Chapter 1 : Genetic Diagnosis of Fanconi Anemia

Fanconi anemia (FA) is a rare inherited disease characterized by developmental defects, short stature, bone marrow failure, and a high risk of malignancies. FA is heterogeneous: 15 genetic subtypes have been distinguished so far. A clinical diagnosis of FA needs to be confirmed by testing cells for.

Published sequence variations in FA genes, with their descriptions conforming to the current nomenclature rules, are listed at http: Most FA genes encode orphan proteins with no known molecular function. This idea is strengthened by the recent extension of the FA pathway with SLX4, a scaffold protein for structure-specific endonucleases involved in unhooking the DNA cross-link [3 , 4]. This cellular phenotype is ascertained using stimulated blood T lymphocytes. The indications for FA laboratory testing are rather broad [32]. Since mutation testing by Sanger sequencing and MLPA is rather laborious, time consuming and therefore expensive, a positive chromosomal breakage test is a prerequisite for starting mutation screening. Confirmation of the FA diagnosis at the DNA level is important in patients in whom the chromosomal breakage test was inconclusive. Furthermore, knowledge about the FA subtype is relevant for the treatment and prognosis of the patients. In addition, identification of mutations allows carrier testing in the family and will enable prenatal DNA testing and preimplantation genetic diagnosis PGD in future pregnancies. Finally, this information can be used to rule out FA in potential donors for bone marrow transplantation. Although simultaneous testing of all FA genes by next generation sequencing will be possible in the near future, this technique will not be available immediately for all laboratories worldwide. In addition, in populations with strong founder mutations, a limited test using Sanger sequencing and MLPA will be a cost-effective alternative [33]. The strategy outlined below has been developed at our DNA diagnostics laboratory to provide a molecular diagnosis of FA. However, depending on the circumstances strategies may differ from case to case. Materials Genomic DNA from e. Screening on cDNA is more efficient but has several drawbacks: In addition, common alternative splice variants will hamper the evaluation of DNA sequences. Therefore, screening on gDNA is the preferred method for mutation screening. However, during the diagnostic process, growing cells from the proband will be helpful in a couple of situations. Growing cells are indispensable for studying the effect of unclassified variants on splicing or to verify the disease gene by functional complementation of the cellular phenotype with a construct expressing a wild type copy of the suspected gene [35 â€” 37]. Finally, if no mutations can be de detected, growing cells can be used to reconfirm the diagnosis FA by checking MMC sensitivity in cell growth or G2-arrest assays [38 , 39]. Mutation Screening Strategy 2. Hints from Ethnic Background or Phenotype Information on the ethnic background of the proband may provide a clue for a specific pathogenic mutation that most likely causes the disease, such as c. More examples of recurrent mutations are shown in Table 3. This is especially worthwhile if confirmed by the cellular phenotype:

Chapter 2 : FANCA | Cancer Genetics Web

There are two main approaches to the prenatal confirmation or exclusion of Fanconi anemia: functional testing and molecular testing. Functional testing involves the determination of crosslink.

