



FA family newsletter

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Summaries of Presentations at Family Meeting

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Bloomington, Minnesota

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N.T. Shahidi, MD, University of Wisconsin, Madison

"Long-Term Management of Fanconi Anemia"

Dr. Nasrollah Shahidi gave a very comprehensive overview of Fanconi anemia. His talk included information on how viral and bacterial infections can affect blood production, how to prevent or treat certain infections, potential benefits of certain vitamins, how transfusions affect bone marrow production, and the use of androgens such as oxymetholone to stimulate the bone marrow. Although he acknowledged that today, there are few answers concerning FA, he was optimistic that eventually we will conquer this disease.

Early Findings Suggestive of FA:

Dr. Shahidi reviewed the physical and skeletal abnormalities associated with FA. Concerning the peripheral blood, even when counts are normal there is "macrocytosis" or enlargement of the red blood cells. In addition, patients have "elevated fetal hemoglobin". In a normal individual fetal hemoglobin declines steadily, and

its concentration is less than 2% after three years; in FA patients much higher levels persist. The bone marrow is morphologically normal at birth, but progressive destruction and production ultimately leads to exhaustion of the marrow. Some red cell precursors have multiple nuclei.

Diagnosis of FA:

Dr. Schroeder from Germany discovered several decades ago that FA patients have chromosome abnormalities, and that the problem involves the nucleus of the cell, the DNA. Certain agents such as DEB cause FA chromosomes to break. This is now the diagnostic test for Fanconi anemia. Carriers of FA, however, do not have more chromosomal breakage nor a demonstrated increased risk of cancer.

Characteristics of FA cells:

FA cells have cellular and biochemical abnormalities. They show a hypersensitivity to bifunctional cross-linking agents. Cells have an inability to detoxify oxygen free radicals and there is a G2 phase cell cycle transit delay. There is a decreased rate of growth and plating efficiency of skin fibroblasts.

Role of toxic oxygen species

Dr. Shahidi reviewed different types of toxic oxygen species. Our bodies produce toxic oxygen species to kill bacteria, but they can exceed their beneficial effects. A patient suffering from an infection produces more of these toxic oxygen species. These, in turn, can damage the DNA in the cell. Cells which reproduce most frequently, such as those in the bone marrow, the gonads or the gastro-intestinal tract are most affected. These are the areas of greatest vulnerability in FA patients.

Potential value of vitamins:

Dr. Shahidi stated that there are non-enzymatic defenses against reactive oxygen molecules which should be considered by FA patients. Nutritionists have studied the use of certain vitamins and have found that they reduce the incidence of cancer and cardiovascular disease in normal individuals. Dr. Shahidi recommends the use of beta carotene, selenium and vitamins C and E in FA patients.

Infections and FA: Suggestions for Treatment

A bacterial or viral infection can cause damage to the bone marrow of an FA patient. After a child gets an infection, there is often a drop in his blood counts. This is because of the generation of superoxides and the production of gamma interferon and tumor necrosis factor. Infection stimulates the macrophages to produce more superoxide anions.

Certain viral agents are associated with bone marrow suppression:

1) Chicken pox: The body produces gamma interferon and tumor necrosis factor, which suppress blood production.

2) Parvovirus B19 causes the 5th disease and aplastic crisis in hemolytic anemias. We're not sure how it affects Fanconi anemia patients.

3) Hepatitis - This can be harmful to FA patients, and can cause aplastic anemia. Every FA patient should be vaccinated against hepatitis B which is transmitted by transfusions. There is no vaccine against hepatitis non-A, non-B and C.

4) Epstein Barr Virus - This causes infectious mononucleosis and can be vicious. There have been cases of frank aplastic anemia following infection with the Epstein Barr virus. If an FA patient becomes very sick and becomes anemic, the physician should check for the presence of this virus.

5) Cytomegalovirus - This is a common complication of transplant patients but also occurs in the absence of a transplant. It can lead to thrombocytopenia and bone marrow graft failure.

How do you protect FA patients from viral agents?

1) The FDA is in the process of approving a vaccine for chicken pox. If it is approved, Dr. Shahidi strongly recommends that all FA patients should get this. Until this is available, a child exposed to chicken pox should be given VZIG (varicella zoster immunoglobulin) within 72 hours of exposure, to prevent or modify the chicken pox. If a child develops chicken pox, he or she should be given acyclovir

2) FA children should be vaccinated against hepatitis B.

Transfusions and FA - Risks and Suggestions:

Transfusions can suppress the bone marrow. It is unwise to give a patient too many red cells. It is usually sufficient to bring the blood count up to 9 or 10 grams of hemoglobin, but is not necessary (and may be harmful) to raise the hemoglobin to high levels consistently. Transfusions can cause the kidneys to produce less erythropoietin which stimulates red cell production, so can actually decrease the ability of the bone marrow to function.

With red cell transfusions one should use a filter to separate the white from the red cells. The filter will remove 98 to 99% of the white cells. White cells can cause a patient to become sensitive to foreign antigens. There have even been reports of graft versus host disease in normal people who have had red cell transfusions! Red cells should also be irradiated, again to eliminate white cells.

Red cell transfusions increase the iron content of the body. Iron overload can cause suppression of the bone marrow and can result in a lack of response to oxymetholone. A high level of iron is toxic to hematopoiesis. If a patient has excess iron, he or she should receive deferoxamine which is a chelating agent to remove iron from the body. This procedure has made at least one FA patient responsive to oxymetholone treatment, and is a pretty safe drug. It is to be used if there is a large amount of iron overload.

