Diabetes Mellitus and the Gene for Fanconi's Anemia

Abstract. *An increased prevalence of diabetes mellitus has been found in relatives of eight probands homozygous for the rare recessive syndrome Fanconi's anemia. Since many of these relatives are expected to be heterozygous for the gene for Fanconi's anemia, this gene may predispose to diabetes in single dose.*

Studies of the familial incidence of diabetes mellitus suggest a multifactorial mode of inheritance for most clinical cases (*1*). In contrast, numerous distinct genetic syndromes, mostly with single-gene autosomal recessive inheritance, are associated with impaired glucose tolerance and an increased incidence of diabetes (*2*). It is possible that the genes for some of these autosomal recessive syndromes may be diabetes-predisposing in heterozygotes and thus be identical with some of the postulated polygenes involved in the multifactorial inheritance of diabetes. If so, for each such syndrome, relatives of homozygous probands may show an increased incidence of diabetes, because some of them are expected to be heterozygous for the gene concerned.

Similar reasoning has been applied to the genetics of cancer and leukemia (*3*). The recessive syndrome Fanconi's anemia (FA) was studied. Patients with FA have an increased likelihood of developing cancer or leukemia, as well as the clinical signs characteristic of the condition. An increased incidence of deaths from malignant neoplasms was found among eight families of FA probands (*3*).

The same families have now been examined for the incidence of diabetes. This second analysis was carried out because it appeared that deaths from cardiac disease in these families were increased among females aged 40 to 69 (*3, 4*), a finding often associated with diabetes. We also wished to learn how to modify the methods of data collection and analysis especially to detect associations of single genes with diabetes before undertaking new studies of inherited recessive syndromes.

Medical information and death certificates were collected for all relatives with a prior probability of heterozygosity (for the FA gene) greater than or equal to 0.125 (*3*). We found the death certificates of 99 of 102 relatives who had died since 1930 in the United States or Canada. The prevalence of diabetes among these deceased relatives was measured by counting all the death certificates on which diabetes was recorded either as one of the underlying causes of death or as an associated condition. This method of computation was used in the 1955 U.S. vital statistics supplement on multiple causes of death (*5*) and is independent of the rules for abstracting the underlying cause of death from the list of conditions on the certificate. We counted as deaths without diabetes those cases where the diagnosis, confirmed in hospital records, was omitted from the death certificate of that individual, and also those of the three individuals for whom death certificates could not be found. The distribution of deaths by sex, age at death, and prior probability of heterozygosity, and the number of certificates with diabetes are given in Table 1.

The observed number of deaths with diabetes was compared to the number expected in a normal white population in the United States, computed from the sex- and age-specific data (*5*). Diabetes was listed among the causes of death on 14 of the death certificates, a number significantly greater ($P < .001$, 1×2 χ^2, with Yates' correction) than the 4.58 expected (Table 2) in a normal population sample. The increase in deaths with diabetes in the overall sample is almost entirely due to the finding of 11 such cases among the 48 female deaths. Only three of the men had diabetes on the death certificate, which is close to the 1.67 expected.

Table 1. Deaths and deaths with diabetes in relatives of FA probands. Blanks indicate no deaths in that category. For each individual the prior probability of heterozygosity is determined by the relationship to the proband in his or her family. The number in parentheses represents death certificates with diabetes.

Age at death	Probability of heterozygosity				
	1.0	0.67	0.5	0.25	0.125
			Males		
0–19				2	
20–39		1	1		2
40–44				1	4
45–49				1	
50–54			1	1	1
55–59			2	3	
60–64	1		5	6(1)	
65–69			3	2(1)	
70–74			2	4	1
75–79			2	4	1
80–84				1(1)	
85 +				2	
All ages	1	1	16	27(3)	9
			Females		
0–19			2	3	1
20–39			1		2
40–44				1	1(1)
45–49					
50–54				1	3
55–59	1		2(1)	5(2)	1
60–64				1	1(1)
65–69			2	7(3)	
70–74			1	2(1)	
75–79			3	1(1)	1
80–84				4(1)	
85 +				1	
All ages	1	0	11(1)	26(8)	10(2)

There were, in fact, 18 diabetics among the 102 deceased relatives, but three of them were men who had the diagnosis of diabetes substantiated in their hospital records although it was not recorded on their death certificates, and one of the three death certificates we could not find was that of a woman said by her daughter to have had diabetes. The three men and 10 of the 11 women who died with diabetes recorded on the death certificate had either an acute coronary occlusion, arteriosclerotic heart disease, or myocardial disease also listed as a cause of death on the certificate.