Research Article Diagnosis of Fanconi Anemia: This is an open access article distributed under the Creative Commons Attribution License , which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Abstract Fanconi anemia FA is a rare inherited disease characterized by developmental defects, short stature, bone marrow failure, and a high risk of malignancies. A clinical diagnosis of FA needs to be confirmed by testing cells for sensitivity to cross-linking agents in a chromosomal breakage test. As a second step, DNA testing can be employed to elucidate the genetic subtype of the patient and to identify the familial mutations. Although simultaneous testing of all FA genes by next generation sequencing will be possible in the near future, this technique will not be available immediately for all laboratories. In addition, in populations with strong founder mutations, a limited test using Sanger sequencing and MLPA will be a cost-effective alternative. In addition, the follow up with respect to genetic counseling and carrier screening in the families is discussed. Introduction Fanconi anemia FA is a rare inherited syndrome with diverse clinical symptoms including developmental defects, short stature, bone marrow failure, and a high risk of malignancies. Fifteen genetic subtypes have been distinguished: These percentages may differ considerably within certain ethnic groups, due to founder effects. The mode of inheritance for all subtypes is autosomal recessive, except for FA-B, which is X-linked. These two different modes of inheritance have important consequences for the counseling of FA families. For all genetic subtypes disease genes have been identified Table 1. Many mutations found in the various subtypes are private, but recurrent mutations are known, particularly in specific ethnic backgrounds Table 2. Fanconi anemia complementation groups, genes, and proteins. Major recurrent mutations in FA. Most FA genes encode orphan proteins with no known molecular function. This idea is strengthened by the recent extension of the FA pathway with SLX4, a scaffold protein for structure-specific endonucleases involved in unhooking the DNA cross-link [3 , 4]. This cellular phenotype is ascertained using stimulated blood T lymphocytes. The indications for FA laboratory testing are rather broad [32]. Since mutation testing by Sanger sequencing and MLPA is rather laborious, time consuming and therefore expensive, a positive chromosomal breakage test is a prerequisite for starting mutation screening. Confirmation of the FA diagnosis at the DNA level is important in patients in whom the chromosomal breakage test was inconclusive. Furthermore, knowledge about the FA subtype is relevant for the treatment and prognosis of the patients. In addition, identification of mutations allows carrier testing in the family and will enable prenatal DNA testing and preimplantation genetic diagnosis PGD in future pregnancies. Finally, this information can be used to rule out FA in potential donors for bone marrow transplantation. Although simultaneous testing of all FA genes by next generation sequencing will be possible in the near future, this technique will not be available immediately for all laboratories worldwide. In addition, in populations with strong founder mutations, a limited test using Sanger sequencing and MLPA will be a cost-effective alternative [33]. The strategy outlined below has been developed at our DNA diagnostics laboratory to provide a molecular diagnosis of FA. However, depending on the circumstances strategies may differ from case to case. Materials Genomic DNA from e. Screening on cDNA is more efficient but has several drawbacks: In addition, common alternative splice variants will hamper the evaluation of DNA sequences. Therefore, screening on gDNA is the preferred method for mutation screening. However, during the diagnostic process, growing cells from the proband will be helpful in a couple of situations. Growing cells are indispensable for studying the effect of unclassified variants on splicing or to verify the disease gene by functional complementation of the cellular phenotype with a construct expressing a wild type copy of the suspected gene [35 â€” 37]. Finally, if no mutations can be de detected, growing cells can be used to reconfirm the diagnosis FA by checking MMC sensitivity in cell growth or G2-arrest assays [38 , 39].

Mutation Screening Strategy 2. Hints from Ethnic Background or Phenotype Information on the ethnic background of the proband may provide a clue for a specific pathogenic mutation that most likely causes the disease, such as c. More examples of recurrent mutations are shown in Table 3. This is especially worthwhile if confirmed by the cellular phenotype: The quantitative multiplex ligation-dependent probe amplification MLPA method [46] is used for this initial screen, which identifies FANCA as the most likely disease gene in 1 out of 4 patients by the detection of aâ€”usually hemizygous-deletion. Most PCRs can be performed under standard conditions. The PCR primers have M13 extensions which allow sequencing of all fragments with universal sequencing primers. MLPA was performed according to the instructions of the supplier. Detailed information about the sequences of the MLPA probes is available from the website of the supplier http: In the small group of patients without mutations no complementation analysis or FANCD2 western blotting was performed. Table 3 does not include prenatal cases, because prenatal testing is only offered in couples in which the FA-causing mutations are already established. Testing was offered as a diagnostic service for which a fee was charged. If this technique is not available, further analysis will depend on the availability of growing cells from the proband. The latter two syndromes can also be excluded by analyzing metaphase spreads for sister chromatid cohesion defects. Mutation Screening in Mosaic Patients If an available lymphoblastoid cell line from an FA patient is phenotypically normal due to genetic reversion at the disease locus, mutation screening is still possible in the reverted cell line, since at least one mutation will be present [49 â€” 51]. The second mutation may be identified through investigating the parents. Alternatively, they can be tested for pathogenicity in a cellular transfection assay to check the ability of the variant gene product to complement the cellular FA defect in a deficient cell line see e. Generally, these tests are only feasible in a setting where a diagnostic laboratory is equipped with a research laboratory with all necessary technology. Functional Assignment to Genetic Subtypes Retroviral constructs have been used to identify the FA subtype by functional complementation, as an intermediate step before a mutation screen is undertaken [36]. Although knowing the disease gene facilitates mutation screening, retroviral transduction has some drawbacks in comparison to direct mutation screening: Genetic Counseling All patients with a diagnosis of FA confirmed by mutation analysis should be referred for genetic counselling, together with their parents and siblings. Mutation testing should be performed in all sibs regardless of any clinical symptoms. A complete pedigree, including a cancer history anamnesis, should be prepared. Mutation carriers might be at increased cancer risk see Section 3. FA patients themselves usually have decreased fertility. Women usually have late menarche, irregular menses, and early menopause. However, pregnancies in women with FA have been described, and therefore women should be adequately informed about the risks for their offspring, which is mainly related to an increase in pregnancy-related complications [53]. Sibs of the parents of an FA patient often request carrier screening to assess their risk of getting a child with FA. If a sib appears to be carrier, this risk is still minimal because of the very low carrier frequency in the population. In the US the carrier frequency has been estimated to be about 1 in [54]. The risk of a proven carrier to get a child with FA is therefore about 1 in However, in small communities or in consanguineous couples this risk is much higher, and mutation screening in spouses of proven carriers may be indicated. Prenatal Diagnosis Prenatal diagnosis of FA is relatively straightforward after the pathogenic mutations in a given family have been identified. Fetal cells can be obtained by chorionic villus sampling CVS during weeks 10â€”12 of the pregnancy or by amniocentesis, which is performed between weeks 14 and However, CVS may be preferred as the diagnosis will be known at an earlier stage. If the mutation is not known, a chromosomal breakage test on fetal material may be performed [55], but this test may be considered less reliable than screening for mutations in the fetal material. Alternatively, flow cytometric testing of MMC sensitivity in amniotic cell cultures might be an option; however this technique is only available in a limited number of specialized laboratories [56]. Occasionally, FA may be suspected by fetal ultrasound imaging and confirmed by parental carrier testing when the family is not yet known to carry a risk for FA [57]. Genotype-Phenotype Correlation FA is considered as one disease, and the question may be raised whether all fifteen genetic subtypes equally conform to the clinical FA phenotype. Genotype-phenotype

