FANCONI ANEMIA

A Handbook for Families and Their Physicians

Third Edition, 2000

Lynn and Dave Frohnmayer

Fanconi Anemia
Research Fund, Inc.

Fanconi Anemia: A Handbook for Families and Their Physicians

by Lynn and Dave Frohnmayer
Editing, design, layout, and cover design: Joyce Owen

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Fanconi Anemia Research Fund, Inc.
1801 Willamette Street, Suite 200
Eugene, Oregon 97401
phone: 541-687-4658 or 800-828-4891 (US only)
fax: 541-687-0548
e-mail: info@fanconi.org
website: http://fanconi.org

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Blanche P. Alter, MD
Formerly, Children's Hospital,
University of Texas Medical Branch
Galveston, Texas

Arleen D. Auerbach, PhD
The Rockefeller University
New York, New York

Grover C. Bagby, Jr., MD
Oregon Health Sciences University
Portland, Oregon

John Hansen, MD
University of Washington and
Fred Hutchinson Cancer Research Center
Seattle, Washington

Joyce L. Owen, PhD
Director Emeritus
Fanconi Anemia Research Fund, Inc.
Eugene, Oregon

Nasrollah T. Shahidi, MD
University of Wisconsin Medical School
Madison, Wisconsin

Fanconi Anemia
Research Fund, Inc.

The Fanconi Anemia Research Fund, Inc. was founded in 1989 to provide support to FA families and to raise money for scientific research. The Fund publishes a newsletter twice a year, sponsors an annual family meeting, and provides telephone, e-mail, and letter support to FA families. To aid research into FA, the Fund gives grants to scientists and sponsors an annual scientific symposium.

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Kevin Shannon, MD

Table of Contents

Appendices

Introduction

This Handbook gives basic information, in plain language, about understanding and coping with a serious disorder known as Fanconi anemia, or FA.

The initial FA diagnosis is always a shock. Once it has been absorbed, families of FA patients and their friends—and even physicians—may search for months for trustworthy information. Families also react emotionally to the grim prognosis. They feel understandable anger, denial, grief, unfounded guilt or withdrawal. As parents of FA children, we personally know each of these reactions.

This Handbook answers common questions in simple terms. We include more technical information in a number of appendices. We are deeply grateful for the experience of researchers, treating physicians and other families in our worldwide support group.

Many medical words—especially those with Latin and Greek origins—confuse families. Complicated words may be a real barrier to your understanding of FA and your discussions with your doctors. Our text defines many confusing terms. We also include a glossary of medical and scientific terms you may see or hear (Appendix Y). Words in **bold** type are defined in this glossary.

This Handbook is the result of many hours of research and consultation, and years of experience. It is written for lay people by lay people. We are not doctors, but we follow progress in FA-related science on a daily basis. *All medical information included here should always be reviewed with your own treating physician*. If you have questions relating to treatment or prognosis, please raise these issues with your doctor or with an appropriate specialist. The Fanconi Anemia Research Fund recently published *Fanconi Anemia: Standards for Clinical Care*, a handbook for treating physicians. Copies are available from the FARF office.

Do not be surprised if world-famous experts disagree. You may read or hear conflicting medical advice. That problem is common even with well-known illnesses. But FA is a rare orphan disease. The biological processes that threaten FA patients are poorly understood. Doctors should welcome your questions. Many might encourage you to get a second opinion.

We know how serious this disorder is. FA has claimed too many lives. We make no exaggerated promises, and we create no false hopes of instant cure. But progress in medical science relating to FA has been very rapid and encouraging in the last decade. Even as we write, new developments in genetic research and improved therapies may make our information out of date. We welcome that progress in every way.

This Handbook is dedicated to the memory of special children and young adults—claimed by FA so unfairly before reaching their full potential. We also dedicate this manual to the physicians and researchers who are working to understand, treat, and cure this hateful affliction.

We hope that this Handbook helps you in your own family struggle. Join us, if you can, in active efforts to seek effective treatments, to obtain funding for medical research, and to find a cure.

Dave and Lynn Frohnmayer
March, 2000

Chapter 1

Definition, Characteristics, and Diagnosis of Fanconi Anemia

What is Fanconi Anemia?

Fanconi anemia (FA) was first described by a Swiss pediatrician, Guido Fanconi. In 1927, Dr. Fanconi published his clinical observations on brothers who had inherited various abnormal physical conditions and who also experienced bone marrow failure. These children suffered severe life-threatening **aplastic anemia**. Their blood systems could not successfully combat infection. In addition, as a result of anemia, they were chronically fatigued. Because their platelet counts were low, they suffered spontaneous bleeding.

Research has established that:

- FA is one of several deadly inherited anemias.
- Both parents must be carriers of a **recessive** FA gene for their child to be born with this disorder. If both parents carry the recessive gene, the chances are one in four that any of their children will inherit the disease. Scientists call this pattern of inheritance "autosomal recessive" (See Appendix F).
- FA patients may have a variety of noticeable birth defects, ranging from minor to serious. These defects may affect every major system of the body. Other FA patients are free from any visible disorder—other than ultimate bone marrow failure.
- FA patients experience a high incidence of leukemia (18%—20%).

- FA patients have a much higher incidence of cancer than the general population.
- The chromosomes in the cells of FA patients, when studied in the lab, break and rearrange easily. Scientists do not yet understand the reason for this chromosome breakage, but can use it as a diagnostic test for the disease.

Studying the FA genes and the proteins they produce should help scientists understand the basic defect in FA.

Is Fanconi Anemia the Same as Fanconi Syndrome?

No. “Fanconi syndrome” is a rare and serious disorder of kidney function, occurring mainly in childhood. In this syndrome, several important nutrients and chemicals are lost in the urine. This leads to failure to thrive, stunted growth, and bone disorders, such as rickets.

FA patients may be born with abnormal kidneys and may experience growth problems, but the treatment of Fanconi anemia is very different from that for Fanconi syndrome. The two disorders should not be confused with each other.

How is Fanconi Anemia Related to Other Types of Aplastic Anemia?

Scientists divide aplastic anemia into two categories: “acquired” and “inherited” (genetic) aplastic anemia. The causes of “acquired” aplastic anemia may be exposure to excessive radiation, toxic chemicals, certain drugs, infections or a host of agents in the environment that damage the bone marrow. In many cases of acquired aplastic anemia, the specific cause is never discovered. These cases are known as “idiopathic aplastic anemia.”

Fanconi anemia is an “inherited” anemia. It is one of several rare genetic conditions that lead to aplastic anemia.

No one has yet explained why FA patients develop bone marrow failure. Understanding can come only after the FA genes have been isolated and studied. However, scientific studies show that almost all FA patients will eventually experience marrow failure.

Some scientists believe that the interaction between toxic environmental factors and an FA patient’s genetic vulnerability to marrow failure may contribute to aplastic anemia. See Appendix D for suggestions on how to reduce exposure to these toxins.

How is FA Diagnosed?

Scientists believe that Fanconi anemia is underdiagnosed. The reason is obvious: FA makes its first appearance in different ways. Some babies are diagnosed at birth. Other children may grow into adulthood before discovering that they are affected by FA. Some FA patients undoubtedly are never correctly diagnosed. Efforts are underway to educate doctors in various medical specialties about the kinds of symptoms and signs that may indicate FA.

1. Chromosome breakage and other tests

Presently the most common test for FA is to take a small sample of blood from the patient and combine the blood’s **lymphocytes** (a type of white cell) with chemical agents such as **diepoxybutane** (**DEB**) or **mitomycin C** (**MMC**). In the laboratory, the chromosomes within FA cells break and rearrange under the influence of these destructive agents; the chromosomes in normal cells are more stable. If the results of the lymphocyte breakage test are negative, but the patient exhibits other symptoms of FA, skin cells (fibroblasts) should be tested. Some patients' blood contains a mixture of normal cells and FA cells. See Appendix M on mosaicism.

A procedure called "cytometric flow analysis" tests for a certain abnormality in the cell cycle. This abnormality is found in FA cells, but not in normal cells.

Scientists can also screen the patient's cells for particular FA mutations, in cases where the disease gene has been discovered.

Highly skilled scientists in properly-equipped laboratories can confirm suspected cases of FA by using these tests. It is absolutely essential to establish an FA diagnosis based on these tests, because the clinical features of many different diseases closely resemble FA.

"There's still a lot we don't know."

from the Rotarian Magazine, December 1988

One or more of these tests should also be performed on the siblings of an FA patient. Even normal-appearing brothers and sisters may also have the condition. If your family is considering a bone marrow transplant from a family member, it is *crucial* to test for chromosome breakage in potential donors as well as testing for matching tissue types.

FA can be diagnosed even before a child is born. The diagnosis can be made based on a **chorionic villus sampling** (**CVS**), performed in the 10th to 12th week of a pregnancy, or by **amniocentesis**, performed most often in the 15th to 17th week of pregnancy. See Appendix G.

2. Existence of birth defects

Birth defects are found in the majority of FA patients. These defects can involve any system of the body. The defects or "anomalies" are sometimes many in number, or very few. There seems to be no predictability about the types of anomalies, even within families where more than one child is an FA patient. Since the clinical variety of these features is so great, doctors often refer to the "heterogeneous" nature of Fanconi anemia.

Among the more common birth defect problems or features are the following:

- *Short stature:*

This feature is common and very striking. One expert has concluded that over 50% of FA patients are below the third percentile in height.

- *Anomalies of the thumb and arm:*

FA is often suspected when a child is born with missing, misshapen or extra thumbs, or an incompletely developed or missing arm bone, the radius. These conditions in the scientific literature are described as "absent, hypoplastic, supernumerary or bifid thumbs" and "hypoplastic or absent radii."

- *Additional skeletal abnormalities:*

About one-fifth of FA patients suffer from a wide range of skeletal defects, such as congenital hip abnormalities, spinal malformations, scoliosis, and rib abnormalities.

- *Kidney (renal) problems:*

Some FA patients are born with a missing kidney, rotated or misshapen kidneys or fused (joined) kidneys. Approximately one-fourth of FA patients have these problems, which the literature refers to as “structural renal malformations.”

- *Skin discoloration:*

Many FA patients develop *café-au-lait* spots, which are patches (larger than freckles) of darker discoloration on the skin. Or the entire body or large portions of the body may have a suntanned appearance, a condition called “hyperpigmentation.”

- *Small head or eyes:*

FA patients may have a small head or eyes, characteristics that the literature calls “microcephaly” and “microphthalmia.”

- *Mental retardation:*

Some FA children are retarded, although this is by no means as common as some of the early literature on FA suggested. However, learning disabilities without retardation may be common.

- *Low birth weight and “failure to thrive”:*

Some cases of FA are detected after parents seek medical advice because their child is born with a low birth weight or does not grow and develop as expected.

- *Abnormalities of the gastrointestinal tract:*

Some FA patients are born requiring immediate surgery to correct serious problems of the stomach, esophagus or intestinal tract. Experts report that a very large number of FA patients with no observable internal defects often experience problems with their digestive systems, including poor appetite. See Appendix N.

- *Heart defects:*

Some FA patients are born with heart defects, usually in the tissues separating chambers of the heart.

This is an incomplete list of birth defects that helps establish the diagnosis of FA. Every cell and every organ of the FA patient may be missing the contribution of an essential gene function. As a consequence, this illness may affect many different systems in the body.

3. Later problems that may affect FA patients

- *Sexual anomalies in FA patients:*

Female FA patients often have a delay in the start of menstrual periods, irregular periods, and a decrease in fertility. Menopause occurs early, often in the 30s. Male FA patients often have undeveloped male organs (hypogonadism), and may have decreased sperm production and fertility. See Appendix Q.

- Solid tumor malignancies:

FA patients, especially those over the age of 20, are at high risk of developing cancers of the head, neck, and esophagus. Women are at high risk of developing cancers of the reproductive tract. See Appendices R and S.

4. Diagnosis through appearance of aplastic anemia or bone marrow failure

In a great many patients, the first sign of FA is the appearance of aplastic anemia, a condition in which the bone marrow does not produce enough red cells, white cells or platelets to protect the body and allow the patient to thrive.

5. Diagnosis through the appearance of myelodysplasia or leukemia

In a small number of cases, the presence of FA was first detected when the patient developed a **myelodysplastic** syndrome. This diagnosis, made by examination of the bone marrow, describes an abnormal production, maturation, and appearance of blood cells, which often leads to a deficiency of red cells, white cells and platelets. Sometimes myelodysplastic syndromes progress to leukemia. A few patients have been diagnosed with FA after first developing **acute myelogenous leukemia** (**AML**).

6. Diagnosis of FA through diagnosis of an affected sibling.

The diagnosis of FA in any patient should lead to testing of brothers and sisters. Those tests may in some cases reveal the chromosome breakage that demonstrates FA, even if the sibling presently is healthy, has no birth defects, and has normal blood counts.

How is Aplastic Anemia Discovered in FA? Why is it Dangerous?

The function of healthy bone marrow

The central portion of bones is filled with a spongy red tissue called bone marrow. The marrow is the site of our body's blood production. Marrow daily produces millions of blood cells that sustain our lives.

The bone marrow harbors or nourishes **stem cells**, which divide and evolve into mature red cells, white cells, and platelets. This process of formation and development of blood cells is called **hematopoiesis**.

Each type of blood cell performs an essential role. Red cells (**erythrocytes**) carry oxygen from the lungs to all areas of the body. White cells (**leukocytes**) help fight infection and disease by attacking and destroying germs. Platelets (**thrombocytes**) help heal wounds and control bleeding by forming blood clots in areas of injury. They also prevent spontaneous internal bleeding.

Stem cells interact with a family of cells in the bone marrow, called **stromal** cells, to provide a continuing supply of new blood cells. (Scientists sometimes refer to this process as the interaction between the "seeds," or stem cells and the "soil," or **stroma**.) New blood production is essential throughout our lifetime. A red cell lives approximately 120 days, platelets live ten days, and some types of white cells live only one day or less. Normally, bone marrow produces the right amounts of all these cells as the body needs them.

Bone Marrow Failure

When normal blood cell production declines because the marrow no longer functions properly in the FA patient, a number of serious conditions can appear, separately or together. These are:

Anemia: When the body lacks adequate oxygen-carrying red cells, the patient experiences weakness, fatigue, shortness of breath, and a visibly pale appearance. The red cell deficiency is known as **anemia**.

Infection: When the body lacks adequate numbers of infection-fighting white blood cells, the patient can be extremely vulnerable to common germs. Fever may be the first sign of a serious infection. The medical term for a low white blood count is **leukopenia**. FA patients are often deficient in a particular class of white blood cells called neutrophils, which are needed to fight bacterial infections. This condition is called **neutropenia**.

Bleeding: Hemorrhage-fighting **platelets** help stop bleeding from wounds. Abnormally low platelet counts lead to easy bruising and sometimes to internal bleeding that can be fatal. A low platelet count is sometimes discovered by the appearance of **petechiae**. These are small red spots that result from spontaneous bleeding in tiny blood vessels under the skin.

The medical term for an abnormally low platelet count is **thrombocytopenia**. In many FA patients, the first of the three major types of blood cells (or lineages) to show a decline in counts is the platelets. When abnormally low counts exist in all three major lineages of blood cells, the condition is described as **pancytopenia**. Another way to describe this condition is **aplastic anemia**.

Other factors that doctors consider

Some doctors look for and measure additional signs of abnormality in FA blood cells. The scientific literature reports many cases where the red cells of FA patients are unusually large (**macrocytic**). Scientists who study FA also note that tests of patients may show large numbers of the kinds of red cells that typically would be found in newborn infants (elevated fetal hemoglobin).

Bone marrow aspiration and biopsy

In making the original diagnosis of aplastic anemia, doctors typically do not rely on blood counts alone. They also use a **bone marrow aspiration** and **bone marrow biopsy** to help with the diagnosis.

Bone marrow aspiration can be a painful procedure. It is eased by local anesthesia. Increasingly, medical centers are using strong sedatives or short-term general anesthesia to eliminate any discomfort. The aspiration involves inserting a sturdy needle into the large pelvic bone of the patient. A small sample of marrow is removed and examined under a microscope. In cases of severe aplastic anemia, the aspiration will show a great reduction of the number of blood-producing cells in the bone marrow. Bone marrow aspirates are used to examine the types of cells in the marrow, and their chromosomal pattern.

The bone marrow biopsy is a procedure in which a needle is inserted in the bone and a very small piece of bone (a plug) containing marrow is removed. The bone marrow biopsy is very helpful in assessing exactly how many cells are present in the bone marrow. It is also useful in determining if the cells have a normal or abnormal shape or size. This information is helpful in predicting possible progression to leukemia.

Physicians usually recommend an annual bone marrow aspiration and biopsy. If the chromosomes in the marrow cells show an abnormal pattern (a "clonal abnormality") or if the cells appear abnormal, doctors may recommend more frequent bone marrow testing.

What Do We Learn from an FA Patient's Blood Counts?

Blood counts usually are measured by what is called a **CBC** (Complete Blood Count). This test can be done from a "finger stick" or from a sample drawn from a vein. Doctors usually prefer a sample drawn from a vein because it can reflect a more accurate platelet count.

Your doctor will interpret your CBC for you. The typical CBC will reveal the count for red cells, white cells, and platelets. The CBC will also show the percentage of the different kinds of white cells that make up the white cell count. Different kinds of white cells perform different functions. **Granulocytes** (neutrophils) mainly fight bacterial infections, but also play a role in controlling fungal infections. **Lymphocytes** are crucial in the eradication of both fungal and viral infections.

An important value is the **absolute neutrophil count** (**ANC**). This number is determined by multiplying the percentage of neutrophils (both mature and immature forms) by the total number of white blood cells. Normal neutrophil counts are over 2,000. The absolute neutrophil count should be between 500 and 1,000 to fight a bacterial infection adequately.

Your doctor may also look at measurements in the CBC that show the size of certain cells and the number of new developing cells. Some of this additional information may be very important in deciding when and how to treat aspects of this disorder.

Many parents have noticed that a bacterial or viral infection can cause a considerable drop in a child's blood counts. Very often, the counts will return to the previous level weeks or even months after the infection has passed. Because infections can be destructive to the FA patient's vulnerable bone marrow, many physicians treat infections early and aggressively. In addition to the usual childhood imunizations, many physicians recommend vaccination to prevent chicken pox, which is devastating to the bone marrow of FA patients, and hepatitis B, because FA patients are likely to need eventual blood transfusions. This is a topic to discuss with your own physician.

Dr. Guido Fanconi with Andrea Lee Kuritzky
Children's Hospital, Los Angeles, 1959

When Does Aplastic Anemia Occur in FA?

No one can predict the age when marrow failure begins in FA patients. The *median* age of onset is approximately 7 years. Most children first experience signs of marrow failure between the ages of 3 and 12. At least 10% of cases were diagnosed after age 16, and one individual was 48 at the time of diagnosis. A few FA patients diagnosed and identified by chromosome breakage tests have had no blood or physical problems into their 30s. Thus, FA is not exclusively a childhood disease.

For many FA patients, blood counts can remain relatively stable over a long period of time, sometimes many years. Remember that any one blood count can be misleading, and that the numbers of cells in different cell lines can go up and down over a period of time. Long-term trends rather than any one CBC will reflect more accurately the status of the FA patient's bone marrow.

What Other Medical Tests Should the FA Patient Undergo? What Reports Should We Collect?

Review this question with your treating doctor. Every FA patient is different. New therapies on the horizon might well change current medical advice. We suggest three important approaches:

First, you should develop a **baseline** medical evaluation of your child. Dr. Ellis Neufeld of Harvard Medical School has offered a helpful and complete checklist which you can review with your treating physician. See Appendix A.

Second, before undergoing any medical procedure or consulting with a new specialist, FA families have found it very helpful to ask their doctor to prepare a brief and readable up-to-date summary of the treatment of their child (vital statistics, CBC results, specialists' reports, surgeries, hospitalizations, etc.) Insist on this diagnostic and treatment summary, if you can. Otherwise, when you see specialists, you will be forced into time-consuming and potentially error-prone recitals of medical information.

Third, siblings and immediate relatives of the patient should be evaluated as potential bone marrow donors. Families without a matched sibling donor should consider a search for an unrelated donor under the guidance of a doctor or transplant center.

Abnormal Clones

FA patients often develop abnormal "clones" that can be detected in studies of their bone marrow aspirations. A clonal abnormality is a change in the structure or in the number of the patient's chromosomes in certain cells of the bone marrow.

Researchers and treating physicians do not agree about the significance of a clonal abnormality in FA patients. Some observe that a clone can disappear, or that sometimes it is replaced by a different abnormal clone. Many FA patients with abnormal clones have remained stable for years, and have not progressed to leukemia. On the other hand, a clone is sometimes the first step in a progression to myelodysplasia or AML.

Most researchers agree that an abnormal clone, or the appearance of multiple clones, may indicate a more aggressive phase in a patient's disease. These developments suggest that more aggressive treatment or more frequent monitoring is needed.

Dr. Richard Harris offers helpful suggestions on monitoring the progression of a clone and guidelines for transplantation in Appendix J. Even in those cases when transplantation is not yet considered appropriate, we recommend initiating a search for a suitable donor immediately upon discovery of the abnormal clone. The search can take months, and in some cases disease progression is rapid.

What is the Prognosis for an FA Patient?

No one is certain how long any given individual with FA will survive. This illness is unpredictable. According to cases reported to the International Fanconi Anemia Registry, the average life expectancy is approximately 22 years. *But life expectancy for any one individual can be quite different from any "average."*

Obviously, this statistic cannot account for recent medical advances. New research discoveries, supported in part by the Fanconi Anemia Research Fund, have led to life-extending treatments and improved bone marrow transplant outcomes. Research continues to address these important issues. As FA patients live longer, more will develop solid tumor malignancies. Scientific studies must address this crucial concern. There is still much to learn about the basic defect in FA patients.

Could an FA Patient Ever Become Pregnant or Father a Child?

The literature reports that at least 110 FA females have reached age 16 or more, and 15% of this population became pregnant. One study reports a total of 26 pregnancies, resulting in 17 births and 16 surviving, normal children. Most women had decreased blood counts during pregnancy and often required transfusions. However, no one died during pregnancy. Most regained their blood counts following the birth of their baby. Tragically, nine mothers died later from FA complications (seven had cancer).

Fertility appears to be reduced in males with FA. The literature reports only three FA men who are fathers.

Chapter 2

Treatments for Fanconi Anemia

What treatments exist for the bone marrow complications of FA? There is no simple answer to this question. For the acutely ill, "supportive care" has been steadily improving in its life-extending potential. Transfusion therapy, antibiotics, and hospital care may be effective in the short run.

Longer-range therapies fall into four categories: bone marrow transplantation, androgen therapy, synthetic "growth factors," and gene therapy.

Bone Marrow Transplantation for FA Patients

A successful bone marrow or stem cell transplant can correct problems related to the bone marrow (anemia, neutropenia, thrombocytopenia, myelodysplasia or leukemia). An FA patient remains at high risk for later development of solid tumors, and can still suffer from problems related to other organs or systems of the body. Recently, the success rate for FA patients fortunate enough to have an **HLA** (human leukocyte antigen) matched sibling has been quite good. Since 1994, 2-year survival has been over 80%. For a more complete explanation of HLA tissue typing, see Appendix I.

Prior to transplantation, a patient's own bone marrow must be destroyed or suppressed to allow new, healthy bone marrow to grow. The existing immune system must be suppressed to avoid graft rejection. A conditioning regimen is used to prepare the patient for transplantation.

The body tissues of FA patients tend to be very sensitive to the radiation and drugs used in the transplant conditioning regimen. Patients with matched sibling donors and uncomplicated aplastic anemia should receive modified (reduced) doses of these conditioning agents. Patients with myelodysplasia and excess **blast** cells may be at risk of post-transplant leukemia. Therefore they may require a more intensive transplant conditioning regimen. This regimen is still greatly reduced from that given to a non-FA patient.

