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Why, What and How Can We Learn from a Rare Disease Like Fanconi Anemia?

Traute Schroeder-Kurth

Department of Human Genetics, University of Würzburg, Würzburg, Germany

Abstract

In a field that embraces multiple aspects of both clinical and basic research and that moves impressively fast, any answers to the questions why, what and how can we learn from a rare disease like Fanconi anemia (FA) must remain tentative and preliminary. However, there are very encouraging advances, most notably at the level of understanding the molecular basis of FA and at the level of treatment via hematopoietic stem cell transplantation. For the sake of our patients we clearly need to arrive at meaningful genotype-phenotype correlations and individualized risk profiles. This requires prospective and longterm studies carried out in close cooperation among patients, clinicians and basic scientists. There are a number of open questions, for example relating to the mechanisms of chromosomal breakage and DNA repair, to the spectrum of genetic changes that herald and promote the emergence of leukaemia and solid tumors, and to the emergence of genetically reverted cells in blood and bone marrow of FA patients. Researchers in the fields of cancer and aging should be encouraged to and are likely to benefit from the study of Fanconi anemia, as are our patients from the welcome results of such studies.

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The year 2007 marks the 80th anniversary of the original description by Guido Fanconi of ‘Familial infantile pernicious-like anemia’, a rare genetic disease that since carries the name of this eminent physician-scientist [1]. Why should we study such a rare disease, what can we learn from its myriad clinical and molecular manifestations, and how should we go about it? I will try to give some brief answers to these questions even though by necessity these answers are tentative and preliminary. Despite undoubted progress, there still are many aspects of this enigmatic disease that we do not fully understand.

Why Study Fanconi Anemia?

First of all, for the sake of the affected patients. Even though there is no definite cure for FA on the horizon, there is encouraging progress regarding diagnosis and treatment. Timely diagnosis is crucial both for the institution of adequate treatment and for the prevention of inadequate medical management that may result from failure to recognize the hereditary and specific nature of the disease. If one looks back at the last monograph on Fanconi anemia that I had the pleasure to edit with my colleagues Arleen Auerbach and Gunter Obe in the year 1989 [2], the progress that has since been made is impressive and encouraging. Two major areas stand out: molecular genetics, and hematopoietic stem cell transplantation. Progress in the understanding of the molecular basis of Fanconi anemia is astounding indeed: even though genetic heterogeneity of FA had been convincingly documented prior to 1989 [3, 4], it was not until 1992 that the first FA gene had been cloned and, as of this writing, 12 FA causing genes have been identified. Several more are in the offing, as there are a number of patients who apparently do not belong to any of the known complementation groups [5]. There is also increasing understanding of how the FA proteins work, how they work together and how they might interact with other proteins in maintaining a stable genome [6, 7].

Although less spectacular than the gene discovery itself, the virtual explosion of knowledge concerning the molecular basis of Fanconi anemia during the past 10–15 years translates into immediate benefits to FA patients and their families. For example, precise knowledge of the affected gene and the specific type of mutation in a given patient will increasingly be of prognostic value and serve as a rational guide for optimized medical management. We clearly need to collect many more data in order to arrive at clinically useful genotype-phenotype correlations. We should strongly oppose arguments that deem the collection of such additional data unnecessary once the clinical diagnosis of FA has been established and confirmed by a chromosome breakage or cell cycle test. The ultimate goal of such efforts is to arrive at individualized risk profiles for FA patients similar to what has already been achieved with congenital abnormality scores [8]. Biallelic mutations in two of the FA genes (*FANCD1/BRCA2* and *FANCN/PALB2*) have so far been associated with very early childhood cancers [9, 10], and awareness of such correlations are of undisputed value in the clinical management of these young tumor patients. Precise knowledge of the disease causing mutations also improves and facilitates prenatal diagnosis that remains an unwelcome but compromise option for some of the FA families. Last but not least, participation in gene therapy trials requires knowledge of underlying genes and mutations.

