New Molecular Diagnostic Tests for Two Congenital Forms of Anemia

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Congenital hypoplastic anemias are a rare and heterogeneous group of disorders. This paper reviews new molecular diagnostic tests in two distinct forms of congenital anemias, anemia due to transplacental infection with B19 parvovirus and Fanconi anemia. In both instances, molecular assays mak-

ing use of amplification of DNA by the polymerase chain reaction have been used to diagnose either a specific viral infection or gene mutation responsible for a disorder. Recognition of these entities has important prognostic and therapeutic implications. *J. Clin. Lab. Anal.* 11:17–22. © 1997 Wiley-Liss, Inc.

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INTRODUCTION

The purpose of this paper is to review new developments in the molecular diagnosis and classification of the congenital hypoplastic anemias, including Diamond Blackfan anemia and the genetic disorder Fanconi anemia. Anemias existing from birth may have diverse etiologies. We consider only the hypoplastic or aregenerative anemias characterized by failure of the hematopoietic or erythropoietic organ. Traditionally, these disorders have been classified and treated without much regard to the etiologic basis of the disorder. With the identification of specific mutant genes or virus infections responsible for the congenital anemias, diagnostic recognition of specific entities such as Fanconi anemia has gained importance in the application of specific therapies. In addition, the spectrum of disease attributed to viral pathogens such as B19 parvovirus has been broadened to include congenital infections. The use of molecular biologic tools has been critical to this new science.

CONGENITAL ANEMIAS: DIVERSE ETIOLOGIES AND COMMON PATHOPHYSIOLOGIC THEMES

The aregenerative anemias of childhood are a heterogeneous group of disorders, including isolated anemia (pure red cell aplasia, also referred to as Diamond Blackfan anemia, or DBA) and pancytopenia resulting from marrow failure (aplastic anemia and Fanconi anemia) (Table 1). Bone marrow examination of DBA typically reveals erythroid aplasia with sparing of the myeloid and mega-

karyocytic series, whereas the Fanconi anemia bone marrow is usually aplastic or hypoplastic in all lineages. As discussed in greater detail below, the genetic disorder Fanconi anemia has, until recently, been diagnosed exclusively on the basis of chromosomal breakage assays. DBA, on the other hand, is diagnosed on clinical criteria and may have both genetic and acquired etiologies. One shared feature between DBA and Fanconi anemia is the variable association with characteristic physical anomalies involving stature, head and face, eyes, and thumbs, as well as renal and cardiac anomalies. The concurrence of stereotypic physical anomalies (especially abnormal thumb development) and hematopoietic failure in some cases of DBA as well as Fanconi anemia suggests a common pathophysiologic mechanism in the genesis of these two very different disorders. However, Fanconi anemia typically involves multilineage hematopoietic failure and can be diagnosed by chromosomal breakage assays, whereas only erythropoiesis is impaired and chromosome studies are normal in DBA. The etiologies of DBA have, until recently, been unknown. As described below, at least one cause of DBA has now been shown to be intrauterine infection with the B19 parvovirus.

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TABLE 1. Fanconi Anemia and Diamond Blackfan Anemia

Congenital hypoplastic anemia	Fanconi anemia	Diamond Blackfan anemia
Etiology	Autosomal recessive	Autosomal recessive Autosomal dominant Acquired variant (B19 parvovirus)
Physical abnormalities	65% of cases	25% of cases
Predominant hematologic manifestation	Pancytopenia	Isolated anemia
Chromosomes	Increased breakage with clastogens	Normal

CONGENITAL ANEMIA DUE TO PARVOVIRUS B19 INFECTION

Background

The human parvovirus B19 (B19), the only known pathogenic parvovirus, was discovered in 1975. The disease manifestations of infection with parvovirus B19 vary widely with the immunological and hematological status of the host (1). In normal immunocompetent individuals, B19 causes erythema infectiosum (EI), also called fifth disease. EI is an innocuous rash disease of childhood, but parvovirus infection in adults may also be associated with an acute symmetrical polyarthropathy that can mimic acute rheumatoid arthritis and persist for many months. In individuals with underlying hemolytic disorders, B19 is the primary cause of aplastic crisis, a self-limiting cessation of erythropoiesis due to parvovirus cytotoxicity to erythroid progenitor cells. In immunocompromised patients, persistent B19 viremia leads to pure red cell aplasia and chronic anemia, and in the fetus (in which the immune response is immature) infection can result in fetal death in utero or hydrops fetalis.