Serious complications can arise in marrow transplantation for FA patients. The likelihood of these complications increases when a bone marrow donor is not a perfect HLA sibling match for the patient. Graft versus host disease (GVHD) occurs when certain lymphocytes (the T cells) from the donor recognize the patient’s cells as foreign and attack them. This attack may result in mild, temporary symptoms, such as skin rash, or in severe, long-term symptoms including multiple organ failure and possibly death. Many transplant centers now remove T cells from the donor marrow before transplantation (a procedure called "T cell depletion"). This greatly reduces the risk of GVHD.

Graft rejection occurs when the patient’s lymphocytes attack the new graft, and prevent engraftment of the transplanted marrow. Transplant centers have recently started using a new drug, fludarabine, to suppress the patient's bone marrow. Early results suggest that this drug dramatically decreases the risk of graft rejection.

The prognosis for a transplant is best for young patients in good clinical condition with uncomplicated aplastic anemia, who have had few or no transfusions. An HLA matched sibling donor increases the likelihood of a successful transplant. Conditions such as myelodysplasia or overt leukemia require more aggressive preparation for bone marrow transplantation, and decrease the likelihood of a successful outcome.

Sanchia Gosztonyi, FA patient (deceased), Basingstoke, England

1. Matched sibling donors

The chances are one in four that another child in the family will have the same tissue type as the affected child. To assist in long-range planning, experts agree that tissue-typing of siblings should be done as soon as possible after the FA diagnosis. The chances are also one in four that another sibling will have FA. That's why it is essential to do diagnostic testing on siblings to make sure that they do not have FA. A family should never agree to proceed with a transplant before making sure that the sibling donor is free from this disease.

Most transplant experts now believe that if the family has a perfectly matched sibling donor, the transplant should be attempted instead of initiating androgen therapy (see below), and preferably before the patient has been given many transfusions. The number and type of transfusions given to an FA patient may have a bearing on the later success of a marrow transplant.

2. Umbilical cord blood transplants

Recent successful experiments in the United States and Europe have demonstrated that umbilical cord blood from a newborn infant can be a source of stem cells for transplantation in an HLA-matched brother or sister who has FA. Tests now exist to determine whether the unborn child is affected by FA and whether the child is an HLA match. The cord blood can be frozen for use at a suitable date.

3. Alternate donor transplants

A number of transplantation centers in the United States and Europe have performed FA bone marrow transplants using unrelated or mismatched related donors. Until recently, results have not been nearly as successful as matched sibling transplants, but new methods appear to improve the outcomes substantially. See Appendix K.

FA patients with unrelated donors or mismatched related donors require more conditioning than those with perfectly matched sibling donors. Dosages still are greatly reduced below those of non-FA patients. If you are considering an unrelated transplant or a mismatched related transplant, *you should confer extensively with medical experts on the latest results.*

A suitable unrelated donor may take many months to find. Therefore, if you, your physician, or a transplant center wishes to consider an unrelated donor transplant, *the ideal time to begin a search is well before a medical crisis that requires this treatment.*

Your physician or transplant center should contact the National Marrow Donor Program (NMDP), 1-800-526-7809. This program has enrolled nearly 4 million partially or totally HLA-typed volunteer donors. It also maintains contact with other registries throughout the world. The NMDP will do an initial computerized search to identify potential donors at no charge. In addition, several umbilical cord blood banks have been established in New York and elsewhere as sources for unrelated donor stem cells.

Numerous transplant centers currently are experimenting with new protocols in attempts to reduce the likelihood and severity of serious complications such as GVHD, graft rejection, and infection. T cell depletion has been effective in reducing or eliminating GVHD; fludarabine appears to be very helpful in preventing graft rejection; and physicians are making new efforts to identify and treat previously undetected infections prior to transplant. Some centers harvest peripheral blood stem cells in an effort to assist engraftment. Centers are trying to modify the conditioning regimen to reduce toxicity. These methods have greatly improved the outcomes of bone marrow transplants.

Experts are not always in agreement about the timing of a bone marrow transplant. Many factors, including the type of donor available, the progression of the patient's disease, and current methods and outcomes must be considered. Parents should confer widely before making this crucial decision.

Dr. Richard Harris, Director, Bone Marrow Transplant Program at Children's Hospital, Cincinnati, has compiled worldwide statistics concerning bone marrow transplantation for FA patients. See Appendix J. Dr. John Wagner, Pediatric Bone Marrow Transplant Program, University of Minnesota, describes the current status and challenges of alternate donor transplants in Appendix K.

Drug Therapy for FA Patients

Between 50% and 75% of FA patients respond to a group of drugs known as **androgens**. Androgens such as oxymetholone (Anadrol®) are artificial male hormones that often stimulate production of one or more types of blood cells for extended periods of time.

Androgens are most effective in improving the red blood cell count. Often they increase platelet production as well. An increase in white cells occurs only in some patients. Androgens prolong the lives of many FA patients, but are not a "cure." Most patients eventually fail to respond to androgens, although some patients experience improved blood counts for many years.

It is not known exactly how androgens work and why they are not successful with every FA patient. Androgens may have serious side effects, which often diminish or disappear if the dose can be lowered significantly. They can cause liver disease and can have masculinizing effects. Use of this drug, dosages, follow-up tests and risks always should be discussed thoroughly with your doctor and other medical experts. See the book *Fanconi Anemia: Standards for Clinical Care.*

Hematopoietic Growth Factors and FA

In the last few years, scientists have identified and manufactured substances known as **hematopoietic growth factors**. Adding these factors—which are already present in the normal body—further stimulates the production of cells that are vital parts of the blood system.

Several growth factors have been used in human trials with FA patients. An early trial suggested that GM-CSF stimulated the white cell count in most FA patients. In 1993-94, eleven FA patients underwent a G-CSF trial. All patients had an increased neutrophil count. Three had an increased platelet count, which was lost when the dose of G-CSF was reduced. Four patients experienced a slight increase in hemoglobin levels. Side effects were minimal.

The 1999 *Fanconi Anemia: Standards for Clinical Care* reports the following conclusions of a group of FA researchers: Patients who have an ANC consistently less than 500/mm^3 or a higher ANC, but significant infectious complications, should be given G-CSF or GM-CSF. Both cytokines have been shown to increase the ANC in patients with FA.

Erythropoietin has been used in some patients who have failed to respond to androgens, but there are no published data on the use of this hematopoietic growth factor in FA. One center reports a 30% response rate using erythropoietin with G-CSF. These data are unpublished, and similar response rates have been reported with G-CSF alone.

In late 1999, one research center reported on the use of interleukin 11 (IL-11) to stimulate platelet production. This growth factor was administered to four FA patients. None had a sufficient response to consider the trial successful. This trial has been discontinued, because the likelihood of patient response was not worth the potential side effects.

Cytokine use is not recommended for patients with a clonal cytogenetic abnormality and should be discontinued if one develops. We do not know the long-term risks of these factors for FA patients, nor their ultimate potential to boost blood production.

The process of obtaining drug company and federal government approval for testing with FA usually has been quite lengthy. Trials in children often must be preceded by trials in adults. Nevertheless, recent progress in getting new products to the trial stage has been encouraging.

The discovery and testing of new hematopoietic growth factors continues. Many experts believe that the combination of two or more growth factors might prove especially effective. Of the few factors in trial to date for FA patients, side effects have been minimal.

We urge interested readers to follow developments reported in the scientific literature or in the *FA Family Newsletter*. On several occasions, scientific investigators have contacted the FA Family Support Group to find FA patients who might meet the design of experimental protocols for these new growth factor trials.

FA Genes and the Potential for Gene Therapy

FA researchers have discovered that although FA patients have much in common clinically, they fall into at least eight different **complementation groups**. From this discovery, scientists conclude that a defect in any one of at least eight different genes can cause the multiple clinical features that we call Fanconi anemia. Scientists are currently investigating how the proteins made by the FA genes interact. Soon we should understand how a defect in any one of these proteins gives rise to the various symptoms of FA.

Each parent must have a defect in the same gene for a child to be affected. There is a one in four chance that any child will be affected if both parents carry the same defective gene. There is a one in two chance that a child will be a "carrier" but not exhibit any symptoms of the disease. And there is a one in four chance that a child will not be a carrier or have the disease. See Appendix F.

How are genes related to chromosomes and the human cell?

Trillions of cells make up the human body. Within each of those cells, 23 pairs of inherited **chromosomes** contain thousands of **genes.** Genes carry the code used by cells to make proteins. These proteins help determine how our bodies look, behave, and cope with life. See Appendix E.

Do we know why FA genes are defective?

When an FA gene is defective, the cells fail to produce a vital protein which is necessary for normal cell function and survival. The exact role of the FA proteins is not known at this time.

Four of the FA genes (for complementation groups A, C, F, and G) have now been isolated. Numerous laboratories are studying the normal FA genes, the normal protein products, and how the proteins or the healthy genes might be introduced into the cells of FA patients. Mutations in the A gene account for about 65% of FA cases; mutations in the C gene account for about 15%; mutations in the G gene account for about 10%, but these percentages vary by population.

What is the status of gene therapy for FA?

We are aware of at least eight laboratories in the United States, Canada, and Europe which are working on gene therapy for FA. Chris Walsh, MD, of the University of North Carolina is currently enrolling patients in clinical trials.

Gene therapy researchers must solve at least three major problems: (1) How can a normal copy of the needed gene be *introduced* into the right type of cells (generally blood stem cells)? (2) How can the new gene be made to *express* itself so that it generates an adequate supply of the missing protein product needed by the FA patient's body and blood system? (3) Can the cells containing the corrected gene *reproduce* in adequate amounts so that their effects on the body are lasting?

As this edition of the *Handbook* goes to press, no final answers to these important concerns are available. Even if the promise of gene therapy is not successful in the short run, greater understanding of the function of the normal FA genes may suggest new drug therapies for FA patients. These drug therapies could correct bone marrow defects. In addition, they have the potential to correct defects in other cells in the body.

A Special Note on Solid Tumor Cancers

FA patients are at high risk for the development of cancers of the mouth, throat, and esophagus, and FA women for cancers of the reproductive tract. These risks are present even in patients who have undergone successful bone marrow transplants.

Cancers of the mouth start out as small ulcers, irritated areas, or whitish or reddish plaques. As we go to press, we are aware of several clinical trials which treat pre-cancerous conditions of the mouth, in an effort to prevent the development of squamous cell carcinoma.

The development of cancer is an area where further research is urgently needed. As more and more FA patients are cured of bone marrow failure, they are living longer, and, thus, are prone to the development of solid tumor malignancies. Patients should be monitored closely for detection of abnormal or early-stage cancerous conditions. Please refer to Appendices O, Q, R, and S.

Chapter 3

Continuing Scientific Study of Fanconi Anemia

How Can I Help Hasten Long-term Scientific Understanding of FA?

There are several ways to hasten scientific progress in understanding this baffling disorder. First, you can help by raising funds for research. Since the incorporation of the Fanconi Anemia Research Fund, Inc. in 1989, concerned families and several foundations have raised over 8.3 million dollars to fund research. Our efforts have supported thirty research laboratories and eleven international scientific workshops. We have also sponsored special workshops on bone marrow transplantation, gene therapy, and clinical care guidelines. FA families should know that their efforts have already made a difference.

Clinical trials of new therapies will continue to accept FA patients, who may benefit from such trials, and scientific understanding of FA will advance. Your physician can help you evaluate the benefits and risks of participating in an experimental trial.

Finally, we are aware of four long-term study projects which benefit by knowing of and studying *every* FA case. *This is not an exclusive listing*. Many major medical centers and researchers may wish to study this illness as it is expressed in *your* family.

1. International Fanconi Anemia Registry (IFAR)

This international registry of FA patients and families is maintained at The Rockefeller University in New York City in conjunction with the laboratory of Arleen D. Auerbach, PhD. The registry includes statistical information and clinical data concerning hundreds of FA patients, and includes cell lines on some patients.

We encourage all families and treating physicians to report diagnosed cases to this registry:

International Fanconi Anemia Registry
c/o Dr Arleen Auerbach
The Rockefeller University
1230 York Avenue
New York NY 10021
(212) 327- 7533

2. Fanconi Anemia Sample Repository at Dana-Farber Cancer Institute and Children's Hospital, Boston

These institutions have just established a new comprehensive Fanconi anemia program, under the direction of Alan D'Andrea, MD, and Eric Nisbet-Brown, MD. Part of this program is the creation of an FA cell repository, including lymphoblastoid and skin fibroblast cell lines, as well as tumor cell lines from FA patients and their families. They have also established an FA patient registry. More information can be found in Appendix U.

Eric Nisbet-Brown, MD or Alan D'Andrea, MD
Dana-Farber Cancer Institute
44 Binney Street
Boston MA 02115
(617) 632-3597 or (617) 632-2080

3. Fanconi Anemia Cell Repository at Oregon Health Sciences University

At the Second International Scientific Meeting on FA held in 1990, scientists concurred that there was no universally accessible FA cell repository, and that such a repository could greatly hasten scientific discovery. Consequently, Markus Grompe, MD, and his colleagues at the Oregon Health Sciences University established a repository of cell lines grown from FA patients and their families. These cells are available to all researchers worldwide. Since these cells will be tested for the effects of different treatments, it is potentially advantageous to each family to contribute cells to the repository.

Information about the cell repository and Grompe's appeal for blood and tissue samples is reprinted in Appendix T. Grompe may be reached as follows:

Fanconi Anemia Cell Repository
Department of Medical and Molecular Genetics
Oregon Health Sciences University
3181 SW Sam Jackson Park Rd, L 103
Portland OR 97201
(503) 494-6888

4. International Collaborative Study on Fanconi Anemia

Hans Joenje, PhD, from The Netherlands, and a group composed primarily of European scientists, are engaged in a collaborative study of Fanconi anemia. They plan to determine the number of FA genes in a large group of European FA families, to determine characteristics of each complementation group at the cellular level, and to isolate and clone new FA genes. Scientists are collaborating with the German, Italian, French and

British FA associations to obtain cells from 100 European patients and their family members. Joenje can be reached as follows:

Institute of Human Genetics
Free University
Van der Boechorststraat 7
NL-1081 BT
Amsterdam, The Netherlands
31-20-444-8270

"The big problem with your illness, Mr. Hawkins, is that nobody famous has caught it yet."

Reprinted by permission of *Punch*

Chapter 4

Coping With Fanconi Anemia

We write this chapter from the vantage point of parents whose child or children have FA. We realize there are adult patients and their families who struggle with many of the same feelings and experiences. We hope this chapter and Appendix W (Support Resources) will be helpful to all who face the challenge of living with FA.

Living with the fact that your child has a potentially life-threatening illness is the most difficult challenge most of us have ever confronted. Your life changes immediately and completely. No longer do you have the luxury of worrying about the things that demand the time and attention of friends and neighbors. Initially, your concerns and energies focus narrowly on the life and health of your child or children.

You probably feel isolated and alone. Maybe you sense that you have little in common with anyone else. Depression, fear, and anxiety about the future are your daily companions. Previously close marriages experience incredible strain, as each spouse copes in his or her own way with this devastating news. Each partner may feel a tremendous need for support, yet have little energy to meet the emotional needs of the other.

What are Common Reactions to the Diagnosis of Fanconi Anemia?

The initial diagnosis of FA causes shock, bewilderment, denial, anger, and feelings of helplessness in the affected family. That is totally understandable. We describe the stages of a family's emotional reaction after diagnosis in Appendix B entitled *Fanconi Anemia: Reactions of Families on the Receiving End*. You may find it helpful.

Many parents also experience intense feelings of guilt and shame over their child's diagnosis. But this is not a time for self-blame. *Every person carries several lethal genes*. Only an unpredictable pattern of inheritance caused this rare event. You had no way to prevent it or know of it, and no reason to apologize for it.

Remember that members of the extended family, such as grandparents, may feel responsible for your child's illness. Just as you should not blame yourself for this diagnosis, others need to know that it is not their fault, either.

What Should the FA Child be Told About the FA Condition and Treatment?

You should consider this issue with care, because there is no "right" answer. The age of the child and his or her interest and ability to understand the diagnosis should be considered. Some young patients are satisfied with basic information and let you know when they no longer want to discuss their illness. Other youngsters want to know much more. Some deeply resent tearful or anxious whispered conferences outside their hearing. Their fears may become even more exaggerated and emotionally destructive when they feel that information is being withheld from them.

As a general rule, we believe that most children need and want to have information about this illness. The subject should be discussed in terms they can understand. Parents and caregivers should answer a child's questions in a supportive, yet direct and honest, fashion. It is helpful to stress the positives as well. Patients need to know that research is moving ahead rapidly and that there will be improved therapies for this disorder. Whenever possible, we must convey our own sense of hopefulness.

When children react to living with this disorder with emotional distress and behavioral problems, a professional counselor can be extremely helpful.

What About the Reactions of Siblings or Other Family Members?

Remember that a serious disorder such as FA *affects the entire family*, not just the patient. Each family member needs his or her own emotional support. Each member of the family may grieve and worry about the FA diagnosis in very different ways: some with tears, some with silence and withdrawal, some with anger. These emotions are all natural. Each person must be allowed to grieve and express feelings in his or her own way.

Each family member needs a good "listening ear" and should be given permission—even encouraged—to express feelings openly. And everyone should look for support systems with friends and community, not just within the family!

What Should the Extended Family be Told?

You must judge this for yourselves. We have disclosed information freely, and gained invaluable emotional support and concrete help from extended family. We recommend that you "take the risk" of disclosure; we think you will be rewarded by family support and understanding.

Where Else Can I Go for Emotional or Other Support?

Families can often take advantage of local resources and support groups. Your physician or hospital should be able to direct you to the appropriate resources in your community. A listing of state or national organizations, phone numbers, and addresses is included in Appendix W.

What Else Can Help in Coping with This Diagnosis?

It is often said, but bears repeating: take care of yourself. Take care of your health (eat well, exercise, avoid destructive habits). Learn to lean on friends and relatives for support, and share your emotions openly, if possible.

Make an effort to enjoy the activities and interests which gave you pleasure before the diagnosis of FA. Try not to make coping with this illness your entire existence (in times of medical crisis, this is impossible). Remember that your spouse and other children need some of your time and attention, too.

If possible, try not to ruin the present with constant worries about the future. Remember that the future is uncertain, that scientific research is advancing rapidly, and that an earlier prediction about your child's future may not prove accurate. Try to maintain a sense of optimism—for yourself and for your family.

Remember that your child is first and foremost a child, who also has FA. Treat your child as you would a child without this diagnosis, to the extent that it is possible. With medical advice and good common sense, allow your child to live as fully and normally as possible.

Finally, we have found it very therapeutic to work on trying to change the course and outcome of this illness. We are not interested in assuming the role of powerless "victim." By playing an active part in the support group and by raising desperately needed funds for research, you may regain some sense of control. You can enjoy the psychological benefits of helping to work on a cure for this devastating illness.

If you would like additional copies of this *Handbook*, issues of the *FA Family Newsletter*, or information about the FA Support Group and Research Fund, contact:

Fanconi Anemia Research Fund, Inc.
1801 Willamette Street, Suite 200
Eugene, OR 97401

Phone:	**(541) 687-4658**
fax:	**(541) 687-0548**
e-mail:	**info@fanconi.org**
website:	**http://fanconi.org**

Appendix A

Medical Review Checklist for FA Families - Suggestions for Baseline Medical Evaluation and Consultant Visits for Patients With FA

by Ellis J. Neufeld, MD, PhD
Director, Clinical Hematology
Children's Hospital, Boston, Massachusetts

As many families are well aware, FA patients are potentially susceptible to many different medical, physical, and developmental problems. Many patients have seen an army of consultants and have accumulated a mountain of test results. This checklist is meant to serve as a reminder of important "baseline" studies and regular consultations.

These studies and consultations are best arranged by the primary physician, who can collect the data in a centralized file and provide copies of results and consultants' suggestions to families and to future consultants. Routine blood counts and many of the other tests may also be done by the primary pediatrician, while a few consultants may be available only at major medical centers.

Baseline Tests

DEB -induced chromosome breakage test:

All brothers and sisters of patients should also be tested. This is the main diagnostic test for FA.

Bone marrow chromosome analysis:

Abnormal clones of cells with similar chromosomal defects may suggest preleukemia. However, this is not always the case in FA patients. Bone marrow chromosome analysis may need to be repeated if blood counts worsen.

HLA typing:

Patients, their siblings and parents should be typed at the time of diagnosis in anticipation of possible bone marrow transplantation. Searching for unrelated donors need not be done at the time of baseline evaluations, but might be considered at a future time. Full blood typing for the patient should also be performed.

Blood chemistry screen:

This should include studies of liver and kidney function, as well as iron status.

Formal hearing testing.

Developmental assessment:

This is particularly important in toddlers or early school-age children.

Ultrasound examination of the kidneys and urinary system.

Complementation group testing:

Either the geneticist or the treating physician should arrange for FA complementation group testing. The genetic defect in cells from FA patients falls into one of at least eight complementation groups, or genes (A through H). Four of these genes have been isolated (A, C, F, and G), and gene therapy trials are underway.

Baseline Consultant Visits

Geneticist

Families with suspected or diagnosed FA should see a trained medical geneticist, who should do the following:

- Perform a complete physical examination of the patient and any siblings.
- Obtain a thorough family history, and document this in a careful pedigree.
- Provide formal genetic counseling about the inheritance of FA and the availability of prenatal diagnosis by DEB testing.

Repeat genetics visits every few years are also a good idea. Physical features develop over time, so some findings of FA, such as pigment changes, may be apparent only later in childhood. Research into the genetic causes of FA is continuing, and new information about diagnosis or treatment may become available over the years.

Hematologist

Every FA child should be closely followed by a pediatric hematologist. If blood counts are normal, visits can be relatively infrequent, but families should establish a relationship with a hematologist in any case. Most pediatric referral hospitals in major medical centers have pediatric hematology consultants, but FA is rare enough that some specialists will never have seen a case. In this event, a second opinion from more experienced hematologists is in order.

Ophthalmologist

A full examination by a pediatric ophthalmologist should be done at the time of diagnosis, with a follow-up as needed for any problems identified.

Endocrinologist

Baseline evaluation should be the rule for every patient. If hormonal abnormalities are present, or if androgen therapy for anemia is considered, follow-up evaluations are also suggested.

Other Specialist Visits

Patients with specific problems may also require baseline assessments and follow-up care from other disciplines.

Hand Surgeon

Serious abnormalities of the thumbs or wrists warrant referral to an expert in surgical reconstruction of the hands. In different institutions, the hand surgeon may be an orthopedist or a plastic surgeon.

Gynecologist

Adult women with FA should be seen regularly. Frequent examinations and pap smears are in order because of increased incidence of gynecologic malignancies.

Urologist/Nephrologist

Abnormalities of the urinary system should be monitored by a urologist/nephrologist.

Notes

1. This list is not all-inclusive. Each specialist will suggest further blood tests, X-rays, or other evaluations.

2. The suggested tests and visits are based on problems in FA which have been reported in the medical literature. It is important to remember that medical journal reports of rare diseases like FA may have an “ascertainment bias” – that is, a tendency to

report cases more severe than average. Thus, a majority of FA patients will have mostly normal baseline studies; obtaining these tests is important nevertheless.

Appendix B

Fanconi Anemia: Reactions of Families on the Receiving End

by Dave and Lynn Frohnmayer
Founders, Fanconi Anemia Support Group
Editors, FA Family Newsletter

This disease doesn't occur very often, but, when it does, it strikes real people. They have names, families, hopes, and plans. Fanconi anemia will affect them profoundly and forever. The experience can be devastating.

1. Denial occurs first. It isn't happening; it can't happen. A lethal childhood illness isn't in our plans. If it is not simply a bad dream or a false diagnosis, there is a quick fix. Medicine—prayer—flight (physical or psychological)—in some combination all might cause the disease to go away.

2. You feel shock. Relief to hear a physician say "It's not leukemia!" is replaced by slowly growing knowledge that this diagnosis could be worse. We are persons accustomed routinely to news reports of medical miracles. Diagnosis of childhood illness is nearly equivalent to promise of cure. Then, painfully, you learn that this life-threatening illness is not yet well understood, and that no therapy promises an easy cure.