Although one may question the extent to which the vastly improved molecular knowledge has so far been of immediate benefit to the majority of

FA patients, such reservations do least apply to the progress in the field of hematopoietic stem cell transplantation (HSCT). The outcomes of matching donor sibling transplantations have continuously improved such that today early HSCT is highly recommended in such families [11]. Unrelated donor transplantation still carries a higher risk, but modified conditioning protocols using fludarabine and T-cell depletion, reduction or complete absence of whole body irradiation, the introduction of minitransplants and improved post-transplant care have contributed to much better longterm survival [12]. The serious problem of posttransplant squamous cell carcinomas remains to be solved [13]. The message from all this progress is to consider the option of HSCT in each patient at a much earlier stage of the disease and with much more optimism than we could have had in the year 1989.

What Can We Learn from Fanconi Anemia?

We have learned that introducing a gene into something as accessible as a bone marrow stem cell does not necessarily result in improvement [14]. There are many reasons for the slow progress in gene therapy, but there is no reason to give up. Improvements in stem cell collection and improved gene transfer protocols are on the horizon [15, 16]. Notwithstanding all previous failures and disappointments, the replacement of a defective gene copy by an intact gene remains an attractive option which should and needs to be further explored.

Looking back at the recent progress of FA research it seems to me a kind of miracle that at first chromosome instability showed up and initiated the search for possible molecular mechanisms causing breaks, gaps and reunions which we saw in the chromosome preparations. Our very first observations reported in 1964 taught us that chromosomal instability was present not only in patients presenting with FA symptoms, but also in their siblings without any clinical manifestation of the disease [17]. We also quickly learned that chromosome instability occurs *in vivo* as it was observed in direct preparations of bone marrow cells [18]. If dividing bone marrow cells exhibit chromosome aberrations, this surely must reflect a fundamental and intrinsic defect of genomic maintenance with severe consequences for the renewal of blood cell lineages. The more we learned about the complex structure that makes up a chromosome, the more naive appeared the simple notion of a DNA lesion that was not properly repaired and therefore manifests as a chromosome break. Why do breaks reunite if there is a repair defect, and what are the prerequisites for a reunion to take place? Why do crosslink-induced radials preferentially form between non-homologous autosomes and are virtually absent from gonosomes? [19]. What is the role of low copy repeats that are increasingly recognized as focal points for

non-allelic homologous recombination? [20]. Does the chromosomal position within the nucleus influence the opportunity for the type of radial formation? There are many unsolved questions, there still is much to be learned.

One of the most impressive lessons we learned from patients with Fanconi anemia is the intimate relationship between genetic instability and cancer. FA patients carry a high risk for acute leukaemia, squamous cell carcinomas and other tumors [21]. Young FA patients with certain gene mutations have a higher risk to develop leukaemia. The older a FA patient gets the higher is the risk to develop a solid tumor. There are impressive case histories describing adult patients in whom a leukaemia or a solid tumor were the first manifestations of FA [22, 23]. With the exception of *FANCF* that is frequently inactivated by an epigenetic mechanism in various types of tumors, the other FA genes seem to be relatively intact in most neoplasias as if they were needed for tumor cell growth [24]. However, monoallelic truncating mutations in at least three of the FA genes (*FANCD1/BRCA2*, *FANCI/BRIP1*, and *FANCG/PALB2*) have been shown to confer variant degrees of breast cancer susceptibility in non-FA patients [25, 26]. Those are important new insights, connecting a rare with a common disease.

In 1971 we postulated that in vivo chromosomal instability leads to multiple somatic mutations which finally may confer a selective advantage to a single mutated cell, giving rise to malignant cell growth [27]. By careful bone marrow studies, this process has been directly observed in FA patients. Specific chromosomal changes in bone marrow cells appear to herald progression to malignancy even prior to any clinical manifestation of leukaemia [28]. It was quite clear in the 1970s that aberrant clones may also appear in peripheral blood lymphocyte cultures. Over time, these cytogenetically aberrant clones were subject to clonal attenuation and clonal succession, but they undoubtedly were bad news for the patient. One of these patients I followed for more than six years with repeated chromosome studies [29]. He died at age 35 of bronchial cancer (unpublished). These types of longterm observations are important for our patients and should be supported as much as possible. There is much to be learned about the close relationship between FA and cancer for the sake of our patients, and for our understanding of the origin of cancer [30].

Even though a fundamental change of the high cancer risk in FA is not in sight, we have already learned that predictive and preventive measures are beneficial for our patients. As already mentioned, regular monitoring for aberrant bone marrow clones, particularly those involving monosomy 7 and 3q duplications, is of great value for both physicians and patient in their difficult decision as to whether and when to proceed with HSCT [28]. Likewise, non-invasive measures for the early detection of oral cavity and genital area lesions as currently developed by the group of Ruud Brakenhoff in Amsterdam [31] will gain even greater importance with more and more patients undergoing and surviving HSCT.

