

## FA Research – A Retrospective and the Road Ahead

**Text of presentation by Hans Joenje, Ph.D., Dutch Cancer Society Professor of Oncogenetics, Vrije Universiteit Medical Center, Amsterdam, The Netherlands, at the Presenters' Dinner, Fanconi Anemia Research Fund Scientific Symposium, September 30, 2005, Geneva, Switzerland.**

I thank the organizers for the invitation to speak during this delightful dinner, even without an approved submitted abstract! I did get a few hints, though, about possible topics. I was asked: first – “please, look back to when you began your search for Fanconi Anemia (FA) genes and mention the roadblocks and successes.” And then: “please, give us your view on what needs to be done now in FA research.” And there was a specific question from Lynn Frohnmayer, who originally thought that: “Once the genes were discovered, we would have the cure in hand. So, how are the perspectives for the families today? And what should be done to get a cure as fast as possible?”

As you see, these are pretty tough questions and I realize that for this audience a simple “don't know” is not good enough.

Looking back is not the most exciting part, of course, so I'll try to be brief on that. We go back to the 1980s. In those years we, like many others, were studying FA at the level of the cellular phenotype, so we were watching the chromosomes break and tried to manipulate that process. But then, in the early 1990s things changed suddenly when Manuel Buchwald's lab came up with the two important breakthroughs that opened up the genetic approach: evidence for four genetic subtypes in a small random sample of only seven patients, suggesting a high level of genetic heterogeneity, *plus* a complementation cloning procedure that was successful to clone the *FANCC* gene and which seemed suitable to get perhaps some of the other genes as well. Now there was a perspective to study the disease at the level of genes, proteins and the molecular processes they are involved in.

Manuel was kind enough to let me come over to his lab for a brief (three months) sabbatical to get familiar with the ins and outs of cell fusion experiments and transfection procedures, and to enter into a collaboration in which we would extend the complementation and gene cloning experiments.

But then: how could you possibly think of embarking on such studies when living in a country where there are maybe one or two new FA patients per year, while we felt we needed at least one hundred patients to study? It was clear that the effort required international collaboration. So we got in touch with the major FA research groups in Europe and were lucky enough to get support from the European Union that allowed us to set up a so-called “Concerted Action” on FA.

The main questions were: 1) How many genes can actually cause FA? and 2) Can we clone those genes? This required setting up a cell repository and doing endless cell fusion experiments. Our lab in Amsterdam was chosen as a suitable site for the repository and the place where the complementation studies should be carried out, because we had already shown that in our hands the protocols actually worked.

Talking about road blocks: the collection of all the patient samples, the generation of cell lines from these, the cell fusion experiments, the library transfections. This meant a tremendous amount of work, not only in the lab, but also in the field. In such circumstances you need full commitment, collaboration, enthusiasm, some luck, and, last but not least, a source of inspiration. A major source of inspiration came from the patient support groups in the European countries and in the United States. Ralf Dietrich from the German FA Hilfe e.V. has been a key figure in the eventual successes. He literally bombarded us with blood samples, not only from families in Europe, but he was also a tireless driving force to help collect samples during the annual family meetings organized by the Fanconi Anemia Research Fund at Camp Sunshine, Maine.

Another road block. Soon it became clear that we needed more hands on the job, so we called upon the Dutch Cancer Society for additional support. They politely characterized our project as “interesting,” but unfortunately “not of sufficient priority.” And, then, we were lucky enough that the FA Research Fund helped us out with a one-year grant to support the project, which actually continued for many more years, because we kept finding new complementation groups.

Another piece of luck. From the beginning we had this idea of each group representing a distinct disease gene, but this was not self-evident. Remember AT, where four complementation groups were all connected to the same gene! This big investment in FA was therefore risky....

But the concept did appear to be valid in FA. Families that we had classified as FA-A were apparently connected to a single disease locus, because they allowed the mapping of *FANCA* by classical linkage analysis. Moreover, Buchwald's complementation cloning procedure was successful in cloning *FANCA*, as well as *FANCG*, *-F*, and *-E*.

So, the classification of patient s by cell fusion appeared pretty robust, although two groups were a problem: group H had to be withdrawn and D actually consisted of two groups, which was finally resolved by the research teams of Markus Grompe [Oregon Health and Science University] and Alan D'Andrea [Dana-Farber Cancer Institute], who identified *FANCD2* and *FANCD1/BRCA2*.