With platelet transfusions one should also use a filter and irradiation. It is best to use a single donor so as not to expose the patient to multiple antigens. If the patient becomes refractory to regular platelets, it is helpful to use HLA matched platelets. However, if one is considering a bone marrow transplant from a relative, should not receive platelets from that relative. Sensitivity to those platelets could develop and could complicate the bone marrow transplantation.

Cancer in FA Patients

FA patients are more prone to cancer. Infection plays an important role in cancer. When a local area becomes infected, the patient produces local free radicals, contributing to the risk of cancer. FA patients develop cancers primarily of the oral cavity and esophagus, and genital tract such as the vagina and cervix. Oral hygiene is extremely important in FA. FA patients should brush and floss their teeth regularly. Regular gynecological exams are crucial, as is good hygiene. If one is careful and vigilant, this could potentially decrease the risk of cancer.

Use and Risks of Androgen Therapy

Androgens such as oxymetholone have been used in FA patients since 1958 and can be effective in increasing blood counts, especially the red count. It should

be used judiciously, however. The initial dosage should not exceed a maximum of 2 mg/kg and should be reduced gradually as blood counts improve. If a patient develops a resistance to oxymetholone, it may be because of a viral infection. Before the dosage is increased, the possibility of infection should be studied. Oxymetholone is degraded in the liver. The more you give, the faster it is deactivated. A larger dosage does not always mean greater effectiveness.

Dr. Shahidi was asked if androgens should be used if the only indication of bone marrow failure is a lowered platelet count. He stated that androgens stimulate primarily red cell production, and could push the red count to unnecessarily high (and unwanted) levels without affecting the platelet count. He would not start this drug because of a low platelet count only. He also would not recommend use of this drug for a patient whose hemoglobin is 9 or higher. Below 8 or 7, he would consider it. Often, patients develop resistance to oxymetholone. It should be administered in order to get the maximum mileage from its use. Some wonder if early administration of oxymetholone prevents late bone marrow failure, but there is no data to support this theory.

Androgens have negative side effects. In addition to their masculinizing effects, they can be harmful to the liver. Adenomas (benign tumors), and even cancerous tumors can result from prolonged usage. It is crucial that the FA patient undergoing androgen therapy have an ultrasound every four to five months in order to detect these complications at an early stage.

Benefits of Combining Oxymetholone and Small Dose Prednisone

A negative side effect of oxymetholone therapy is that this drug speeds up bone maturation. Prednisone, on the other hand, delays maturation of the bone. When androgens are combined with a low dose of prednisone, this results in unhindered linear growth.

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

"Fanconi's Anemia in 1993: The Hematologist's Perspective"

Fanconi's anemia is complicated by Aplastic anemia in almost all patients, and by leukemia in approximately 10%, with an increasing risk of leukemia with age. Patients with leukemia (who do not have FA) may have clonal cytogenetic abnormalities. We wished to determine whether clonal cytogenetic abnormalities in cells from the marrow of FA patients means that leukemia is inevitable. In the medical literature, 30 FA patients were reported with clonal markers. Six of these developed leukemia and the others had either died by 6 years or were still alive up to 13 years later without leukemia. Thus the literature evidence that a clonal marker means leukemia is not terribly compelling. In addition, in 3 of the cases in the literature the clonal markers were transient, and disappeared (and sometimes reappeared) over time.

At Mount Sinai Medical Center in New York and the University of Texas Medical Branch in Galveston, we studied the bone marrows from 17 FA patients, of which 11 were adequate for cytogenetic analysis. Three had clonal markers, for a frequency of 30%. In 2 patients the markers disappeared or were replaced by new markers, and then reappeared. None of our 17 patients has overt leukemia, although follow-up is short. We suggest that a clonal marker may reflect the small number of stem cells which comprise the marrow of an FA patient. Serial studies must be done in a large number of patients and over a long period of time to determine whether there is any association of clonal cytogenetic markers and development of leukemia in FA.

Conventional treatment of marrow failure in FA currently involves bone marrow (or cord blood) transplantation, or androgens. Recent experimental trials have included interleukin-3 (IL-3) or granulocyte macrophage-colony stimulating factor (GM-CSF). Future trials may involve granulocyte-colony stimulating factor (G-CSF), PIXY 321 (plasmid insert 321, a fusion product from IL-3 and GM-CSF), and perhaps other interleukins, such as IL-11. Other factors to consider include erythropoietin (Ep), and stem cell factor (SCF). Factor trials in FA require preliminary toxicity studies in non-FA patients.

We now have data to begin to answer questions with regard to the laboratory studies of FA which we began 3 years ago. (1) Does erythropoiesis in the bone marrow and peripheral blood cultures from FA patients correlate with their clinical status? Many patients in clinical groups 6 and 5 (no hematologic signs, or mild ones) do have normal or at least some erythroid colony formation, while those with more severe marrow involvement have fewer or none. (2) Is oxygen toxic to marrow function? Studies are not conclusive, although there is slightly better colony growth in low oxygen. (3) Do changes in colony growth over time correlate with, or predict, clinical changes? Perhaps, but more studies are needed over longer times. (4) Can we identify factors which might be beneficial? The laboratory studies suggest that stem cell factor may increase colony growth. This agent is not yet ready for clinical trials, because it has toxic side effects.

