DCCT Protocol

- 1,441 unrelated patients were recruited from 29 centers across North America in two cohorts between 1983-9:
  - primary prevention cohort (n=726) to determine if intensive therapy prevented development of diabetic retinopathy in patients with no retinopathy
  - secondary intervention cohort (n=715) to determine whether intensive therapy would affect the progression of early retinopathy
- Intensive therapy consisted of three or more daily insulin injections, or use of an insulin pump, with frequent blood glucose monitoring
- Conventional treatment consisted of one or two daily insulin injections (usual treatment)

Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC)

Andrew Paterson

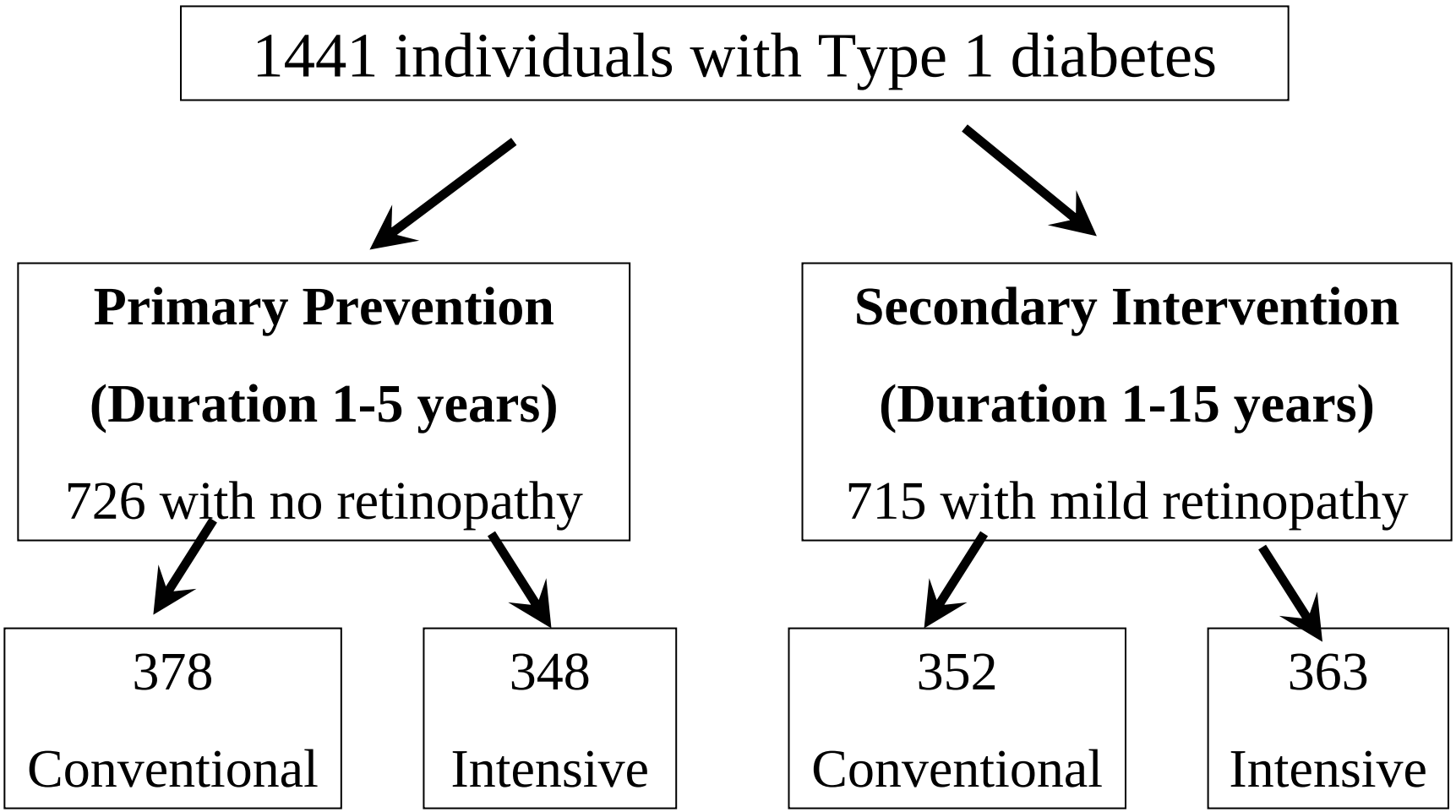
Scientist, Program in Genetics and Genomic Biology, Hospital for Sick Children, Toronto