correlation studies comparing the 3 most common groups A, C, and G indicated modest phenotypic differences, which were rather correlated with the relative severity of the mutations [23]. However, bias due to the ethnic distribution of the studied population is very well possible. The observations that one of the pathogenic mutations in BRCA2 in FA-D1 patients is hypomorphic and that mice with biallelic null alleles in Brca2 are embryonic lethals suggest that the BRCA2 protein serves a function that is essential for survival. Different mutations in the same gene may be associated with divergent phenotypes, as illustrated by the two FANCC mutations, c. The former splice-site mutation is associated with a relatively severe phenotype in Ashkenazi Jewish people [19] although the associated phenotype was reportedly less severe in patients of Japanese ancestry [20]. The carrier frequency for this mutation in the Ashkenazi population is relatively high 1 in 87 , which has led to the recommendation of carrier detection to prevent disease [59]. The phenotype associated with this mutation, like other exon 1 mutations, seems relatively mild, as these patients rarely have skeletal abnormalities and show a relatively late age of onset of their marrow failure [24]. Awareness of such genetically determined phenotypic differences may help in clinical decision making, including the counselling of patients and families. Cancer Risk in Heterozygous Mutation Carriers An important issue is whether FA mutation carriers are at increased risk to develop cancer or other types of disease. Overall, there is no increased risk for cancer among FA heterozygotes [60 , 61]. However, the situation is different in some of the less prevalent FA subtypes. Whether the parent with the hypomorphic mutation is also at increased risk is unknown: Although cancer patients have been identified with germ-line mutations in these genes, an accurate estimate of the relative cancer risk for mutation carriers is still lacking. Nevertheless, in the few female FANCB mutation carriers studied so far, inactivation appeared strongly skewed towards the mutated allele [67]. This suggests that FA cells have a poor chance to survive next to unaffected cells in the same tissue, and these FA cells may therefore not give an increased cancer risk. However, the data are scarce at present so that no firm conclusions can be drawn regarding the cancer risk of female FANCB mutation carriers [60]. Conflict of Interests The authors do not declare any conflict of interests related to this study. View at Google Scholar S. View at Google Scholar A. Guidelines for Diagnosis and Management, M. View at Google Scholar M. View at Google Scholar N. Lo Ten Foe, M.

Chapter 3 : Carrier Screening for Genetic Conditions - ACOG

Fanconi anemia (FA) is a rare genetic disorder affecting multiple body systems. Genetic testing, including prenatal testing, is a prerequisite for the diagnosis of many clinical conditions.

