Christmas Disease in One of a Pair of Monozygotic Twin Girls, Possibly the Effect of Lyonization

THOMAS RÉVÉSZ, DEZSŐ SCHULER, BÉLA GOLDSCHMIDT, and
SUSAN ELÖDI

Second Department of Paediatrics, Semmelweis University Medical School and Central Institute of Haematology and Blood Transfusion, Budapest, Hungary

Christmas disease (clotting factor IX deficiency) being, like haemophilia (factor VIII deficiency), a rare X-linked recessive character is very seldom found in females. A case is reported here of a young girl who suffers from Christmas disease but whose monozygotic twin sister does not.

Case Report

The probanda, a girl of 18 months, was sent to our department in May 1970 from Jászberény County Hospital. She had been admitted previously on 2 occasions to the hospital for excessively severe bleeding following minor trauma. She was in severe shock both times and required multiple transfusions. After the 2nd episode she was transferred to us for further investigations. Her parents said that she always bruised easily after slight injury and developed sizeable swellings. There had been bleeding from the nose and gums on a few occasions. A swelling of the elbow joint was noted once, but it subsided spontaneously after a couple of weeks.

We found her to be of average build and development. Several bruises were seen on her arms and legs. Her blood count was normal, without any gross anaemia present, probably as a result of her recent transfusions. There was no indication of organic disease on physical examination. No abnormality was found in the chest or abdomen. Musculature was adequately developed, joints appeared to be of normal shape and movements were unlimited in all extremities. She was a somewhat shy, but intelligent little girl.

Family History

The pedigree of the family is given in Fig. 1. On taking the family history, we could not trace a single relative or progenitor with bleeding episodes or with a tendency to easy bruising. There was information about the last 5 generations but no indication of any consanguinous marriage.

Of particular interest is the fact that the probanda (III.3) has a twin sister (III.4) who proved to be mono-

zygotic. The twin sister has never shown any tendency to bleeding, nor does she bruise easily after falls or small injuries. The father (II.3), a blacksmith by profession, appeared to be healthy and completely free of bleeding symptoms, as did the mother (II.4), grandmother (I.4), and other members of the family who were investigated. Neither the mother nor the grandmother had ever experienced unusually long or severe menstruations.

Methods

Coagulation Studies. Whole blood clotting time was determined in glass tubes by the method of Lee and White (1913). The bleeding time was measured according to Duke. The platelets were counted in the phase contrast microscope as described by Brecher and Cronkite (1950/1951). Adhesiveness of platelets *in vitro* was studied with Hellem's technique (1960). Prothrombin time was measured by Quick's method, kaolin-activated partial thromboplastin time (PTT) was carried out according to the method described by Proctor and Rapaport (1961). The thromboplastin generation test was performed by the method of Biggs and Douglas (1953). Quantitative determination of factor IX activity was performed by means of kaolin-activated PTT, based principally on the method of Langdell, Wagner, and Brinkhous (1953). Factor IX deficient plasma served as substrate plasma in this assay.

FIG. 1

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HL-A Typing was carried out using the standard NIH microlymphocytotoxicity test.

Mixed Lymphocyte Cultures were performed according to the method of Schellekens and Euijsvoogel (1968).

Glucose-6-phosphate Dehydrogenase was measured in the blood of the twins by the spectrophotometric method of Marks and Gross (1959).

Chromosome Studies were performed in the leucocyte cultures using the method of Moorhead *et al* (1960). On one occasion the fluorescent pattern of the chromosomes was also studied by a modified version of Caspersson's method (Caspersson *et al*, 1968).

Results

Zygosity of the Twins. We observed a striking resemblance between the patient and her twin sister. They both have fair hair, blue eyes, and similarly shaped heads, noses, and ears. Their heights were the same on all occasions, but the proposita was usually a little under-weight. It was in fact extremely difficult to tell which was which, except for the bruises on the girl with Christmas disease.

Unfortunately no information was available about the placenta or the amniotic sac. Detailed analysis of red cell groups, serum groups, and isozymes showed the twins to be identical for these characters, and to conform with those of their parents (Table I).