andrew.paterson@utoronto.ca

DCCT Eligibility and exclusion criteria

- insulin dependence as evidenced by deficient C peptide secretion
- age 13-39 years old at entry (1983-9)
- duration of type 1 diabetes
  - 1-5 years (primary prevention)
  - 1-15 years (secondary prevention)
- $HbA_{1c} > 6.6$
- absence of hypertension, hypercholesterolemia, severe diabetic complications and other medical conditions
- Numerous other exclusion criteria
  - ~ 7,000 individuals screened for eligibility

The Diabetes Control and Complications Trial (DCCT)



Primary outcomes of DCCT

- Terminated after a mean study duration of 6.5 years (range 3-9 years) because of significant findings
- Benefits of intensive therapy
  - Adjusted mean risk for development of retinopathy in the primary cohort reduced by 76% by intensive treatment
  - In the combined cohorts, intensive therapy reduced micro-albuminuria and albuminuria by 39% and 54%, respectively
  - Clinical neuropathy reduced by 60% in the intensive group
- Adverse outcomes of intensive therapy
  - 3-fold increase in hypoglycemia requiring assistance
  - 3-fold increase in incidence of coma/seizure due to hypoglycemia
  - mean weight gain of 4.6 kg after 5 years in the intensive compared with the conventional group

EDIC

- After DCCT closeout all participants invited to participate in EDIC
- Advised to practice intensive therapy
- By January 1994 1375/1425 (96%) of DCCT subjects agreed to participate in EDIC
- Annual visits for measurement of complications/risk factors

EDIC

Added:
Cystatin C
Carotid US x 2
Coronary calcium
Neurocognitive

Examinations (outcomes)	EDIC year 1	2	3	4	5	6	7	8	9	10
Cardiovascular (CABG, MI, angina, CHF, stroke, TIA)										
Standardized history (including family) and physical exam	X	X	X	X	X	X	X	X	X	X
ECG	X	X	X	X	X	X	X	X	X	X
Duplex carotid ultrasonography central review	X					X				X
Peripheral vascular (foot ulcer, amputation, bypass graft)										
Standardized history and physical exam	X	X	X	X	X	X	X	X	X	X
Ankle/arm index by Doppler	X	X	X	X	X	X	X	X	X	X
Lipoprotein levels (hypercholesterolemia, hypertriglyceridemia)										
Total cholesterol										
HDL cholesterol	Scheduling of visits is a function of randomization date (alternate years)									
Triglycerides										
Calculated LDL cholesterol										
Nephropathic (renal failure, transplant, dialysis, elevated serum creatinine)										
Standardized history and physical exam	X	X	X	X	X	X	X	X	X	X
Serum creatinine	X	X	X	X	X	X	X	X	X	X
Glomerular filtration										
Albumin excretion rate	Scheduling of visits is a function of randomization date (alternate years)									
4-h standard creatinine clearance										
Neuropathy										
MNSI	X	X	X	X	X	X	X	X	X	X
10-g filament examination	X	X	X	X	X	X	X	X	X	X
Retinopathic (photocoagulation, vitrectomy, blindness, vitreous hemorrhage)										
Standardized history	X	X	X	X	X	X	X	X	X	X
Ophthalmological exam*										
Visual acuity*										
Fundus photographs*	Scheduling of visits is a function of randomization date									
Hypoglycemia (mortality/morbidity)										
Standardized history	X	X	X	X	X	X	X	X	X	X
Metabolic (DKA, chronic glycemia)										
Standardized history	X	X	X	X	X	X	X	X	X	X
HbA_{1c}	X	X	X	X	X	X	X	X	X	X
Psychological										
Quality of life questionnaire (DQOL)	X		X		X		X		X	X
Health status questionnaire (SF-36)	X		X		X		X		X	X
Health care delivery										
Standardized questionnaire	X	X	X	X	X	X	X	X	X	X
Dietary										
Food frequency recall questionnaire	In conjunction with lipids									

*Ophthalmological exam, visual acuity, and fundus photographs to be done on the patient's 8th, 12th, and 16th anniversaries of randomization. CABG, coronary revascularization; CHF, congestive heart failure; DQOL, Diabetes Quality of Life; SF, short form; TIA, transient ischemic attack.