The prevalence of diabetes among living relatives was determined from questionnaires and from diagnoses recorded on hospital records. The short questionnaire sent to living family members asked, "Were you ever seriously ill?" and, "Were you ever in any hospital?"; but it did not ask about any specific disease, test, or symptom. The distribution of living relatives by sex, age, and prior probability of heterozygosity, and the number of diabetics in each category are given in Table 3.

As shown in Table 2, the total number of diabetics among living relatives was significantly greater ($P < .05$, 1×2 χ^2, with Yates' correction) than the number expected in three different normal populations (*6–8*). This increased prevalence of diabetes was due almost entirely to the female cases, just as it was for the deceased relatives. The expected number of diabetics among the living females falls outside the 95 percent confidence limits of the observed value for two out of the three controls. The expected number (3.54) of female diabetics, based on the 1964 U.S. Health Survey (*6*), falls just within this confidence interval. However, the published age- and sex-specific data from this survey is not given separately by race, and, since diabetes is much more prevalent among adult black females, this expected number is higher than it would have been if data for white females alone had been used.

It is likely that we did not find all the diagnosed diabetics among the living relatives because we asked only

Table 2. Observed and expected numbers of FA relatives with diabetes. Numbers in parentheses indicate confidence limits for the observed values above.

Sex	Deceased relatives			Living relatives				
	Total No.	With diabetes		Total No.	With diabetes			
		Observed	Expected*		Observed	Expected†		
						I	II	III
M	54	3 (0.63–8.32)‡	1.67	169	4 (1.10–10.06)‡	2.43	1.76	4.33
F	48	11 (4.58–20.09)§	2.91	202	8 (3.48–15.46)‡	3.54	3.05	1.86
Both	102	14 (6.45–25.17)§	4.58	371	12 (6.24–20.71)‡	5.97	4.81	6.19

* 1955 examination of multiple causes of death on death certificates in the United States (*5*). †Expected for I: 1964 U.S. Health Survey (*6*); for II: Prince Edward Island, Canada, 1966 (*7*); for III: Sudbury, Massachusetts, 1965 (*8*). ‡ 95 percent confidence limits. § 99 percent confidence limits

about serious illnesses and not specifically about diabetes. For example, the diagnosis was discovered only through hospital records for three of the four diabetic men. The experimental and control data for the living diabetics are thus not strictly comparable, since the latter came from surveys in which each person was asked directly whether or not he had diabetes.

It may be estimated from our data, with the use of the prior probabilities of heterozygosity in a maximum likelihood method (*9*), that female FA heterozygotes are about six times more likely than normal controls to have or die with diabetes, whereas the male heterozygote's risk is not significantly different from the normal. This increased risk for female FA heterozygotes may be compared to those Simpson (*10*) estimated for first-degree relatives of diabetics (parents 2 to 4 times, siblings 2 to 14 times, child 2 to 40 times the age- and sex-specific risk for the normal population).

We can also estimate how important numerically the FA gene may be in predisposing to diabetes. If a female FA heterozygote is about six times more likely to develop diabetes than a member of the general population and FA heterozygotes are 1 in 300 in the population (*3*), then $6 \times (1/300) = 0.02$—or 1 in 50—is the estimated proportion of FA heterozygotes among all female diabetics.

An association of the FA gene and diabetes mellitus is surprising, since we do not know of any instances of diabetes among FA homozygotes. However, many of the FA homozygotes die before adolescence, so that there is a limited opportunity for genetic predisposition to diabetes to be expressed. It was easier to accept that the FA gene predisposes the heterozygote to cancer and leukemia because of (i) the prevalence of malignancies in homozygotes, (ii) the clinical reports of leukemia in presumptive heterozygotes, and (iii) the increased susceptibility of FA heterozygous cell cultures to transformation by the oncogenic virus SV40 (*11*). Whatever our expectations, the association we have found between diabetes and the FA gene is stronger statistically ($P < .001$) than the association between the gene and death from a malignant neoplasm ($P < .05$) (*3*).