The twins' fingerprints showed a striking resemblance in ridges and angles. The *atd* angles, for example, were equal on the corresponding hands of the twins. The difference between the total ridge counts was 8.

TABLE I
BLOOD GROUP STUDIES OF THE FAMILY

Blood Group System	Type			
	Father	Mother	Proposita	Sister
ABO	A ₁	O	A ₁	A ₁
Rh	R ₁ R ₂	R ₂ R ₂	R ₁ R ₂	R ₁ R ₂
MNSs	MN ₂ Ss	MN ₂ Ss	MN ₂ Ss	MN ₂ Ss
P ₁	+	+	+	+
Lutheran	a-b+	a-b+	a-b+	a-b+
Kell	-	-	-	-
Lewis	a+b-	a-b-	a+b-	a+b-
Duffy	a+b+	a+b+	a+b+	a+b+
Kidd	a-b+	a+b+	a-b+	a-b+
Xg	a+	a+	a+	a+
Dombrock	a-	a-	a-	a-
PGM	1	2-1	1	1
ADA	1	2-1	2-1	2-1
Gc	2-1	1	2-1	2-1
Hp	2-2	2-2	2-2	2-2
Gm	a-x-b+	a-x-b+	a-x-b+	a-x-b+
InV 1	-	-	-	-

On the basis of the above findings, using the method of Maynard Smith and Penrose (1954/1955), we calculated the total chance of the twins being dizygotic as 0.0006 and of being monozygotic as 0.9994 (Table II).

HL-A typing also showed complete identity between the girls, and as both parents were also tested,

TABLE II
CALCULATED RELATIVE CHANCES OF THE TWINS BEING DIZYGOTIC

Character	Independent Relative Chances
Initial odds	2:3333
Likeness in sex	0.5000
Likeness in ABO	0.6113
Likeness in MNS	0.5000
Likeness in P	0.9034
Likeness in Rh	0.5000
Likeness in Kell	1.0000
Likeness in Lewis	0.7634
Likeness in Duffy	0.5000
Likeness in Kidd	0.5000
Likeness in Xg	1.0000
Likeness in Dombrock	1.0000
Likeness in Hp	1.0000
Likeness in Gm	1.0000
Likeness in PGM	0.5000
Likeness in ADA	0.5000
Likeness in Gc	0.5000
Difference of total ridge count	0.2574
Difference of <i>atd</i> angles	0.5800
Total relative chance (pD)	0.0006
Total chance (pD/[1 + pD])	0.0006

TABLE III
HL-A TYPING OF THE FAMILY

Series	Father	Mother	Proposita	Sister
1st haplotype, 1st series	HL-A9	HL-A9	HL-A9	HL-A9
2nd haplotype, 1st series	HL-A9 or X ₁	HL-A3	HL-A9	HL-A9
1st haplotype, 2nd series	Mapi	Mapi	Mapi	Mapi
2nd haplotype, 2nd series	LND	R ^x	Mapi	Mapi

TABLE IV
MIXED LYMPHOCYTE CULTURE (MLC) TEST OF THE TWINS

1	$P + S_m$	$\bar{x} = 408 \text{ rpm}$
	$\text{MLC index} = \frac{P + S_m}{P + P_m} = 0.8$	
2	$S + P_m$	$\bar{x} = 527 \text{ rpm}$
	$\text{MLC index} = \frac{S + P_m}{S + S_m} = 1.09$	

P = proposita's lymphocytes; S = twin sister's lymphocytes; m = mitomycin treated cells.