"Growth and Physical Development in FA Patients"

Dr. Gertner discussed growth problems in the general population, in patients with Fanconi anemia, and some factors one should consider when trying to determine if an FA patient should be given synthetic growth hormone.

Factors Which Cause Growth Problems:

1. Genetic factors
2. Environmental factors

Environmental factors which can lead to growth retardation include pre and perinatal malnutrition, later malnutrition (including gastro-intestinal diseases which lead to malabsorption of important nutrients), systemic disease, hormone diseases and psychological factors.

Short Stature and Fanconi Anemia

Approximately 50% of Fanconi anemia patients are significantly short. The primary cause of shortness is genetic. Approximately 25% of FA patients are moderately deficient in growth hormone; another 10 to 15% are quite deficient in growth hormone. When shortness is seen in FA it is usually not a consequence of growth hormone deficiency. Thyroid deficiency is very uncommon in FA patients. Treatment with androgen might limit ultimate height in some FA patients, especially if higher doses are used.

General Observations about Synthetic Growth Hormone:

Growth hormone is most effective in children who are growth hormone deficient. It can also boost height in children who are not deficient in this hormone, although the results are much less spectacular. Not every child will benefit, but some might gain an additional two to three inches. It is also most effective when started at a younger age (5 or 6 would be preferable to 12 or 13).

There are concerns, however, related to giving this hormone to "normal" children. Some of these concerns are psychological. Regular injections lead to "medicalization" of the child. Taking this hormone may lead to frustration due to the realization that the end result is not as promising as one had hoped (the patient is still short). There may be a conflict between two different fears: the fear of being short and the fear of the needle. And growth hormone can be exceedingly expensive.

The main concerns, however involve the safety of this hormone. In children who are not at risk for a malignancy, growth hormone is believed to be quite safe. There is increased worry if a child is considered "susceptible" to a later malignant transformation, however. For example, if a child has been irradiated to eliminate a brain tumor, this child might be more susceptible to the later development of leukemia. It is doubtful that growth hormone would increase this potential but it is still a worry.

Use of Growth Hormone in FA Children

Dr. Gertner made the following recommendations:

1. Growth hormone could be used, but with caution, in selected FA cases.
2. Possible indicators include:
 - a) The FA patient has been confirmed as growth hormone deficient.
 - b) The FA patient is not growth hormone deficient but is short and has had a successful bone marrow transplant.
3. If a patient has a clonal abnormality, growth hormone would not be recommended due to the concern that it might stimulate a potential for leukemia.
4. We need to pool all of our data concerning FA children who are taking synthetic growth hormone. Effectiveness and safety of this therapy need to be ascertained by studying a large group of patients.

“Gene Therapy for Fanconi Anemia”

Overview

Dr. Nienhuis gave a brief overview of hematopoiesis and gene transfer therapy, and discussed the potentially exciting implications of gene therapy for FA patients. He described recent progress made at the National Institutes of Health in laboratory studies of gene therapy and FA. Subject to government approval, human gene therapy trials for FA patients in complementation group “C” are being planned in the near future (potentially early in 1994).

Dr. Nienhuis observed that the Fanconi anemia cell defect affects all the body tissues but especially the bone marrow. The bone marrow is renewed regularly. The FA defect primarily affects rapidly dividing cells. Consequently, the bone marrow is particularly susceptible to this disorder.

Stem cells are the earliest appearing cells in the bone marrow. These cells have the capacity to self-renew. They also give rise to progenitor cells which produce the precursors that ultimately populate the bone marrow. For example, every stem cell leads to the creation of one billion circulating red cells! Stem cells regenerate the whole pathway of the bone marrow on a continuing basis. For this to occur, a continual process of cell division is required.

The process of cell division is highly organized by the body. Before a cell divides, it stops and checks to see if it has done everything right. This newly discovered process is called a “checkpoint”. If a cell needs to fix its DNA, the correction usually occurs at the checkpoint. The Fanconi anemia cell is defective because it does not repair its DNA properly. The cell's progression toward division may therefore be arrested at this checkpoint.

FA and Gene Therapy

Fanconi anemia is an appropriate candidate for early gene therapy experiment. If the FA stem cell can be corrected with the normal gene, the cell will be rescued

from this state of checkpoint arrest. Hopefully, healthy stem cells will proliferate and fill up the empty bone marrow. At the present time, this approach can only be attempted for the 10% to 15% of the FA patients who are in complementation group “C”. This is the one group for which a gene has been discovered. Gene therapy would be targeted to the bone marrow only. It would not affect the other cells of the body.

Gene therapy is a technique that allows a healthy gene to be inserted into stem cells to correct an inborn genetic error. Scientists at the NIH began to work on this technology in a serious way in the mid-1980s. Several problems still must be resolved.

In the last seven months, new information indicates that gene therapy is a potential reality for FA patients. Plans are now underway to develop a protocol and seek FDA approval for a clinical trial.

For gene therapy to succeed, scientists need a method to get the normal gene into a stem cell. They then must develop ways to preserve the stem cells in the laboratory and retain their capacity to regenerate the entire bone marrow when normal cells are returned to the patient. To get the gene into a stem cell, scientists use retroviral vectors. These are engineered viruses which carry normal FA genes into patient cells. They are made in a clinically certifiable way so that they can be approved by the FDA.