Recently, congenital anemia was described in three children following maternal B19 infection during the second trimester (2). One patient died within the first year of life with respiratory, wound, and gastrointestinal infection, but had evidence of B19 infection in multiple tissues at autopsy. After immunoglobulin therapy (containing anti-B19 antibodies), the other two patients no longer had detectable virus DNA in the bone marrow but remained persistently anemic.

B19 DNA Assays

The most sensitive assays for B19 virus are based on nucleic acid hybridization. B19 DNA can be detected in serum at the time of transient aplastic crisis using simple dot-blot hybridization, and in situ hybridization has been used to identify B19 DNA within specific cells. However, the detection of DNA alone does not indicate that the cell is permissive for infection (B19 may have been taken up by phagocytosis) or that the DNA is infectious (non infectious DNA can be detected by DNA assays).

The sensitivity level of detection of B19 has been greatly increased by the use of polymerase chain reaction (PCR) amplification. This can be a very useful tool but, as with all

PCR, there is also great propensity for cross-contamination and false positive results. The B19 parvovirus genome encodes a nonstructural protein from the left side and two major structural (capsid) proteins from the right side. The assay used in the diagnosis of congenital B19 infection makes use of specific primers in the nonstructural gene of the virus. DNA from serum or tissues was tested for B19 by single-round PCR with 50 μ mol of primer pair F (nt1490-1520) and I (nt2088-2065). Negative samples were retested by nested PCR, using primer pairs M (nt1419-1435) and L (nt2139-2123), followed by re-amplification of the product with the F and I primers. Following PCR amplification, the DNA product is analyzed by Southern transfer and hybridization with an internal sequence probe (2).

Clinical Features

All patients included in the study had similar intrauterine histories, with maternal B19 infection during pregnancy and the subsequent development of hydrops fetalis. There was no family history of congenital anemia in any of the families. Two of the fetuses were treated with intrauterine blood transfusions, and the third case was delivered at 32 weeks due to fetal distress and the development of hydrops. The infants were all anemic from birth, and all cases were associated with hypogammaglobulinemia.

The first child developed meconium ileus during the perinatal period, requiring removal of a cecal meconium plug, partial ileal resection, and primary re-anastomosis. During the following months she developed recurrent abdominal wound infections, chronic diarrhea and two episodes of pneumonia. She died of respiratory, wound, and gastrointestinal infections at 9 months of age, but treatment with gammaglobulin shortly before her death resulted in marked reticulocytosis. Bone marrow obtained at autopsy showed pure red cell aplasia with giant pronormoblasts, and B19 parvovirus DNA was detected in the bone marrow, kidney, liver, brain, and spleen. The serum was negative for parvovirus B19 DNA, and there were no specific IgM or IgG antibodies to the virus.

In the other two cases, despite severe anemia the children were maintained with regular blood transfusions. The bone marrow appearance was abnormal in both children, with abnormalities of the erythroid lineage but apparently normal

myelopoiesis and megakaryopoiesis. In both children, multiple serum specimens lacked B19 whereas viral DNA was detected in the bone marrow prior to immunoglobulin treatment. After immunoglobulin therapy (and viral clearance), virus could no longer be detected in the marrow although the patients remained persistently anemic.