This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed. Carrier screening is a term used to describe genetic testing that is performed on an individual who does not have any overt phenotype for a genetic disorder but may have one variant allele within a gene s associated with a diagnosis. Information about carrier screening should be provided to every pregnant woman. Carrier screening and counseling ideally should be performed before pregnancy because this enables couples to learn about their reproductive risk and consider the most complete range of reproductive options. A patient may decline any or all screening. When an individual is found to be a carrier for a genetic condition, his or her relatives are at risk of carrying the same mutation. The patient should be encouraged to inform his or her relatives of the risk and the availability of carrier screening. If both partners are found to be carriers of a genetic condition, genetic counseling should be offered. What follows is a detailed discussion of some of the more common genetic conditions for which carrier screening is recommended in at least some segments of the population. Recommendations and Conclusions The American College of Obstetricians and Gynecologists the College makes the following recommendations and conclusions: General Recommendations Information about genetic carrier screening should be provided to every pregnant woman. After counseling, a patient may decline any or all screening. Carrier screening and counseling ideally should be performed before pregnancy. Concurrent screening of the patient and her partner is suggested if there are time constraints for decisions about prenatal diagnostic evaluation. Prenatal diagnosis and advanced reproductive technologies to decrease the risk of an affected offspring should be discussed. The obstetricianâ€”gynecologist or other health care provider should not disclose this information without permission from the patient. It is important to obtain the family history of the patient and, if possible, her partner as a screening tool for inherited risk. The family history should include the ethnic background of family members as well as any known consanguinity. Individuals with a positive family history of a genetic condition should be offered carrier screening for the specific condition and may benefit from genetic counseling. Because of the rapid evolution of genetic testing, additional mutations may be included in newer screening panels. The decision to rescreen a patient should be undertaken only with the guidance of a genetics professional who can best assess the incremental benefit of repeat testing for additional mutations. Prenatal carrier screening does not replace newborn screening, nor does newborn screening replace the potential value of prenatal carrier screening. If a patient requests carrier screening for a particular condition for which testing is readily available and which reasonably would be considered in another screening strategy, the requested test should be offered to her regardless of ethnicity and family history after counseling on the risks, benefits, and limitations of screening. The cost of carrier screening for an individual condition may be higher than the cost of testing through commercially available expanded carrier screening panels. When selecting a carrier screening approach, the cost of each option to the patient and the health care system should be considered. Recommendations for Specific Conditions Spinal Muscular Atrophy Screening for spinal muscular atrophy should be offered to all women who are considering pregnancy or are currently pregnant. In patients with a family history of spinal muscular atrophy, molecular testing reports of the affected individual and carrier testing of the related parent should be reviewed, if possible, before testing. If the reports are not available, SMN1 deletion testing should be recommended for the low-risk partner. Cystic fibrosis carrier screening should be offered to all women who are considering pregnancy or are currently pregnant. For couples in which both partners are unaffected but one or both has a family history of cystic fibrosis, genetic counseling and medical record review should be performed to determine if CFTR mutation analysis in the affected family member is available. Hemoglobinopathies A complete blood count

with red blood cell indices should be performed in all women who are currently pregnant to assess not only their risk of anemia but also to allow assessment for risk of a hemoglobinopathy. Ideally, this testing also should be offered to women before pregnancy. A hemoglobin electrophoresis should be performed in addition to a complete blood count if there is suspicion of hemoglobinopathy based on ethnicity African, Mediterranean, Middle Eastern, Southeast Asian, or West Indian descent. If red blood cell indices indicate a low mean corpuscular hemoglobin or mean corpuscular volume, hemoglobin electrophoresis also should be performed. Fragile X Syndrome Fragile X premutation carrier screening is recommended for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome and who are considering pregnancy or are currently pregnant. If a woman has unexplained ovarian insufficiency or failure or an elevated follicle-stimulating hormone level before age 40 years, fragile X carrier screening is recommended to determine whether she has an FMR1 premutation. Prenatal diagnostic testing for fragile X syndrome should be offered to known carriers of the fragile X premutation or full mutation. DNA-based molecular analysis eg, Southern blot analysis and polymerase chain reaction is the preferred method of diagnosis of fragile X syndrome and of determining FMR1 triplet repeat number eg, premutations. In rare cases, the size of the triplet repeat and the methylation status do not correlate, which makes it difficult to predict the clinical phenotype. In cases of this discordance, the patient should be referred to a genetics professional. Genetic Conditions in Individuals of Eastern and Central European Jewish Descent When only one partner is of Ashkenazi Jewish descent, that individual should be offered screening first. If it is determined that this individual is a carrier, the other partner should be offered screening. However, the couple should be informed that the carrier frequency and the detection rate in non-Jewish individuals are unknown for most of these disorders, except for Tayâ€”Sachs disease and cystic fibrosis. Tayâ€”Sachs Disease Screening for Tayâ€”Sachs disease should be offered when considering pregnancy or during pregnancy if either member of a couple is of Ashkenazi Jewish, Frenchâ€”Canadian, or Cajun descent. Those with a family history consistent with Tayâ€”Sachs disease also should be offered screening. When one member of a couple is at high risk ie, of Ashkenazi Jewish, Frenchâ€”Canadian, or Cajun descent or has a family history consistent with Tayâ€”Sachs disease but the other partner is not, the high-risk partner should be offered screening. If the high-risk partner is found to be a carrier, the other partner also should be offered screening. Enzyme testing in pregnant women and women taking oral contraceptives should be performed using leukocyte testing because serum testing is associated with an increased false-positive rate in these populations. If Tayâ€”Sachs disease screening is performed as part of pan-ethnic expanded carrier screening, it is important to recognize the limitations of the mutations screened in detecting carriers in the general population. In the presence of a family history of Tayâ€”Sachs disease, expanded carrier screening panels are not the best approach to screening unless the familial mutation is included on the panel. Referral to an obstetricianâ€”gynecologist or other health care provider with genetics expertise may be helpful in instances of inconclusive enzyme testing results or in discussion of carrier testing of an individual with non-Ashkenazi Jewish ethnicity whose reproductive partner is a known carrier of Tayâ€”Sachs disease. Introduction Carrier screening is a term used to describe genetic testing that is performed on an individual who does not have any overt phenotype for a genetic disorder but may have one variant allele within a gene s associated with a diagnosis. Information about genetic carrier screening should be provided to every pregnant woman. Carrier screening and counseling ideally should be performed before pregnancy because this enables couples to learn about their reproductive risk and consider the most complete range of reproductive options, including whether or not to become pregnant and whether to use advanced reproductive technologies such as preimplantation genetic diagnosis or use of donor gametes. Knowledge during pregnancy allows patients to consider prenatal diagnosis and pregnancy management options in the event of an affected fetus. Ideally, information on the specific mutation will be available to aid testing and counseling. Although several different strategies for screening are available and reviewed in Committee Opinion No. The different sections collect topics that had previously been discussed in separate Committee Opinions to show how the aforementioned general principles are used and reflected in carrier