Finding *FANCE* was pretty much the end of the successful application of the library transfections. But, thanks to Weidong Wang's protein association approach, two complementation groups were added that were not previously defined by cell fusion studies, groups L and M, and this approach was also crucial to finally identify *FANCB*.

Where are we today? With *FANCI* also being identified, we have now twelve complementation groups, with all the genes known, except for the still elusive *FANCI*. And perhaps the best of all (but not entirely unexpected): the gene products collaborate in a pathway, a pathway that most likely plays an important role in the handling of a very basic problem in biology, which is: the resolution of a blocked replication fork. And last but not least: a pathway that appears to communicate with the major breast cancer susceptibility gene products. This connection is interesting,

not only scientifically, but also because it will hopefully motivate the major research funding organizations to now start investing in basic FA research.

And now: the road ahead.

The FA pathway is a complex puzzle that will, however, in the long run be solved by the basic scientists, one way or another. FA research is no longer a field for FA scientists only. The basic scientists who study the fundamental processes of DNA replication and repair will now adopt the FA pathway, and this will greatly accelerate progress—as well as increase the competition!

But what role could the Fanconi Anemia Research Fund play in the increasingly turbulent FA research field? The *raison-d'être* for the Fund has always been clear, their mission statement being “to find effective treatments and a cure for FA.” And Lynn’s question: “are we any closer to a cure now that we have the genes at hand?” directly follows from this mission statement.

I think the answer to Lynn’s question must be: “yes, we certainly are!” Not because a cure is within reach today or tomorrow, but because we are well underway to reach a definite understanding of the process that goes wrong in FA patients. Without having the genes, we would be really very far away from such understanding. There will be no guarantee that full understanding will bring us a cure, but insight is essential to be at least in a position to think of productive ways that can lead to a cure.

On the other hand, there are ways towards curative treatment of FA patients for which full understanding of the FA pathway is not absolutely essential, although knowing the genes certainly will be a great help in many instances. The five Research Priorities that are listed on the Fund’s website (and which you all know by heart, I’m sure) aim at improving the perspectives for FA patients by eliminating the main life-threatening symptoms: the marrow failure and cancer risk. All these priorities can be better studied with the genes at hand, because the phenotypic heterogeneity of FA patients may now be correlated with genetic subtype and/or mutations. And, of course, the options of gene therapy and preimplantation genetic diagnosis would be non-existent without having the genes available.

Let’s talk a bit more about research priority number one, which is my personal bias. This reads: “To identify the FA genes and understand the function of their products.” Suppose we had all twelve genes, including *FANCI*. Are we in need of knowing any more genes? The answer to this question is most certainly: “yes, we are!” Remember that, in retrospect, genes from the most common complementation groups (A, C, and G) have given us only poorly informative orphan proteins, in contrast to the much rarer subtypes, D1, D2, L, M, and J, whose proteins turned out to be much more informative key-players in the pathway! So, if we want to have the complete picture, we obviously shouldn’t stop here!

Even if any new genes may not be genuine FA genes (in the sense that they are found mutated in FA patients), their role in the pathway may be enlightening and perhaps crucial for understanding. Powerful tools are now available to get to such genes:

the protein association studies, as well as the chicken DT40 system, have already proven their unique potential, while the cell-free DNA replicating extracts from *Xenopus laevis* offer another unique and powerful approach.

Perhaps we should now also start to pay more attention to patients who look somewhat like FA but who do not quite fit the clinical phenotype, although they do share some of the symptoms. Think of the patients in the D1 group, with biallelic mutations in the *BRCA2* gene, who seem to have a syndromic association that makes them distinct from classical FA. Call them distinct or overlapping, but see how much we can learn from identifying the disease gene in this rare group of patients. Something similar has been the case with Seckel syndrome and Nijmegen breakage syndrome patients.

In our cell repository there is a cell line from a female FA-like patient, confirmed by mitomycin C-induced chromosomal breakage testing, who is atypical in that she is now over 65 and has shown still no sign of malignancies. Think of what we might be able to learn once we know the gene that is defective in this patient!

I hope to have convinced you that there is ample reason to go ahead and find the remaining genes that act in the FA pathway. We, the scientists, are ready for that!

And, finally, what can the Fanconi Anemia Research Fund do?

Please, do continue to inspire us! Please, do continue to challenge our brains so one day we will come up with the solutions you need!

We strongly feel it is our moral obligation to help FA patients and families, and we would appreciate it if you won't stop reminding us of this task.

Thank you for your attention.