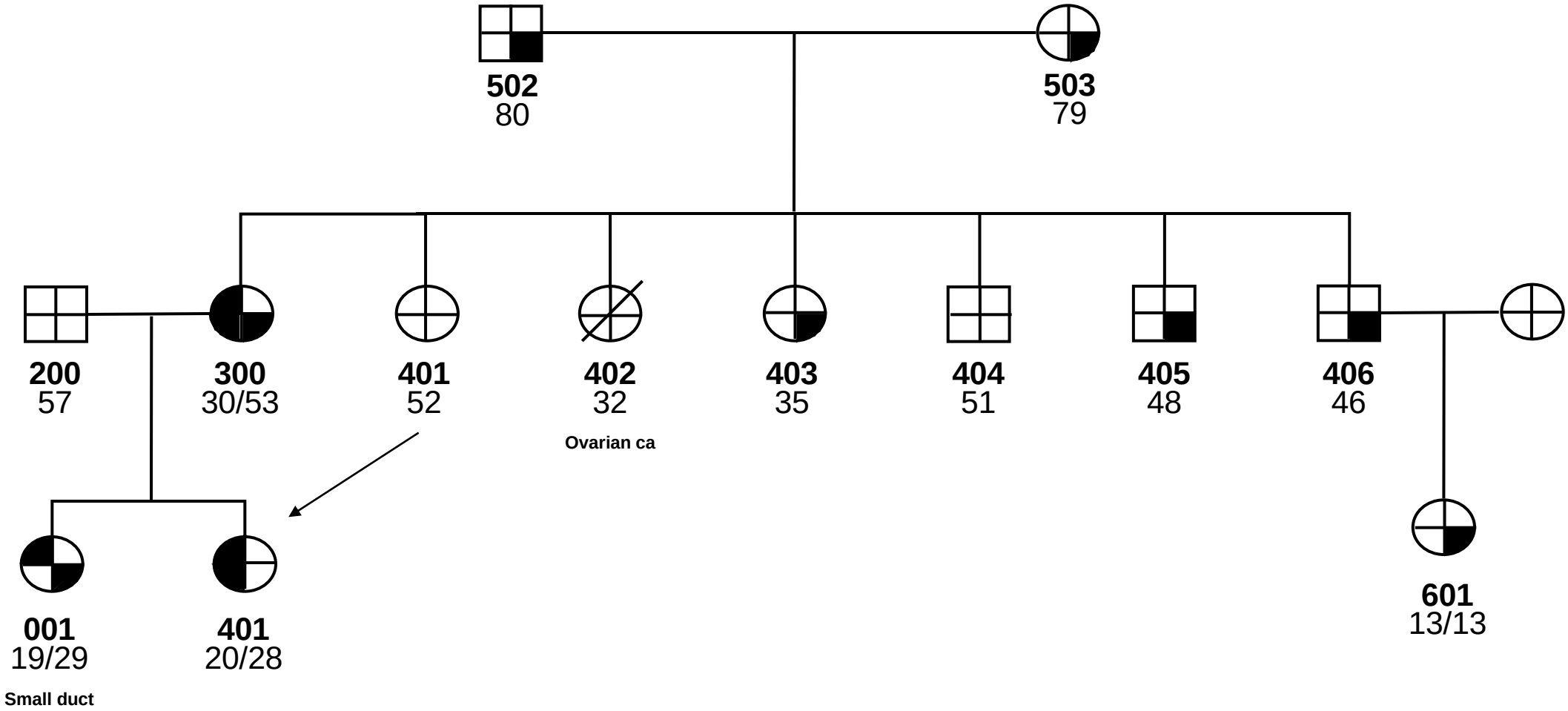
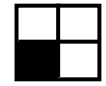


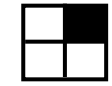
Pedigree #5139



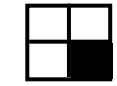
Primary Sclerosing Cholangitis (PSC)



Orthotopic Liver Transplantation (OLT)



Inflammatory Bowel Disease (IBD)



Gallstone Disease (GSD)

Conclusions from Pedigree #5139

- The R595X mutation in ABCB4 is likely a strong contributor to the severe liver disease in this family

- Exome sequencing of mother's siblings will help to better define the contribution of the R595X mutation to PSC

- Exome or Whole Genome Sequencing in the near future will improve the diagnosis and therapy of PSC

PROGRESS Future Studies

- Whole Exome Sequencing in Selected TRIOs (affected patient and unaffected parents)
- Gene x Environment interaction studies
- Genomic-based disease outcome studies (prediction of disease progression)

Acknowledgements

- PSC patients and family members
- PSC Partners Seeking A Cure
- NIDDK RO1 grant (2011-2015)
- A. J. Sigismunda Palumbo Charitable Trust
- American Liver Foundation
- Mayo Clinic College of Medicine
- Division of Gastroenterology and Hepatology Mayo Clinic

Exome Sequencing

- **Exome enrichment:** Agilent SureSelect system
- **Sequencer:** Applied Biosystems SOLID v4
  (All 4 DNAs sequenced on single slide, 50bp run)
- **Alignment to reference genome** (hg18): BioScope
- **Polymorphism calling:** BioScope diBayes and SAMtools pileup
- **Filtering and Annotation:** In-house tools

Filtering

Filter #1: SNPs present in all 3 affected individuals
Non-synonymous cSNP or splice-site
Not in dbSNP, 1000 genomes freq <0.01
Total # SNPs – 84

Filter #2: SNPs in cholestasis candidate genes2
Total # SNPs – 61

Overlap: 1 nonsense SNP/variant in MDR3 or ABCB4 (R595X)


Primary
Sclerosing
Cholangitis