3. Helplessness accompanies this news. Your fate is in the hands of medical professionals, experts who themselves do not have answers and sometimes do not agree with one another. Your child is alive, but threatened. Where do you turn?

4. You probably experience profound anger. You are outraged by the visitation of an undeserved and unanticipated lethal condition. There was no warning, and—really—no possibility of one. You had other plans for your life. You still do. How can you be expected to cope?

5. Guilt feelings may be profound, though not always near the surface. You, together with your spouse, unknowingly passed this disease, hidden deeply within your genes, to a blessed innocent child. No matter that it's no one's "fault" and that it cannot routinely be detected by the unsuspecting. The knowledge of genetic responsibility lies deeply in the subconscious, but it can weigh heavily on the soul.

6. Isolation overwhelms almost all FA families. No one you know, usually even experts, has routinely treated (or sometimes even heard of) this orphan disease. No one else exists to share the anguish, the occasional hopes, and the peaks and valleys of crisis after crisis.

Isolation has two other aspects. First, your problems are different in quality and dimension from those of other parents. A friend worries understandably about behavior problems, or frets that her child's grades will not be competitive for entrance to a top college. Your concerns are whether the last platelet count is telling you that the downward spiral has continued, that your child may not live long enough to experience a single year of college. Will there be another birthday, another Christmas? Will health insurance—if there is any—enable you to secure every possible lifeline of hope?

The second aspect is your own potential victimization. You are apart, and people sense it. The wisest of them ask and help, but sometimes even the most sensitive of friends is at a loss for words. You both know it. Without meaning for it to happen, it can wrench you from your community roots and family support.

7. Grief and sadness are a continuing part of your life from the start. We would happily trade places with our children because we've had our chance. There is some completeness in our lives, joys, tragedies, and experience of life-fulfillment.

How cruel that a child bright with talent and promise might not possess the genetic constitution to know and learn of this vast and wonderful world; to make the choices that shape life; and to have at least the fleeting moments of mature reflection we have known. Any parent wants to be his child's companion and share the experiences of a child's maturity. It wrenches. "You cry for them, and you cry for yourself," one FA parent remarked.

But there are helpful ways to cope.

1. Take things one day at a time and one step at a time. One blood count does not necessarily tell a disastrous story. Counts can ebb and flow, sometimes unpredictably.

This disease runs a course, usually a long course (and the more FA is studied, the longer it seems to be). Plan tasks to deal with this illness in small, manageable doses. Do not project tomorrow into today.

2. Fight back. This is the strongest, most helpful attitude and reaction we can advise. Do not yourself become a "victim," even though forces pushing you in that direction seem irresistible.

Ongoing research should lead to a greater understanding of the mechanisms of this disease and to possible therapies. Bone marrow outcomes are improving for all patients. Gene therapy holds hope for the future.

Raising funds to promote research is a positive way to fight back. You can also fight back by learning more.

Increased knowledge helps you to become a strong advocate for the needs of your child.

3. Do not accept the blame or guilt. Every person carries lethal genes. This disease was not predictable, and neither parent is at fault. Marriages have been needlessly damaged because of self-blame or spousal blame.

4. Work with and talk to other FA families. This network, more than anything, can break down isolation and help relieve grief.

5. Retain optimism. Children do this better than adults. Remember that these conditions are hard on all family members, not just those who are ill. Stress and depression can be contagious; the family needs to concentrate on the positives. Set up occasions that reinforce normal family life. To the extent that it's medically advisable, treat your child as a normal child who is capable of enjoying a wide range of activities. Enjoy each and every day to the fullest.

Live with the knowledge that the future is uncertain, but still might be happy. A healthy 12-year-old brother of two FA sisters told his parents: "The girls are doing fine, now. They are enjoying this time. You need to enjoy it, too. Don't ruin the good time by being depressed and fearful about the future. It's not here yet, and you don't know for sure what it will hold."

It's good advice. We try to live by it.

Appendix C

The Physician's Role: A Mother's Perspective

Lynn Frohnmayer, MSW
Co-founder, Fanconi Anemia Reserach Fund

The Physician's Role

A patient's physician is not expected to "treat" the emotional distress of the grieving parents or spouse, although it may be appropriate for the physician to refer the parents or spouse to a support group, grief counselor or other appropriate professional. The power of the patient's physician to affect the emotional state of the caregivers is nonetheless enormous. The physician can play a crucial role in helping the family move from the depths of despair, anger and self-blame into understanding the disease, making and participating in a treatment plan, and maintaining hope.

How Physicians Can Help

Physician Characteristics Which Help
Very few pediatricians or family doctors, and not all hematologists, have had prior experience in treating FA patients. The treating physician needs to be willing to learn, eager to explore current literature and seek out information from experts, and able to invest the time to learn of new therapeutic approaches. It is also helpful if he or she is a caring, warm individual, concerned about the welfare of this patient and the stress the family is experiencing.

Treating physicians must be good at both explaining and listening. They must communicate in a language the family will understand. Physicians need to listen to fears and concerns, and answer questions in understandable terms. It is all right for doctors to admit they don't know all the answers, but they will try to find out.

Maintaining Hope

The treating physician must be honest, straightforward, and frank in discussing the diagnosis of Fanconi anemia. The family needs to know that this is a very serious, life-threatening disorder. False reassurances are not helpful. At the same time, doctors should encourage families to be hopeful. The literature on Fanconi anemia and the dire statistics presented reflect past treatment approaches. Statistics do not include the possibility that bone marrow transplant outcomes will improve, that new methods of gene therapy could change life expectancies, and that future discoveries could improve overall survival rates. Families need to know that scientific discovery concerning this rare disorder has progressed at a very rapid pace over the past few years and that many laboratories are actively pursuing new, hopeful approaches. When appropriate, they need to know that new discoveries could greatly improve the prognosis for their child or spouse.

Depressed parents (and FA parents have reason to be depressed) must work harder than most to be great parents. They can unwittingly create an atmosphere of sadness and worry which permeates every day. As a result, the time a patient has may not be quality time at all. By emphasizing progress and helping to instill hope, physicians can greatly assist in improving the patient's quality of life.

Entering into a Partnership with Families

Family members should be encouraged to educate themselves about this disorder and to play an active role in the treatment plan. Becoming a part of the decision-making process enables many to cope with the anxiety, depression, and loss of control they are experiencing. The relationship between physician and family should be one of mutual respect, shared information, and joint decision-making. Caretakers know the patient well, are aware of subtle or abrupt changes in the patient’s condition, and can be an invaluable source of information.

Family members may need permission to voice their concerns or disagreements. Some are intimidated by medical authority, or fear appearing foolish by asking inappropriate questions. But parents or spouses must live with the result of any medical intervention, so they must understand and agree with decisions. Usually decisions are not clear-cut. Outcomes are unknown and risks are enormous. Parents must believe that the most appropriate decisions were made, given what was known at the time. When parents are ill-informed and have never voiced their questions or concerns, they may forever feel guilty if the outcome is not good.

Being Responsive to Patient Needs

A doctor’s responsiveness and empathy with the patient helps foster a good relationship with other family members. When the physician is warm, caring and concerned about the patient, parents feel positively towards that provider. Whether the patient’s immediate concerns are about pain, nausea, fear, or side effects of treatment, these concerns need to be addressed in a caring manner. Parents are terrified that their child will experience unmanageable pain. It is this writer’s belief that a great deal of pain can be eliminated when pain management is a priority. Bone marrow aspirations and biopsies can be performed under very short-term, total anesthesia, leaving the patient with a painless experience. Bone marrow transplant centers have done this routinely for years. But outpatient clinics, aware of the importance of this issue, can offer the same service. Even though total anesthesia is more costly, and the assistance of an anesthesiologist is mandatory, the children who must experience these procedures on a regular basis should not have to endure unnecessary pain. On very rare occasions, a patient's clinical status makes total anesthesia unusually risky. However, in almost every case in which patients are not provided with total anesthesia, it is because it is not suggested or offered.

Communicating Diagnostic Results in a Timely Way
Much of the distress family members experience occurs while waiting for the results of tests. From a simple CBC to a full-body CAT scan or MRI, parents or spouses wait with excruciating anxiety for results which may tell them if their loved one is doomed to die soon or has dodged a terrible diagnosis. For many, the waiting process is more painful than dealing with the results. Once you know the extent of the problem, you can begin to deal with it. The treating physician should make sure that family members get crucial information as soon as possible. If the news is catastrophic, it is important that the patient’s primary doctor deliver the bad news if at all feasible.

Encourage Normalcy While Remaining Alert to Unusual Symptoms
When appropriate and within prudent medical guidelines, physicians should encourage patients to live as normally as possible. Sometimes it is necessary to

curtail physical activity, but simple measures such as a protective helmet might make normal activities possible. Consideration should always be given to maximizing the quality of a patient’s life.

On the other hand, physicians need to be alert to a wide variety of symptoms which seem unusual, and should be more aggressive in pursuing a diagnosis. For example, physicians should inform patients and their families of changes which might suggest a malignancy, and work together to monitor a patient's clinical status.

Being “There” for a Family When a Patient’s Condition Worsens

When a patient's condition worsens suddenly or when he or she approaches death, a physician should not suddenly withdraw from the family. Many families believe this occurs regularly, and suspect that doctors need to protect themselves from the family’s emotional response and their own feelings of grief. But families desperately need support at this time, and are deeply appreciative when physicians are able to empathize with them during the hardest times.

Attitudes and Behaviors Which Do Not Help

Family members are well aware of physicians' behaviors which have not been helpful to them. The doctor who knows little or nothing about Fanconi anemia and has no time to become informed is not helpful. Doctors who appear cold, distant, and unsympathetic do not gain the family’s confidence. Physicians who speak in complicated medical terms, have little time to answer questions, are rushed or impatient, deal with families in a condescending way, or do not consider the family’s input are not appreciated.

Many parents tell stories of doctors who informed them that their child would probably die within a specific period of time or before reaching a certain age. These comments have devastated parents and have frequently proven to be untrue. Too much is unknown about how any one individual will progress. The positive impact of future therapies is obviously unknown and cannot be addressed in the medical literature available today.

Doctors who are noticeably missing when bad diagnostic news is delivered or who never come to see a dying patient bring additional pain to a grieving family.

The physician with endless time to research an orphan disease and provide ideal patient care may be difficult to find in these times of work overload, HMOs, and pressures from other patients equally in need of quality care. But having dealt with this illness for fifteen years, this writer has experienced enormous variance from one physician to another in terms of ability to work with families burdened with a life-threatening, chronic illness. It behooves families to try to locate physicians who can best meet the patient's physical and emotional needs. It behooves physicians to become more aware of and responsive to the needs of this unique group of families.

Appendix D

Toxic Chemicals to Avoid

by Joyce L. Owen, PhD
Director Emeritus
Fanconi Anemia Research Fund, Inc.
Eugene, Oregon

Many parents have asked for information about toxic chemicals which FA children should avoid, because of their susceptibility to chromosome breakage and leukemia and other cancers. Here are some suggestions:

Tobacco smoke

This contains many carcinogenic (cancer-causing) chemicals, including benzene, formaldehyde, heavy metals, radioactive particles, benzpyrenes, and free radicals. Secondhand smoke increases cancer risk significantly, even in people not affected with FA. Of particular interest to FA families, tobacco smoke increases the incidence of leukemia, a cancer especially prevalent in those with FA. *Do not let anyone smoke in your home or around your child.*

Organic solvents

These include paint thinner, paint remover, gasoline, benzene, wood preservatives (for instance, pentachlorophenol), and cleaning solvents. *They are absorbed through the skin as well as through the lungs*. Many are highly carcinogenic.

Herbicides (weed killers), pesticides (bug killers) and other killers

These are highly toxic; some are carcinogenic, and many are contaminated with small amounts of far more deadly and carcinogenic chemicals (like dioxins). Don't let your child play in an area that has recently been treated (home, field, lawn).

Formaldehyde

Present in tobacco smoke, new foam insulation, new particleboard. Present in any new construction. Especially dangerous in new mobile homes or tightly sealed buildings. Carpets manufactured in the United States no longer contain formaldehyde.

Gasoline

Contains benzene, and is one of the major sources of exposure to benzene by the general public (tobacco smoke is the other major source). Elevated levels of benzene were found in the blood of children who were in the family car when it was being filled with gasoline. Try to fill your tank when your child is not present. If your child is in the car, be sure to close the windows while the tank is being filled. *Never let your child handle gasoline.*

Fumes of all kinds

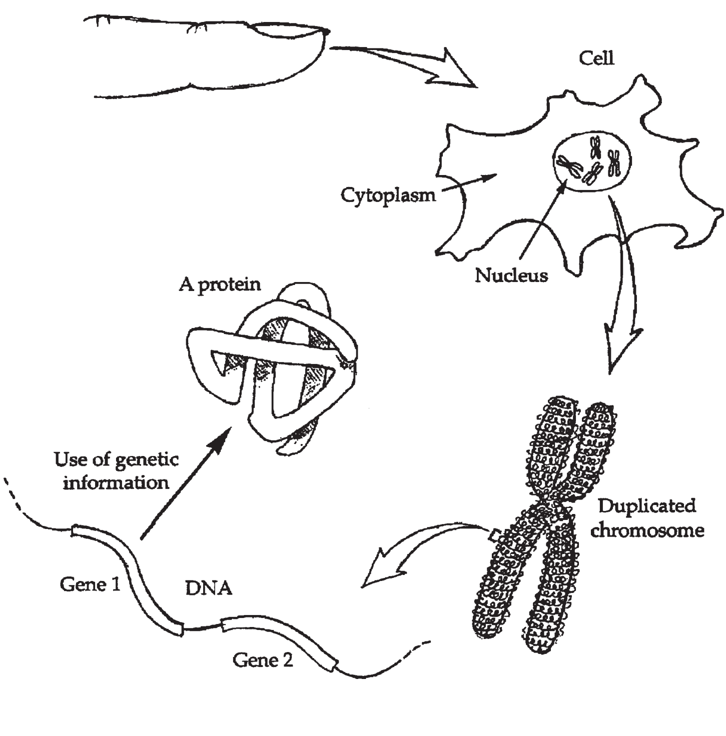
Fumes from automobiles, lawn mowers, boats, snowmobiles, or any gas or oil burning engine are toxic. Burning of almost any organic material (gas, oil, leaves, wood, plastic) produces carcinogens which are easily absorbed by the body.

Appendix E

Cells, Chromosomes and Genes

The human body contains more than 100 trillion cells. Each cell (except the red blood cell) contains the entire human genome—that is, all the genetic information necessary to build a human being. This information is "encoded" in the DNA.

Inside the cell's nucleus, six feet (if unraveled) of DNA are tightly twisted and packed into 23 pairs of chromosomes (one chromosome in each pair comes from each parent).



The 46 human chromosomes are estimated to contain about 100,000 individual genes (current estimates are between 130,000 and 142,000) that determine each person's special inherited characteristics.

Each gene is a segment of double-stranded DNA that holds the information for making a specific molecule, usually a protein. This information (or code) lies in varying sequences of vast numbers of pairs of the four chemical bases that make up the DNA. A change in the sequence (a mutation) or missing sequences of these bases may result in an altered protein that does not work properly, or in failure to produce that protein.

Appendix F

Basic Information on Autosomal Recessive Inheritance

by Sandra Grilliot, MS

[This article is excerpted, with our thanks, from the Spring, 1991 issue of TEXGENE.]

How hard it is to answer a parent's bewildered cry of "but there's no history of it on either side of our families" when a child is diagnosed with a genetic disorder. The sporadic inheritance of most chromosomal anomalies and multifactorial conditions is quickly understood, if not liked, by most parents. But the "hidden gene" concept behind autosomal recessive conditions is less clear to the public. Frequently, parents learn the autosomal recessive risk that affects their family, but are not aware of how this risk was derived. Here, then, is a brief primer of autosomal recessive inheritance.

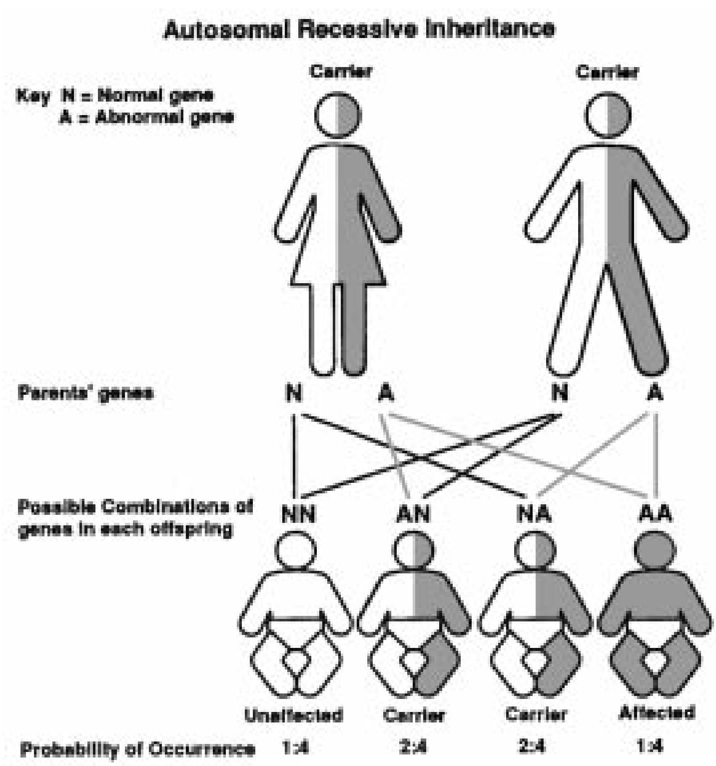
To begin with the basics, the genes are the individual functional units of heredity. Each chromosome is made up of thousands of genes. The two can be thought of together, the genes like beads on a string which form a chromosome. Chromosomes come in pairs and each person has 23 pairs, one member of each pair coming from each parent. The two chromosomes in the pair look very much alike and are formed so that for every gene on one member of the chromosome pair, there is a gene that does the same job on the other chromosome of that pair. Therefore, we have two genes that function together to provide each bit of genetic information. While each of the two genes provides information for the same job, they provide it in different ways. This is the subtle variation that keeps us all a little different from each other even though each of our bodies works in basically the same way.

It is estimated that 50,000 - 100,000 genes make up the 46 chromosomes. Biostatisticians have calculated that among these 100,000 working genes, everyone has 5 - 8 genes which are "non-working", that is, they do not properly relay the information on how to do their job. Fortunately, since we have inherited two genes for almost every job, if one does not work correctly, the other one can often compensate and still get the job done. We call a person who has one working and one non-working gene making up any particular pair a "carrier" for that non-working gene. Because this person's working gene compensates and gets the job done, this person has no health problems related to the non-working gene. But a carrier does need to be aware of his or her status because of the implications it could have for his or her offspring.

If two carriers decide to have a child together, they could each pass on their copy of the non-working gene to the child. This child then does not have a working gene to compensate for the non-working one. Therefore the body has no working gene to do whatever job that gene pair controlled. That job then is not performed properly and a disease state results in the child. Genetic diseases inherited in this autosomal recessive fashion include Tay-Sachs disease, sickle cell anemia, PKU, and cystic fibrosis.

For a couple who each carries a recessive gene, there is a 25 percent (one in four) chance with each pregnancy that the child will inherit the recessive gene from each parent and have the disease. There also then is a 75 percent (three in four) chance with each pregnancy that the child will inherit at least one working gene and so not have the disease.

Autosomal recessive diseases can occur only if both parents carry the same non-working gene. If one parent is a carrier for a non-working gene, but the other parent carries two working genes for the same job, then their offspring are not at risk for the disease since they will always inherit at least one working gene.



The availability of prenatal diagnosis for a carrier couple depends on the disorder involved. Some conditions are not prenatally detectable at this time. Others may be detected either by looking directly for the gene (as in DNA testing for cystic fibrosis) or by looking to see if the gene's job is being done properly by looking for the gene's products (as in biochemical analysis to see if babies at risk for Tay-Sachs disease are making enough Hex A).

There are other prenatal counseling points to which couples should be alerted. First, certain disorders may be diagnosed by certain techniques at certain stages of pregnancy and this may determine the appropriate testing. For instance, if a condition is diagnosed by biochemical analysis of amniotic fluid obtained at 16 weeks gestation then CVS at 10 weeks gestation may not be the test of choice. Second, prenatal diagnosis is usually offered only for the diseases for which a couple is known to be at risk. Most labs do not routinely run tests for specific recessive disorders unless notified in advance that both parents are documented carriers of the non-working gene and are requesting the testing. Therefore, no series of prenatal tests can look for all potential genetic disorders. Finally, the most important point, of course, is that the decision to pursue prenatal diagnosis must be made by each individual couple based upon their own values and beliefs.

Appendix G

Prenatal Diagnosis of FA

Susan Olson, PhD
Oregon Health Sciences University

Prenatal Diagnosis

Prenatal diagnosis is the process of determining whether or not a fetus has any detectable disorders. Currently, there are more than 300 different disorders that can be detected during pregnancy. Some of these are chromosome abnormalities, such as Down syndrome; some are single gene defects, such as Fanconi anemia (FA). These disorders may be diagnosed by examining fetal cells collected through amniocentesis or chorionic villus sampling (CVS) or by removal of a cell from the early embryo. Some structural and growth abnormalities can be detected prenatally with the use of ultrasonography.

Screening for large numbers of disorders at one time is not yet possible. Therefore, prenatal diagnosis is available if there are indications or suspected abnormalities based on family history, maternal age or other significant risk factors.

Genetic Counseling

Genetic counseling is an important part of any prenatal diagnostic experience. A family may have already been through counseling related to the diagnosis of a family member with FA or may have discussed the availability of prenatal diagnosis. However, there are specific issues related to prenatal diagnosis options, risks of procedures, and pregnancy management that should be explored when prenatal diagnosis is the focus of attention.

Ultrasound

Ultrasound examination (ultrasonography) uses certain sound waves to create a picture of the fetus on a television screen. The results of many well-respected studies have shown no ill effects of these sound waves on the fetus or the mother. From the ultrasound image, measurements of the fetus are made to determine the age, by week of the fetus (gestational age). The gestational age is calculated from the beginning date of the last normal menstrual period.

Some major birth defects, such as open spine (spina bifida) and hand and limb defects, may also be detected. Following a pregnancy by ultrasound over time allows monitoring of appropriate growth.

A special type of ultrasound procedure (fetal echocardiography) provides images of the heart. This examination is available to families who are at risk for certain types of heart defects or for fetuses who have inconclusive heart examinations on routine ultrasonography.

An early ultrasound examination is offered to all pregnant women from 11 through 13 weeks gestation to measure the width of the fluid filled space behind the fetus's neck (nuchal translucency). This measurement is used as a screening test to assess the risk for certain chromosome abnormalities, such as Down syndrome. Because it is only a screen, an abnormal result simply means there is a higher than normal chance for a chromosome problem. Further testing is required to make the specific diagnosis.

Amniocentesis

Amniocentesis involves withdrawing a small amount (about one ounce) of the fluid surrounding the fetus (amniotic fluid) by means of a thin needle inserted through the abdominal and uterine walls and through the amniotic sac. During amniocentesis, the fetus and the amniotic fluid are visualized by ultrasonography to guide the needle placement. Amniocentesis is generally performed between 14 and 16 weeks gestational age.

Amniotic fluid contains loose cells shed by the skin of the fetus and eliminated in the fetal urine. These living cells are grown into cultures sufficiently large for diagnostic tests. Chromosomes are analyzed and chemical assays and DNA studies are performed as indicated.

Chorionic Villus Sampling (CVS)

Chorionic villus sampling (CVS) is carried out by passing a thin plastic catheter through the opening of the cervix (opening of the uterus) or by inserting a thin needle through the abdominal and uterine walls. Cells are withdrawn from a specific part of the placenta. Ultrasonography is used to guide placement of the catheter or needle. CVS is performed between 10 and 12 weeks gestation.