Scientists have been working on a monkey model at the NIH. They can put genes into the stem cells of monkeys. These cells were expressed persistently for a year or two following insertion. One drawback is that scientists are presently able to modify between 1% and 5% of the cells. Achieving greater efficiency is a challenge which must be overcome before gene transfer technology can be broadly applied. However, researchers believe that correcting only a small percentage of FA cells could potentially lead to great benefit for the FA patient.

Laboratory trials suggest that gene therapy might be very effective with FA. Drs.

Johnson Liu, Christopher Walsh and Neal Young have succeeded in putting normal genes into FA cells. These cells were then resistant to mitomycin C. The chromosomal breaks characteristic of FA disappeared. Researchers were able to get corrected FA progenitor cells to grow in the laboratory.

Protocol for FA Gene Therapy Trial

A protocol soon to be presented for FDA approval will include the following elements:

- 1) A patient will be treated with G-CSF for several days to mobilize the stem cells and bring them from the bone marrow to the circulating blood
- 2) Stem cells will be harvested over a period of 2 to 3 successive days. They will be taken from the blood, not the bone marrow, using an apheresis (platelet donor) process.
- 3) Stem cells will be purified in the laboratory
- 4) Stem cells will be cultured for 3-4 days with a retrovirus vector containing the normal FA gene, using conditions which have proven successful in an animal model
- 5) The genetically modified cells will be returned to the patient through the bloodstream. The entire process may be repeated several times over a period of several months.

It will take at least another six months to get approval for this trial. Candidates for this trial will be FA patients who are not candidates for bone marrow transplantation and who have some bone marrow failure.

The potential downside to this approach:

- 1) It might not work
- 2) By putting a vector in, you are making a change in DNA. You may damage a potential gene. This risk is estimated at one in a billion and is considered acceptably low.
- 3) If there are cells that could become leukemic, could you potentially augment their chance to grow? This is a big uncertainty.

"Mutational Analysis at the Oregon Health Sciences University; Fanconi Anemia Repository"

I will discuss three topics in this article. The first is an update on the activities of the OHSU FA repository. Second, I will give a general introduction on the topic of mutations and third, I will discuss the specific results of the mutation analysis work that has come out of the OHSU repository.

Update on the Repository

The OHSU FA cell repository was founded in early 1992 with the help of the FA research fund. Many families reading this newsletter will have contributed to it by sending in blood and sometimes skin samples.

The goal of the repository is to create and store cell lines, which are needed for FA research, and to make them available upon request and without restrictions to any qualified researcher. The cells can be used for the cloning and mapping of FA genes, to define mutations in FA genes and to test genetic and other therapies in cultured cells. When blood or skin is sent in we a) attempt to create a cell line, b) isolate DNA, c) isolate RNA and d) collect clinical information on the patient/family.

Before the family meeting in Orlando last year, we had 72 samples in the repository. At the present time our total sample number is 253. Fifty-two new samples were collected during this year's family meeting and are included among the 253. We have therefore taken in a total of 181 specimens in the past year. Samples have been donated by both FA patients (63) and their family members (171). Other investigators have sent us already established cell lines (19). Some of the 63 FA patients have donated both skin and blood and are therefore counted twice. We have already sent out cells and/or DNA samples to 6 laboratories around the world, which have requested them.

In summary, the work of the FA repository is going well and we are moving towards our goal of collecting samples from the majority of FA families in the US.

Introduction of Mutations

At the meeting in Minneapolis, Dave Frohnmayer likened my lecture to a "101 course in Molecular Biology". Obviously, it is beyond this article to provide you with a comprehensive report on this topic, but I hope to be able to highlight some of the basic concepts step by step.

1. How do genes work?

Before I can talk about mutations, I need to remind you of some of the basics of how genes work. Humans have an estimated 50,000-100,000 different genes, packaged in 46 chromosomes. Genes are the basic unit of genetic information, the "blueprints", which tell our body how to function. In Fanconi anemia one of these genes is defective, an important blueprint is missing, thereby causing the disease. Genes consist of DNA, which is a chemical compound made up of 4 basic ingredients, simpler chemicals (bases) given the abbreviations A,C,G and T. Millions of these 4 bases are connected to each other in strings, thereby creating DNA and chromosomes. The particular sequence in which these 4 bases are strung together is of crucial importance and it is this sequence which contains the genetic information.

How does this information get used? The first thing that the cell does with a gene is to convert it from DNA to RNA, in a process called transcription (see figure 1). During this step, unessential parts of the gene (introns) are removed by splicing. Only the essential portions of DNA (called exons), which actually contain information on how to build a protein, are retained. Thus, after transcription, the gene has been trimmed down in size (usually 10 -100 fold) and its information now exists in a different chemical form, RNA. RNA also consists of a string of the 4 bases. RNA is "ready to use" information, which is converted to a protein by translation (see figure 1). Proteins are the chemicals in our cells which actually perform functions and are also called gene-products, indicating that they are the

result of the information contained within a gene. Proteins are also long strings of individual compounds, the amino acids. In contrast to DNA, there are not only 4, but 20 different building blocks. How can 4 bases code for 20 different amino acids? This problem is solved by using 3 bases together (a triplet) to code for a single amino acid. There are 64 possible combinations of triplets of the 4 bases. Individual triplets encode either an amino acid or stand for a stop signal. Stop signals tell the cellular machinery to quit translation at this point in the RNA.