TABLE V
COAGULATION RESULTS OF THE FAMILY

Tests	Members of the Family							Normal Values
	I.2	I.3	I.4	II.3	II.4	III.3	III.4	
Whole blood clotting time (min)			10	8	7	18	10	7-12
Bleeding time (sec)	160	120	180	110	140	150	160	60-180
Thrombocyte count (1000/mm ³)			280	350	280	600	300	150-400
<i>In-vitro</i> thrombocyte adhesiveness (%)						38	25	20-50
Fibrinogen (mg %)	320	380	280	300	250	280	250	200-400
Prothrombin time (sec)	16	15	17	16	14	18	17	14-18
Partial thromboplastin time (sec)	40	40	42	44	38	68	45	35-45
Thromboplastin generation test	Normal	Normal	Normal	Normal	Normal	Delayed	Normal	
Factor IX activity (%)			44	77	28	< 1	15	60-160

the HL-A types given in Table III represent the girls' genotypes.

In the one-way mixed lymphocyte culture (Table IV) the lymphocytes of each twin failed to stimulate those of the other, a fact strongly in favour of monozygotism.

Coagulation Results are summarized in Table V. The prolongation of clotting time and partial thromboplastin time revealed a coagulation disorder in the proposita (III.3).

Deficiency of factor IX in the serum and plasma of the patient was demonstrated by the delayed thromboplastin generation test and by the PTT correction test. The prolonged PTT of the patient's plasma could be shortened by the addition of normal plasma, or normal serum, containing factor IX. On the other hand, Al(OH)₃ treated plasma, which does not contain factor IX, and plasma from a patient with severe Christmas disease failed to correct the prolonged clotting time. In this way factor IX deficiency seemed to be verified. Quantitative tests showed that the proposita was completely lacking in factor IX, the activity being below 1%. The diagnosis of Christmas disease was therefore established. Her bleeding time, thrombocyte count and thrombocyte adhesiveness *in vitro* was found to be normal.

X-linked Genetic Markers. Of the other X-linked characters studied, G6PD level was normal in both the proposita and her twin sister, and their colour vision was normal.

Chromosome Studies. Analysis of peripheral blood samples of both girls was carried out on 4

occasions. On the first occasion a deletion of the short arm of one of the C group chromosomes of the proposita was found in 2 out of 10 cells analysed, the other 8 cells were normal 46,XX. On subsequent occasions, however, we found only one more cell with a deleted C group chromosome out of 50 examined: all other analyses showed normal karyotypes. Thus the original deletion could well have been an artefact *in vitro*. The karyotypes of the twin of the proposita were normal on all occasions.

In the course of the last study the fluorescence pattern of the X chromosomes was found to be normal in both girls.

Discussion

As shown in Table V, a coagulation disorder was found in the proposita, caused by the lack of factor IX activity in her plasma. As her bleeding time and thrombocyte adhesiveness gave normal, and in the case of platelets, higher than normal values, von Willebrand's disease with factor IX deficiency can be ruled out. That the factor IX level of the proposita is below 1% could mean either that she cannot produce it or that the substance is produced but is inactive; this question will be subject to further studies.

Routine coagulation tests gave normal results for all other members of the family. The quantitative factor IX assay, however, showed the twin sister of the proposita to have a level of 15%, her mother 28%, and her maternal grandmother 44%. All 3 can be regarded as heterozygotes, their factor levels falling within that range.

There is good evidence that the mean factor values for heterozygotes in haemophilia and Christmas

disease are considerably lower than the normal values (Rapaport, Patch, and Moore, 1960; Didisheim and Vandervoort, 1962). Frota-Pessoa, Gomes, and Calicchio (1963) found the mean factor IX level for obligatory heterozygotes to be about half that of normal males and females.

The father of our patient had a normal factor IX level of 77%. It seems that the Christmas trait was present in the maternal side of the family for at least the last 3 generations, without ever manifesting itself in a male member. That the father is healthy and has an absolutely normal factor IX level excludes the possibility that the *proposita* is homozygous for the trait, by straightforward inheritance.

The most curious finding is that, although the twins are identical in all other investigated genetic characters, they give significantly different values for factor IX activity. The factor IX levels of the various members of the family showed no change when re-tested after several months, thus excluding the possibility of an error in determining factor IX activity.