The cells removed are contained in structures called chorionic villi that are genetically representative of the fetus. The villi are either processed immediately or grown in culture, depending upon the type of genetic test to be performed.

Preimplantation Genetic Diagnosis (PGD)

A newer method of diagnosis involves sampling one or two cells from an embryo soon after fertilization. The process of preimplantation genetic diagnosis (PGD) requires the use of assisted reproductive technologies (ARTs). Following *in vitro* fertilization, the early fertilized egg may have one or more of the extruded polar bodies examined to assess the genetic content of the maturing egg. In another approach, embryos are grown in culture until they reach the 6-10 cell stage on day 3 post-insemination. One or two cells are then removed from these embryos. Following biopsy, the embryos are kept in culture until they reach a more mature stage on day 5 or 6.

The cell or cells removed from the fertilized egg or embryo are analyzed for chromosome or DNA defects. Only embryos without the specific disorder are transferred back to the mother. Traditional prenatal diagnosis by amniocentesis or chorionic villus sampling is still recommended to confirm the results of preimplantation genetic diagnosis. Only families with known FA mutations are candidates for preimplantation diagnosis of FA.

Risks

Amniocentesis and chorionic villus sampling are relatively safe procedures. There is, however, a slight possibility that a miscarriage or other complication will occur after the procedure. Currently, the risks are believed to be low enough to warrant their use for patients who are at risk for children with genetic disease.

Preimplantation genetic diagnosis is still very new and assessment of risks to the embryo is ongoing. So far, there has not been an increase in babies born with birth defects following this procedure nor has there been a predominance of any specific type of birth defect.

Laboratory Tests

Chromosomal Breakage Analysis
The standard for diagnosis of FA has been chromosome breakage analysis. The test involves the exposure of cells to DNA damaging agents, in particular mitomycin C (MMC) and diepoxybutane (DEB). The diagnosis is made after observation of increased chromosomal breakage and radial formations over normal controls. Since this type of analysis requires visualizing chromosomes, it is also possible to determine whether the fetus has a chromosome abnormality, such as Down syndrome.

DNA Diagnosis
With the identification and characterization of genes for each complementation group will come the ability to diagnose an embryo or fetus with the specific genetic defect resulting in FA. If the specific gene defect is known in a family, DNA can be extracted from amniotic fluid or chorionic villus cells for mutation analysis. It is critical to the diagnosis that the specific mutation(s) within a family be delineated. Not every family has the same defect. The DNA testing is dependent on knowing the mutation(s).

Other Tests
Families may have additional genetic risks that can be tested for using the same sample retrieved for FA testing. Delineating these risks is one of the important functions of the genetic counseling session. In addition, some families may be interested in determining the HLA compatibility of the embryo or fetus with an affected FA sibling. Genetic counselors and medical geneticists are available to address any questions or concerns related to the process of prenatal diagnosis.

Appendix H

Mutation Analysis of Cloned FA Genes

Arleen Auerbach, PhD
The Rockefeller University, New York, NY

Cell hybridization studies have revealed extensive genetic heterogeneity in Fanconi anemia (FA), with the demonstration of at least eight complementation groups. The genes for complementation groups FA-C (*FANCC*), FA-A (*FANCA*) FA-G (*FANCG*) and FA-F (*FANCF*) have now been cloned, and the sequences for these four genes have been placed in the public domain. Elucidation of the DNA sequence and molecular organization of these genes have enabled us and others to detect modifications in the DNA of FA patients and their family members. Such DNA variants which are responsible for the disease are known as mutations.

Currently we are using gene therapy vector technology in the laboratory to test whether a patient is in one of these four complementation groups. We will then know which FA gene to screen for mutations in a particular family. Our data on the frequency of the different complementation groups indicates that FA-A, FA-C and FA-G together account for approximately 90% of all FA cases; thus the remaining groups are quite rare. This means that mutation screening should now be feasible for most FA families. Once the mutation(s) in an FA patient are identified, this information can be used for carrier screening in their extended family, as well as for prenatal and postnatal diagnosis in that family. Currently, FA diagnosis in families for which no mutation information is available is done by exposing cultured cells in the laboratory to a DNA cross-linking agent such as diepoxybutane (DEB), and then analyzing for induced chromosomal breakage; carrier testing is not feasible using this method.

Our analysis of genomic DNA prepared from peripheral blood specimens or from cell lines from FA patients in the International Fanconi Anemia Registry (IFAR) indicates that approximately 15% of these patients exhibit mutations in the *FANCC* gene. These patients can be classified as FA-C on this basis. After screening the entire coding region of *FANCC* from genomic DNA from a large number of patients, we found three common mutations (IVS4+4A>T, R548X and 322delG) and several rare mutations (Q13X, R185X and L554P) in IFAR patients affected with FA. Based on this knowledge we have developed mutation-specific tests for the rapid detection of these six mutations in the *FANCC* gene.

We have used these assays for: (1) testing for mutations in newly diagnosed FA patients; (2) prenatal testing in FA-C families; (3) carrier testing in FA-C families; and (4) carrier testing in healthy individuals in high risk populations. From these studies we have confirmed that approximately 15% of FA families in the IFAR have mutations in the *FANCC* gene. IVS4+4A>T (IVS4, intron 4) was found exclusively in individuals of Ashkenazi Jewish origin, and most Ashkenazi Jews affected with FA have this specific *FANCC* mutation. IVS4, R548X (exon 14) and L554P (exon 14) patients in our study usually had multiple major birth defects associated with the classical form of FA, and an early onset of hematologic abnormalities. Patients with 322delG (exon 1) and Q13X (exon 1) usually do not exhibit any major birth defects, and bone marrow failure may progress at a slower rate than in patients with IVS4 or R548X. Carriers of 322delG, R185X, R548X and L554P have Northern European ancestry; the Q13X mutation is of Southern Italian origin. Since the population that we study in the United States is racially and ethnically very diverse, and different mutations in FA genes appear to have occurred in different ancestral groups (Founder Effect), the frequency of these various *FANCC* mutations found in our study reflects the diversity of our study population. The relative frequency of the different FA complementation groups, as well as the frequency of the specific mutations within a complementation group, would be different in studies of less ethnically diverse populations, such as is found in some European countries.

We also performed a study in which we screened DNA samples from approximately 3,200 healthy Jewish individuals primarily of Ashkenazi ancestry, in order to determine the carrier frequency of the IVS4 mutation in this population. These samples, screened for Tay-Sachs, cystic fibrosis, and other genetic diseases with a high frequency in the Jewish community, were tested for both IVS4 and 322delG mutations; 41 IVS4 carriers were identified, for a carrier frequency of greater than 1%. No 322delG carriers were found in this population.

Linkage studies in 49 IFAR families in which there are two or more affected siblings, or in which the parents are related to each other, was performed with DNA markers from a region on chromosome 9q that is tightly linked to the FA gene. Results from these studies confirmed our data from the mutation-specific assays identifying families as FA-C. Linkage analysis also enhances our ability to perform molecular-based prenatal diagnosis and carrier detection in FA-C families in which one mutation has been identified but the second mutation is still unknown.

Over 85 mutations in *FANCA* have been reported worldwide; our laboratory has identified at least 50 unique germline mutations in *FANCA* that are likely to be disease related, rather than normal variants. These include missense, nonsense, splicing and frameshift mutations, which are widely distributed over the gene. A large number of the mutations are microdeletions/microinsertions associated with sequence-specific mutation “hot spots.” Except for the two most common mutations, 3788-3790del and 1115-1118del, carried on about 5% and 2% of *FANCA* alleles in the IFAR populations, few *FANCA* mutations are shared between affected individuals. IFAR patients with the 1115-1118del mutation have Northern European ancestry, whereas the ancestry of the 3788-3790del carriers is varied; our studies show that this mutation has occurred in at least two different founders.

The mutation spectrum of *FANCA* also includes a variety of large deletions within the gene that are difficult to detect by simple molecular screening methods; these mutations probably account for at least 50% of all mutations in *FANCA*. The heterogeneity of the mutation spectrum and the frequency of large deletions in the gene make the molecular diagnosis of FA a formidable task. Even if the FA complementation group is identified by complementation methods, the subsequent identification of the specific mutations in a family is still very time consuming, but is necessary in order to offer rapid prenatal diagnosis and carrier detection as well as genetic counseling. The presence of sequence-specific hypermutable regions in *FANCA* suggests that *FANCA* may have a higher mutation rate than the genes for the other FA complementation groups, which could explain why FA-A accounts for at least two-thirds of all FA patients. This also raises the possibility that *FANCA* may be susceptible to increased somatic mutation, which would have implications regarding cancer risk for FA-A heterozygotes. The epidemiological and molecular implications of this hypothesis are currently being tested.

Results of a study to correlate genotype with phenotype in *FANCA* patients from the IFAR show that the median age of onset of hematologic manifestations in these patients was 7 years. This is similar to the median age of onset reported for the IFAR patients with mutations in exon 1 of *FANCC*, who have a better prognosis than other *FANCC* patients. There were no significant differences in the age of onset for males vs. females. Most of the IFAR patients with *FANCA* mutations had short stature, café-au-lait spots, and microphthalmia, but few major congenital malformations.

We have screened genomic DNA from a panel of 307 racially and ethnically diverse unrelated FA patients from the IFAR for mutations in *FANCG*. IFAR patients with known mutations in *FANCA* and *FANCC* were excluded from this study. A total of 19 different mutations likely to cause FA were identified. We estimate that FA-G accounts for ~10% of all FA. The most frequent pathogenic mutations found in the IFAR population are: IVS3+1G>C (Korean/Japanese); IVS8-2A>G (Brazilian); IVS11+1G>C (French Canadian); 1184-1194del (Northern European); and 1794-1803del (Northern European). The median age of onset of hematologic abnormalities in FA-G patients was 4.8 years. Most FA-G patients have a severe phenotype, while a small subset display a milder phenotype with few major congenital malformations and a later onset of hematologic abnormalities. This is similar to our findings for *FANCC*; IVS4 and exon 14 subgroups are associated with a severe phenotype compared to the exon 1 subgroup, which has a better prognosis.

Now that the genes accounting for the majority of FA patients have been identified, we expect that mutation screening and further analysis of genotype/phenotype correlations will aid in prediction of clinical outcome and in decision-making regarding therapeutic modalities. We are happy to provide information to FA families or their physicians regarding mutation screening for FA by our laboratory.

Appendix I

Tissue Typing and Donor Selection for Hematopoietic Cell Transplantation: The HLA System and Genetics of Transplantation

by John A. Hansen, MD
Fred Hutchinson Cancer Research Center
University of Washington
Seattle, Washington 98109-1024

Introduction

Tissues transplanted from one individual to another elicit reactions similar to the immunological responses that occur following infection or vaccination. If sufficiently strong, these transplant reactions can lead to graft rejection or graft-versus-host disease (GVHD). Graft rejection can occur when sufficient numbers of the patient's functional immune cells survive the pre-transplant conditioning therapy. Prevention of rejection in most patients requires some form of immune suppression. Immune competent T cells present in the hematopoietic cell graft can cause GVHD. GVHD can be prevented or substantially modified by immune suppression therapy following transplantation, or by deletion of donor T cells from the graft prior to transplant. The strength of transplant reactions can be minimized when donor and recipient are matched for tissue antigens encoded by genes of the HLA system.

The HLA System

HLA antigens are controlled by a family of closely linked genes known as the major histocompatibility complex (MHC) (Figure 1). HLA molecules are found on the cell surface; they can be recognized by T cells and identified by HLA typing sera. The individual genes encoding HLA antigens are referred to as alleles. Conventional serological typing methods are useful for classifying HLA antigens into distinct groups. However typing sera can not recognize all the unique types encoded by different alleles. For example, DRB1*0401 and 0405 are distinct alleles, but the molecules they encode are both typed by serology as DR4. DRB1*0401 and *0405 can be distinguished by T cells; therefore the differences between these two closely related alleles are likely to be functionally important. Matching for the DR4 antigen alone is not adequate to assure genetic identity.

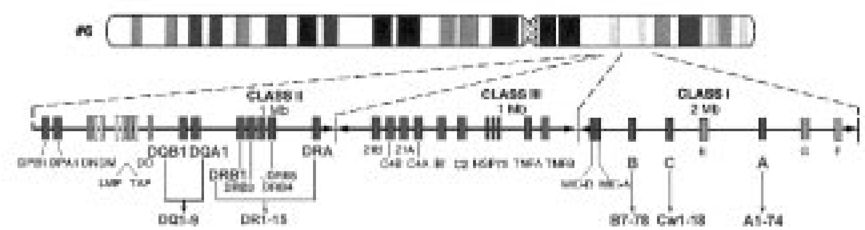


Figure 1. *The genes of the HLA system are found within a 4 megabase stretch of DNA on the short arm of chromosome number 6. The HLA genes inherited from each parent comprise a haplotype. There are at least six different sets of cell-surface antigens known to function as trasnplant antigens: the class I HLA-A, B and C antigens and the class II HLA-DR, DQ and DP antigens (HLA-A, B, C, DR, DQ and DP). The class I antigens are formed by two proteins, beta-2-microglobulin and a heavy chain encoded by the HLA-A, B or C gene. The class II antigens are also formed by two proteins, one encoded by the DR, DQ, or DP alpha gene and one encoded by the DR, DQ, or DP beta gene.*

The HLA genes inherited from each parent comprise the HLA *haplotype*. Segregation of HLA *haplotypes* within a family is illustrated in Figure 2. In this example the parental haplotypes are identified as "a" and "b" (paternal, I.1) and "c" and "d" (maternal, I.2). Each of the four HLA haplotypes can be readily identified because the parents are heterozygous for all HLA loci tested (A, B, C, DR, and DQ), and each locus expresses a distinct antigen. Sibling II.1 (the patient) and II.5 have inherited the same two parental haplotypes ("a" and "c"); by definition these two siblings are HLA identical.

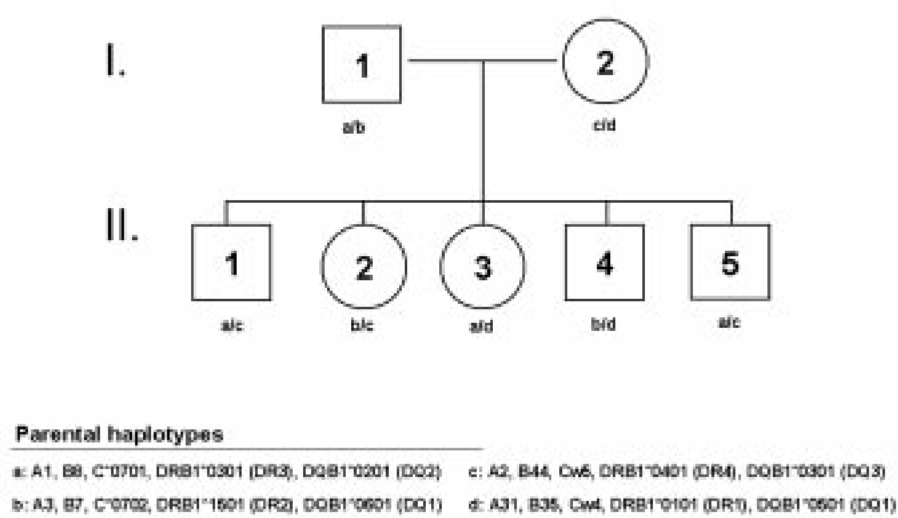


Figure 2. *Segregation of HLA haplotypes within a family. In the family illustrated here the four parental haplotypes (paternal = a and b, maternal = c and d) can be clearly identified among the parents and offspring. Individuals II.1 and II.5 have inherited the same two haplotypes. Thus they are HLA genotypically identical.*

There are six sets of HLA cell-surface antigens (A, B, C, DR, DQ, and DP)(Figure1). These can be subdivided into class I and class II molecules based on their structure and biological function. The class I HLA-A, B and C antigens are formed by union of two molecules, a small protein known as beta-2-microglobulin (,2m) and a heavy chain encoded by the HLA-A, B or C locus. The gene for ,2m does not vary from individual to individual. HLA-A, B, and C genes, however, are highly polymorphic. The class II HLA-DR, DQ, and DP antigens consist of two heavy chains each encoded by homologous genes (Figure 1). The HLA-DR1-18 antigens are the product of the DRA gene, which is the same in all individuals and the DRB1 gene, which is highly polymorphic. A second DRB gene (DRB2, DRB4 or DRB5) can be found on certain haplotypes. DRA/ DRB3 encode the HLA-DR52 antigens, DRA/ DRB4 encode HLA-DR53, and DRA/DRB5 encode HLA-DR51. HLA-DQ molecules and DP antigens are also encoded by a pair of genes, DQA1/DQB1 and DPA1/DPB1, respectively.

HLA antigens and alleles have their own separate nomenclature. Each HLA locus is designated by a capital letter (A, B, C, DRA, DRB1, DRB3, DQB1, etc.). Antigens defined by serology are identified by a one or two digit number (e.g. A2, DR4, DR11, etc.). Alleles are identified as 4 or 5 digit numbers proceeded by an asterisk (e.g. A*0201, A*0202, DRB1*0401, DRB1*0405, etc.). DNA types can be defined either at an intermediate level of resolution (e.g. A*02, DRB1*04) equivalent to the type assigned by serology, or defined as alleles (A*0201 or *0202) by high resolution typing.

HLA Typing

The technology for HLA typing and donor matching has evolved substantially over the last two decades. There are two basic methods used in most clinical tissue typing laboratories: serology, and DNA or genomic typing.

Serology

Serology is based on the use of antibodies or antisera collected from transfused patients or multiparous females immunized to paternally inherited fetal HLA antigens during pregnancy. Serology has been the mainstay of HLA typing for more than 30 years. The antibodies induced by HLA antigens, however, do not detect all the structural differences that distinguish HLA molecules from different individuals. Many of these differences can be recognized by T cells; therefore matching for specific alleles may be important in transplantation. Because of this limitation in detecting genetic variation, serology alone provides only for *low to intermediate resolution* typing.

Genomic Typing

Genomic typing involves the detection of nucleic acid variation by DNA sequencing, or indirectly by typing DNA with sequence specific primers (SSP) or sequence specific oligonucleotide probes (SSOP). A method known as the polymerase chain reaction (PCR) is used to amplify selected segments of DNA for hybridization assays with SSOP, or the PCR is used to amplify DNA sequences detected by SSP. SSOP and SSP can yield *high resolution* typing, depending on the overall strategy and the number of primers and probes used. Sequencing of HLA genes is definitive for detecting variants and matching; however, it is relatively expensive and not yet easily adapted to high volume typing.

Today, both serology and genomic methods are employed in clinical laboratories for routine HLA typing. Typing for HLA-A, B, and C antigens is still done mostly by serology, while DNA typing is increasingly done for DR, DQ, and DP. Allele level DNA typing remains relatively labor-intensive and time-consuming, but the importance of comprehensive high resolution typing is increasingly recognized as necessary for optimal donor selection. DNA typing technology is steadily improving; it is likely that all clinical HLA typing and donor matching will be done by high resolution DNA-based methods within a few years.

Mixed Lymphocyte Culture (MLC)

The mixed lymphocyte culture assay has been an important confirmatory test for demonstrating HLA identity. DNA typing, however, appears to have sufficient power to make MLC testing unnecessary.

HLA Polymorphism and Diversity of the Human Population

Given the large number of HLA alleles, and the vast number of possible combinations of these alleles forming unique haplotypes, the theoretical number of distinct HLA-A, B, C, DR, DQ, and DP genotypes exceeds the number of people on earth. HLA antigens, however, are not randomly distributed. Some occur more frequently than others (Figure 3), and certain antigens encoded by different loci are found together on the same haplotype more frequently than expected. For example, the predicted frequency of the HLA- A1, B8, DR3 haplotype in Caucasians is 0.02%, but the observed frequency is 6.1%. This preferential association between *A1, B8, DR3* is known as positive linkage disequilibrium. An individual with common HLA haplotypes where the alleles show a significant association is more likely to find a well matched donor than patients with less common haplotypes.

HLA Matching and Donor Selection

HLA Identical Sibling

The ideal donor for a hematopoietic cell transplant is a normal HLA-identical sibling. The chance that any two siblings may be HLA identical is 25%. In North America approximately 30% of patients have an HLA

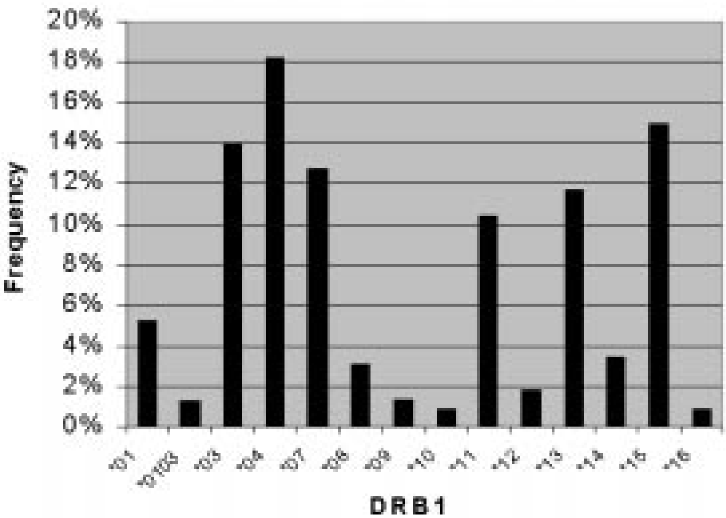


Figure 3. *Frequency of HLA-DRB1 genes in Caucasians. DRB1*04 is the most frequent HLA-DRB1 gene in Caucasians from the Pacific Northwest, and DRB1*10 and DRB1*16(2) are least frequent. DRB1*04 however can be further subdivided into at least 32 different allelic forms (DRB1*0401-32)(Table 2) each encoding a DR4 antigen, but each sufficiently different that they can be distinguished by T cells.*

identical sibling, but in other countries where the average family has fewer children the chance of finding an HLA identical sibling may be much lower. If the patient has an inherited disease, an HLA identical sibling may be excluded as a donor if this individual also carries the abnormal gene(s).

Haploidentical HLA Partially Matched Related Donors

The risk of graft rejection and severity of GVHD is greater with increasing degrees of HLA disparity. Nevertheless, transplants mismatched for only one HLA-A, B, or DR antigen have been successful in some cases. Unfortunately, patients with aplastic anemia, thalassemia, and FA have not done as well with mismatched transplants. The more intense conditioning regimens which are necessary to assure engraftment and the additional immunosuppressive therapy needed to control GVHD are not well tolerated.

HLA Matched Unrelated Donors

An unrelated donor search should begin as early as possible before the patient's condition becomes urgent. Searches are usually directed first to a national donor registry and extended if necessary to registries in other countries. There are currently more than 6 million HLA-A and B typed volunteers available worldwide. Approximately 40% have also been typed for HLA-DR. In 1999, the probability of finding at least one HLA-A, B, DR matched donor on the initial search was greater than 80% for Caucasian patients. Although these statistics are encouraging, unrelated donor searches are less successful for Blacks, Asians, and Hispanics because the racial groups are not as well represented among the available donors. This discrepancy can be remedied only by greater participation of racially diverse volunteers in donor registries.

Many of the potential donors identified during a preliminary search will be excluded in the process of confirmatory typing. If one HLA-A, B, DR matched donor is available, the chance that subsequent *high resolution* DNA typing will prove the donor to be matched for DRB1 alleles is approximately 40%. If two or three HLA-A, B, DR identical donors are available, the chance of finding a DRB1 match is 65% and 90% respectively. Approximately 50% of patients who started an unrelated donor search in 1998 have found a sufficiently matched donor. The average duration of the unrelated donor search for patients transplanted in 1993 was 5.5 months (range, 1-48 months). More recently, the average search time has been reduced to 4 months, an interval of time that is probably reasonable for most stable patients. Unfortunately, 3 months or longer may be too long a delay for patients with severe marrow failure, myelodysplasia with excess blasts, or high risk acute leukemia. The indications and feasibility of a transplant and potential availability of an HLA matched unrelated donor should be considered as part of the overall treatment strategy for each patient early in the course of disease.