Implications

These patients all had congenital hypogammaglobulinemia, intrauterine infection with B19 parvovirus, and congenital pure red cell aplasia. Although not proven, congenital parvovirus infection is likely to have been the cause of the patients' congenital anemia. The maternal infection was similar in all the cases, and B19 infection during pregnancy was documented by serology in two cases. All the patients had similar presentations and immunological defects. Bone marrow is the target organ for B19 parvovirus, and B19 DNA was only detectable in the bone marrow from the patients. Bone marrow was also the only organ to demonstrate pathologic changes consistent with B19 infection. Finally, in two patients, reticulocytosis followed the administration of gammaglobulin, consistent with viral clearance by anti-B19 antibodies.

Congenital pure red cell aplasia or DBA is a syndrome of heterogeneous manifestations and etiologies. Most affected children develop anemia by the third month of life. Affected children may have cellular and humoral immune deficiencies (3). The above cases indicate that persistent B19 parvovirus infection should be suspected in infants with DBA exposed to B19 parvovirus during fetal life or with bone marrow findings compatible with B19 parvovirus infection. The primary target organ in congenital B19 parvovirus infection is the bone marrow; serum viremia can be undetectable, and viral antibody studies are not diagnostic. Because of the very low viral concentration in tissues, nucleic acid amplification by PCR may be necessary to establish a diagnosis. Cases of congenital pure red cell aplasia suspected in intrauterine infection with B19 parvovirus should be investigated with PCR amplification of the viral genome from bone marrow prior to therapeutic intervention.

FANCONI ANEMIA

Background

Fanconi anemia (FA) is an autosomal recessive disorder characterized clinically by diverse congenital abnormalities and a predisposition to bone marrow failure and malignancy, particularly acute myelogenous leukemia (AML) (4–6). FA cells exhibit an abnormally high level of baseline chromosomal breakage (7) and are hypersensitive to both the cytotoxic and clastogenic effects of DNA cross-linking agents, such as diepoxybutane (DEB) and mitomycin C (MMC) (8–10). The relationship of this DNA instability to the potential for FA patients to develop cancer is unknown. It is now rec-

ognized that the FA phenotype is so variable, with considerable overlap with that of a variety of genetic and nongenetic diseases, that diagnosis on the basis of clinical manifestations alone is difficult (11,12). Although the primary biochemical defect in the syndrome has not yet been identified, diagnosis is facilitated by a laboratory test measuring the sensitivity of cells to chromosomal breakage induced by DEB (13–16). This unique cellular characteristic can be used to identify the preanemic patient as well as the patient with aplastic anemia or leukemia who may or may not have the classic physical stigmata associated with FA (17).

FA is found in all races and ethnic groups and has been reported to have a carrier frequency of 1:300 (18). The true gene frequency may be considerably higher than this; a low estimate would result from a lack of ascertainment of cases due to the occurrence of extreme phenotypic heterogeneity in the syndrome, leading to difficulty in establishing the clinical diagnosis of FA. Heterozygotes for FA cannot be detected by the DEB test. We suggest that at least 0.5% of the general population is heterozygous for the FA gene.

Clinical heterogeneity in FA is both interfamilial and intrafamilial. There is lack of concordancy for specific congenital malformations among affected siblings, and even monozygotic twins may exhibit phenotypic heterogeneity (19). Extensive genetic heterogeneity has also been reported in FA. At least five different complementation groups have been defined (20,21); the full extent of genetic heterogeneity in FA remains to be determined. Only one of these genes, for complementation group C (FAC) has been cloned (22); this gene, which maps to chromosome 9q22.3 (20), corrects the DNA cross-link hypersensitivity of a complementation group C cell line. The DNA sequence of this gene has no substantial homology with any known gene family. This review highlights recent advances in the molecular diagnosis of FA.

FA Diagnosis and Family Resource

The International Fanconi Anemia Registry (IFAR) was established at The Rockefeller University in 1982 to collect clinical and genetic information from a large number of FA patients. The primary source of case material for the IFAR is voluntary physician reporting. Once a potential case is identified, an IFAR questionnaire form is completed by the referring physician and copies of laboratory reports and other patient records are obtained with the consent of the patient or guardian. Diagnosis of FA is confirmed by a positive DEB test performed on a peripheral blood sample (13,14). DNA is extracted from peripheral blood of patients with positive DEB tests, for the identification of FAC mutations.