Both twins have 2 X chromosomes, so the disease in the *proposita* cannot be explained by her being 45,X (Turner's syndrome) as in the cases of Gilchrist, Hammond, and Melnyk (1965) or Bithel, Pizzaro, and MacDiarmid (1970) or 46,XY (testicular feminization syndrome [Nilsson *et al*, 1959]). The principal assumption is that our patient has inherited a factor IX deficient X chromosome from her mother, just as her twin sister has. For some reason, however, her other X chromosome is also incapable of factor IX production.

Why is one twin a heterozygote, while the other gives the signs and laboratory findings of true Christmas disease? During the first chromosome study a deletion of the short arm of a C group chromosome was found in some of the karyotypes examined. If the deletion had involved one of the X chromosomes this would have offered a rather clear explanation. Namely, that the girl had inherited a factor IX deficient X chromosome from her mother, and from her father, an X with a deletion involving the factor IX locus and thus preventing it from counteracting the effect of the maternal allele. As we could not confirm this first finding in subsequent cultures, but instead found normal chromosome complements and a normal fluorescent pattern of the X, we have to consider other explanations for this puzzling case, though it should be remembered that in spite of the negative chromosome findings, a very faint chance still remains that there is a mosaic deletion: the proportion of mosaicism could be quite different in tissues other than lymphocytes.

The loss of a paternal X chromosome during cleavage at an early mitotic division with subsequent non-disjunction of the remaining maternal X chromosome, which bears the gene for Christmas factor, could also be a possible, but not very likely, explanation.

Another theoretically possible, though very unlikely explanation is that a fresh mutation has happened to the plasma thromboplastin component (factor IX) locus of the paternal X of the *proposita*; this could only have occurred postzygotically, for the twin sister is certainly capable of PTC production, even if at a reduced rate. The mutation could be pictured as happening in an early ancestral cell of the primitive streak from which the *proposita* was to grow; or perhaps later in a cell which was about to differentiate and give rise to a line normally responsible for factor IX production. The mutation rate at the Christmas disease locus has been estimated to be $(0.55 \pm 0.04) \times 10^{-6}$ by Barrai *et al* (1968), but this was the rate in gametes—what the somatic cell rate may be is unknown.

It seems to us that the chance of Lyonization, or the subsequent chance migration of Lyonized cells, can best explain the monozygotic twins, one affected with Christmas disease and the other a carrier. According to an investigation at Birmingham (Edwards, 1968) 3 kinds of monozygotic twins can be distinguished, the result of the following mechanisms: (1) the doubling of blastocyst—28% of all monozygotic twins; (2) the early doubling of embryonic disc (61%), and (3) the late doubling of embryonic disc (11%).

Were the twins of the first type their ancestral cells could have separated after the first mitotic division of the zygote and when both lines were subjected by Lyonization it could have happened that in one, by somewhat rare chance, all the paternal Xs carrying the normal allele were inactivated in the cells growing to form the blastocyst, and later the embryonic disc, of the affected twin. In the other cell line inactivation would probably affect nearer the mean expected 50% paternal and 50% maternal Xs and the observed carrier would result. This is a simple explanation but, according to Edwards (1968) there is only a 28% chance that our twins are of this kind.

As 72% of monozygotic twins are of types 2 and 3 it is more likely that our twins are also of these types. In these instances the chances of Lyonization of paternal or maternal Xs play no part: chance would lie in the migration of cells carrying active or inactive Xs into the primitive streaks and thence into the embryonic discs growing therefrom.

Whichever type of monozygotic twins they may

represent the great difference between them, one having Christmas disease and the other not, can most satisfactorily be attributed to the consequences of Lyonization.

Summary

The case of a 3-year-old girl is presented, who showed severe bleeding tendency and on laboratory investigations was found to have Christmas disease. Her twin sister, who proved to be identical to the proposita on zygosity studies, has never shown any bleeding symptoms. The quantitative factor IX assay, however, showed that the twin sister, as well as the mother and maternal grandmother, are asymptomatic carriers of the disease, while the father is completely healthy. The chromosome complement of the proposita was normal 46,XX. A number of explanations for the occurrence of the disease in this girl have been considered: of these, Lyonization and its consequences are thought to be the most probable.

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