Summary

Donor availability and HLA matching are limiting factors for many patients who need a hematopoietic cell transplant. Timing of transplantation can be critical to ultimate success, and a donor search can be time-consuming. The possibility of a transplant should be considered as part of the overall treatment plan soon after diagnosis to assure timely identification of a suitable donor. Criteria for donor matching are evolving, but whenever possible the donor search should be continued until an optimal match is identified.

Mismatching for HLA alleles increases the risk of graft failure and GVHD. The hazards of HLA incompatible transplants have discouraged this approach, especially for FA patients. Improvements in the safety and effectiveness of immunosuppression therapies, and less toxic approaches to eliminating malignant cells, are needed to improve both the safety and effectiveness of transplantation, and to make this life saving therapy possible for a greater number of patients.

Appendix J

Matched Sibling Donor Transplant For Patients with FA

Richard E. Harris, MD
Medical Director, Stem Cell Transplantation Program, Children's Hospital Medical Center, Cincinnati, Ohio

Background

Fanconi anemia is a rare disorder with only about 1000 reported cases in registries and in the literature to date. Most children with FA develop severe aplastic anemia on average around age 6 to 7 years. However, the onset of the severe aplastic anemia may be as early as one or two years of age. At the opposite end of the spectrum are adults who still have normal or near normal blood counts.

With the onset of severe aplastic anemia, children with FA generally first develop an increase in the size of their red blood cells (high mean corpuscular volume; MCV > 100). Then, with time, anemia develops, then a low platelet count (thrombocytopenia), then finally a low white blood cell count. These cytopenias lead to anemia, bleeding and bruising problems, and infections.

As the disease progresses over the years, a disorderly production of blood cells in the bone marrow occurs, in which most of the marrow cells die off in the marrow space before even being released from the marrow as mature blood cells (myelodysplastic syndrome - MDS). This is often associated with abnormalities of the chromosomes in the marrow cells (a clone, such as monosomy 7, only one copy of chromosome 7). The disordered marrow cell production may eventually lead to leukemia, cancer of the bone marrow. Children with FA may thus die from severe aplastic anemia, bleeding, infections, myelodysplastic syndrome, or leukemia.

The aplastic anemia can at present be cured only with stem cell transplant. The transplant will also prevent the later development of abnormal cytogenetic clones, myelodysplastic syndrome, and leukemia. A stem cell transplant is unlikely to alter the incidence of other malignancies frequently seen in patients with FA, such as squamous cell carcinoma of the head and neck region and genito-urinary tumors [1]. In fact, their incidence may be increased due to exposure to the chemotherapy drugs and radiation given to the child as part of the conditioning therapy for the transplant.

What is a Stem Cell Transplant?

A stem cell transplant is performed by giving preparative therapy, i.e., high dose chemotherapy and radiation, designed to destroy the residual weak bone marrow of the patient and to suppress the immune system of the patient to prevent the rejection of the donor stem cells. This preparative therapy for children with FA commonly consists of a few days of cyclophosphamide (Cytoxan® or CTX, a cancer chemotherapy drug) and one day of radiation therapy. Additionally, immune suppressive drugs are often given to prevent rejection of the graft and, also, to prevent graft-vs-host disease (GVHD). GVHD is an immune attack of the donor immune cells against the tissues of the patient, leading often to a rash, diarrhea, and inflammation of the liver (hepatitis). The immune suppressive drugs which have been used are cyclosporine and anti-thymocyte globulin (ATG).

The donor stem cells are generally obtained from the donor under general anesthesia in the operating room, in a procedure termed a marrow harvest. This procedure usually takes 1-2 hours and is performed by putting needles into the pelvic bones and suctioning back marrow with a syringe. The marrow is then placed into a blood bank bag and infused into the patient through an intravenous line like a regular blood transfusion. The new marrow stem cells circulate through the blood stream of the patient, then settle out in the patient's own bone marrow space. There they take up residence and start to produce more marrow cells. After about 2-3 weeks, the blood counts begin to rise. First, the white blood cell count rises, then the red cells are produced by the new marrow, then finally the platelet count rises. The blood counts are often normal about 1-2 months after the transplant.

Umbilical cord blood cells may also be used as the stem cell source for the transplant[20-21]. The blood in the placenta at the time of birth contains large numbers of blood-forming stem cells. If these cells are collected at the time of delivery of a sibling who does not have FA, and if those cells are an HLA match with the FA child, then these cells can be given as the stem cell source for the transplant. Unrelated donor cord blood cells may also be used if they are closely matched with the patient. There may be a higher risk of non-engraftment with these cord blood cells, though the risk of GVHD may be lower. The blood counts may also take longer to come up after a cord blood transplant when compared to a standard bone marrow transplant.

During the first 2-4 weeks after the transplant, the patient will require transfusions of red cells and platelets and antibiotics to fight off infections. The chemotherapy drugs and the radiation are likely to cause mouth sores and diarrhea, which will lead to some pain and an unwillingness to eat. Thus, patients will require supplemental pain medication and nutrition. The pain medication is often given as a continuous infusion of a narcotic such as morphine. The nutrition is often given as an intravenous infusion (total parenteral nutrition - TPN) or via a naso-gastric tube (NG feeds). This supplemental nutrition is often required for 2 or more weeks until the child is able to eat again.

The other major complication of a transplant is GVHD. This immune attack of the donor immune cells against the tissues of the host can often be prevented by giving GVHD prophylaxis—immune suppressive drugs such as cyclosporine and anti-thymocyte globulin (ATG)—to the patient post-transplant. However, in spite of prophylaxis, some patients will develop evidence of GVHD such as a skin rash, diarrhea, and/or hepatitis. Such patients generally need to be treated with high dose corticosteroids, such as prednisone or methylprednisolone, for several weeks. Such therapy often controls the GVHD, but often results in weight gain, high blood pressure, spilling of glucose in the urine (sometimes requiring insulin to control), thinning of bones, and an increased risk of infections.

Patients usually stay in the hospital for about 4-6 weeks for the transplant, then are followed closely as outpatients at the transplant center for a few weeks after discharge. Most patients return to their local community about 2-3 months after the transplant.

The donor for the transplant may be a sibling or close relative of the child or may be an unrelated person. The donor is chosen by performing a special test on the donor and the patient, known as human leukocyte typing—HLA typing. Fewer than 25% of patients can be expected to have an available matched sibling donor who does not have FA. Clearly, the safest and most successful type of transplant for FA is with a matched sibling donor (MSD).

How Successful are Matched Sibling Donor Transplants for FA?

Currently, over 70% of children with FA who have a MSD transplant can be expected to survive the transplant and to have normal blood counts afterwards. The first MSD transplants for FA were performed over 20 years ago.

Patients with FA were initially prepared for transplant with the usual regimen utilized for patients with idiopathic aplastic anemia—50 mg/kg/day of cyclophosphamide for 4 days. Such transplants generally resulted in severe mouth sores and diarrhea in the FA patients, and the patients usually died of multi-organ failure or severe GVHD [2,3].

Dr. Eliane Gluckman [3-5] in Paris was the first to investigate the use of a milder conditioning therapy for patients with FA. She showed that the transplant could be safely performed with doses of only 10% of the usual dose for patients with aplastic anemia. Gluckman also demonstrated a hypersensitivity to radiation [6,7] and pioneered the use of low dose (500 cGy) radiation to the chest and abdomen (thoraco-abdominal irradiation or TAR). With a median follow-up of 5 years, 13 of 19 patients (68%) were surviving with normal blood counts [6]. More recent follow-up of the Paris transplant results show a survival of about 76% with matched sibling donor transplantation for FA among 45 patients [8,9]. The major complication of transplant was GVHD, seen in 58% of patients.

A modification of the Paris regimen was published from the Children's Hospital Medical Center in Cincinnati [10] which used the same dose of cyclophosphamide, a lower dose of TAR (400 cGy), and the addition of anti-thymocyte globulin (ATG) to the preparative regimen. The results showed a survival of 94% in 18 patients, with very little GVHD and no graft rejections. A follow-up of 26 patients at CHMC-Cincinnati transplanted from matched sibling donors shows an actuarial survival of 84% from 6 months to 12 years, with one graft rejection (4%) and no cases of severe GVHD. The Paris regimen or the modified Cincinnati regimen is today utilized at most centers transplanting children with FA. A copy of the recommended protocol and consent form is available through the FARF or by directly contacting Dr. Richard Harris at richard.harris@chmcc.org.

Another promising preparative regimen is that used at the Fred Hutchinson Cancer Research Center in Seattle as well as in Curitiba, Brazil. This regimen utilizes no radiation, but higher doses of cyclophosphamide. The combined Brazil and Seattle experience is outlined in a series of three articles [11-13]. Currently, this regimen is studying doses of 80 mg/kg of cyclophosphamide, and survival is presently in excess of 70%. So far, there have been no graft rejections on the Seattle/Brazil study, even though no radiation has been given. Toxicity seems higher than with the Paris or Cincinnati regimens, but radiation and its potential late side effects are avoided. The principal late effect of radiation of greatest concern is the possible development of a radiation-induced cancer.

In a summary of results from 151 matched sibling donor transplants for FA reported to the International Bone Marrow Transplant Registry[14,15], post-transplant secondary cancers were seen in 3 patients; all three were squamous cell carcinomas occurring outside of the radiation port. Such tumors are known to occur with increased frequency in patients with FA [16,17]. Since the tumors were outside of the radiation port, it is unlikely the radiation caused the tumors. Chronic GVHD causing damage to the tissues of the mouth may have contributed to their development.

However, other studies in patients with idiopathic aplastic anemia as well as FA who have undergone transplant have implicated radiation in the cause of the secondary cancers[18-19]. There were 23 secondary malignancies among 700 patients with aplastic anemia or FA who received an allogeneic transplant. The median time to development of the second malignancy was 7-8 years. Not surprisingly, the risk of developing such a secondary cancer was higher in the FA patients than in the patients with idiopathic aplastic anemia. Five of 79 patients with FA developed a secondary malignancy, all head and neck carcinomas. None of the FA patients developed leukemia.

What if a Matched Sibling Donor is Not Available?

The results on transplants using donors other than matched sibling donors (termed alternative donor transplants) have not been nearly as good—only about 35% of such patients have survived the transplant in the past. Such alternative donors may include unrelated donors or partially-matched relatives. Newer approaches, though, are being studied to improve upon this rather disappointing outcome, including use of a highly immunosuppressive drug known as fludarabine[22-24]. Several centers are investigating this new approach to alternative donor transplants for FA. Preliminary results were reported at the 11th International Fanconi Anemia Scientific Symposium in December 1999. Of thirteen such alternative donor transplants incorporating fludarabine into the preparative regimen, twelve patients were alive and eleven of the twelve were fully engrafted. Only two patients had developed significant GVHD. However, results are too premature at this point to give solid recommendations for how the transplant should be performed for the patient who lacks a matched sibling donor transplant. The median length of follow-up of the patients was less than one year at the time of the presentation.

Most patients with FA who lack a matched sibling donor are first offered non-transplant therapy to boost the poorly working marrow, such as anabolic steroids (Anadrol®) or cytokines (G-CSF, erythropoietin, Neumega®). An unrelated donor transplant is generally not offered until the patient has become transfusion dependent in spite of Anadrol or cytokine therapy.

A Flow Chart for Recommended Treatment of FA is Available

A group of parents with the help of some physicians within the Fanconi Anemia Research Fund have developed a flow diagram which outlines the currently recommended approach to the therapy of FA. You may want to ask the Fund for a copy of this flow diagram, and utilize it in discussions with your child's own physician.

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Appendix K

Alternate Donor Transplant for Patients with FA

Margaret MacMillan, MD and John Wagner, MD
University of Minnesota Medical Center

Introduction

As of 1999, allogeneic hematopoietic cell transplantation (HCT) remains the only proven treatment with the potential of correcting the hematologic complications common to most if not all patients with FA[1]. While HCT from HLA identical sibling donors is generally associated with excellent outcome (i.e., survival >85% for children <10 years of age; and survival >65% for all patients)[2-8], HCT from an alternate (i.e., mismatched related or unrelated) donor is complex and challenging, and until recently has been associated with relatively poor survival (~30%)[6,9-11]. For this reason, it is recommended that HCT from alternate donors be performed at transplant centers experienced with FA in the context of a clinical trial designed to reduce the high incidence of graft rejection and regimen related toxicity.

This appendix summarizes the indications for alternate donor HCT, pre-transplant evaluation process (including assessment of eligibility for HCT), approach to identifying a donor, general treatment plan as it pertains to HCT, a list of potential transplant related complications, late effects, and other associated issues.

Indications for Alternate Donor HCT

Indications for alternate donor HCT are the same as those described for sibling donor HCT. However, the timing has been different. Alternate donor HCT has been delayed because of the high risk of early death observed with earlier treatment protocols. Supportive care, such as with androgens or hematopoietic growth factor therapy, was attempted first. Once such therapies failed or could not be administered due to excess toxicity (side effects) and the patient developed persistent and severe cytopenia(s) (i.e., hemoglobin [Hgb] <8 g/dL, absolute neutrophil count [ANC] <5 x 10^8/L and/or platelets [PLT] <10 x 10^8/L) or evidence of myelodysplasia or leukemia, the option of alternate donor HCT was considered. However, it is anticipated that this recommendation to delay alternate donor HCT will change once new regimens are proven safe and effective.

Indications for Alternate Donor HCT

- Patient age <35 years
- Severe cytopenia (Hgb <8 g/dL, ANC <5 x 10^8/L, PLT 10 x 10^8/L)
- Myelodysplasia with or without clonal cytogenetic abnormality
- Leukemia
- Absence of an HLA-A, B, DRB1 identical sibling donor

Transplant Evaluation: Assessment of Eligibility

Past Medical History

FA is a genetically and phenotypically heterogeneous autosomal recessive disorder characterized by multiple congenital malformations as well as progressive marrow failure and predisposition to malignancy[12-17]. Congenital malformations may range from many to none and may involve any of the major organ systems[18]. Because certain malformations and treatments may interfere with HCT, a thorough history needs to be obtained. The patient and family should be prepared to answer the questions in the table below.

Patient History

- Date of diagnosis
- Results of diepoxybutane (DEB)/mitomycin C (MMC)
- Evidence of somatic mosaicism (i.e., presence of DEB/MMC resistant cells)
- Results of complementation group or mutation analysis (if known)
- List of congenital malformations and treatments (kidneys, liver, bladder, heart, lungs)
- Chronic pain and management
- List of medications and responses to treatments (e.g., androgens, steroids, hematopoietic growth factors, chemotherapy, radiotherapy, hormonal replacement)
- Transfusions (e.g., how many and frequency of red cells and platelet transfusions, any reactions)
- Details on prior infections (organism, antibiotic sensitivities, sites, response to treatment; history of prophylaxis)
- History of cancer (site, treatment)

Family History

- Congenital malformations (e.g., abnormal thumbs)
- Blood disorders*/transfusions
- Early death
- Spontaneous abortions
- Infertility
- Cancer at young age
- Consanguinity (marriage within the extended family)

**Other hematological disorders that may be confused with FA include Diamond-Blackfan anemia, dyskeratosis congenita, amegakaryocytic thrombocytopenia, thrombocytopenia absent radius (TAR) syndrome, aplastic anemia.*

Physical Examination

Prior to HCT, it is necessary to assess what factors might be present that could alter the risk or plan of transplant procedure. Careful attention should be paid to the mouth and throat area (precancerous lesions, infection), ears (hearing), nose and sinuses (infection), respiratory system (infection, reactive airway disease) and urogenital system (infection, bladder access). The general examination should carefully document preexisting skin changes (e.g., café-au-lait spots, areas of hyper or hypopigmentation, nail abnormalities), heart sounds/murmurs, liver and spleen size, and scars from prior surgeries. This documentation is important to differentiate abnormalities related to FA from complications associated with alternate donor HCT (i.e., GVHD).

FA potentially affects every organ of the body. The more common abnormalities that might be detected on physical examination or radiographic/laboratory evaluation are described in Chapter 1[18-20].

Laboratory Evaluation

In addition to the "routine" laboratory evaluations used to assess a patient's general status prior to transplantation, FA patients require more individual attention due to heterogeneity of the syndrome. Depending upon the results of the past medical history and physical examination, the laboratory tests required will vary from one patient to the next. However, all FA patients should have the tests described on the next page performed prior to HCT.

Exclusion Criteria

Not all patients referred for alternate donor HCT will be offered transplant therapy. While exclusion criteria may differ somewhat between Transplant Centers, in general, patients will be considered unacceptable transplant candidates if the transplant evaluation indicates that the patient has:

1) Active uncontrolled infection;
2) HIV seropositivity;
3) Active extramedullary leukemia at time of HCT;
4) History of malignant solid tumor within 2 years of HCT;
5) Severe end-organ dysfunction;
6) Karnofsky performance status <70% or Lansky status <50%.

Donor Identification

Principles of the Donor Search

In general, a search for an alternate donor should be initiated when the patient has worsening marrow failure or evidence of a clonal cytogenetic abnormality. The median time from search initiation to HCT according to the National Marrow Donor Program (NMDP) is approximately 4.1 months (communication from

Laboratory Evaluation

FA Genotype
- Mutation analysis (research)
- Cell repository (research)

Hematological
- Complete blood count and differential
- Bone marrow aspiration and biopsy
- Cytogenetic evaluation
- Repeat diepoxybutane or mitomycin C screen (if only performed once elsewhere)
- Coombs test

Hepatic
- Liver enzymes, total bilirubin
- Ultrasound (R/O adenomata, size)

Renal
- Serum electrolytes and creatinine
- 24 hour creatinine clearance or glomerular filtration rate
- Ultrasound (R/O renal dysplasia, hydronephrosis)

Cardiac
- Electrocardiogram
- Echocardiogram with ejection fraction

Infectious Disease
- Chest radiograph
- Chest CT with high resolution inspiratory/expiratory films
- Sinus CT
- Panorex

NMDP, 1998). Therefore, it is recommended that a search be initiated well before the need for transfusions or development of leukemia.

Prior to an unrelated donor search, it is necessary to have complete HLA typing by a recognized HLA laboratory. While serological HLA typing is often adequate for finding a related donor, it is not adequate for finding a suitable unrelated donor. Furthermore, there can be inconsistencies in HLA typing between two HLA laboratories. For these reasons, it is always necessary to repeat the HLA typing prior to HCT, which is most often performed by the identified Transplant Center.

At the time the patient is referred to a Transplant Center, blood will be requested and sent to the HLA laboratory for testing. The minimal acceptable level of testing is serologic HLA typing of the A and B antigens and high resolution DNA-based HLA typing of the DR antigens. Although not proven to be important in patients with FA undergoing unrelated donor HCT, it is recommended that HLA-C and DQ also be tested.

Once the HLA type on the patient is known, a search of the unrelated bone marrow donor registries (e.g., National Marrow Donor Program and Worldwide Marrow Donor Registry) and umbilical cord blood banks (e.g., New York Blood Center and Netcord) should be performed. After the preliminary search is completed (<1 week), registered marrow donor(s) are notified that they have been identified as a potential donor and asked to have their HLA type confirmed by the FA patient's Transplant Center. In the case of umbilical cord blood, the banked blood is retested for HLA confirmation.

Other factors might also be considered in the selection of an unrelated marrow donor: donor age and female parity[21]. It has been shown that younger donor age is associated with improved survival and male or nulliparous female donors are associated with lower risk of chronic graft-versus-host disease (GVHD) after unrelated donor HCT in general. These factors, however, are important only if multiple donors with the same level of HLA match are available.

Transplant Therapy

Once it has been determined that the patient and donor meet the eligibility criteria, the patient is scheduled for the transplant admission. The exact therapeutic plan may vary depending upon the hematopoietic cell source (marrow, peripheral blood, or umbilical cord blood), degree of donor and patient HLA disparity, and presence of specific end organ dysfunction.

Preparative Therapy

The pre-transplant therapy or preparative therapy most often consists of cyclophosphamide and total body irradiation. The preparative therapy not only destroys the diseased marrow but also suppresses the patient's immune system so that the hematopoietic stem cells from the alternate donor will engraft and not be rejected. In general, the preparative therapy in FA patients is significantly reduced as compared to non-FA patients because of the FA patient's unique hypersensitivity to alkylating agents[22-25] and irradiation[26]. In the past, higher dose therapy was administered and was associated with extreme morbidity and mortality. While lower dose therapy in recipients of sibling donor HCT[3,5-8] was highly successful, similar therapies in recipients of alternate donor HCT were associated with high rates of graft rejection[6,10,11]. Preparative therapy prior to alternate donor HCT today is somewhat more intense than that used in FA patients with an HLA-identical sibling donor. New therapeutic strategies are being developed to reduce graft rejection and regimen-related toxicity associated with alternate donor HCT.

Graft-versus-Host Disease (GVHD) Prophylaxis
GVHD occurs after HCT because the donor immune system is transplanted along with the hematopoietic stem cells responsible for marrow recovery. While GVHD can occur in all patients undergoing an allogeneic HCT, it is particularly common and severe after alternate donor HCT because of the greater degree of HLA disparity. GVHD occurs when the immune system of the donor recognizes the patient's tissues as “foreign” and tries to reject the patient's tissues. The signs and symptoms of acute and chronic GVHD are described on the next page.

The optimal preparative therapy has not yet been developed for FA patients undergoing alternate donor HCT, and neither has the optimal GVHD prophylaxis[8,10,11]. At this time, no one strategy has been found to be superior. While marrow T cell depletion clearly reduces the risk of acute and chronic GVHD after alternate donor HCT, it has not clearly been translated into improved disease-free survival[10]. Of the various immunosuppressive regimens and T cell depletion procedures available, there is no clearly superior method for any disease. While early data with umbilical cord blood looks promising in terms of low rates of acute and chronic GVHD, it is unknown whether cord blood transplantation offers any advantages for patients with FA[27].

Regardless of the source of hematopoietic cells, most patients receive cyclosporine A or tacrolimus (FK506) for approximately 6 months to reduce the risk of GVHD. Cyclosporine A and tacrolimus, however, are associated with numerous side effects, such as nephrotoxicity, which is particularly common in patients with FA, who often have renal insufficiency at the outset.

Acute GVHD

- Skin (maculopapular rash to generalized erythroderma to desquamation and bullae)
- Liver (hyperbilirubinemia)
- Gastrointestinal System (secretory diarrhea, abdominal pain, ileus, hemorrhage, nausea/vomiting)
- Pancytopenia
- Ocular (photophobia, hemorrhagic conjunctivitis, pseudomembrane formation and lagophthalmos)
- Fever

Chronic GVHD

- Skin (lichen planus, scleroderma, maculopapular rash, hyperkeratosis, hair and nail loss)
- Liver (cholestasis, absent bile duct syndrome, cirrhosis, portal hypertension, hepatic failure)
- Gastrointestinal System (dysphagia, failure to thrive, aperistalsis, malabsorption syndrome)
- Obliterative Bronchiolitis (restrictive/obstructive airway disease)
- Sicca Syndrome (keratoconjunctivitis sicca with burning, photophobia, irritation, pain; oral dryness, pain, lichenoid lesions, gingival atrophy, dental caries)
- Vaginitis, vaginal dryness/strictures
- Pancytopenia; eosinophilia
- Serositis (pleural, pericardial, joint effusions)
- Myofasciitis

Regardless of the prophylactic approach used, GVHD can still occur. The more severe the GVHD, the higher the risk of death, most often from opportunistic infection. If GVHD occurs, the mainstay of treatment is methylprednisolone. Other agents successfully used in the management of acute and chronic GVHD include mycophenolate mofetil (MMF), thalidomide, and psoralens with ultraviolet light (PUVA).