SSCP Analysis

Results of our SSCP study of DNA samples from FA patients enrolled in the IFAR indicate that approximately 15% of patients exhibited pathogenic mutations in FAC (23). These

patients can be classified as complementation group (FA-C) on this basis. The specific mutations detected in our study and/or published by others are shown in Table 2 (23,24). In addition to the disease-causing mutations, two variants were found that did not segregate with the disease allele in multiplex families, indicating that they represent polymorphisms. One additional mutation was found in only one family with one affected individual; the possibility that this mutation represents a normal variant cannot be ruled out. The most frequent mutations in FAC that were found in the IFAR population by SSCP were IVS4A→T (IVS4), an A-to-T transversion four bases into the intron 4 donor splice site, and 322delG, a deletion in exon 1 resulting in a frameshift and a premature stop codon. These two mutations were present in 84% of FA-C families. IVS4 is found exclusively in individuals of Jewish ancestry while the ancestry of carriers of 322delG is primarily northern European. Our analysis (23) showed a correlation between genetic and clinical features in FA-C patients; all patients with IVS4 +4 A→T exhibited a severe phenotype with multiple birth defects and early onset of hematologic abnormalities, while 322delG was associated with a milder form of the syndrome.

ARMS Assays

Verlander et al. recently developed amplification refractory mutation system (ARMS) assays (25) for the pathogenic mutations in FAC found in the SSCP study of the IFAR population, as well as other published mutations (23,24). These assays provide a means for rapid, nonradioactive testing and we have applied these assays (a) to assign newly diagnosed FA patients to group C, (b) to provide rapid carrier testing for FA-C families, and (c) to provide prenatal diagnosis for FA-C families. In addition, we have applied these ARMS assays to screen a healthy Jewish population for IVS4 and 322delG, the two most common FA alleles, to determine the frequency of these alleles in this population (25).

Mutation-specific primers anneal to the plus strand and were designed so that the 3' base matched the point of the mutation (Fig. 1). All mutation-specific primers anneal to the plus strand. The second primer for each assay was the forward

Fig. 1. Primer binding in ARMS assays. A. On chromosomes with the mutant allele, the 3' base of the mutation-specific primer anneals to the base changed and the PCR reaction proceeds normally. B. On chromosomes with the normal allele, the 3' base of the mutation-specific primer is mismatched and the PCR reaction is blocked.

primer for the exon containing the mutation. Primers were designed to have a predicted annealing temperature of 56°C. In order to reduce background, a second mismatch was introduced at the third base from the 3' end in some primers, so that two of the last three nucleotides would be mismatched in an individual lacking the mutation. The ARMS assays can be combined in three reactions as shown in Table 3. Assay A includes primers for two common mutations, IVS4 and 322delG; assay B includes primers for Q13X, R185X, and L554P, and assay C includes primers for R548X. Each assay includes a set primers for FAC exon 13 as a positive control for the PCR reaction.

Recent results of ARMS assays show that a total of 47 probands in the IFAR were in group C. The distribution of FAC alleles seen in this study are shown in Table 4. Of 23 probands from the IFAR for whom both parents have Ashkenazi Jewish ancestry, 21 were homozygous for IVS4; the two remaining patients had no detectable FAC mutation. Five additional probands with Ashkenazi ancestry on only one side of their family were also tested; only one carries IVS4, from the Jewish parent. The fact that 100% of FA-C chromosomes of Jewish origin carry the IVS4 mutation is strong evidence for a founder effect.