DCCT Family Study (1991-3) Aims

- Identify first degree diabetic relatives of probands
- Measure complications in diabetic relatives
- Assess evidence for clustering and correlation of complications
- Collect DNA, cells for immortalization, serum, plasma for future studies on probands, diabetic relatives, and other first-degree relatives in families with a diabetic relative

Familial clustering and correlation analysis (DCCT family study)

- Microalbuminuria
  - Albumin excretion rate of > 40 mg / 24 hours
- Any retinopathy
  - ETDRS score >=20
- Severe retinopathy
  - ETDRS score >=47 or clinical significant macular edema or laser treatment
- Type 1 and 2 diabetes considered together
- Covariates
  - age, sex, diabetes duration, type of diabetes, recent HbA1c, percentage of ideal body weight and their pairwise interactions

Diabetes, 1997

Clustering of AER>40

	Subjects		Relatives		OR (95% CI)	
	Status	*n*	*n*	Percentage with event	Unadjusted*	Covariate Adjusted†
Microalbuminuria††						
Intensive	With event	3	6	66.7	3.48 (0.73–16.60)	4.67 (1.13–19.32)§
	Without event	57	63	36.5		
Conventional	With event	10	12	58.3	7.00 (2.01–24.36)‖	5.67 (1.57–20.44)‖
	Without event	44	48	16.7		
Combined‡	With event	13	18	61.1	5.33 (2.01–14.13)¶	5.46(2.17–13.72)¶
	Without event	101	111	27.9		

*Based on analyses of 2 × 2 tables, using Rao and Scott's adjustment for clustering. †Based on a GEE model with logit link adjusting for the covariates listed in Table 6. ‡OR adjusted for treatment group. §$P < 0.05$; ‖$P < 0.01$; ¶$P < 0.001$. # ETDRS score ≥20; **ETDRS score ≥47 or clinically significant macular edema or laser treatment. ††AER >40 mg/24 h.

Diabetes, 1997

Clustering of severe retinopathy

	Subjects	Relatives			OR (95% CI)	
	Status	*n*	*n*	Percentage with event	Unadjusted*	Covariate Adjusted†
Primary cohort						
Any Retinopathy#						
Intensive	With event	24	27	37.0	0.36 (0.12–1.07)	0.51 (0.10–2.74)
	Without event	28	31	64.5		
Conventional	With event	31	33	42.4	0.78 (0.24–2.55)	0.63 (0.10–4.04)
	Without event	9	10	50.0		
Combined‡	With event	55	60	40.0	0.52 (0.23–1.14)	0.59 (0.21–1.64)
	Without event	37	41	61.0		
Secondary cohort						
Severe retinopathy**						
Intensive	With event	10	12	41.7	2.42 (0.72–8.09)	1.77 (0.41–7.55)
	Without event	50	57	22.8		
Conventional	With event	14	15	40.0	4.33 (1.01–18.60)§	5.17 (1.22–21.91)§
	Without event	40	45	13.3		
Combined‡	With event	24	27	40.7	3.07 (1.21–7.77)§	3.12 (1.12–8.76)§
	Without event	90	102	18.6		

*Based on analyses of 2 × 2 tables, using Rao and Scott's adjustment for clustering. †Based on a GEE model with logit link adjusting for the covariates listed in Table 6. ‡OR adjusted for treatment group. §$P < 0.05$; ‖$P < 0.01$; ¶$P < 0.001$. # ETDRS score ≥20; **ETDRS score ≥47 or clinically significant macular edema or laser treatment. ††AER >40 mg/24 h.