Another lesson we are beginning to appreciate from Fanconi anemia is the discovery of reverse mutations in blood cells [32]. One in four or five patients displays MMC resistant cells among the original MMC sensitive cells. These patients are mosaic FA patients who have a chance to escape BM failure and, possibly, the development of leukaemia. Much research has to be done to clarify the underlying mechanisms. The likelihood of ‘natural gene therapy’ leading to revertant mosaicism appears to be higher in patients belonging to certain complementation groups and in the presence of compound heterozygosity. Longterm observations of these mosaic FA patients will ultimately teach us whether there is more than a temporary benefit of this type of ‘natural gene therapy’ to the individual, and what the prerequisites are for somatic reversions to occur. And, of course, could there be ways to enhance the occurrence of such reversions for the benefit of our patients?

How Should We Go About It?

First and foremost, FA research needs the cooperation of the affected families and patients as well as the unrelenting interest and commitment of their doctors. A well established (and well funded!) FA registry such as pioneered by Arleen Auerbach in New York is essential for both clinical care and basic research [33, 34]. It should include regular follow-ups with complete information on the natural history of the disease in each individual family. For practical purposes, such registries should be organized at the regional or national levels, but they should and must communicate with each other. Today we have every reason to believe that most FA families are aware of the importance of FA research for the sake of their children and thus are willing to cooperate with such registries. Many research projects require the participation and involvement of the patients themselves, and there is little doubt that the affected patients and families can still teach us a lot about their disease. On the technical side, new tools such as retroviral complementation, knockdown of FA genes via RNA interference, or MLPA for the detection of large deletions have contributed both to diagnosis and research. With such technical advances at hand the time might have come to tackle the old question of what really causes the oxygen sensitivity of FA cells [35, 36].

We now know that at least some of the FA genes are highly conserved during evolution and that they play a major role during DNA replication and recombination in premeiotic stages and meiosis. Some of the FA genes have been recognized as very ancient ‘caretaker’ genes that have emerged even prior to the vertebrate lineage [37]. In addition to valuable insights gained from vertebrate model organisms such as zebrafish, mouse and birds, scientists working

on ancient model organisms like yeast, *C. elegans* and *Drosophila* should be invited and encouraged to participate in FA-oriented basic research. Last but not least, Fanconi anemia should be introduced as a human model system into basic cancer research. It has already been shown that targeted inactivation of FA genes in cancer cells may offer specific therapeutic options [38, 39]. I am convinced that both, our patients and our colleagues involved in cancer research will benefit from inclusion of Fanconi anemia into current research paradigms.

Concerning the premature aging phenotype of Fanconi anemia, it is obvious that truly progeroid features (such as in the Hutchinson-Gilford or Werner syndromes) are far less conspicuous in FA. The decline of bone marrow function in FA clearly is a much more progressive and devastating event compared to what happens during normal aging. This is the likely reason why Martin and Oshima did not include FA in their presentation of human genetic instability syndromes with progeroid features [40]. However, squamous cell carcinomas of the oral cavity and genital area are typical tumors of advanced age that occur very prematurely in FA patients. Likewise, endocrine abnormalities including hyperinsulinemia, growth hormone deficiency and hypothyroidism as typical endocrine abnormalities of older individuals affect more than 80% of FA patients during young adulthood [41]. Impaired gametogenesis and premature reproductive aging are additional features of FA patients that are reminiscent of an accelerated aging process. Collectively, the premature manifestation, in FA patients, of clinical features also encountered during normative aging seems to indicate that genetic instability per se may be one of the most significant factors that contributes to and promotes aging. From this point of view, scientists involved in aging research might benefit from the study of Fanconi anemia. Conversely, Fanconi anemia patients may ultimately benefit from the insights gained from the ongoing efforts of serious and scientifically sound anti-aging research.