In order to determine the frequency of IVS4 carriers in the Jewish population, genomic DNA samples ascertained anonymously from 3104 Jewish individuals, primarily of Ashkenazi descent, were obtained from Dor Yeshorim, an organization that provides genetic testing services to the religious Jewish community in New York. DNA samples from 563 Iraqi Jews from Israel were supplied by Dr. Uri Seligsohn, in order to determine whether the IVS4 mutation is present in the

TABLE 2. FAC Mutations and Variants

	Location	Base	Base change	Amino acid
Mutations				
Q13X	Exon 1	292	C→T	Gln→Stop
322 delG	Exon1	322	deletion	frameshift
IVS4	Intron 4	712+4	A→T	splice site
R185X	Exon 6	808	C→T	Arg→Stop
R548X	Exon 14	1897	C→T	Arg→Stop
L554P	Exon 14	1916	T→C	Leu→Pro
Variants				
D195V	Exon 6	839	A→T	Asp→Val
S26F	Exon 1	332	C→T	Ser→Phe
G139E	Exon 4	671	G→A	Gly→Glu

TABLE 3. ARMS Assay Primers^a

Mutation	Primers	Product (bp)
Assay A		
332delG	5'-ACCATTTCCCTTCAGTGCTGG-3' 5'-AAAGTGGAAGCCTGATACA-3'	147
IVS +4 A→T	5'-GTAGGCAATTGTACATAAAAG-3' 5'-AAACATTTCAAAAAGTGATAAATATTAAATTC <u>A</u> -3'	220
Control	5'-CCTAGAAGTATGTCTGTCCTG-3' 5'-CTCTCCTTGACTAGGATGCTG-3'	303
Assay B		
Q13X	5'-ACCATTTCCCTTCAGTGCTGG-3' 5'-AGCTTCTGCATCCAAA <u>A</u> CTA-3'	121
R185X	5'-GTCCTTAATTATGCATGGCTC-3' 5'-AATAAGTGGGACACAAACGCA-3'	111
L554P	5'-GGATAGGGCTTCTTTTCAGGG-3' 5'-CTTCTAGACTTGAGTTCAC <u>G</u> -3'	258
Control	5'-CCTAGAAGTATGTCTGTCCTG-3' 5'-CTCTCCTTGACTAGGATGCTG-3'	303
Assay C		
R548X	5'-GGATAGGGCTTCTTTTCAGGG-3' 5'-CAGCTCTTTAAGGAGCTCGCA-3'	310
Control	5'-CCTAGAAGTATGTCTGTCCTG-3' 5'-CTCTCCTTGACTAGGATGCTG-3'	303

^aBold indicates a base that is mismatched when annealed to the wild-type sequence; underline indicates a base that matches a mutation.

Sephardic Jewish population as well. Thirty-five IVS4 carriers were identified in the Dor Yeshorim population, for a carrier frequency of >1%; no 322delG carriers were found. No carriers for IVS4 were detected in the Iraqi Jewish sample (25) (Table 5).

Prenatal DEB testing can be performed on cells obtained by chorionic villus sampling and by amniocentesis. However, prenatal diagnosis of FA can be difficult, and the ability to support a positive DEB test with a molecular diagnosis is helpful for those families that have been assigned to complementation group C. ARMS assays have been used to confirm a prenatal diagnosis of FA in several cases in which the affected fetus was homozygous for the IVS4 mutation of FAC. Unfortunately, some mutations have yet to be identified; 14 IFAR probands have been assigned to group C based on the identification of a single FAC mutation, while the second mutation remains unknown (Table 4). For these families, linkage analysis may be used to supplement the ARMS assay for molecular diagnosis of FA. FAC has been demonstrated to lie within the 5-cM interval between the microsatellite markers

D9S196/D9S197 and D9S176, and linkage analysis using these markers can be used as an aid to prenatal diagnosis. If the microsatellite markers are sufficiently informative in a given family, identification of a haplotype associated with the unknown mutation can be used to distinguish a carrier from an affected fetus.

Implications

Little is currently known about the genes for the other FA complementation groups. A nuclear localization for the non-group C proteins cannot be ruled out, although FAC has been shown to form a complex in which it is bound to at least three other cytoplasmic proteins (26). The similarity in the phenotype of FA patients from different complementation groups suggests that mutations in a single member of a multisubunit complex could lead to FA, and that perhaps these FAC-associated proteins are the products of genes for the other FA complementation groups. Alternatively, the various FA complementation groups may represent different enzymes in a multistep biochemical pathway.