2. What are mutations?

Genes are passed on from generation to generation and can be altered in the process of reproduction. Mutations are such DNA alterations and can be detected as differences between the DNA of 2 individuals. If this difference causes a problem in the function of a gene, it is called a mutation.

Many different kinds of DNA alterations can be harmful to a gene. Examples include deletions, premature stop codons, missense mutations and splicing mutations.

In a deletion (figure 2) a portion of the gene is simply missing, leading to a damaged protein. In premature stop codons, a base triplet, which normally encodes an amino acid is altered to encode a stop signal. The synthesis (translation) of the protein is prematurely stopped, leading to a shortened, non-functional protein. In a missense mutation, a triplet is altered, so that it codes for a different amino acid than originally was located in this spot. The wrong amino acid in the wrong spot in a protein can severely damage its ability to function. And finally, splice mutations (see figure 3) are DNA changes that damage the process which removes non-essential parts of DNA. If a gene is not spliced right, its RNA can either be too long (still contain non-essential parts) or too short (important parts are skipped either of which may be bad).

In Fanconi anemia, all of the above kinds of mutations have been identified in some families.

3. Why mutation analysis?

Why might this fairly academic and dry topic be of interest to a family with FA? There are at least 4 different kinds of Fanconi anemia genes, only one of which, the FACC gene, has been isolated so far. A lot of research has been performed on this gene since it was cloned by Dr. Buchwald's group in Toronto last year. This research pertains to improved carrier and prenatal diagnosis as well as gene therapy. It is therefore of interest for families to find out whether they have a mutation in the already cloned FACC gene. In the future, we may also learn that certain mutations have different clinical courses than others and may be able to make clinical predictions from mutation-analysis.

4. Technical aspects

I will be very brief on this. In the lab, we can look for mutations in a gene in either the DNA itself or in the RNA produced from the gene. DNA is easier to isolate than RNA and does not require a growing cell line. However, the essential (coding) parts of genes often represent only a small portion of all the DNA in a gene and are often split into many different exons. Each exon has to be analyzed individually. The RNA, on the other hand, consists of only essential sequence and can be analyzed in one piece. The disadvantage of this approach, however, is that one first needs growing cells to isolate RNA. The process is therefore more time-consuming (see figure 4).

The OHSU repository used RNA analysis to look for unknown mutations (all possible mutations of the FACC gene) and DNA analysis to look for already known FACC mutations that are common.

Results of our FACC Mutation Analysis

When we first began the mutation analysis work, the true proportion of Fanconi anemia due to mutations in the FACC gene was unknown. We therefore set up a pilot study to analyze the RNA of 17 FA patients of unknown complementation groups. We used a technique called chemical mismatch cleavage to search for differences in the FACC gene between a healthy control and the 17 FA cells. In 4 cell lines (24%) we found clear differences, i.e. potential mutations. Upon closer analysis 3/4 were indeed mutations indicating a total incidence of 18% among our initial families.

1. The Ashkenazi Jewish FACC Mutation

Interestingly, 2 of the 3 patients had identical mutations and upon further questioning (that's why the questionnaires are important!) both families were of Eastern European Jewish descent (Ashkenazi indicates Eastern European origin). This led us to suspect that this particular mutation, which causes defective splicing, may be common among Jewish FA patients. We have since had the opportunity to examine 10 additional (for a total of 12) Jewish FA families. Ten out of 12 (83%) were positive for the same splice mutation. This indicates that this mutation causes the majority of Fanconi anemia in Ashkenazi Jews and that almost (but not quite) all Jewish FA patients belong to complementation group C.

There are some immediate benefits to this discovery. First, we can now provide accurate carrier detection and prenatal diagnosis in these families. Second, the NIH is very close to a gene therapy protocol for FA in FACC families.

2. Carrier frequency among Ashkenazi Jewish individuals

In order to determine how frequent FA carriers are among Jewish people, we set up a collaboration with the Tay-Sachs carrier detection program in California. We used a DNA test to test for the Jewish splice mutation among 314 individuals. Two out of 314 (0.6%) were positive, indicating a carrier frequency of 1/155. From a practical standpoint, this means the following. Should a known FACC carrier marry a spouse of Jewish descent, their chances of having a baby with FA would be 1/600.

3. Proportion of FACC mutations in all families

We have now completed the analysis of a total of 43 families in the OHSU cell repository. Among these, we have found 12 (28%) FACC families, of which 10 are Jewish. Only 2/31 (6%) non-Jewish families belong to complementation group C.

Future Directions

Much progress has been made in the last year in the understanding of Fanconi anemia. Yet it is clear that much work is yet to be done, especially in the isolation of additional FA genes. It is the commitment of the OHSU FA cell repository to provide all families who have submitted samples to us with up-to-date information on our results, especially those obtained with samples from your family. We will continue to perform mutation analysis for FACC and in the future other newly identified Fanconi anemia genes, so that you will be able to have access to improved diagnosis and hopefully therapy for this disease.