Cyclosporine A Toxicities

- Nephrotoxicity (elevations in creatinine to renal failure and dialysis)
- Neurotoxicity (seizures, confusion, coma, paresthesias, tremor)
- Electrolyte imbalances (↓K, ↓Mg, ↓Ca)
- Gingival hyperplasia
- Hirsuitism
- Hypertension
- Thrombotic thrombocytopenic purpura

Infectious Disease Prophylaxis

Infectious complications after alternate donor HCT continue to be a major problem. Based upon 1) the peculiar sensitivity of FA patients to chemoradiotherapy, 2) the resultant breakdown of mucosal barriers after treatment, and 3) extensive periods of neutropenia and considerable transfusion exposure prior to HCT and the resultant exposure to infectious agents, FA patients are at high risk of opportunistic infection during the early HCT period. For this reason, strategies are needed to prevent infection in the early period after alternate donor HCT. Infectious disease prophylactic regimens may include itraconazole one month prior to HCT. It has not yet been proven that this is effective in prevention of later fungal infections.

The length of infection prophylaxis therapy depends upon the degree of immunosuppression, development of acute or chronic GVHD and development of infectious complications and responses to therapy after alternate donor HCT.

Late Effects

All recipients of chemoradiotherapy and allogeneic HCT are subject to numerous late effects that are not necessarily peculiar to patients with FA. These include: late graft failure, recurrent acute and chronic GVHD and the effects of prolonged steroid therapy, such as hypertension, hyperglycemia and aseptic necrosis of bone. Other late effects such as short stature and sterility have not been formally evaluated in patients with FA since these are preexisting problems in most patients with FA. As more FA patients are surviving HCT, it is becoming increasingly important to document the patient's endocrine status before transplantation and to consider the use of growth hormone therapy prior to the use of agents such as TBI and steroids that could interfere with later growth.

One of the most important late effects is the high incidence of carcinoma in patients with FA. Although there is no known method of prevention, recognition of the problem and close monitoring of the head and neck and frequent dental evaluations is the most important strategy toward reducing the morbidity and mortality associated with this late effect. Deeg et al.[28] published one report which suggested that the one risk factor associated with the development of carcinoma was a history of chronic GVHD and use of azathioprine.

Therefore, it has been recommended that azathioprine not be used in this patient population and that patients diagnosed with chronic GVHD be followed more closely.

Transplant Results at the University of Minnesota

Graft rejection
To date, nine FA patients with alternate donors have been enrolled on the protocol combining fludarabine with cyclophosphamide and total body irradiation at the University of Minnesota. All nine have successfully engrafted. These excellent results with fludarabine indicate that we may have overcome the barrier of graft failure which had been a major obstacle to the success of unrelated donor HCT. However, it is important to point out that only three of the nine patients demonstrated T cell mosaicism prior to transplantation, and only two of the nine were mismatched. Additional data are needed to confirm these early positive results.

Graft-versus-host disease
To date, none of the FA patients enrolled on the fludarabine, cyclophosphamide and total body irradiation protocol and transplanted with T cell depleted unrelated marrow at the University of Minnesota has developed acute GVHD. One recipient of HLA 2 antigen mismatched umbilical cord blood developed moderate (grade 2) GVHD. While no patient has developed proven chronic GVHD, one patient has developed autoimmune hemolysis (antibodies directed against the red cells) which may be a manifestation of chronic GVHD.

Infections
Notably, we have already observed a high rate of "occult" infections that we previously would not have known about. More patients are delayed from going to

HCT while they first receive treatment for their infection. Despite these measures, infectious complications are still common. In most cases, the infections have been manageable.

Survival
While follow-up is short (the longest is one year after transplantation), seven of nine patients are alive. One patient had had leukemia prior to transplant and had been aplastic (after chemotherapy) and on a ventilator for several months before the initiation of the preparative therapy. The second patient did well early after transplant but had a refractory CMV infection.

In summary, HCT is the only treatment with curative potential for patients with the hematological complications of FA. Fludarabine has been shown to promote engraftment and T cell depletion of the marrow has been shown to reduce the risk of GVHD in the unrelated setting. New research is directed toward the development of novel approaches for reducing the risks of infection and cancer after HCT.

Collection of Autologous Hematopoietic Stem Cells

Although not uniformly accepted, the collection of autologous hematopoietic stem cells may be recommended. In many instances, patients with FA have very poor marrow cellularity, thereby eliminating this option. However, earlier consultation regarding the future need for transplantation has led to renewed consideration of this procedure. At this time, it is unknown whether the infusion of autologous hematopoietic stem cells collected at an earlier time would benefit patients either as a method of rescue after graft rejection or as a source of hematopoietic stem cells for future gene therapy.

Psychosocial Issues

During the initial hospitalization for the transplant procedure, all patients will be kept in a single occupancy room equipped with a high efficiency air filtration system to reduce exposure to infectious agents. Once the marrow has recovered sufficiently, patients will be allowed out of their hospital room unless problems (e.g., GVHD) prevent this. After discharge, patients will still be expected to avoid crowded enclosed spaces and to wear masks in an attempt to reduce exposure to viral pathogens.

Most Transplant Centers expect that patients undergoing alternate donor HCT will remain near the facility for a minimum of 100 days. While major complications can occur after this period, the first 100 days is considered the highest risk period for the development of the immunologic complications (i.e., graft rejection, GVHD and opportunistic infection) associated with alternate donor HCT.

Late effects

While HCT can cure the hematological abnormalities in FA patients, unfortunately FA patients remain at risk for cancers, especially of the head and neck, and cervix in females. Because two factors appear to be associated with risk of late malignancy in FA patients (i.e., use of irradiation and development of chronic GVHD), we have developed a regimen that does not include radiation for patients receiving matched sibling (brother or sister) donor transplantation and takes advantage of T cell depletion to reduce the risk of GVHD even in patients with HLA matched sibling donors. While we do not advocate the elimination of radiation for patients requiring unrelated donor HCT, we are piloting such a regimen in specific subpopulations of FA patients at this time. Clearly, the elimination of irradiation from the preparative therapy for all FA patients is a future goal.

Summary

At this time, there remain many challenges in terms of improving the outcome of allogeneic HCT for the treatment of the hematologic manifestations of FA: 1) determining the optimal time for HCT; 2) predicting individual patient sensitivity to chemoradiotherapy; 3) understanding the effect of the mosaic phenotype on risk of graft rejection and natural history of the disease; and 4) reducing late effects, particularly risk of malignancy.

The recent cloning of several of the FA genes has also provided new opportunities for understanding the molecular basis of FA as well as having a potential impact on diagnosis and therapeutic options. While gene therapy may not be readily applicable to FA patients today, the hematologist should consider hematopoietic stem cell collection early in the course of the disease, optimally at a time preceding the development of marrow hypoplasia and MDS. Even if such a strategy does not result in the infusion of genetically modified hematopoietic cells, these cells may serve as an autologous backup should graft failure occur after allogeneic HCT.

Clearly, all patients with FA should be followed routinely by a hematologist, even prior to the onset of marrow failure. To understand better the natural history of this disease and significance of cytogenetic clonal abnormalities, patients must have marrow examinations annually and more frequently after the onset of marrow failure and/or development of clonal hematopoiesis. Moreover, the hematologist must be aware of the importance of family genetic counseling and the availability of prenatal diagnosis. However, the hematologist must also be aware that the availability of predictive testing has led to complex ethical issues, such as deliberate conception of a fetus and, more recently, embryo selection for the possibility of providing an unaffected HLA-identical sibling HSC donor. These issues are beyond the scope of this chapter.

In summary, a number of obstacles remain preventing the successful use of HCT from alternate donors. However, new treatment protocols are now being evaluated in a cooperative group setting, for the first time allowing new therapies to be tested in larger cohorts of FA patients in a shortened period of time. It is anticipated that new therapies will reduce the risks of alternate donor HCT, making this treatment option more acceptable in the not too distant future.

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Appendix L

Gene Therapy: Risks and Potential

Chris Walsh, MD, PhD
University of North Carolina

Genetic correction for Fanconi anemia conjures up different images to patients, families, and physicians. I will try to address some basic concepts concerning the potential risks and benefits of gene therapy.

The hematologic complications of FA are life threatening and currently the target for genetic correction. Low blood counts are the direct result of the inability of the bone marrow to resupply the blood with the normal number of cells. Blood counts should be viewed as a monitor of the daily birth and death of blood cells. This process is exquisitely regulated so that neither too many nor too few cells are in the blood stream at any given time.

The process of generating blood cells is quite complex, but an overall view is simple. The biblical account of the *loaves and fishes* is apt. The generation of enough food to feed thousands from only a few loaves of bread and fish is analogous to the few *stem cells* which generate all the white blood cells, platelets, and red blood cells each day. Each one of these cell types has its own life span ranging from hours to weeks. Thus stem cells are unique due to their capacity to regenerate themselves while differentiating into the mature cells found in the blood.

The bone marrow stem cells are rare and are described in a number of ways. By definition, stem cells must be able to restore normal hematopoiesis (blood cell production) completely . This criterion is paramount and is easily demonstrated in the laboratory. Using mice, dogs, and monkeys, which have received lethal doses of irradiation or chemotherapy, infused stem cells set up shop in the marrow. Normal blood counts, sustained for the life of the animal, demonstrate the ongoing regeneration of blood cells. Bone marrow transplantation (BMT) for patients with a variety of diseases, such as FA, illustrates the use of stem cells. Donor bone marrow cells containing a contingent of stem cells are infused into a recipient patient following the destruction of the patient's bone marrow by chemotherapy and irradiation. Bone marrow reconstitution occurs by the donor stem cells.

Are bone marrow stem cells defective in FA? Yes. How do we know? Bone marrow cells obtained from FA patients do not grow normally in vitro (in the lab). Using the same conditions that allow growth of bone marrow obtained from normal individuals, bone marrow cells from FA patients do not grow. More importantly, we know that BMT can be curative for the blood problems of FA patients. We do know that the FA genes must contribute to the survival and growth of either stem cells or their progeny (descendents). We believe that the lack of fully functional FA proteins over time contributes to the eventual loss of the stem cell pool. This leads to the subsequent loss of circulating blood cells. The correction of a patient's stem cell pool would therefore reconstitute the ability to generate normal numbers of blood cells.

If the defect in FA is the early death of the stem cell pool, how does leukemia develop? Leukemia is the overproduction of a particular type of cell which overruns the marrow much as weeds overrun a garden. Leukemias develop by the inappropriate positioning of pieces of chromosomes next to each other. These apparent random acts of genetic juggling produce a hybrid gene not normally expressed. These hybrid genes alter the regulation and growth rate of the cells. No such particular hybrid has yet been identified in FA cells. But the loss or gain of particular chromosomes (monosomy 7, for example) suggest that other genetic insults are required for leukemia to develop. It may be that the mechanisms which control stem cell viability are also tied to chromosomal maintenance. When these mechanisms are not functioning properly, either marrow failure or cancer is the outcome.

Despite the gaps in our knowledge of FA cells, we have shown that FA cells can be corrected by the simple act of using altered viruses to transfer the normal FA genes into FA bone marrow cells. Bone marrow stem cells are the target of these viruses. Unlike other organs, bone marrow cells are relatively easy to obtain, and the discovery of peripheral blood stem cells has made this easier.

Decades of viral research are now applied to gene therapy. Gene therapy resulted from the use of modified viruses as delivery vehicles for genes. The viruses used, under normal conditions, cause disease. For example, retroviral vectors are based on the work of virologists over 100 years ago on leukemia-producing viruses in animals. Molecular biology now allows modification of the virus so it can carry genes of interest without producing any disease. Viral vector production for clinical use requires strict monitoring for any "bandit" or wild-type virus which could promote disease.

The field of gene therapy has grown rapidly, and with it have come newer viruses for gene delivery. Each virus vector has its own unique properties. Normally it will infect only a particular cell type.

Viruses can then deposit their genetic payload in different ways. Some viruses can sequester themselves (or "integrate") within the DNA of the cell and remain there for the life of the cell. Other viruses may deliver genes as "episomes," which remain separate from the rest of the cell's DNA. Both methods have their advantages for gene therapy. If the constant expression of a gene product (protein) is required, then the integrating viruses are preferred. If a temporary or limited production of protein is needed, the episomal viruses (non-integrating) are preferred.

The risk of foreign DNA insertion into cells is a reasonable concern. The insertion of genetic material into the genome (all the DNA in the cell) is random. The new genes could be placed in such a manner that they either turn on or turn off adjacent genes. Due to the vast amount of genetic material, the odds of such an occurrence are viewed as possible, but unlikely. Several hundred patients have received such integrating vectors without tumor development. In fact, patients with solid tumors receiving viral vectors directly placed into the tumor have not developed adverse affects. This suggests that either the original concerns were unfounded or that the rate of vector transfer is so low that adverse effects will not occur. As more efficient methods of gene transfer are developed, this concern will again resurface.

No adverse immunologic outcomes have been reported to either the retroviral vector or the transferred gene product. Other viral vectors, adenoviruses in particular, are extremely good in stimulating host immunity against the vector, which causes destruction of the cells exposed to the virus. Immune response to the transferred gene product is always a question, especially in patients who totally lack the protein being made. Some proteins easily arouse the immune system. Many FA patients, despite the different mutations, make some FA protein, even if it doesn't work properly. This suggests that gene-corrected cells making the correct FA protein may avoid the immune response. This issue has not been adequately studied in gene transfer trials, particularly in FA patients.

Potential risks for FA patients considering gene therapy include the risks mentioned above, in addition to features particular to the disease. A theoretical risk might include the development of a clone of stem/progenitor cells which has started down the path toward malignancy (leukemia). The introduction of a normal FA gene into stem/progenitor cells destined for malignancy may lead to an acceleration of the tumor process. On the other hand, the normal gene may prevent or slow the process of malignancy. Results from the NIH FANCC gene therapy trial address this question. One patient who received the FANCC vector went on to develop leukemia. Molecular analysis of that individual's cells indicated that the blood and bone marrow did not contain the transferred gene or any viral contaminant that could have directly triggered the leukemia. Protocols are designed to allow the entry of patients without indicators of overt malignancy.

Will I Be Able to Undergo BMT if Gene Therapy Fails?

If one enters a gene therapy trial prior to BMT, there is the possibility of creating gene-corrected lymphocytes. These cells are now resistant to the doses of chemotherapy or irradiation commonly used for FA bone marrow transplantation. In this setting, the patient's lymphocytes may not be destroyed. The lymphocytes may then attack the donor cells, leading to graft failure. Once again, current gene transfer protocols are designed to include patients who are not eligible for BMT or are unwilling to undergo BMT.

The risks appear daunting, but in retrospect many worries may be unfounded. The potential for gene therapy in FA is significant. Why? FA is a syndrome with multiple complexities, but the hematologic aspect can be boiled down to stem cells which don't work well. Stem cells can now be easily captured. Several methods for correcting these cells are available, and it remains for investigators to put the two systems together. Other non-hematologic complications of FA may some day be treated using gene transfer. *In utero* gene transfer studies could potentially treat many of the manifestations of FA before they develop.

As with any scientific undertaking, new discoveries will challenge how we think about FA. Patients and families should realize that gene therapy is a young field which relies on a number of different basic sciences. The idea is worth pursuing and will require the continued support of patients and families.

Appendix M

Mosaicism in Fanconi Anemia: An Example of Spontaneous Gene Therapy

Hans Joenje, PhD
Department of Clinical Genetics and Human Genetics, Free University Medical Center, The Netherlands

Mosaicism refers to a condition in which an individual has two or more cell lines of different genetic makeup. Mosaicism results from alterations in DNA that occur during an individual's lifetime. The DNA contained in the cells of an organism is not 100% stable. Alterations may originate from incorrectly-repaired DNA damage or from errors during DNA replication. Such events, when occurring in germ line (sperm or egg) cells, have contributed to the *mutational load* of the human species, which causes people to have variant forms of many genes. Sometimes, but not always, a variation causes the gene to become defective (to lose its functionality).

Individuals who carry two defective copies of a certain FA gene suffer from FA. Individuals with only one mutated copy of the gene are healthy (see Appendix F). New mutations are generally "bad" in the sense that they lead to loss of function. However, there are "full-fledged" FA patients who suddenly present with lymphocytes in their blood which appear normal in a chromosomal breakage test. Such patients are described as being *mosaic*, as they have two types of cells, one FA and one non-FA.

Two types of observation suggest that a particular FA patient may be mosaic:

1. A lymphoblastoid cell line (derived from a B lymphocyte) has a normal (non-FA like) MMC or DEB sensitivity.

2. In the standard diagnostic chromosomal breakage test (carried out with T lymphocytes), a significant proportion of the cells behaves as normal (non-FA).

The occurrence of normal cells in an FA patient may be explained by secondary DNA alterations in the mutated FA genes giving rise to at least one normal copy of the gene. Generation of a normal FA gene might result from *mitotic recombination* in compound heterozygous patients or from a secondary mutation that somehow corrects the primary pathogenic mutation.

We are only just beginning to understand the molecular basis of mosaicism in FA patients and are still far from knowing its precise clinical implications. In some patients, but not in others, the level of mosaicism has reached a point where the standard chromosomal breakage test would no longer indicate FA. Some mosaic cases seem to be associated with relatively mild hematological symptoms. On the other hand, clear examples exist of non-mosaic (100% FA-like) patients who have only mild symptoms despite a relatively advanced age. Such cases might represent relatively subtle changes of the affected FA gene, i.e., mild mutations.

Much research remains to be done to answer important questions, such as: which other cell types (besides lymphocytes) are reverted to normal? Is mosaicism associated with improved hematopoiesis? Is the presence of a significant proportion of non-FA lymphocytes in an FA patient a complicating factor for bone marrow transplantation or the treatment of leukemia?

Once experimental methods have been worked out to determine the type of progenitor cell where the reversion has taken place in mosaic patients, these and other questions may be answered.

Understanding the occurrence and development of mosaicism in FA patients may be important for the design of effective gene therapy protocols. Current protocols aim at transferring an intact FA gene into hematopoietic progenitor (stem) cells of an FA patient and, in that way, creating a mosaic bone marrow, a situation that may be similar to the spontaneously occurring mosaicism observed in some FA patients.

Appendix N

The Gastrointestinal Tract and FA

Sarah Jane Schwarzenberg, MD
University of Minnesota

Fanconi anemia is associated with both anatomic and functional disorders of the gastrointestinal (GI) tract.

Anatomic abnormalities include esophageal atresia, duodenal atresia, and anal atresia. Atresia is a birth defect involving complete loss of some portion of the lumen of the GI tract.

Functional abnormalities include poor oral intake, nausea, abdominal pain, and diarrhea.

The major function of the gastrointestinal tract is to provide good nutrition, reflected by normal growth, energy to meet demands of daily living, and adequate reserves to face short-term malnourishment during acute illness.

Poor growth in FA can be the result of multiple endocrine abnormalities and/or poor oral intake. Problems resulting in poor oral intake include lack of appetite/interest in food, nausea, and/or pain caused by eating (cramps).

Causes of poor oral intake include gastrointestinal abnormalities, chronic inflammation or infection, medication side effects, and/or neurologic abnormalities or behavioral problems. Some gastrointestinal problems are related to complications of congenital abnormalities in FA. After esophageal atresia repair, patients frequently have gastroesophageal reflux; 30-50% require anti-reflux surgery. Esophageal replacement is associated with pain eating solids and vomiting.

Complications of duodenal atresia repair include >25% symptomatic with abdominal pain, chronic alkaline reflux, blind loop syndrome, poor duodenal motility above the repair, and recurrent obstruction-like episodes. Some of these complications are seen less frequently in patients who have duodenal tapering at the time of their repair. Complications of anal atresia repair include the following: 30% have fecal incontinence, 50% have occasional soiling, and some have constipation with or without encopresis (soiling as a result of leakage of stool around a chronic stool impaction).

Evaluation of poor feeding will begin with a good history and physical exam, which may take up to an hour. The child's previous records and a three-day diet history should be available to the physician two weeks before the visit.

Other tests which might be ordered include:

- Barium study of gastrointestinal tract;
- Gastric emptying study;
- Blood for CRP, ESR, Helicobacter pylori antibody, zinc level;
- Stool for ova and parasites, cryptosporidium;
- Urine culture;
- Endocrine studies;
- Endoscopy with biopsy.

Some clinical situations suggest certain problems. Abdominal pain plus nausea suggest:

- Mechanical obstruction;
- Abnormal gastrointestinal motility;
- Small bowel overgrowth;
- Gallbladder disease.

Nausea alone suggests:

- Infection;
- Urinary tract infection;
- Sinusitis;
- Behavioral problem;
- Medication side-effect;
- Gastric emptying delay.

If a diagnosis cannot be made, a trial of therapy to improve symptoms is warranted. Treatment options include:

- Trial of acid suppression: ranitidine, famotidine, omeprazole;
- Trial of motility-promoting agents: cisapride, metoclopromide, erythromycin. *Note that cisapride (Propulsid) must be used with caution (if at all) in FA, as many FA children have cardiac abnormalities.*
- Trial of antinausea agents: ondansetron;
- Trial of small bowel overgrowth treatment: metronidazole;
- Supplemental nutrition.

Supplemental nutrition can be administered by two methods: supplemental enteral feeds (delivered orally or by a tube) or supplemental parenteral feeds (delivered into a vein).

Supplemental parenteral feeds require placement of a central line, and are associated with increased risk of infection and metabolic disorders. Their use is limited to patients unable to meet their nutritional needs enterally. Supplemental enteral feeds are used when a child persistently is less than 85% expected weight for height *or* fails to gain weight over 3-6 month period. Lasting benefits may require long-term therapy.

Enteral feeds are supplemented at night, over 8-10 hours, to allow for appetite during the day. After the child reaches the "goal" weight, flexibility is possible with "nights off" for teenagers, for example. Problems include development of heartburn, decrease in daytime appetite, vomiting, or tubes may become dislodged. Routes for enteral feeds include nasogastric tube, nasojejunal tube, or gastrostomy tube. Nasogastric tubes are soft feeding tubes passed through the nose into the stomach. They may be removed daily or left in place. They may become dislodged at night (this is a higher risk in infants). They are unattractive and may cause sinusitis, but are good for short term (<3 months) supplementation or to determine if a gastrostomy tube feeding would be successful.

Nasojejunal tubes are soft feeding tubes, passed through the nose into the small intestine by a radiologist. They cannot be removed daily, but they reduce the risk of reflux.

A gastrostomy tube is a flexible tube placed into the stomach through the abdominal wall. Placement requires a minor surgical procedure. Complications are generally limited to local irritation and/or infection. Rarely, disruption of the tube can cause a more serious infection. The choice of enteral methods must be made by the family and child together after they are educated about their options. Before gastrostomy tube placement, it is important to do a trial of NG feeding to show that feeds will work. The method should have minimal impact on the child's social situation and the family's lifestyle when possible.

Appendix O

Dental Care for FA Patients

Elise Bolski, DDS
Private Practice, Weston, FL

Dentists treating patients with FA should become familiar with the problems common to all FA patients and should ask that the patient's primary physician or hematologist provide a summary of the medical problems specific to the patient he or she is treating. The following is a general guideline for evaluating and treating FA patients, which must be adapted to each individual patient.

Problems Common to All Patients with FA

1. Patients are at an increased risk of cancers of the gastrointestinal tract, including oral mucosal and tongue carcinoma. These problems are usually seen after the first decade of life, but base-line head and neck examination and oral cancer screening should be started at the first visit and continued on a semiannual basis. Suspicious lesions, including ulcerations, persistently swollen tissue, and leukoplakia should be subjected to biopsy. If a biopsy is contemplated, contact the patient's hematologist to see if special processing is required.

2. Patients are at an increased risk of leukemia. Persistent gingival swelling, oral bleeding or loose teeth without apparent cause may be symptoms of leukemia and should be reported to the patient's hematologist.

3. Patients may have low platelet counts from an early age. A simple examination and uncomplicated

prophylaxis might be done when the patient has mild thrombocytopenia, but the same patient may need a platelet transfusion before an extraction, a biopsy, a procedure that requires a mandibular block anesthetic, or other procedures with a significant risk of bleeding. Therefore, the patient's hematologist should be consulted to check the status of the blood counts and review possible treatment plans several days before each visit.