Diabetes, 1997

Correlations of log AER

Type of family relationship	Number of families	Number of family members	Number of parent-offspring pairs	Correlation*	95% CI
Intensive					
All members	114	242	—	0.104	0.000–0.272
Parent-child	61	130	69	0.090	0.000–0.324
Father-child	33	68	35	0.103	0.000–0.424
Mother-child	28	62	34	0.022	0.000–0.368
Sib-sib	59	126	—‖	0.176	0.000–0.406
Conventional					
All members	99	206	—	0.013	0.000–0.200
Parent-child	60	126	66	0.188	0.000–0.411
Father-child	35	72	38	0.232	0.000–0.517
Mother-child	26	55	30	0.191	0.000–0.521
Sib-sib	44	90	—¶	0.000	0.000–0.288
Combined					
All members	213	448	—	0.063	0.000–0.188
Parent-child	121	256	135	0.138	0.000–0.301
Father-child	68	140	73	0.170	0.000–0.387
Mother-child	54	117	64	0.103	0.000–0.343
Sib-sib	103	216	—#	0.107	0.000–0.287

*Intraclass correlation for all members and sib-sib family relation, pairwise correlation for the other types of family relations. †$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$. ‖52, 6, and 1 families have 2, 3, and 4 siblings, respectively; ¶42 and 2 families have 2 and 3 siblings, respectively; #94, 8, and 1 families have 2, 3, and 4 siblings, respectively.

Diabetes, 1997

Correlation of retinopathy grades (log ETDRS)

Type of family relationship	Number of families	Number of family members	Number of parent-offspring pairs	Correlation*	95% CI
Intensive					
All members	116	246	—	0.124	0.000–0.291
Parent-child	63	134	71	0.311‡	0.083–0.507
Father-child	34	70	36	0.236	0.000–0.524
Mother-child	29	64	35	0.371†	0.043–0.627
Sib-sib	59	126	—‖	0.000	0.000–0.233
Conventional					
All members	103	214	—	0.256‡	0.082–0.430
Parent-child	64	134	70	0.344‡	0.117–0.538
Father-child	36	74	39	0.262	0.000–0.538
Mother-child	29	61	33	0.413†	0.071–0.669
Sib-sib	44	90	—¶	0.148	0.000–0.431
Combined					
All members	219	460	—	0.187‡	0.067–0.308
Parent-child	127	268	141	0.327§	0.171–0.467
Father-child	70	144	75	0.249†	0.022–0.452
Mother-child	58	125	68	0.391‡	0.166–0.577
Sib-sib	103	216	—#	0.060	0.000–0.239