screening for specific genetic conditions. The disorder is caused by a mutation in the gene known as the survival motor neuron gene SMN1 , which is responsible for the production of a protein essential to motor neuron function. Because of the severity and relatively high carrier frequency, there has been increasing interest in carrier screening for spinal muscular atrophy in the general prenatal population 3. The genetics of spinal muscular atrophy are complex and, because of limitations in the molecular diagnostic assays available, precise prediction of the phenotype in affected fetuses may not be possible. The incidence of spinal muscular atrophy is approximately 1 in 6, to 1 in 10, live births, and the disease is reported to be the leading genetic cause of infant death. Carrier frequencies in most populations are estimated at 1 in 40 to 1 in 60, but carrier frequencies appear to be lower in the Hispanic population 1: Carrier frequencies and residual risks are outlined by ethnicity in Table 1. There is no effective treatment for the disease. There are several types of spinal muscular atrophy based on age at symptom onset. Earlier onset is correlated with more severe manifestations. The most severe and most common form of the disease, type I Werdnigâ€"Hoffman , has symptomatic onset before 6 months of age and causes death from respiratory failure within the first 2 years of life. Type II spinal muscular atrophy is of intermediate severity, with typical onset before 2 years of age. Affected children are able to sit, but few are able to stand or walk unaided. Respiratory insufficiency is a frequent cause of death during adolescence; however, the lifespan of patients with spinal muscular atrophy type II varies from age 2 years to the third decade of life. However, the symptom profile is quite variable. Affected individuals typically reach all major motor milestones, but function ranges from requiring wheelchair assistance in childhood to completely unaided ambulation into adulthood with minor muscular weakness. Many patients have normal life expectancies. Type IV has onset in adulthood. There is an additional Type 0 proposed, which has onset in the prenatal period. There is generally one copy of SMN1 per chromosome, but occasionally two can be located on the same chromosome. A variable number of SMN2 gene copies ranging from zero to three may be present, but the SMN2 gene produces only a small amount of functional survival motor neuron protein. A higher number of SMN2 copies correlates with generally milder clinical phenotypes, but accurate prediction of the spinal muscular atrophy phenotype based on SMN2 copy number is not possible 5. However, this approach is not sufficient to identify patients who are heterozygous, or carriers, for the SMN1 deletion. Carrier testing requires a quantitative polymerase chain reaction assay that provides a measure of SMN1 copy number. Detection of a single normal copy of SMN1 would indicate the carrier state Figure 1. There are limitations, however, to the use of this assay to determine carrier status. These individuals are carriers because one of their chromosomes is missing the SMN1 allele. Therefore, the counseling of patients who are tested for carrier status must account for the residual risk present when carrier screening assay results are negative, particularly in patients from families affected by spinal muscular atrophy. Carrier Screening Screening for spinal muscular atrophy should be offered to all women who are considering pregnancy or are currently pregnant and have had appropriate counseling about the possible range of severity, carrier rate, and detection rate. Posttest counseling should reiterate residual risk after negative screening based on the number of SMN1 copies present. In patients with a family history of spinal muscular atrophy, molecular testing reports of the affected individual and carrier testing of the related parent should be reviewed, if possible, before testing to determine the residual risk for the patient with a negative screen. If this individual is found to be a carrier, the couple should be referred for further genetic counseling and consideration of further genetic testing in the high-risk partner. The goal of cystic fibrosis carrier screening is to identify individuals at risk of having a child with classic cystic fibrosis, which is defined by significant pulmonary disease and pancreatic insufficiency. Cystic fibrosis is more common among the non-Hispanic white population compared with other racial and ethnic populations; however, because of the increasing difficulty in assigning a single ethnicity to individuals, in , the American College of Obstetricians and Gynecologists recommended offering cystic fibrosis carrier screening to all patients. Cystic fibrosis is the most common life-threatening, autosomal recessive condition in the non-Hispanic white population. The disease incidence is 1 in 2, individuals in the non-Hispanic white population and considerably less in other ethnic groups. It is a progressive, multisystem disease that primarily