It is hoped that the elucidation of the function of the FAC gene product and the polypeptides mutated in the other FA complementation groups will lead to more effective treatment for FA patients. One approach that is being explored is gene

TABLE 4. Distribution of FAC alleles

Allele 2	Allele 1				
	Q13X	322delG	IVS4	R548X	L554P
Q13X	1	2			
322delG		1			
IVS4			21		
R185X		2			
R548X		2		4	
Unknown	1	6	1	5	1

TABLE 5. IVS4 Carrier Frequency in Jews

	Carriers	Noncarriers	Total	Frequency
Ashkenazi	35	3,069	3,104	1.1
Iraqi	0	563	563	0

therapy, which would allow a patient's own multipotential hematopoietic progenitor cells to be returned to the body after ex vivo correction by transfection with a normal copy of FA cDNA in a retroviral vector. A clinical trial of gene therapy for FA-C patients is currently being conducted at the NIH Clinical Center (27).

A better understanding of the function of the FAC polypeptide, and the cloning of the genes for the other FA complementation groups should lead to improved understanding of the basic problems of birth defects and cancer predisposition, and the interaction of genetic and epigenetic factors in their pathogenesis.

CONCLUSIONS

This paper describes the use of molecular diagnostic tests for two specific forms of congenital anemias. In both instances, the appropriate ascertainment of either a characteristic viral infection or genetic mutation in a patient suffering from a clinical syndrome has important prognostic and therapeutic consequences.

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NOTED ADDED IN PROOF

Recently, the FAA gene was cloned by both positional cloning and functional complementation (*Nature Genet* 14:320–323 and 324–328, 1996). The 5.5 kb cDNA contains a predicted ORF of 4683 nucleotides encoding a protein of 162 kD. The predicted FAA polypeptide has no homology to known proteins including FAC. In contrast to FAC, FAA contains a putative nuclear localization signal, but actual localization analysis has yet to be performed. Further studies on FAA expression may reveal some information as to the specific function of the FA genes.