References

- 1 Lobitz S, Velleuer E: Guido Fanconi (1892–1979): a jack of all trades. *Nat Rev Cancer* 2006;6: 893–898.
- 2 Schroeder-Kurth TM, Auerbach AD, Obe G (eds): *Fanconi Anemia. Clinical, Cytogenetic and Experimental Aspects*. Berlin, Heidelberg, New York, Springer-Verlag, 1989.
- 3 Zakrzewski S, Sperling K: Genetic heterogeneity of Fanconi's anemia demonstrated by somatic cell hybrids. *Hum Genet* 1980;56:81–84.
- 4 Duckworth-Rysiecki G, Cornish K, Clarke CA, Buchwald M: Identification of two complementation groups in Fanconi anemia. *Somat Cell Mol Genet* 1985;11:35–41.
- 5 Hanenberg H, Batish SD, Pollok KE, Vieten L, Verlander PC, et al: Phenotypic correction of primary Fanconi anemia T cells with retroviral vectors as a diagnostic tool. *Exp Hematol* 2002;30: 410–420.

- 6 Taniguchi T, D'Andrea AD: Molecular pathogenesis of Fanconi anemia: recent progress. *Blood* 2006;107:4223–4233.
- 7 Gurtan AM, D'Andrea AD: Dedicated to the core: understanding the Fanconi anemia complex. *DNA Repair (Amst)* 2006;5:1119–1125.
- 8 Rosenberg PS, Huang Y, Alter BP: Individualized risks of first adverse events in patients with Fanconi anemia. *Blood* 2004;104:350–355.
- 9 Alter BP, Rosenberg PS, Brody LC: Clinical and molecular features associated with biallelic mutations in *FANCD1/BRCA2*. *J Med Genet* 2007;44:1–9.
- 10 Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, et al: Biallelic mutations in *PALB2* cause Fanconi anemia subtype FA-N and predispose to childhood cancer. *Nat Genet* 2006;39:162–164.
- 11 Rosenberg PS, Alter BP, Socie G, Gluckman E: Secular trends in outcomes for Fanconi anemia patients who receive transplants: implications for future studies. *Biol Blood Marrow Transplant* 2005;11:672–679.
- 12 Wagner JE, Eapen M, Macmillan ML, Harris RE, Pasquini R, et al: Unrelated donor bone marrow transplantation for the treatment of Fanconi anemia. *Blood* 2007;109:2256–2262.
- 13 Alter BP: Fanconi's anemia, transplantation, and cancer. *Pediatr Transplant* 2005;9:81–86.
- 14 Williams DA, Croop J, Kelly P: Gene therapy in the treatment of Fanconi anemia, a progressive bone marrow failure syndrome. *Curr Opin Mol Ther* 2005;7:461–466.
- 15 Jacome A, Navarro S, Casado JA, Rio P, Madero L, et al: A simplified approach to improve the efficiency and safety of ex vivo hematopoietic gene therapy in Fanconi anemia patients. *Hum Gene Ther* 2006;17:245–250.
- 16 Kelly PF, Radtke S, Kalle C, Balcik B, Bohn K, et al: Stem cell collection and gene transfer in Fanconi anemia. *Mol Ther* 2007;15:211–219.
- 17 Schroeder TM, Anschütz F, Knopp A: Spontane Chromosomenaberrationen bei familiärer Panmyelopathie. *Humangenetik* 1964;1:194–196.
- 18 Schroeder TM: Cytogenetische und cytologische Befunde bei enzymopenischen Panmyelopathien und Pancytopenien. *Humangenetik* 1966;2:287–316.
- 19 Newell AE, Akkari YM, Torimaru Y, Rosenthal A, Reifsteck CA, et al: Interstrand crosslink-induced radials form between non-homologous chromosomes, but are absent in sex chromosomes. *DNA Repair (Amst)* 2004;3:535–542.
- 20 Erdogan F, Chen W, Kirchhoff M, Kalscheuer VM, Hultschig C, et al: Impact of low copy repeats on the generation of balanced and unbalanced chromosomal aberrations in mental retardation. *Cytogenet Genome Res* 2006;115:247–253.
- 21 Rosenberg PS, Greene MH, Alter BP: Cancer incidence in persons with Fanconi anemia. *Blood* 2003;101:822–826.
- 22 Liu JM, Auerbach AD, Young NS: Fanconi anemia presenting unexpectedly in an adult kindred with no dysmorphic features. *Am J Med* 1991;91:555–557.
- 23 Huck K, Hanenberg H, Gudowius S, Fenk R, et al: Delayed diagnosis and complications of Fanconi anaemia at advanced age – a paradigm. *Br J Haematol* 2006;133:188–197.
- 24 Neveling K, Kalb R, Schindler D: Cancer in Fanconi anemia and Fanconi anemia genes in cancer; in Schindler D, Hoehn H (eds): *Fanconi Anemia*, Basel, Karger-Verlag, 2007, pp 59–78.
- 25 Seal S, Thompson D, Renwick A, Elliott A, Kelly P, et al: Truncating mutations in the Fanconi anemia J gene *BRIP1* are low-penetrance breast cancer susceptibility alleles. *Nat Genet* 2006;38:1239–1241.
- 26 Rahman N, Seal S, Thompson D, Kelly P, Renwick A, et al: *PALB2*, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* 2006;39:165–167.
- 27 Schroeder TM, Kurth R: Spontaneous chromosomal breakage and high incidence of leukemia in inherited disease. *Blood* 1971;37:96–112.
- 28 Tonnies H, Huber S, Kuhl JS, Gerlach A, Ebell W, Neitzel H: Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: gains of the chromosomal segment 3q26q29 as an adverse risk factor. *Blood* 2003;101:3872–3874.
- 29 Schroeder TM, Drings P, Beilner P, Buchinger G: Clinical and cytogenetic observations during a six-year period in an adult with Fanconi's anemia. *Blut* 1976;34:119–132.
- 30 Mathew CG: Fanconi anaemia genes and susceptibility to cancer. *Oncogene* 2006;25:5875–5884.