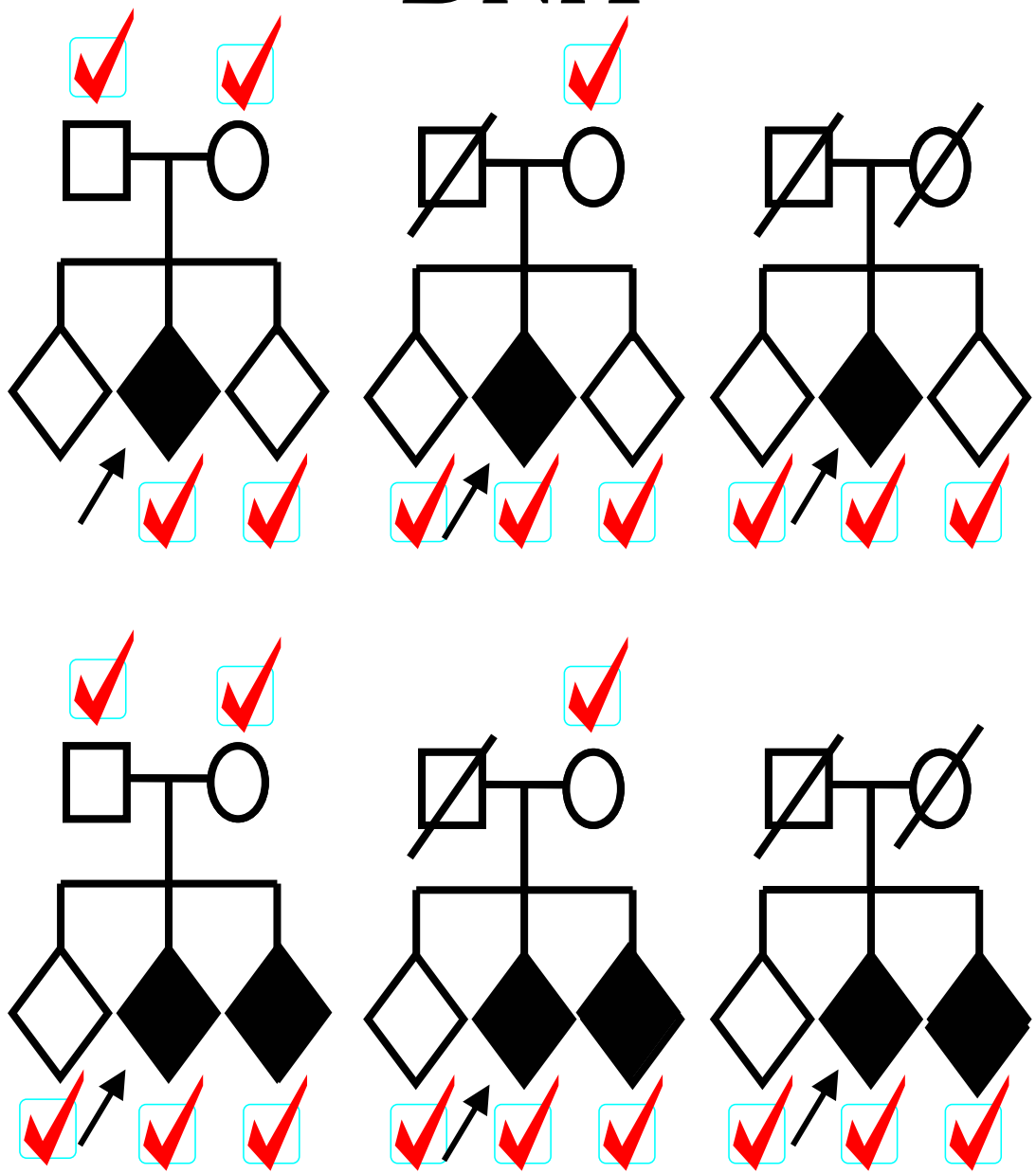
*Intraclass correlation for all members and sib-sib family relation, pairwise correlation for the other types of family relations. †$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$. ‖52, 6 and 1 families have 2, 3, and 4 siblings, respectively; ¶42 and 2 families have 2 and 3 siblings, respectively; #94, 8, and 1 families have 2, 3, and 4 siblings, respectively.

Diabetes, 1997

EDIC Genetics Study (2000-6) Aims

- 1.1 Complete collection of DNA, cells, serum and plasma on all available parents and at least one non-diabetic sib
- 1.2 Measure diabetic complications on diabetic siblings
- 1.3 Repeat familial clustering and correlation of diabetic complications
- 2. Establish DNA repository for genetic studies of Type 1 diabetes and it's complications
- 3. Perform family-based association studies of diabetic complications with candidate gene polymorphisms

DNA



	DNA & Cells	Basic Biochem*	C-peptide	4 hour AER	Retinal Photos	Cardio-vascular	Neuropathy
Probands	-	-	-	-	-	-	-
Parents	+	+	-	-	-	-	-
Siblings							
Previously Studied TIDM	+	+	+/-②	+	+	+	+
Previously Studied T2DM	+	+	+/-②	+	+	+	+
Diabetic Siblings Not Studied in 1991 - 1993	+	+	+	+	+	+	+
Identified Non-diabetic①	+	+	-	-	-	-	-

*Basic biochemistry: HbA1c; fasting blood glucose; serum creatinine; fasting lipids; cystatin C; random urine creatinine and albumin; serum, plasma and urine for storage

DCCT and EDIC DNA (26 Jan, 2005)

- 1,419 DCCT/EDIC probands
- 2,960 relatives of DCCT/EDIC probands
  - 806 mothers with DNA
  - 582 fathers with DNA
  - 1,572 siblings with DNA
    - Of which 140 have diabetes and complications measured
- 4,379 individuals in total

Effective # of trios

(Oct 2004)