4. FA patients often develop a low white blood cell count, making them susceptible to bacterial infections. Therefore, aggressive preventive care is important. Bottle weaning should be encouraged at one year of age and dental checkups should begin by the age of 18 months and continue semiannually.

Specific Problems Seen in Some FA Patients

1. Some patients will have an indwelling venous access device or a cardiac defect. Such patients should be given prophylaxis against subacute bacterial endocarditis (SBE) during dental procedures, according to American Heart Association guidelines.

2. Patients may have upper extremity deformities that interfere with the maintenance of daily oral hygiene. In such cases, the parents should be given the responsibility for daily brushing and flossing. An electric toothbrush may be an aid to maintenance of oral hygiene in such patients.

Appendix P

Controlling Nose and Mouth Bleeding with Amicar®

Wayne Rackoff, MD, Richard Harris, MD, Jeff Lipton, MD, and Blanche Alter, MD

Amicar® (aminocaproic acid) is a drug used to help control bleeding. It is most effective in bleeding from the mucosa of the nose and mouth. It works by blocking the breakdown of clots that are formed naturally in the body. It does not work for bleeding from all sites in the body, because it works by being secreted into the lining of body cavities (e.g., it is secreted into the saliva).

This drug should be used only after consulting your hematologist. There are situations, such as bleeding in the urinary tract (kidneys/bladder), when Amicar should *not* be used. If bleeding is related to low platelets, Amicar may be helpful, but a platelet transfusion may also be needed. Amicar may be useful to *prevent* bleeding after dental procedures, but always consult your hematologist before using this drug.

Amicar may cause nausea and vomiting. It is expensive, but may be stored at home for a fairly long time. You should speak to your hematologist about whether he or she recommends keeping Amicar at home. Never take more than the prescribed dose, because an excessive amount may cause harmful blood clots.

Appendix Q

Gynecology and Pregnancy in FA Patients

Blanche P. Alter, MD
Former Chief, Pediatric Hematology/Oncology
University of Texas Medical Branch
Galveston TX

Menarche

FA females often are older than their peers when they begin to have menstrual periods. Their menses are irregular and may not be accompanied by ovulation. However, close to two dozen FA women have had children; thus some menses are clearly functional.

Pregnancy

Close to two dozen FA women have been pregnant. The rate of spontaneous abortions may be increased in these women. In half, the mother's hematologic status worsened, often requiring red blood cell and platelet transfusions. Preeclampsia or eclampsia was increased, as was the need for cesarean sections for the former or for failure of labor to progress. FA pregnancies are high risk, and should be managed by experts in maternal-fetal medicine. Consideration should be given to cryopreservation of the baby's placental blood, as a possible source of stem cells for the mother.

Menopause

Menopause is uniformly early in FA, mostly before age 40 years. FA women are thus very likely to develop estrogen insufficiency prematurely, with the risks of osteoporosis and heart disease. Estrogen replacement is indicated, with the caveat that estrogens may be bone marrow suppressive; thus blood counts need to be monitored closely.

Gynecologic Cancer

See Appendix R. Vaginal, anal, and cervical cancers occur earlier than in normal women and may be associated with human papillomavirus (HPV). Gynecologic exam and Papanicolaou smear should be done every year after age 16 or menarche, whichever is first. Breast self-exam should be done monthly; a breast exam should be done by a physician annually.

Reference

Alter BP, Frissora CL, Halpérin DS, Freedman MH, Chitkara U, Alvarez E, Lynch L, Adler-Brecher B, Auerbach AD: Fanconi's anemia and pregnancy. Br J Haematol 77:410, 1991.

Appendix R

Malignancies in FA Patients

Blanche P. Alter, MD
Former Chief, Pediatric Hematology/Oncology
University of Texas Medical Branch
Galveston TX

FA patients develop characteristic hematologic and solid malignancies at an earlier age than in the general population, with an unexplained excess in females compared to males. Although surveillance does not guarantee early detection of a cancer, it does increase the possibility of detection at a stage which might require less aggressive management than in advanced disease.

Myelodysplastic Syndrome (MDS) or Leukemia

Overall, the incidence of MDS is ~5% and of leukemia is ~10%. The cumulative risk of development of MDS or leukemia, or both, may be as high as 50%, among those who survive into later adulthood. Since few patients lived to adulthood in the past, these data are only estimates. So far, our data do not indicate that an abnormal cytogenetic clone in the bone marrow *per se* is a bad prognosis (many patients have had clones for many years). However, if the bone marrow has microscopic features diagnostic of MDS, the prognosis may not be good. Currently we recommend **unrelated** bone marrow transplant only for MDS or leukemia, not for an abnormal clone by itself. A matched **related** transplant might be done for leukemia, MDS, or a clone (or for aplastic anemia).

We recommend a routine complete blood count (CBC) every 4 months, unless it is needed more frequently because of a hematologic abnormality. We also recommend an annual bone marrow examination, including an aspirate for examination for MDS morphology, a biopsy for cellularity and MDS, and cytogenetics for clonality. If available, the laboratory should include special stains and flow cytometry for markers of MDS or leukemia. This analysis should be done at a center with experience in MDS and leukemia.

Gynecologic Cancer

FA females have an increased risk of breast, cervical, and vulvar cancer, occurring in their 20's and 30's. We suggest that they have a gynecologic exam and Papanicolaou smear every year after age 16 or menarche, whichever is first. Human papilloma virus (HPV) testing can be done on vaginal or cervical scrapings. The Pap smear and HPV tests may only need to be done in sexually active FA females. Colposcopy can be reserved for patients with abnormal Pap smears. Patients should be taught how to do a breast self-exam monthly, and a physician (usually the gynecologist) should do one annually .

Head, Neck, and Upper Esophagus (HNE) Cancer

This location for cancer is usually seen in 40-year-old males who smoke, but we see it in 20-year-old females with FA. It is important for the FA patient to notify his/her physician if there is throat pain, throat or neck soreness, ear pain, pain on swallowing, difficulty swallowing, hoarseness, or unexplained weight loss. The FA patient should have a physical examination every 4 months, with careful attention to the mouth, mucous membranes, throat, neck, and lymph nodes.

Gastrointestinal Cancer

The majority of these cancers are in the middle or lower esophagus, although stomach cancer has also been reported. The symptoms usually include dietary changes (loss of appetite), nausea, vomiting, weight loss, and/or blood in stools.

Liver Tumors

Most (but there are exceptions) of the FA patients who have developed liver tumors had been receiving androgen treatment. The patient may notice decreased appetite, jaundice, pain on the right side of the abdomen, or an enlarging abdomen. The doctor should examine for liver size, tenderness, or masses. Laboratory tests should include liver function tests: enzymes, bilirubin, and alpha-fetoprotein. We do all of these annually, and enzymes and bilirubin every 3-4 months in patients who are undergoing androgen therapy. We also recommend a liver ultrasound examination every 6 to 12 months in patients on androgen therapy.

Reference

Alter BP: Fanconi's anemia and malignancies. Am J Hematol 53:99, 1996.

Appendix S

Squamous Cell Cancers of the Head and Neck

Frank Ondrey, MD
University of Minnesota

Squamous cell aerodigestive cancers are malignant tumors of the linings of the lips, gums, oral cavity, and a variety of structures of the throat, including the larynx (voice box) and entrance to the esophagus. These cancers affect 40,000 people in the United States yearly. Most patients are over 45 years of age. Men are affected about twice as often as women. This disease is most common in individuals who smoke or chew tobacco and is also more common in those who drink alcohol. *But FA patients are unusually susceptible to these cancers, whether or not they smoke or drink.* Once a patient has been diagnosed with a head and neck squamous cancer, he or she is more likely to get additional tumors of the aerodigestive tract (throat, lung, and esophagus).

Squamous cell cancers start out as small ulcers, irritated areas, or whitish or reddish plaques with a sandpaper-like texture. These lesions are slow growing; many individuals with these tumors do not notice them until they become painful or interfere with eating and drinking. Because these lesions are most curable at early stages and because they are recognizable by Ear, Nose and Throat surgeons, they can be effectively screened and usually treated on an outpatient basis, if they are less than the size of a quarter. However, many lesions go unnoticed because of hesitation to seek attention or a variety of other social and psychological factors, and the tumors may go on to affect speech, breathing, and eating. These cancers can progress in size and spread to the lymph glands of the neck and the lung. Large tumors or tumors that spread to the neck usually require extensive treatments, involving surgery, radiation, and sometimes chemotherapy. In spite of great advances in treatment, survival for these more advanced-stage lesions is less than 50% at 5 years. This rate of cure has not essentially changed for over 25 years. Furthermore, there is considerable morbidity and rehabilitation after the successful removal of these tumors, since these tumors affect organs of communication and eating. It is possible that treatment of these tumors could involve complete removal of the voice box or removal of significant portions of the roof of the mouth or the tongue. Interfering with the function of these organs often requires difficult rehabilitation.

After successful treatment of these malignancies, head and neck cancer patients require extremely close follow-up, with immediate attention to any abnormalities of the throat and lungs. Any symptom referable to these organ systems (e.g., hoarseness, chronic cough, coughing up blood) may indicate that another tumor is forming.

A variety of factors may contribute to the growth and spread of these tumors, but it has been well recognized that defects in immunity of head and neck cancer patients exist and are associated with decreased success in treating these lesions. It is not known whether the immune defects noted in patients with head and neck cancer are secondary to the tumor growth itself, a lack of good nutrition, or some other factor. It is known, however, that certain immune-compromised patient groups are more susceptible to developing head and neck cancer whether they smoke or not. There is considerable evidence that patients who undergo transplantation of organs, like a kidney, seem to get more squamous cell cancers. Often these tumors occur in the skin and the aerodigestive tract. Squamous cell cancers in transplant patients also seem to be more virulent. There appears to be decreased survival of transplant patients who contract squamous cell cancer.

It has long been recognized that individuals with FA are predisposed to developing squamous cell cancers, particularly of the aerodigestive tract, skin, and cervix. *These cancers represent one of the most common malignancies affecting FA patients.* Unfortunately, there are no good markers for predicting who may develop squamous cell aerodigestive cancer that can be used for screening purposes in the FA population. At the present time, the following are recommended for individuals with FA:

- Decrease or eliminate smoking and alcohol consumption;
- Have periodic head and neck examinations by an Ear, Nose and Throat surgeon, primary care physician or dentist;
- Seek evaluation of any new abnormalities in speech or swallowing, or abnormal-appearing areas of the tissues lining the mouth and throat.

We are interested in studying any biopsy material in patients being evaluated for potential squamous cell aerodigestive cancers. Prior to surgery, please notify

Dr. John Wagner or Dr. Margaret MacMillan at the University of Minnesota so that arrangements can be made to obtain part of the biopsy for research studies. Clinical trials, including radiation therapy as well as novel preventative agents, are open or are being planned for FA patients.

Appendix T

FA Cell Repository at OHSU

Markus Grompe, MD
The Oregon Health Sciences University
Portland, Oregon

Scientists in the fields of genetics, blood diseases (hematology) cancer (oncology), DNA repair and pharmacology contribute to the study of Fanconi anemia. Many of these researchers use blood and skin cells from patients and family members for their experiments. A supply of these cells, easily accessible to any interested investigator, is now available. Encouraged and supported by the FA Research Fund, we decided in early 1992 to establish a repository or cell bank of Fanconi anemia cell lines at the Oregon Health Sciences University.

The cells in the repository are available to any Fanconi anemia researcher upon request. Cells in the repository can be used to perform family linkage studies, to clone yet unknown FA genes, to find mutations in already known FA genes, to test new drugs, to test gene therapy, and to study the function of FA genes. The repository carries out the following: creation of immortal white blood cell lines from patients and family members, establishment of fibroblast (skin) cell lines, immortalization of fibroblast cells from FA patients with rare complementation groups, isolation of DNA and RNA from patient cells, and collection of clinical information on all families in the repository.

Many families have already contributed cells to the repository. We have collected samples from 129 families since the repository opened in February, 1992.

Although the FA genes A, C, F, and G have now been isolated, the other FA genes (at least four others) remain to be cloned. Several laboratories are working hard to understand the functions of these genes and their protein products, and to improve therapy for the disease. The cells in the repository will be useful for all of these studies.

Complementation group testing

Recently, we have started performing retroviral complementation group testing for all FA skin fibroblast lines submitted to the repository. This is a new test which is done in collaboration with Dr. Alan D'Andrea. It allows the rapid detection of patients who belong to complementation groups A, C or G; these groups include about 85% of all patients. In this test, the patient's cells are infected with a gene therapy virus carrying the FANCA, the FANCC, or FANCG gene. Chromosome breakage testing is then performed; if the patient's cells are corrected (complemented) by the virus, then this identifies the complementation group. This result will then be shared with the family and the physician.

Why should you contribute cells to the Repository?

Your family may benefit directly. There is a good chance that the mutation causing FA in your family will be found in the cells you submit. If you submit skin cells, we will determine whether you belong to complementation groups A, C, F, or G. Families with mutations in the FANCC gene may be eligible for the

NIH gene therapy protocol. Gene therapy for other complementation groups will become available in the future. For this therapy to help your family, it is essential to know which gene is defective in your family (in other words, your "complementation group"). As FA genes are discovered, we will analyze cell lines and assign families to their appropriate complementation groups.

Fanconi anemia cells in the repository will be tested for the effects of different treatments. It is to your advantage to have those tests performed on your cells. In addition, contributing cells to the repository might also help other FA families.

Who in the family should contribute samples?

Samples are needed from the patients, all their brothers and sisters, and the parents and grandparents on both sides.

What kinds of samples are needed?

It would be best for research to be able to obtain both a blood sample and a skin sample from all individuals with the disease. Blood cells from patients with FA grow very poorly in the test tube,, and sometimes the cell lines cannot be successfully isolated. Skin cells, on the other hand, grow quite well. If you can submit only one sample, we prefer skin cells from patients and blood from relatives of FA patients.

How do you furnish a skin sample?

A dermatologist (or surgeon, if an individual is already undergoing a surgical procedure) can obtain a skin sample. This is a minor procedure, no more painful than a blood draw. The skin is numbed with local anesthetic, and a tiny (1/10) inch) circle of skin is removed. The wound is covered with a band-aid, and it's done.

Skin samples are sent at room temperature in a special fibroblast tissue culture medium (Dulbecco's Minimal Essential Medium with 10% fetal calf serum and antibiotics, or equivalent). This medium can be obtained from us if your physician or laboratory does not have it available. The samples are sent via overnight Federal Express to our address (see below).

How do you furnish a blood sample?

Make an appointment with your physician and show him/her this article. It is easiest to bring the whole family for one session rather than drawing everyone's blood on separate days. Two 10 cc tubes of blood drawn in a sodium heparin (not lithium heparin!!) test tube are required for all family members. The samples need to be sent at room temperature (not refrigerated!!) via overnight Federal Express to our address (see below).

Additional Information

We need the full name and birth-date of every person. It is also very important that you indicate who the parents, siblings, and patients are. If chromosome testing has been done, we also need the reports of those tests. Please include your phone number and that of your physician, so that we can contact you for additional questions. We will send a more detailed medical questionnaire at a later time.

When and Where?

Samples should be sent to arrive Tuesday, Wednesday or Thursday in Portland, OR. Samples can therefore be drawn on Monday, Tuesday or Wednesday. It is crucial that the samples take no longer than 24 hours to arrive.

Dr. M. Grompe
Department of Medical and Molecular
Genetics, L103
Oregon Health Sciences University
3181 SW Sam Jackson Pk Rd
Portland, OR 97201
Tel: (503) 494-6888
FAX: (503) 494-6882
e-mail: <grompem@ohsu.edu>

Who pays for it?

Federal Express charges and laboratory costs which are not covered by your insurance will be picked up by us. Please send the appropriate documentation. We sincerely hope that many more of you will participate in this effort. Many thanks!

Appendix U

New Fanconi Anemia Center in Boston

Alan D'Andrea, MD
Dana-Farber Cancer Institute and
Children's Hospital, Boston

Dana-Farber Cancer Institute and Children’s Hospital in Boston have established a comprehensive Fanconi Anemia Center. This program will support a broad range of activities and services and will ultimately lead to a better understanding of the molecular and cellular basis of Fanconi anemia, as well as improved methods for its diagnosis and treatment.

As an important part of the establishment of the Center, Dr. Alan D’Andrea and Dr. Eric Nisbet-Brown are conducting a survey of Fanconi anemia patients and their families. The data gathered in this survey (which has been approved by Human Subjects Review Boards at both Institutions) will provide the basis for a Fanconi anemia patient registry and database. This initiative has been made possible in part through the generous support of the Charles H. Hood Foundation of Boston.

The following are some of the activities in the Center:

- A basic research program in the molecular and cellular biology of Fanconi anemia, conducted in the laboratory of Dr. D’Andrea.
- The creation of a Fanconi anemia patient cell repository, including lymphoblastoid cell lines, skin fibroblast cell lines, and tumor cell lines from patients

and family members. These samples will be used to further research into the different complementation groups of FA and their significance. Instructions for the preparation and shipment of these samples is provided below.

- A Registry of FA patients from across the United States and Canada. Based on a brief initial questionnaire, we will establish a database of basic demographic information. A second questionnaire will collect treatment information, and further questionnaires will be used to gather information about family history, susceptibility to different cancers, and other areas of concern. These data will also be used to try to identify correlations between complementation groups and different clinical forms of the disease, so they can be studied further. Note that all questionnaires will be approved by the Human Subjects Review boards at both Dana-Farber Cancer Institute and Children's Hospital, and that complete confidentiality is assured.

- The development of a Cytogenetics Core Laboratory at the Dana-Farber, headed by Lisa Moreau, for diagnosis, DEB testing, complementation group analysis, and carrier detection.

- A Diagnostic and Evaluation Center for new and known patients with Fanconi anemia, which will be directed by Dr. Eric Nisbet-Brown. This program will provide both consultative services and comprehensive care for patients with FA. A broad spectrum of treatment options will be supported, including androgens, transfusion therapy, hematopoietic growth factor therapy, and bone marrow transplantation. Additional consultations from the endocrine clinic and orthopedics clinic at Children's Hospital will also be available on a case-by case-basis.

• The development of a gene therapy protocol for treatment of selected patients with FA.

A number of patients and family members have already participated in the first stage of this program, by completing questionnaires and providing skin and blood samples at the Lake Geneva Family Conference this past August 1999. Other persons who wish to participate, or who would like to obtain further information about the Center, should contact one of us (see the end of this appendix).

Patients and family members may participate in any or all of the above activities of the Center. We hope that collection of these data will allow us to gain a better understanding of the mechanisms of this disease, its course and treatment.

Materials and information gained from these studies will be available to other FA researchers on request, provided appropriate ethical protections are met.

Instructions for shipment of tissue samples

Tissue samples from patients with FA or from their family members have proven again and again to be critical for productive scientific research. For example, the judicious use of FA patient-derived cell lines has led to the identification of discrete complementation groups (subtypes) of FA, to the cloning of several FA genes, and to the molecular characterization of the FA proteins (encoded by the FA genes). Our laboratory in Boston, in collaboration with the laboratory of Dr. Markus Grompe at Oregon Health Sciences University, has generated an FA cell repository and patient database. The procedure outlined below will allow for the efficient establishment and transport of more of these critical materials for our research programs.

Tumor and leukemia samples from FA patients or their family members who develop cancer will be critical to our understanding of the process by which normal human cells transform into tumor cells. Understanding these processes may lead to more rational diagnostic procedures and drug treatments of FA patients with cancer.

Skin Sample Collection

A skin sample can be obtained by your local physician. The skin is numbed with a local anesthetic and a tiny (1/10 inch) circle of skin is removed. Skin samples are sent at room temperature in a special fibroblast tissue culture medium (Dulbecco's Minimal Essential Medium with 10% fetal calf serum and antibiotics or equivalent). This medium can be obtained from us if your physician or laboratory does not have it available. The samples are sent via overnight Federal Express to our address (see below). The cost of shipping will be paid by our office.

Blood Sample Collection

Your physician should collect two 10cc tubes of blood drawn in a sodium heparin *(not lithium heparin!!)*; this can be done while you are undergoing other blood work for diagnostic or treatment reasons. The samples need to be sent at room temperature *(not refrigerated!!)* via overnight Federal Express to our address (see below). The cost of shipping will be paid by our office.

Tumor and Leukemia Collection

We are extremely interested in obtaining fresh samples of tumors or fresh leukemic bone marrows from FA patients who develop cancer. Because of the special care required for preparing and transporting these samples, please contact Alan D’Andrea directly. We would be happy to discuss appropriate procedures directly with your physician or surgeon.

For additional information, please contact Alan D'Andrea or Eric Nisbet-Brown.

Dr. Eric Nisbet-Brown	**Dr. Alan D’Andrea**
Room D308	**Room M640**

Dana-Farber Cancer Institute
44 Binney Street
Boston, MA 02115

Tel: 617 632 3597	**Tel: 617 632 2080**
FAX: 617 632 5757	**FAX: 617 632 5757**

E-mail: Eric_Nisbet-Brown@dfci.harvard.edu
E-mail: Alan_Dandrea@dfci.harvard.edu

Appendix V

Lead FA Families and Organizations Throughout the World

Argentina

Cesar and Irma Lucero
Associacion Civil Argentina de Anemia de Fanconi
Palpa 3162 - 50 C
C.P.: (1426)
Buenos Aires, Argentina
011 54 11 4 551-8815 (Home and Fax)

Brazil

Antonieta Medeiros and Maria de Fatima
R. Humberto Fernando
Fortes, 260 Bloco 47, Apto. 01
Vila Sao José Sao Caetono • Brasil CEP: 09580.060
011 5511 4238-1883 (Home)
011 5511 4238-6687 (Fax)

Eastern Canada

Annette Waxberg and Lorne Shelson
72 Castlewood Road
Toronto, Ontario • Canada M5N 2L2
(416) 489-5502 (Home)
(416) 598-5837 (Lorne's Work)
(416) 599-8341 (Fax)
lornette@interlog.com (e-mail)

Mid-England

David and Christine Westmoreland
4 Pateley Rd.
Woodthorpe, Nottingham • England NC3 5QF
011 44 115 926-9634

Northern England

Rick and Anne Dudarenko
82 Parkhills Road
Bury, Lancashire • England BL9 9AP
011 44 161 797-4114 (Home)
011 44 161 873-5015 (Rick's Work)
011 44 161 873-7534 (Fax)

France

Sylvette and Alain Silverston
Association Française de la Maladie de Fanconi
10 Rue Emile Zola
94400 Vitry sur Seine • France
011 33 1 4680-1083 (Home)
011 33 1 4244-8983 (Alain's work)
011 33 1 4244 9897 (Fax)

Germany

Ralf and Cornelia Dietrich
Deutche Fanconi-Anaemie-Hilfe e.V.
- Bundesgeschaeftsstelle -
Boeckenweg 4
59427 Unna-Siddinghausen • Germany
011 49 2308-2324 (Home)
011 49 2308-2111 (Work)
011 49 2308-2143 (Fax)
FAHilfe01Ralf.Dietrich@T-Online.de (e-mail)

India

Marzban and Daisy Ardeshir
F & I Bone Marrow Foundation
24 Ratanbai Tata Bldg.
38th Road, Bandra
Mumbai, 400 050 • India
011 91 22 640-4989 (Home)
011 91 22 651-6544 (Fax)
Marzi@vsnl.com (e-mail)

Italy

Giovanni Pagano
AIRFA • Italian Assoc. for Fanconi Anemia Research Instituto Nazionate Tumori
Fondazione Pascale Via San Rocco, 14
80078 Pozzuoli • Italy
011 39 33 7860250 (Home)
011 39 81 5903205 (Work)
011 39 81 3031140 (Fax)
fanconiass@tin.it (e-mail)

Mexico

Rocio Gomez Gutierrez
Asociacion Anemia Fanconi
Esmerald No. 3066-1
Residencial Victoria
Zapopan, Jalisco • Mexico 44560
011 52 3 641-6849 (Home)
011 52 3 642-9417 (Work)
afanconi@foreigner.class.udg.mx (e-mail)

South Africa

Charles and Dawn Church
No. 5 DeHoop Street
Edgemead, Capetown • South Africa 7441
011 27 21 588628 (Home and Fax)

Spain

Damaris Perez and Jesus Cabrera
Obispo Benitez de Lugo #4
Apto 1-A
La Orotava, Tenerife • España 38300
011 34 922 323297 (Home)
011 34 922 331862 (Fax)

The Netherlands

Ron and Monique Baas
Nederlandse Stichting Fanconi Anemia
Hoofdstraat 32
9989 AN
Warffum • Holland
011 31 595 423277 (Home and Fax)

Appendix W

Support Resources for FA Families

Cancer Fund of America

2901 Breezewood Lane, Knoxville, TN 37921-1009
(423) 938-5281
Supplies and financial aid for low income cancer patients.

Children's Leukemia Foundation of Michigan

29777 Telegraph Road, #1651, Southfield, MI 48034
(810-353-8222; 800-825-2536; Fax 810-353-0157)
e-mail: <leukemiamich@voyager.net>
<http://leukemiamich.org>
Provides compassionate personalized support to people in Michigan affected by leukemia and other related disorders. Some financial aid available.