- 31 Bremmer JF, Braakhuis BJ, Ruijter-Schippers HJ, Brink A, Duarte HM, et al: A noninvasive genetic screening test to detect oral preneoplastic lesions. *Lab Invest* 2005;85:1481–1488.
- 32 Lo Ten Foe JR, Kwee ML, Rooimans MA, Oostra AB, Veerman AJ, et al: Somatic mosaicism in Fanconi anemia: molecular basis and clinical significance. *Eur J Hum Genet* 1997;5:137–148.
- 33 Auerbach AD, Schroeder TM: First announcement of the Fanconi Anemia International Registry. *Blood* 1982;60:1054.
- 34 Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, et al: A 20-year perspective on the International Fanconi Anemia Registry (IFAR). *Blood* 2003;101:1249–1256.
- 35 Pagano G, Degan P, d'Ischia M, Kelly FJ, Nobili B, et al: Oxidative stress as a multiple effector in Fanconi anaemia clinical phenotype. *Eur J Haematol* 2005;75:93–100.
- 36 Mukhopadhyay SS, Leung KS, Hicks MJ, Hastings PJ, Youssoufian H, Plon SE: Defective mitochondrial peroxiredoxin-3 results in sensitivity to oxidative stress in Fanconi anemia. *J Cell Biol* 2006;175:225–235.
- 37 Nanda I, Buwe A, Wizenman A, Takata M, Haaf T, Schartl M, Schmid M: Fanconi anemia genes in vertebrates: evolutionary conservation, sex-linkage, and embryonic expression of *FANCC* and *FANCG* in avian cells; in Schindler D, Hoehn H (eds): *Fanconi Anemia*, pp 183–199. Basel, Karger-Verlag, 2007.
- 38 van der Heijden MS, Brody JR, Dezentje DA, Gallmeier E, Cunningham SC, et al: In vivo therapeutic responses contingent on Fanconi anemia/BRCA2 status of the tumor. *Clin Cancer Res* 2005;11:7508–7515.
- 39 Gallmeier E, Calhoun ES, Rago C, Brody JR, Cunningham SC, et al: Targeted disruption of *FANCC* and *FANCG* in human cancer provides a preclinical model for specific therapeutic options. *Gastroenterology* 2006;130:2145–2154.
- 40 Martin GM, Oshima J: Lessons from the human progeroid syndromes. *Nature* 2000;408:263–266.
- 41 Wajnrajch MP, Gertner JM, Huma Z, Popvich J, Lin K, et al: Evaluation of growth and hormonal status in patients referred to the International Fanconi Anemia Registry. *Pediatrics* 2001;107:744–754.

Prof. Dr. med. Traute Schroeder-Kurth
Wilhelm-Doles-Str. 7
D-97246 Eibelstadt (Germany)
Tel./Fax +49 9303 8762