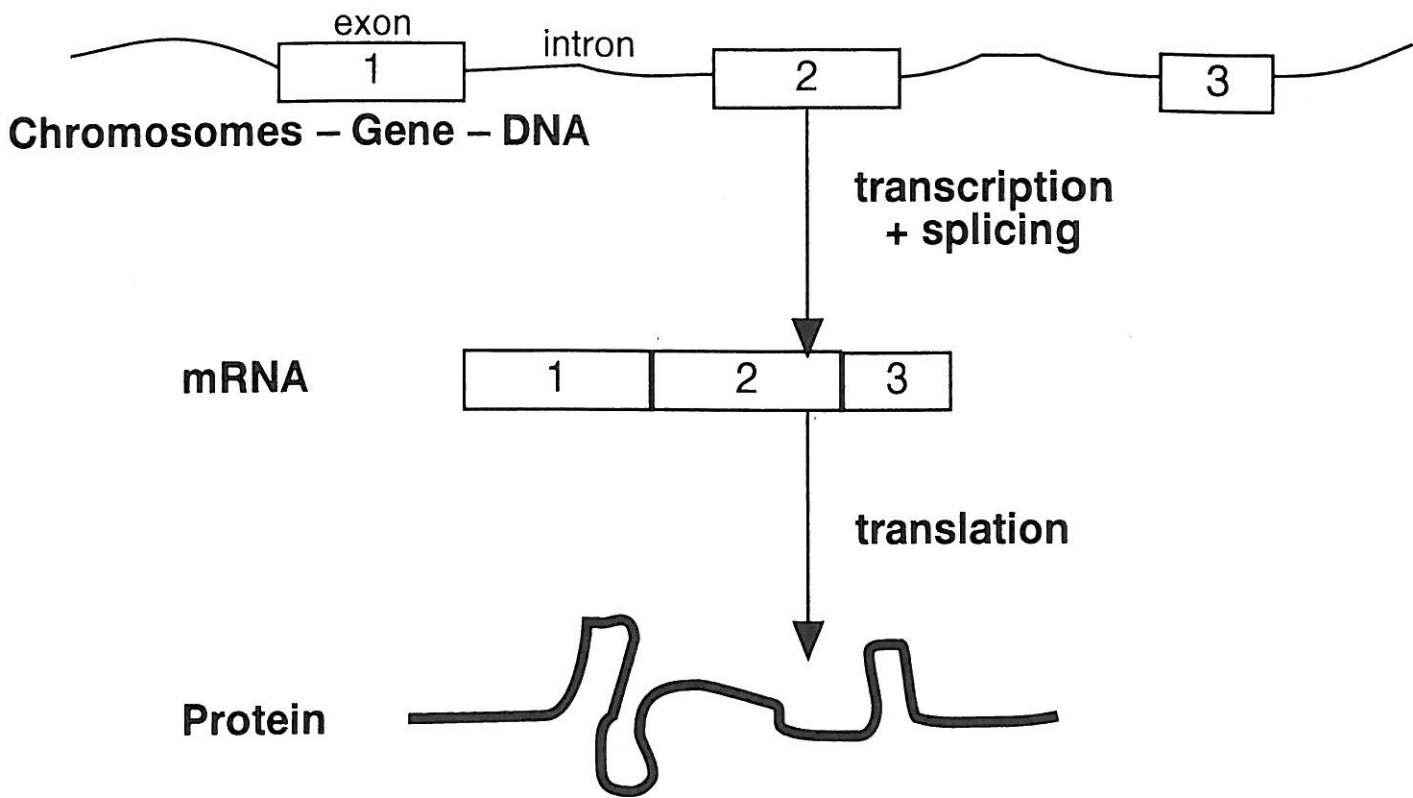


Figure 1

ATGCCCGCTTATCGAAAAGAATGA

Met Pro Ala Tyr Arg Lys Glu stop



ATGCCCGAAAAGAATGA

Met Pro Arg Lys Glu stop

Figure 2

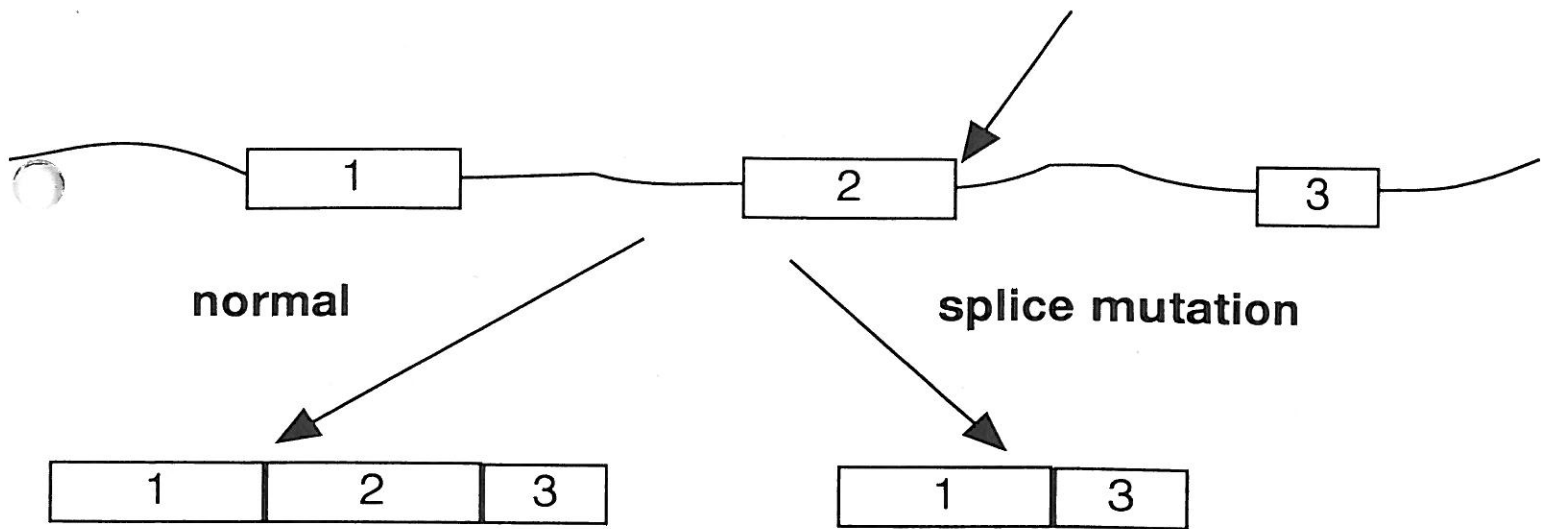


Figure 3

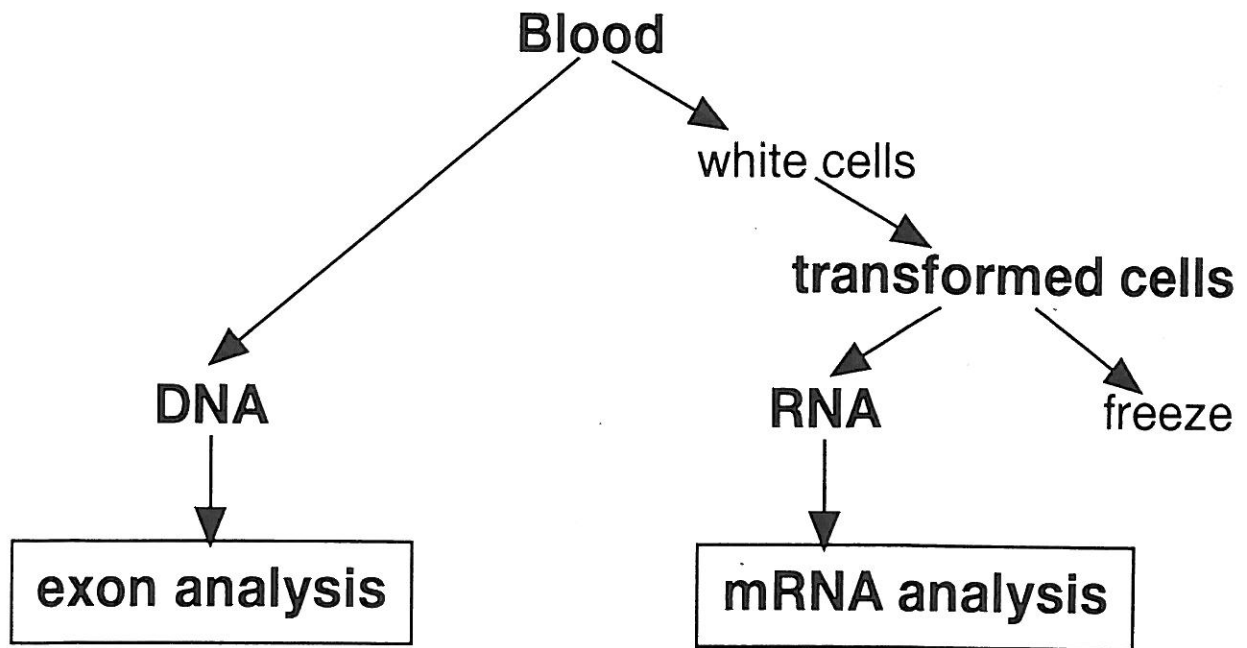


Figure 4

“Unrelated Bone Marrow Transplants”

Dr. Ramsay cautioned that there is very limited data on unrelated transplants with Fanconi anemia, and that one therefore has to be very careful in interpreting results.

Transplant centers began doing FA unrelated transplants in 1987. Although the numbers have grown steadily over the last six years, they are still very small.

As of 7-5-93 the National Marrow Donor Program had 912,000 potential donors. A preliminary search is free of cost, identifies the number of potential donors for a given patient (usually donors who match the patient at the “A” and “B” locus) and is available within 24 hours. A formal search takes a median of four months and can cost a significant amount, due to the need to DR type donors who have already been identified as A, B matches. If a donor has not been located, the search should be rerun every three to six months.

Most patients (97.9%) can find donors who match at the A and B locus only. However, as of 7/93 only 51.5% can find an A, B and DR match through the National Bone Marrow Donor Registry.

Process of Transplantation

The patient’s own bone marrow must be destroyed in order to make space for the new marrow. It is also necessary to suppress the patient’s immune system so that he or she will accept the new marrow. A common protocol used with Fanconi anemia patients consists of two days of low dose cyclophosphamide (about 10% of the dosage used for non FA patients) and low dose radiation. Then the new marrow is infused. It “seeds” the bone marrow and begins to produce new circulating blood cells in several weeks.