REFERENCES

1. Brown KE, Young NS, Liu JM: Molecular, cellular and clinical aspects of parvovirus B19 infection. *Crit Rev Oncol Hematol* 16:1–31, 1994.
2. Brown KE, Green SW, Antunez de Mayolo J, Bellanti JA, Smith SD, Smith TJ, Young NS: Congenital anemia after transplacental B19 parvovirus infection. *Lancet* 343:895–896, 1994.
3. Lipton JM: Congenital pure red cell aplasia. In *Hematology: Basic Principles and Practice*. 2nd ed. R Hoffman, EJ Benz, SJ Shattil, B Furie, HJ Cohen, LE Silberstein, eds. Churchill Livingstone, New York, 1995, p 365.
4. Fanconi G: Familial constitutional panmyelocytopenia, Fanconi's anemia (F.A.). I. Clinical aspects. *Semin Hematol* 4:233–240, 1967.
5. Auerbach AD, Allen RG: Leukemia and preleukemia in Fanconi anemia patients: A review of the literature and report of the International Fanconi Anemia Registry. *Cancer Genet Cytogenet* 51:1–12, 1991.
6. Young NS, Alter BP: Clinical features of Fanconi's anemia. In *Aplastic Anemia Acquired and Inherited*. NS Young, BP Alter, eds. WB Saunders, Philadelphia, 1994, pp 275–324.
7. Schroeder TM, Anschultz F, Knoff A: Spontane Chromosomenaberrationen bei familiärer Panmyelopathie. *Humangenetik* 1:194–196, 1964.
8. Sasaki MS, Tonomura A: A high susceptibility of Fanconi's anemia to chromosome breakage by DNA cross-linking agents. *Cancer Res* 33:1829–1836, 1973.
9. Auerbach AD, Wolman SR: Susceptibility of Fanconi's anaemia fibroblasts to chromosome damage by carcinogens. *Nature* 261:494–496, 1976.
10. Weksberg R, Buchwald B, Sargent P, Thompson MW, Siminovich: Specific cellular defects in patients with Fanconi anemia. *J Cell Physiol* 101:311–324, 1979.
11. Glanz A, Fraser FC: Spectrum of anomalies in Fanconi anaemia. *J Med Genet* 19:412–416, 1982.
12. Giampietro PF, Adler-Brecher B, Verlander PC, Pavlakis SG, Davis JG, Auerbach AD: The need for more accurate and timely diagnosis in Fanconi anemia. A report from the International Fanconi Anemia Registry. *Pediatrics* 91:1116–1120, 1993.
13. Auerbach AD, Adler B, Chaganti RSK: Prenatal and postnatal diagnosis and carrier detection of Fanconi anemia by a cytogenetic method. *Pediatrics* 67:128–135, 1981.
14. Auerbach AD, Rogatko A, Schroeder-Kurth TM: International Fanconi Anemia Registry: Relation of clinical symptoms to diepoxybutane sensitivity. *Blood* 73:391–396, 1989.
15. Auerbach AD, Sagi M, Adler B: Fanconi anemia: Prenatal diagnosis in 30 fetuses at risk. *Pediatrics* 76:794–800, 1985.
16. Auerbach AD, Zhang M, Ghosh R, Pergament E, Verlinsky Y, Nicholas H, Boue J: Clastogen-induced chromosomal breakage as a marker for first trimester prenatal diagnosis of Fanconi anemia. *Hum Genet* 73:86–88, 1986.
17. Auerbach AD: Fanconi anemia diagnosis and the diepoxybutane (DEB) test. (Editorial.) *Exp Hematol* 21:731–733, 1993.
18. Swift M: Fanconi's anaemia in the genetics of neoplasia. *Nature* 230:370–373, 1971.
19. Giampietro PF, Verlander PC, Maschan A, Davis JG, Auerbach AD: Fanconi anemia: A model for somatic gene mutation during development. *Am J Med Genet* 52:36–37, 1994.
20. Strathdee CA, Duncan AMV, Buchwald M: Evidence for at least four Fanconi anaemia genes including FACC on chromosome 9. *Nature Genet* 1:196–198, 1992.
21. Joenje H, Lo Ten Foe JR, Oostra AB, van Berkel CGM, Rooimans MA, Schroeder-Kurth TM, Wegner R-D, Gille JJP, Buchwald M, Arwert F: Classification of Fanconi anemia patients by complementation analysis: Evidence for a fifth genetic subtype. *Blood* 86:2156–2160, 1995.
22. Strathdee CA, Gavish H, Shannon WR, Buchwald M: Cloning of cDNAs for Fanconi's anaemia by functional complementation. *Nature* 356:763–767, 1992.
23. Verlander PC, Lin JD, Udono MU, Zhang Q, Gibson RA, Mathew CG, Auerbach AD: Mutation analysis of the Fanconi anemia gene FACC. *Am J Hum Genet* 54:595–601, 1994.
24. Murer-Orlando M, Llerena JC, Birjandi F, Gibson RA, Mathew CG: FACC gene mutations and early prenatal diagnosis of Fanconi's anemia. *Lancet* 342:686, 1993.
25. Verlander PC, Kaporis A, Liu Q, Zhang Q, Seligsohn U, Auerbach AD: Carrier frequency of the IVS4 +4 A T mutation of the Fanconi anemia gene FAC in the Ashkenazi Jewish population. *Blood* 86:4034–4038, 1995.
26. Youssoufian H, Auerbach AD, Verlander PC, Steimle V, Mach B: Identification of cytosolic proteins that bind to the Fanconi anemia polypeptide FACC in vitro. Evidence for a multimeric complex. *J Biol Chem* 270:9876–9882, 1995.
27. Walsh CE, Grompe M, Vanin E, Buchwald M, Young NS, Nienhuis AW, Liu JM: A functionally active retrovirus vector for gene therapy in Fanconi anemia group C. *Blood* 84:453–459, 1994.