The significance of the difference, in this sample, between male and female FA relatives in the prevalence of diabetes is an open question. It is reminiscent of male-female asymmetry in other genetically directed observations concerning cancer and diabetes (*12*) and may, although there is no biological explanation for it at present, represent the real situation. We may, however, have failed to detect a significant association between the FA gene and diabetes in men because (i) the size of the sample was inadequate, (ii) diabetes was not mentioned on three of the six death certificates of known diabetic men, or (iii) the diagnosis of diabetes was poorly reported on the questionnaires received from living relatives. For these reasons, the question of a true difference between male and female FA heterozygotes in the prevalence of diabetes is still unresolved.

Table 3. Diabetes in living relatives of FA probands. For each category, the number of diabetics is in parentheses.

Age	Probability of heterozygosity				
	1.0	0.67	0.5	0.25	0.125
			Males		
0–19		8		15	25
20–39			5	16	13
40–44	1		2	2	11(1)
45–49	4		3	2	4(1)
50–54	1		4	3	7
55–59	1		3	1	9
60–64	1		1	2	5
65–69			3	3	3
70–74				3	1
75–79			2	2	
80–84				3(2)	
85 +					
All ages	8	8	23	52(2)	78(2)
			Females		
0–19	1	5	2	19	18
20–39			8	21	27
40–44	4		2	1	11
45–49	2(1)		1	2	16
50–54			6	1	6
55–59	1		5	1	5
60–64	1		3	2	6(1)
65–69			4(2)	4	3(1)
70–74			3(1)	4(1)	
75–79			3	3(1)	
80–84				1	
85 +					
All ages	9(1)	5	37(3)	59(2)	92(2)

Any interpretation of our study must be provisional because the data were analyzed retrospectively. The remarkable prevalence of diabetes in these eight FA families might be due to factors other than the FA gene—they could have been chosen unwittingly from some subpopulation with a high incidence of diabetes. There was no such stratification obvious in this sample. The families were of diverse social class and ethnic origin (Eastern European Jewish, British, Scotch, Irish, Danish, Puerto Rican, and Italian ancestors). The finding of an increased prevalence of diabetes in another set of FA families would provide additional, still indirect, evidence for an association of diabetes with the FA gene; but ultimately the association of diabetes with the FA or any other gene in this category should be measured directly, when there is a test or unique set of criteria to identify the heterozygous carrier.

MICHAEL SWIFT
LAURA SHOLMAN
Department of Medicine,
Child Development Institute,
Chapel Hill, North Carolina 27514
DOUGLAS GILMOUR
Department of Pathology,
New York University Medical Center,
New York 10016

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13. Supported by American Cancer Society grant EP-7 and NIH grant CA 12520. We thank R. Pinkham and J. Cohen for statistical discussions.

10 April 1972

Adenosine 3′,5′-Monophosphate: Electrophysiological Evidence for a Role in Synaptic Transmission

Abstract. *Synaptic potentials and changes in resting membrane potentials of superior cervical ganglia of the rabbit were measured in the presence of adenosine 3′5′-monophosphate and agents that affect its metabolism. Adenosine 3′5′-monophosphate and its mono- and dibutyryl derivatives caused a hyperpolarization of the postganglionic neurons. Theophylline potentiated the slow inhibitory postsynaptic potential that follows synaptic transmission, as well as the hyperpolarization of postganglionic neurons caused by exogenous dopamine. Conversely, prostaglandin E_1 inhibited both the slow inhibitory postsynaptic potential and the dopamine-induced hyperpolarization. We hypothesize that the slow inhibitory postsynaptic potential as well as the dopamine-induced hyperpolarization result from increased amounts of adenosine 3′5′-monophosphate in the postganglionic neurons. The dibutyryl derivative of guanosine 3′5′-monophosphate caused a depolarization of the postganglionic neurons, which is consistent with the possibility that guanosine 3′5′-monophosphate mediates synaptic transmission at muscarinic cholinergic synapses.*