affects the pulmonary, pancreatic, and gastrointestinal systems butdoes not affect intelligence. The current median predicted survival is approximately 42 years, with respiratory failure as the most common cause of death 7. Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane regulator CFTR gene, located on chromosome 7. Two copies of deleterious mutations in this gene cause cystic fibrosis. Therefore, screening is most efficacious in non-Hispanic white and Ashkenazi Jewish populations.

Chapter 4 : Poster Presentation on Fanconi Anemia

Fanconi anemia (FA) is a rare inherited disease characterized by developmental defects, short stature, bone marrow failure, and a high risk of malignancies. FA is heterogeneous: 15 genetic subtypes have been distinguished so far.

Research Article Diagnosis of Fanconi Anemia: This is an open access article distributed under the Creative Commons Attribution License , which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Abstract Fanconi anemia FA is a rare genetic instability syndrome characterized by developmental defects, bone marrow failure, and a high cancer risk. Fifteen genetic subtypes have been distinguished. Since FA patients need special clinical management, the diagnosis should be firmly established, to exclude conditions with overlapping phenotypes. Identification of the pathogenic mutations is also important for adequate genetic counselling and to facilitate prenatal or preimplantation genetic diagnosis. Here we describe and validate a comprehensive protocol for the molecular diagnosis of FA, based on massively parallel sequencing. Introduction Fanconi anemia FA is a recessive chromosomal instability syndrome with diverse clinical symptoms and a high risk for acute myeloid leukemia and squamous cell carcinoma of the head and neck region [1]. Clinical suspicion of FA is mostly based on growth retardation and congenital defects in combination with life-threatening bone marrow failure thrombocytopenia and later pancytopenia , which usually starts between 5 and 10 years of age. Currently, mutations in 15 different genes are known to cause FA, and their gene products act in a pathway that takes care of specific problems that may arise during the process of DNA replication [2]. The conventional Sanger sequencing-based mutation screening approach for FA is time-consuming, costly, and most importantly may not detect all types of disease-causing aberrations, such as deep intronic mutations, large deletions, and amplifications. Here, we demonstrate a comprehensive mutation detection approach for FA based on massively parallel sequencing MPS [3]. Methods We designed an in-solution oligonucleotide hybridization capture kit SureSelect, Agilent targeting the open reading frames of all FA genes, except for regions that contain repetitive and low complexity DNA sequences as assessed by RepeatMasker http: BED format, are available upon request. In addition, a number of other genes involved in cancer predisposition and routinely screened in our diagnostic lab were included in the enrichment kit. To assess the performance of the custom target kit and the massively parallel sequencing method, we selected FA samples with a spectrum of different types of known variations Table 1. The pathogenic mutations in these samples have previously been identified either by Sanger sequencing or by multiplex ligation-dependent probe amplification MLPA [4]. One of the samples included in the study was from a carrier of a BRCA1 mutation. Unique barcode sequences were used for 11 DNA libraries to allow distinction between the samples that were run on a single Illumina flow-cell lane. In addition, to evaluate the sensitivity of the approach, two DNA samples were pooled before library preparation to mimic a mosaic condition. Selected FA samples with mutations previously identified by Sanger sequencing and multiplex ligation-dependent probe amplification MLPA , used for validation of the next-generation sequencing approach. An in-house variation detection pipeline, including a novel tool for large deletion detection, was used to score for relevant mutations. Library Preparation For each sample, 1. MS, USA using the following settings: Next, DNA library that hybridized to the baits is captured using magnetic beads Dynabeads, Invitrogen , washed, and eluted in elution buffer. Primers containing unique barcode sequences are used to amplify captured libraries, and equimolar pooling is performed after quantification on a bioanalyzer. The eleven pooled DNA libraries were then sequenced on a single flow cell lane of an Illumina GAIIx using a 72 cycle multiplex paired-end sequence protocol. An average of 2. Several types of disease-causing genetic aberrations were present in the assayed DNA samples including single nucleotide substitutions, small deletions 1â€”8 nucleotides , and large deletions multiple exons. We developed a variation detection pipeline detecting all these types of aberrations. Numbers on top of the bars indicate the average sequencing depth obtained for the individual samples. Blue bars indicate total number of unique reads, red