Children's Organ Transplant Association

2501 Cota Drive, Bloomington, IN 47403
(800-366-2682, 812-336-8872)
e-mail:< cota@cota.org> < http://www.cota.org>
Helps patients in need of a BMT organize fundraisers, and maintains accounts to which tax-deductible contributions can be made on a patient's behalf.

Dexter Johnson Trust (Oklahoma residents)

PO Box 26663 Oklahoma City, OK 73125
(405-232-3340)
Provides financial aid for children who need a BMT.

Leukemia Research Foundation
(Illinois/Indiana residents within a 100-mile radius of Chicago)

20 Davis St., Suite 420, Evanston, IL 60201
(847-424-0600)
Provides limited financial aid, counseling, and support groups for patients with leukemia.

Leukemia Society of America

600 3rd Avenue, New York, NY 10016
(800-955-4572)
<http://www.leukemia.org>
Offers brochures, a newsletter, and videos on leukemia, myelodysplasia, lymphomas and other diseases. Spanish materials available. Financial aid also available.

My Friends Care Bone Marrow Transplant Fund
(Michigan residents)

6743 Highland Road,. Suite 9, Waterford, MI 48327
(313-666-5936)
Helps patients throughout Michigan in need of a BMT organize fundraisers. Also funds and sponsors bone marrow donor drives.

National Cancer Care Foundation

1180 Avenue of the Americas, New York, NY 10036
(212 221-3300)
Offers counseling and guidance, financial assistance for patients and families.

National Children's Cancer Society

1015 Locust, #1040, St. Louis, MO 63101
(800-532-6459)
e-mail: <nccs@cybergate.org>
http://www.children-cancer.com
Provides financial aid to children who need a BMT, as well as fundraising advice, education, information, and advocacy.

National Leukemia Association

585 Stewart Ave., Suite 536, Garden City, NY 15530
(516-222-1944)
Provides information for leukemia patients, as well as financial assistance for drugs, x-rays, and laboratory fees.

National Patient Air Transport Hotline

(800-296-1217)
e-mail: <npathmsg@aol.com>
<http://www.npath.org>
Information about free or discount air travel for transplant patients and donors.

National Foundation for Transplants
(Formerly the Organ Transplant Fund)

1102 Brookfield Street #202, Memphis, TN 38119
(800-489-3863, 901-684-1697)
e-mail: otfnatl@aol.com
http://www.otf.org
Helps patients in need of a BMT organize fundraisers, and maintains accounts to which tax-deductible contributions can be made on a patient's behalf. Medical grants for transplant related emergencies.

The HLA Registry Foundation

70 Grand Avenue, River Edge, NJ 07661
(201-487-0883)
Provides fundraising and public relations help to groups and persons organizing bone marrow donor recruitment drives.

The Jeffrey Katz Bone Marrow Fund for Children

4560 Fountain Avenue, Los Angeles, CA 90029
(213-666-6400)
e-mail: <info@katzfund.org>
<http://katzfund.org>
Provides financial assistance to BMT patients from anywhere in the US who are transplanted at hospitals in southern California.

The National Transplant Assistance Fund

6 Bryn Mawr Ave., PO Box 258, Bryn Mawr, PA 19010
(800-642-8399 or 610-527-5056)
e-mail: NTAF@transplantfund.org
<http://www.transplantfund.org>
Provides fundraising assistance and donor awareness materials to transplant patients nationwide.

Aplastic Anemia Foundation

PO Box 613, Annapolis, MD 21404
(800-747-2820)
e-mail: <aafacenter@aol.com>
<http://www.aplastic.org>
Publications about aplastic anemia, myelodysplastic syndromes, and other disorders. Support groups.

BMT Family Support Network

PO Box 845, Avon, CT 06001
(800-826-9376)
Links patients with survivors who can provide emotional support.

Blood & Marrow Transplant Newsletter

2900 Skokie Valley Road, Highland Park, IL 60035
(847-433-3313; 888-597-7674)
e-mail: help@bmtnews.org
http://www.BMTNews.org
Publications, attorney referrals for patients with insurance problems, links patients with survivors.

Candlelighters Childhood Cancer Foundation

7910 Woodmont Ave. #460, Bethesda, MD 20814
(800-366-2223)
e-mail: info@candlelighters.org
http://www.Candlelighters.org
Publications, including a book about pediatric BMT, support groups, penpal program for children.

Children's Hopes and Dreams

280 Rt. 46, Dover, NJ 07801
(201-361-7366)
Penpal program for children ages 5-17.

Fanconi Anemia Research Fund, Inc.

1801 Willamette St, Suite 200, Eugene, OR 97401
(800-828-4891) Family Support Line
(541-687-4658) Information)
e-mail: <info@fanconi.org>
<http://www.fanconi.org>
Provides semiannual newsletters, annual family meetings, regional networks, telephone, e-mail, and letter support.

National Association of Hospital Hospitality Houses

(800-542-9730)
Provides information about lodging for people who need medical treatment away from their home communities.

National Cancer Institute Cancer Information Service

9000 Rockville Pike, Building 31, Room 10A24
Bethesda, MD 20892
(301 496-5583, 800 422-6237) Monday through Friday from 9am - 7pm.
Specialists who can search the NCI's Physician Data Query data base for information on state of the art treatments and clinical trials. Information and publications.

National BMT Link 29209

Northwestern Hwy. #624, Southfield, MI 48034
(800-546-5268)
<http://www.comnet.org/nbmtlink>
Publications, links patients with survivors who can provide emotional support.

National Marrow Donor Program

3433 Broadway St. NE, #400, Minneapolis, MN 55413 (800-627-7692) general information
(800-548-1375) donor search information
<http://www.marrow.org>
Transplant center directory, patient advocate, information on recruiting and becoming a donor.

INTERNET RESOURCES

BMT-TALK (Internet Mailing List)

To subscribe, send an e-mail message to: <listserv@listserv.acor.org>. Enter the message: Subscribe bmt-talk (your first name) (last name). Send messages others can read to: <bmt-talk@listserv.acor.org>. If you have trouble, e-mail: <laurel@ai.mit.edu>.

Fanconi Anemia Research Fund egroup

E-mail list for FA patients, families, friends, and physicians. High-volume list. To join, contact Leslie at the FA office: <leslie@fanconi.org>.

Internet BMT Support Group

E-mail: <kendrabmt@aol.com> for information.
<http://users.aol.com/kendrabmt/bmtonli.htm>
Interactive support CHAT on American Online.

Outlook <http://www.outlook-life.org>

A web resource for childhood cancer survivors and their families. Sponsored by the University of Wisconsin.

INSURANCE AND LEGAL PROBLEMS

CANCER CARE

(212 221-3300)
Provides professional counseling, insurance and entitlement counseling, education programs, and referrals, and a limited amount of financial aid, but just for NY, CT and NJ.

National Children's Cancer Society

(800 532-6459)
Negotiates with insurance companies and hospitals on children's behalf and offers financial assistance for bone marrow transplant expenses.

National Patient Advocate Foundation

739 Thimble Shoals Blvd. #704, Newport News, VA 23606 (757-873-6668)
e-mail: <ndepaf@pinn.net> <http:www.medinfo.org>
Publications, help with insurance problems, referrals to attorneys.

Patient Advocacy Coalition

3801 E. Florida Avenue, Suite 400, Denver, CO 80210
(303-512-0544)
Helps resolve insurance reimbursement problems utilizing a non-adversarial mediation-based approach.

Appendix X

Additional Reading

Johnson Liu, MD
Hematology Branch, NHLBI, Bethesda, MD

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Appendix Y

Glossary

absolute neutrophil count (ANC): This number is important in determining the body's capacity to fight a bacterial infection. To determine the ANC, multiply the percentage of neutrophils (found in the "differential" section of the CBC—see below) by the total number of white blood cells. Include both mature neutrophils (usually designated as "segs") and more immature forms, (often called "bands").

AML (acute myelogenous leukemia): A malignant disease of the blood-forming cells of the bone marrow, which frequently develops in FA patients. Anemia and low platelet counts and variable white blood cell counts characterize this disease. Common symptoms are weakness and fatigue, easy bruising and petechiae, and sometimes frequent infections. The diagnosis is made by taking a sample of bone marrow for microscopic analysis. The cells that dominate the bone marrow of patients with AML are known as "blasts."

aplasia: Lack of development of an organ or tissue, or of the cellular products from an organ or tissue. In the case of FA, this term refers to lack of adequate blood cell production from the bone marrow. Also refers to the lack of thumb and radius in some FA patients.

aplastic anemia: Failure of bone marrow cell and peripheral blood cell production. Bone marrow biopsy reveals an "empty" marrow space which lacks normal marrow cells.

amniocentesis: A prenatal test usually performed in the 15th to 17th week of pregnancy. A needle is inserted through the abdomen or through the cervix into the uterus, and amniotic fluid is extracted. Cells are studied for the detection of chromosome abnormalities. These fetal cells can also be tested for HLA matching.

androgens: Artificial male hormones that may stimulate production of one or more types of blood cells for extended periods of time in FA patients.

anemia: Decrease in the oxygen-carrying capacity of the blood; indicated by a low red blood cell count, low hemoglobin, low hematocrit.

antibody: A complex molecule produced by certain blood cells (see “lymphocyte”) in response to stimulation by an antigen (see below). Antibodies bind to antigens, causing the cells bearing the antigens to clump. These clumps are then destroyed by other blood cells.

antigens: Proteins present on the surface of all cells and bacteria and viruses. Our bodies are used to their own antigens and usually don’t attack them. But the body considers foreign antigens (such as bacteria, viruses, or grains of pollen) dangerous and will attack them. Bone marrow transplant specialists look for “matching” HLA antigens on the white cells. These antigens can help predict the likely success of a marrow transplant.

autosome: Any non-sex-determining chromosome; in humans there are 22 pairs of autosomes.
autosomal, adj.

B cells: Lymphocytes responsible for humoral (fluid based) immunity and antibody production.

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baseline test: Test which measures an organ's normal level of functioning. Used to determine if any changes in organ function occur following treatment.

basophil: Type of white blood cell; a type of granulocyte (see below); involved in allergic reactions.

blast cell: An immature cell. Too many blast cells in the bone marrow or blood may indicate the onset of leukemia.

bone marrow: Soft tissue within the bones where blood cells are manufactured.

bone marrow aspiration: Test in which a sample of bone marrow cells is removed with a sturdy needle and examined under a microscope. Aspirates are used to examine more specifically the types of cells in the bone marrow, and the chromosomal pattern.

bone marrow biopsy: Procedure in which a special type of needle is inserted in the bone, and a piece of bone (a plug) with marrow is removed. This test is very helpful in assessing the number of cells in the bone marrow.

CBC: Complete blood count; gives the number and/or percentage of certain blood cells, primarily white cells, red cells, and platelets.

chorionic villus sampling (CVS): An early prenatal diagnostic test. In the 10th to 12th week of a pregnancy, an instrument is inserted vaginally or through the abdomen into the uterus. Villus cells, which later form part of the placenta, are removed. These cells are then studied for early detection of chromosome abnormalities. These cells may also be tested for HLA matching.

chromosomes: Structures in the cell nucleus which contain the genes responsible for heredity. Normal human cells contain twenty-three pairs of chromosomes. One of each pair is inherited separately from a person’s father and mother (see Appendix X).

colony stimulating factors (also known as hematopoietic growth factors or cytokines): Substances produced naturally by the body (and also synthetically) which stimulate the production of certain blood cells. Examples are G-CSF, GM-CSF, various “interleukins”, stem cell factor (or steel factor), erythropoietin, etc.

complementation groups: When a mutant (or defective) cell is able to restore normal function to (or complement) another defective cell, the mutations in those cells are said to be in different complementation groups. That means the mutations are in different genes. If a mutant or defective cell is not able to restore normal function to another defective cell, the mutations are said to be in the same complementation group (in other words, in the same gene).

culture: A specimen of blood, urine, sputum or stool which is taken and grown in the laboratory. This culture is then tested to determine whether infection is present and which antibiotic to use.

cytokines: (see **colony stimulating factors**).

diepoxybutane (DEB): A chemical agent that damages DNA in cell culture and is used in a diagnostic test for FA, either before or after birth.

differential: Percent of different types of white blood cells in the blood.

DNA: This abbreviation stands for deoxyribonucleic acid. DNA is the component of the chromosomes that carries the genetic code (see Appendix I).

eosinophil: Eos; a type of white blood cell; a type of granulocyte (see below).

erythrocyte: Red blood cell; red blood cells go through various stages, starting out as erythroblasts, changing to reticulocytes, and finally becoming erythrocytes.

erythroblast: An immature red blood cell.

erythropoietin (EPO): A colony stimulating factor which influences red cell production in some conditions.

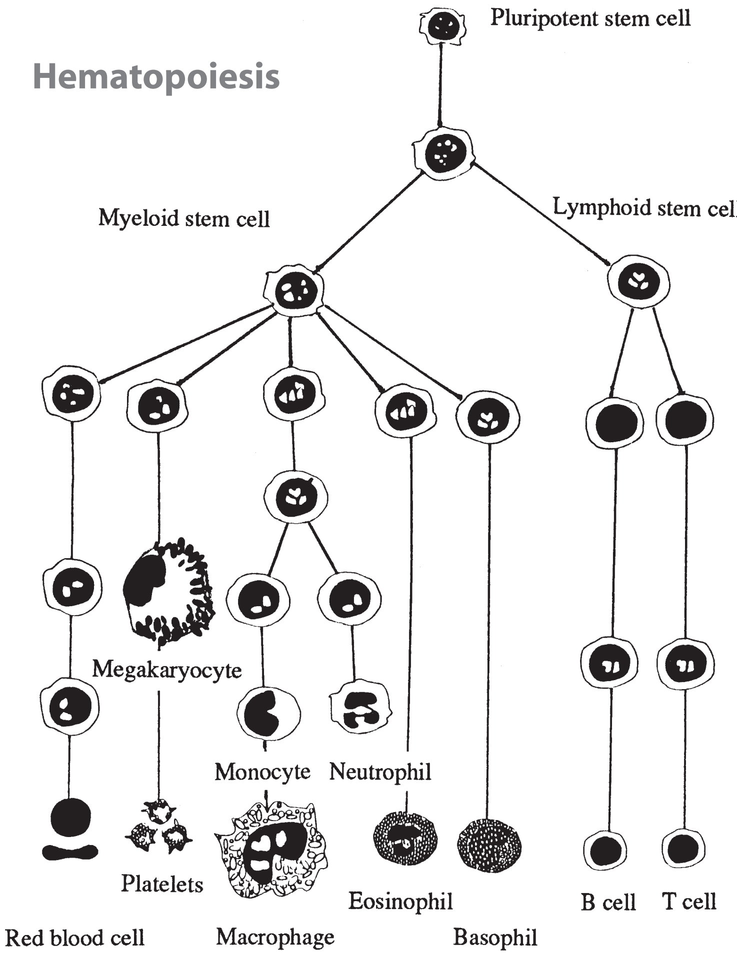
febrile: Feverish.

gene: Hereditary unit. Each gene carries the genetic code, or blue print, for a specific protein. Each human cell has about 100,000 genes, but most of these are not active in a given type of cell (see Appendix X).

graft-versus-host disease (GVHD): A complication of bone marrow transplantation which occurs when donor T cells attack the patient's cells. GVHD is more likely to occur when there is HLA mismatching. GVHD is classified in stages from Grade I (minor) to Grade IV (extrememly serious).

granulocyte: Type of white blood cell; includes the basophil, eosinophil, and neutrophil (or poly), which is the infection-fighting cell.

Hematopoiesis



hematocrit: Ratio of red blood cells to plasma in the blood; portion of the blood's total volume that is made up of red blood cells.

hematopoiesis: The formation and development of blood cells. *See diagram.*

hematopoietic growth factors: (see **colony stimulating factors**).

hemoglobin: The oxygen-carrying pigment of the red blood cells; combines with oxygen from the lungs and carries it to the body's cells.

hemorrhage: Excessive bleeding.

HLA (human leukocyte antigen) tissue typing: The tissue-typing test done on white cells to determine if a bone marrow donor and recipient are compatible.

immunosuppression: Decrease in the ability of the body's normal immune response to fight the invasion of foreign material. In transplantation, the patient must be immunosuppressed to prevent graft rejection.

immune response: The body's defense against disease and foreign substances, including transplanted bone marrow; substances may be recognized as "foreign" and then killed by other cells.

intravenous (IV): Injection directly into the vein.

leukocytes: White blood cells.

leukopenia: Low white cell count.

lymphocyte: Type of white blood cell that fights infection by producing antibodies and other protective substances; occurs in 2 forms: B cells that recognize specific antigens and produce antibodies against them, and T cells that are essential agents of the immune system. Lymphocytes are produced in the lymph system, not in the bone marrow.

macrocyte: An abnormally large erythrocyte. **macrocytic**, adj.

macrophage: A type of white blood cell that assists in the body's fight against bacteria and infection by engulfing and destroying invading organisms.

matched platelet transfusions: Transfusions from a donor who has been HLA matched to a particular patient.

megakaryocyte: Large cell in the bone marrow from which pieces break off to form platelets.

mitomycin C (MMC): A chemical which, in sufficient doses, causes destruction and rearrangement of the chromosomes in cells. Because Fanconi anemia cells are unusually sensitive to MMC, it is used to diagnose this condition.

mixed lymphocyte culture (MLC): A special tissue typing test that determines if lymphocytes from one person are HLA compatible with lymphocytes from another; has been used to identify matched bone marrow donors. Recently, the MLC test is being replaced by more precise DNA typing methods.

myelodysplasia: Abnormal production, maturation, and appearance of blood cells; often leading to deficiency of red cells, white cells and platelets; sometimes leading to bone marrow failure or leukemia. The condition is sometimes called myelodysplastic syndrome (MDS).

neutropenia: Low neutrophil (poly) count.

neutrophil: Type of white blood cell; also called a poly; granulocyte; the body's primary defense against harmful bacteria.

pancytopenia: Abnormally low number of red and white cells and platelets.

petechiae: Tiny red dots on the skin due to bleeding under the skin caused by low platelet count.

peripheral blood: The blood in the bloodstream.

phagocytosis: Cell-eating. The engulfment and destruction of dangerous microorganisms or cells by certain white blood cells, including neutrophils (see ANC).

plasma: A colorless fluid which contains water and other components in which red cells, white cells, and platelets are suspended.

platelets: Blood cell fragments containing clotting factors which prevent bleeding and bruising.

recessive: A mutation is said to be recessive if an individual must inherit two copies of the mutant gene, one from each parent, to show the mutant trait. Individuals with one mutant and one normal gene appear normal. They are called "carriers."

red blood cell (erythrocyte): Oxygen-carrying cell in the blood which contains the pigment hemoglobin; produced in the bone marrow.

reticulocyte: An immature red blood cell.

stem cell: Original cell from which megakaryocytes (giant cells from which mature blood platelets originate), red blood cells, and white cells develop in the bone marrow.

stroma: The supporting tissue of the bone marrow. This tissue provides the growth environment for blood cells. **stromal**, adj.

T cells: Lymphocytes responsible for "cell-mediated" immune reactions; critical for immune resistance to viruses, fungi, parasites and certain bacteria; important cells in transplant (graft rejection and GVHD) reactions.

thrombocyte (platelet): Cell fragment which releases clotting factors in the blood.

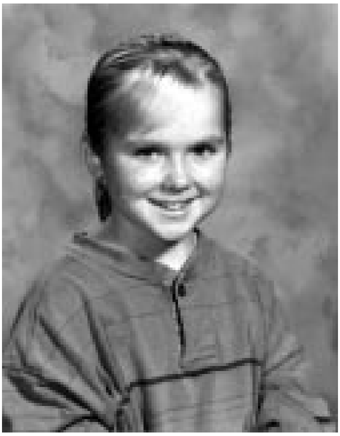
thrombocytopenia: Low platelet count.

thymocytes: T cells.

white blood cells: Blood cells which fight infection.

About the Authors

Dave and Lynn Frohnmayer are parents of three children born with Fanconi anemia. They have lost two daughters to this illness. Katie died from complications of FA in 1991 at the age of 12. Kirsten died in 1997 at the age of 24, two and a half years after a bone marrow transplant. She graduated Phi Beta Kappa in biology from Stanford University and planned to do graduate work in Public Health Administration. Daughter Amy is 13, affected by FA but presently stable. The Frohnmayers have two sons, Mark, age 25 and Jonathan, age 15, who are unaffected.



Katie: 1978-1991

Kirsten: 1973-1997

The Frohnmayers founded the FA Family Support Group in 1985 and have edited the semiannual FA Family Newsletter since that time. In 1989, they helped incorporate the Fanconi Anemia Research Fund, Inc. (a tax-exempt non-profit corporation) in order to further scientific research. Over the past eleven years, the

Frohnmayers have worked tirelessly to raise funds for research and family support. Their efforts and those of other FA families have been rewarded by the discovery of FA genes, progress toward implementing gene therapy trials, instigation of experimental trials of new therapies, and the development of strategies to improve outcomes of unrelated or mismatched bone marrow transplants.

Dave Frohnmayer is President of the University of Oregon. He served as Attorney General of Oregon from 1981 to 1991 and was Dean of the University of Oregon School of Law from 1992 to July, 1994. He was educated at Harvard College and holds an MA degree from Oxford University, where he studied as a Rhodes Scholar. He received his JD from the University of California, Berkeley. Dave was a founding Director of the National Marrow Donor Program and currently serves on the Board of Trustees of the Fred Hutchinson Cancer Research Center and the Board of Directors of the Fanconi Anemia Research Fund. Inc.

Jonathan, Dave, Amy, Lynn, and Mark

Lynn Frohnmayer is a graduate of Stanford University and received her Master in Social Work degree from Smith College. She has been a caseworker and manager for the Oregon Children’s Services Division and a national consultant and trainer on foster care issues. She is the co-founder of a child abuse prevention program in Eugene. Lynn volunteers her time to write the FA Family Newsletter, consults with families by phone or e-mail on a regular basis, and raises funds for the FA Research Fund. She serves as Advisor to the Fund’s Board of Directors.

In 1999, Dave and Lynn were selected as Eugene’s First Citizens for their volunteer and professional contributions to the community.

In 2000, Lynn and Dave received two national awards in recognition of their work on behalf of families affected by Fanconi anemia, and for their support of medical research. Research! America bestowed on them its 1999 Advocacy Award, for their "exceptional contributions as volunteer advocates for medical research." The Americans for Medical Progress Educational Foundation bestowed on them its Albert B. Sabin Heroes of Science Award, given annually to people who have made significant contributions to scientific research and medical advancement.