Studies in our laboratory have implicated adenosine 3′,5′-monophosphate (cyclic AMP) in the physiology of synaptic transmission in the mammalian superior cervical sympathetic ganglion (*1–4*). Stimulation of preganglionic fibers causes an increase in the amount of cyclic AMP in this ganglion (*1, 2*). In addition, dopamine, a putative neurotransmitter in the ganglion (*2–6*), increases the amount of ganglionic cyclic AMP (*3*) and causes a hyperpolarization of the postganglionic neurons (*5, 6*) (see Fig. 1). The effects of dopamine, both on cyclic AMP (*3*) and on postganglionic membrane potential (*5, 6*), are antagonized by α-adrenergic blocking agents. To account for these and other results, we have suggested (*2–4*) that dopamine, released from interneurons during activity, causes an increase in the amount of cyclic AMP in the postganglionic neurons and that it is this increased cyclic AMP which is responsible for the slow inhibitory postsynaptic potential (slow-IPSP) that follows preganglionic stimulation. We now report the results of electrophysiological studies designed to test certain predictions made by this hypothesis, namely, (i) that cyclic AMP would hyperpolarize the postganglionic neurons; (ii) that theophylline, a phosphodiesterase inhibitor which potentiates the accumulation of cyclic AMP in the ganglion (*7*), would also potentiate the slow-IPSP as well as the hyperpolarization due to dopamine; and (iii) that prostaglandin E_1 (PGE_1), which has been shown to affect adenylate cyclase activity of almost all tissues studied, might alter the slow-IPSP as well as the hyperpolarization due to dopamine.

Changes in membrane potential of postganglionic neurons in superior cervical sympathetic ganglia of the rabbit were measured by the sucrose gap technique (*8*). Preganglionic stimulation of this ganglion results in the generation of an initial brief excitatory postsynaptic potential (initial EPSP), followed successively by a slow-IPSP that reaches a maximum within 600 msec, and a slow excitatory postsynaptic potential (slow-EPSP) lasting

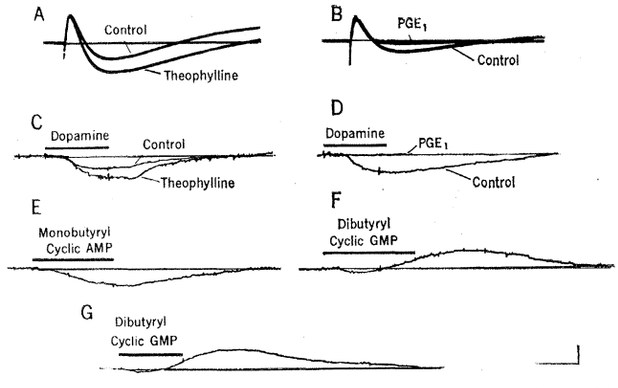

Fig. 1. (A to F) Effect of cyclic nucleotides, and of agents which affect cyclic AMP metabolism, on synaptic and resting membrane potentials recorded by means of the sucrose gap technique from postganglionic neurons of the superior cervical sympathetic ganglion of the rabbit. (A and B) Oscillographic traces of electrotonically conducted synaptic potentials elicited in response to a single supramaximum stimulus to the preganglionic nerve. Hexamethonium chloride (600 μ*M*) was present to abolish propagated responses. (A) Responses obtained in Locke solution and after 30 minutes of superfusion with Locke solution containing 1.5 m*M* theophylline are superimposed. (B) Responses obtained in normal Locke solution and after 15 minutes of superfusion with Locke solution containing 3×10^{-7} *M* PGE_1 are superimposed. Results similar to those illustrated here were obtained when *d*-tubocurarine (125 μ*M*) was used instead of hexamethonium chloride to abolish propagated responses. This dose of *d*-tubocurarine also abolished the initial EPSP. (C and D) Resting membrane potential changes in response to a brief period of superfusion with dopamine. (C) Responses to 50 μ*M* dopamine before (control) and 30 minutes after the start of superfusion with 2 m*M* theophylline are superimposed. (D) Responses to 200 μ*M* dopamine before (control) and 20 minutes after the start of superfusion with 6×10^{-7} *M* PGE_1 are superimposed. (E and F) Changes in membrane potential in response to a brief period of superfusion with 2.5 m*M* monobutyryl cyclic AMP (E) or 25 μ*M* dibutyryl cyclic GMP (F). (G) Change in membrane potential of the cervical vagus nerve in response to a brief period of superfusion with 200 μ*M* dibutyryl cyclic GMP. The duration of superfusion with Locke solutions containing dopamine or cyclic nucleotides is indicated by the solid bars. All records are d-c recording, hyperpolarization downward. Calibration marks: (A and B) 1 second, 800 μv; (C to G) 2 minutes, 400 μv.