bars indicate reads that fall on target of the bait design, green bars indicate reads that fall in target regions covered with at least 20x depth. Sample 4 is a mix of genomic DNA from two individuals 4a and 4b. The resulting list of variations is annotated with Annovar [17] that utilizes information from external databases to generate context around the mutations, such as amino acid change consequence, location to canonical splice site regions, and information about reference to dbSNP and frequencies if available. Finally, a manual filtering step is executed to prioritize relevant mutations. Low-frequency frameshifts and truncating mutations are considered pathogenic. Unreported nonsynonymous amino acid variations are analyzed in silico by the pathogenicity predicting programs, Align-GVGD, Polyphen-2 and SIFT [7 â€” 9] to help assess the damaging effect. The total number of variations detected in the samples and the subsequent reduction in number of variations through filtering is depicted in Table 2. Pathogenic mutation detection through filtering. Using the variation detection pipeline and filtering procedure 12 SNVs out of the total of 13 were detected. The variation that escaped the initial filtering procedure resided deep in the intronic region; nucleotide upstream of the exon start site. However, the variation was present in the initial variation list. Firstly, a reference local read depth is established by binning read counts in a preset sliding window using data from all pooled samples. The local read depth is also determined for each sample separately, using the same preset sliding window used for the reference. A Log2 ratio is calculated for each window by dividing the local read depth of the sample by the reference. Normalization is performed through a mean shift to zero. The copy number data is projected on the open reading frame ORF of the gene and also projected on an exon scale, where the mean read count is aggregated on a per exon basis. Interestingly, in sample 11 only one pathogenic nucleotide substitution in FANCI has previously been identified while the other mutation remained undetected. Here we show the deletion of the last exon exon 38 of the gene as detected by our large indel analysis tool Figure 2 d. We confirmed the deletion by PCR and Sanger sequencing by using a SNP in the last unaffected exon 37 and two sets of primers amplifying up- and downstream of the breakpoint Figure 3. Large detection using next-generation sequence data. Copy number data are projected on an open reading frame ORF and on an exon scale. The ORF scale upper panel shows log2 ratios M for all exons and introns. Red segments indicate an overlap with an exon and black segments indicate no overlap with exons. The exon scale lower panel only shows the mean log2 ratio per exon with their 25th and 75th percentile. R plots of large deletion analysis. A part of exon 37 with a SNP was amplified with primers designed either up- or downstream of the deletion breakpoint. Sequence analyses resulted in the detection of the SNP red arrow as heterozygous or hemizygous, respectively. Identification of Pathogenic Mutations in Unclassified FA Patients To investigate if our next-generation sequencing method also identifies mutations in unclassified FA patients, we investigated five patients that were sent in for FA mutation screening, without prior knowledge about their gene defect. In two other patients, novel homozygous mutations were detected. The other patient Unc3 showed a missense mutation in exon 28 of FANCI that changed codon from a cysteine to a tyrosine. The affected amino acid is highly conserved, up to fruit fly, suggesting that it may have an important function. Moreover, in silico analysis by SIFT [9] and Polyphen 2 [8] predicted the amino acid change to be damaging. All the identified variations were confirmed by Sanger sequencing, and the novel variations had a proper segregation within the family. In one patient no pathogenic variants were detected, although the coding regions of all known FA genes were covered deep enough to call variants. This suggests that the patient has a defect in a new FA gene or is not a true FA patient. All the FA mutations identified by Sanger sequencing were also detected by next-generation sequencing. Moreover, we discovered a novel large deletion in the FA-I patient, for whom only one truncating mutation was previously identified [4]. The exact breakpoint at nucleotide level could be distinguished as also the intronic regions of the FA genes were enriched and sequenced. We confirmed the deletion by PCR and Sanger sequencing, using a SNP in the last unaffected exon and two sets of primers amplifying the regions up- and downstream of the breakpoint Figure 3. The sensitivity of the method was demonstrated by mixing two DNA samples prior to library preparation and enrichment. A deletion of one allele in a background of four alleles can be detected, suggesting that the