Problems Resulting from Transplantation

Bone marrow transplantation entails several risks:

1. Graft vs. host disease (GVHD) may develop. This happens when the new bone marrow reacts against the patient. GVHD can affect the skin, gut, liver and immune system. Drugs are given to prevent its occurrence and to treat its symptoms.
2. Graft failure. The patient’s immune system sees the new bone marrow as foreign and rejects the graft.
3. Infection. This can be viral, bacterial or fungal. The first two can be prevented or treated, the latter presents more of a problem.
4. Interstitial pneumonia
5. Later malignancy

Fanconi Anemia and Unrelated Transplants

Dr. Ramsay presented data on 24 Fanconi anemia patients who had received transplants from unrelated donors. Median age was 11.6 years. Seventeen patients had a 6/6 match; five had a 5/6 match. No data were available on two patients. Four had developed leukemia at the time of transplantation.

Of the 24 transplanted, ten or approximately 40% were alive from one month to 110 months post transplant, with a median survival of 2 1/2 years. The greatest complication was graft vs. host disease. Most deaths occurred in the first three months post transplant. Dr. Ramsay cau-

tioned again that these numbers are preliminary and the data are incomplete and very preliminary.

Dr. Ramsay concluded that proceeding with an unrelated transplant is a very personal question. Ideally, a transplant should be performed before conditions such as platelet sensitization or leukemic conversion are present. Other indicators that an unrelated transplant should be considered include the presence of an abnormal clone (especially a clone which is indicative of myelodysplasia progressing to leukemia). Other clinical conditions (refractory to androgens, transfusion dependent, showing evidence of androgen toxicity such as adenomas or liver dysfunction) would also be important indicators. To offer the patient the best outcome, bone marrow transplant should be performed before the patient is in poor clinical condition.

Dr. Ramsay cautioned that a patient’s status going into transplantation helps predict the outcome. One should not wait too long before proceeding. If the patient is too sick, it will be much harder to survive the procedure.

"A Worldwide Overview of Bone Marrow Transplants for FA Patients, Statistics and Success Rates"

Dr. Harris has compiled statistics concerning bone marrow transplantation for Fanconi anemia patients from the International Bone Marrow Transplant Registry, the National Bone Marrow Donor Registry, the Fred Hutchinson Cancer Research Center, the FA support group survey and various bone marrow transplant physicians. He presented a wealth of data at the Family Meeting concerning transplant outcomes for 255 FA patients. These include 186 matched sibling transplants, 44 haploidentical (meaning any fully or partially matched relative other than a fully matched sibling) transplants and 25 unrelated donor transplants.

Much of the data he presented were obtained from the International Bone Marrow Donor Registry. Of 116 FA patients transplanted using HLA identical siblings, the overall survival was 60%. Of 37 patients who had alternative donors (unrelated donors, or haploidentical donors), 30% were alive and well two years after transplant.

As one would expect, fully matched sibling transplants did the best. However, if the alternative donor was a 6/6 match, the results were almost as good as if the donor were a matched sibling. Fully matched haploidentical donor transplants had a 50-55% success rate, and fully matched unrelated donors succeeded 45-50% of the time. There was no statistically significant difference between these outcomes and those for a matched sibling donor transplant.

However, a significant difference is observed if the donor is a mismatched alternative donor. These clearly do not do as well. For example, if the haploidentical donor is a 6/6 match, 50-55% of the patients are alive and well two years post transplant. However, if the haploidentical donor is a 5/6 match, only 20-30% survive. If the haploidentical donor is a 4/6 or

3/6 match, only 2 out of 14 (or 15%) were alive and well two years post transplant. Dr. Harris concludes that a significant mismatch from a haploidentical relative does not suggest a good outcome. The more the mismatch, the worse the results.

Families sometimes have to choose between a 6/6 unrelated donor and a 5/6 haploidentical (related) donor. In this case, Dr. Harris advises choosing the matched unrelated donor. With a matched unrelated donor, the chance of success is approximately 50%, compared to 20-30% with a 5/6 haploidentical donor.

Factors which Influence Transplant Outcomes:

1) Transfusions are a risk factor. The best results occur when the patient has never been transfused, or has received few transfusions.

2) Younger patients (ten or younger) tend to do better than older patients.

3) Patients treated with low dose cyclophosphamide (cytoxan) do better than those given a higher dosage of cytoxan. Survival rate for patients receiving low dose cyclophosphamide was 70% compared to 50% for those receiving high dose cytoxan without radiation.

4) There is also a suggestion that patients treated with ATG to prevent graft vs. host disease fare better than those treated with other drugs. This difference is not statistically significant, but it is nonetheless intriguing.

To date, all 21 FA patients transplanted using the protocol developed at Cincinnati Children's Hospital who (1) had an HLA identical sibling donor and (2) had not developed leukemia at the

time of transplant are alive and well. Dr. Harris observed, appropriately, that he "may be onto something!"

Recommendations

1) For the FA patient with a matched sibling donor, Dr. Harris recommends that transplantation occur before age 10 and before transfusions or treatment with androgens when at all possible. He would transplant using low dose cytoxan, low dose radiation and ATG.

2) For the FA patient without a matched sibling donor, Dr. Harris again recommends that transplantation occur before age 10, before transfusions and before the development of leukemia if possible. The best alternative to a matched sibling donor is a fully matched haploidentical donor (a father, mother or other relative who is a 6/6 match). If that is unavailable, a 6/6 unrelated donor will still do quite well (45-50% success rate). Least likely to succeed is a mismatched haploidentical donor or mismatched unrelated donor. Here, the success rate drops to 30-20%.

Editors' Note and Disclaimer

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FA family *newsletter*

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