Structure	# families	Relative Information	Effective Trios
Both parents	499	100%	499
One parent, ≥ 2 sibs	175	85%	149
One parent, 1 sib	119	70%	83
One parent, 0 sibs	72	50%	36
No parents, ≥ 2 sibs	65	75%	49
No parents, 1 sibs	93	50%	47
No relatives	403	-	-
TOTAL	1,023		862

Knapp (1999); Lange & Laird (2002)

DCCT probands

(from Baseline 002.4)

Group	**N**	**(%)**
White, not of Hispanic origin	1391	97
Black, not of Hispanic origin	29	2
Hispanic	14	1
Asian or Pacific Islander	6	0.5
American Indian or Alaskan Native	1	0

EBV cell transformations: CBL

(18 Feb 2005)

Status	EDIC Genetics	DCCT family study	Total
Completed	466	2,354	2820
Pending	1,593	5	1598
Failures	0	39	39
Success rate	100%	98.3%	98.6%

Progress in Genotyping

- 55 DNA variations genotyped in 29 genes
- Genes selected because of previous association with diabetic complications or involved in pathways responsible for glucose homeostasis
- Markers selected either from previous studies, and to capture common haplotypes across the gene
  - Published studies
  - International Haplotype Map
- Example of ACE and renal outcomes in press Boright et al., Diabetes (April 2005)

Renal outcomes

Persistent microalbuminuria is defined as the time (yrs) from DCCT baseline to the first of two consecutive visits with Albumin Excretion Rate >30 mg/day up to EDIC year 8

(Left censoring introduced for probands with Albumin Excretion Rate >30mg/day at DCCT baseline and DCCT year 1, n=66)

Severe nephropathy is defined as the time (yrs) from DCCT entry to either Albumin Excretion Rate >300 or dialysis or transplant up to EDIC year 8 (plus two consecutive visits with Albumin Excretion Rate >30 mg/day)

Table 2.1.2. Frequencies of renal outcomes obtained up to EDIC year 8 in 1389 "White" probands.

Outcome	Total data		Primary Prevention				Secondary Intervention			
			Intensive		Conventional		Intensive		Conventional	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Any two consecutive AER >30 mg/day	318	1071	25	308	76	288	91	262	126	213
Any two consecutive AER >30 mg/day and any AER >300 mg/day, dialysis or transplant	116	1273	5	328	31	333	19	334	61	278

Retinal outcomes

- **>3 step progression:** Time from DCCT entry to ≥ 3 step progression from DCCT baseline, up to EDIC year 4

- **Severe Retinopathy:** Time from DCCT entry to severe non-proliferative retinopathy (ETDRS patient level at least 53<53) or scatter laser, up to EDIC year 4

- **Macular Edema:** Time from DCCT entry to macular edema or focal laser, up to EDIC year 4

Table 2.1.1. Frequencies of retinal outcomes obtained up to EDIC year 4 in 1390 "White" probands.

Outcome	Total data		Primary Prevention				Secondary Intervention			
			Intensive		Conventional		Intensive		Conventional	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Any ≥3 step ETDRS progression from DCCT baseline	628	762	86	248	217	147	128	225	197	142
Any severe non-proliferative retinopathy (ETDRS≥10) or scatter laser	168	1222	5	329	19	345	37	316	107	232
Any macular edema or focal laser	124	1252	2	329	23	338	30	316	69	269

Families

- Positive genetic association from individual-based analysis are followed by family-based association analysis
  - Deviance residuals as a function of Martingale residuals from Cox PH
  - Haplotype analysis more powerful cf. unrelated

Familial clustering of diabetic complications

- Nephropathy (DCCT/EDIC plus others)
- Retinopathy (DCCT/EDIC)
- ? Neuropathy (EDIC has MNSI on probands and diabetic relatives)
- Cardiovascular disease or surrogates (IMT in T2D: Lange et al., 2002)
  - Genetic factors associated with CVD and surrogates in DCCT/EDIC probands likely to have general relevance for the population

Value of DCCT/EDIC

- Repeated measures of the four main diabetic complications
  - Reduce measurement error
  - Accurate time-to-event
  - Rates of change
- Measurement of all appropriate covariates
  - Medication, diet, other complications