method is even applicable for the classification of mosaic FA patients. However, a thorough assessment of the method using serial dilutions with samples harboring large deletions is required to determine the detection limit of the assay. As large deletions have also been demonstrated for FANCI this study and FANCN [6], it is plausible that these types of aberrations are present in other FA samples, which were previously unclassified by conventional molecular screening methods. Therefore, these types of mutations should be examined in the standard molecular diagnostics of FA. The presence of FANCD2 pseudogenes can complicate the identification of pathogenic mutations in this gene, as variations will tend to have a reduced frequency due to the occurrence of multiple highly similar copies. However, this difficulty can be resolved with bioinformatics by flagging variations that tend to have lower frequencies than expected within those regions. In cases where all other FA genes are excluded for mutations, careful inspection is required for flagged variations. Deep intronic mutations represent another type of variation, which are not analyzed with the classical molecular diagnostics approach. When we applied our novel molecular diagnostics approach on unclassified FA patients, we identified pathogenic mutations in four individuals. All the variations were confirmed with Sanger sequencing and demonstrated proper segregation with the disease. The frameshift mutation in FANCL results in a stop codon in the last exon of the gene, which likely produces an mRNA targeted for nonsense mediated decay. Nevertheless, functional studies are required to ultimately classify this variation as pathogenic. In one FA sample we could not detect the disease causing mutations in any of the known FA genes. This patient might represent a novel FA subtype and whole-exome sequencing might be a useful approach to identify the affected gene. Mutation detection in unclassified FA patients. Altogether, inspection of different variation types and inclusion of intronic regions warrants a comprehensive molecular FA diagnosis. Given the average number of variations of around per patient, it appears a difficult task to recognize the disease-causing mutation s. Here we propose a prioritization approach following the recessive mode of inheritance Figure 4. When no large deletions are identified, an initial filtering for nonsynonymous and canonical splice site variations should be performed. In cases where only one heterozygous pathogenic mutation is found, close examination of variations in intronic- and UTR regions in the same gene is required. When no pathogenic variations have been detected, assessment of all unique intronic and UTR variations is needed. With an ever expanding variant database of characterized FA patients, the identification of pathogenic mutations will become less complicated. Nevertheless, the necessity for functional tests, such as retroviral complementation or transfection, will remain essential to help assess the pathogenic status of unclassified missense variants. Prioritization scheme for the detection of disease-causing mutations by next-generation sequencing. In conclusion, multiplexed next-generation sequencing based on massively parallel sequencing is an effective molecular diagnostics approach for FA. The procedure, performed on genomic DNA, reduces the turnaround time, number of assays, and costs for a reliable detection of the disease-causing mutation. With the ever decreasing costs of enrichment and sequencing procedures, we expect that in the near future this will be the first test for patients clinically suspected of FA, thus avoiding labor-intensive chromosomal breakage assays and reducing turnaround time for FA diagnosis. To increase the efficiency of the molecular diagnosis, genes involved in other bone marrow failure syndromes e.

Chapter 5 : A Case Report of Fanconi Anemia Diagnosed by Genetic Testing Followed by Prenatal Diagno

FANCONI ANEMIA (FA) is a rare autosomal recessive disease characterized by multiple congenital abnormalities, bone marrow (BM) failure, and cancer susceptibility. The mean age of onset of anemia is 8 years, and the mean survival is 16 years. Death usually results from complications of BM failure.

This article has been cited by other articles in PMC. Abstract Fanconi anemia FA is a rare genetic disorder affecting multiple body systems. Genetic testing, including prenatal testing, is a prerequisite for the diagnosis of many clinical conditions. However, genetic testing is complicated for FA because there are often many genes that are associated with its development, and large deletions, duplications, or sequence variations are frequently found in some of these genes. This study describes successful genetic testing for molecular diagnosis, and subsequent prenatal diagnosis, of FA in a patient and his family in Korea. Multiplex ligation-dependent probe amplification analysis was performed to detect large deletions or duplications in the FANCA gene. The amniotic fluid cells were c. This is the first report of genetic testing that was successfully applied to molecular diagnosis of a patient and subsequent prenatal diagnosis of FA in a family in Korea. FA shows severe genetic heterogeneity, although the proteins encoded by FA-related genes are considered to work together in a common pathway that regulates cellular resistance to DNA cross-linking agents [2]. At least 15 genes have been identified that are responsible for FA complementation groups: Molecular diagnosis of FA is quite complicated, not only because at least 15 genes are associated with its development, but also the mutation spectra of most FA-associated genes are very diverse and some of these genes frequently contain large deletions or duplications [7 - 9]. Mutational information is a prerequisite for genetic counseling of family members, screening of potential bone marrow transplantation donors who are phenotypically and hematologically normal, and prediction of clinical prognosis on the basis of genotype-phenotype correlations. We describe the successful application of genetic testing to the molecular diagnosis of FA, and subsequently to prenatal diagnosis of FA, in a patient and his family in Korea. The patient was the first child of unrelated healthy parents born after 41weeks of pregnancy. The patient had a history of recurrent pneumonia, epistaxis, easy bruising, urinary urgency, and perineal area pain. He had no eyeball abnormalities or ear problems. Urological examination was unremarkable. His initial complete blood cell count results were as follows: Repeat complete blood cell counts indicated persistent thrombocytopenia. No bone marrow examination was included in the initial study. No family members both parents and a younger sister had experienced symptoms and manifestations that were similar to those of the patient.

Chapter 6 : Diagnosis of Fanconi Anemia: Mutation Analysis by Next-Generation Sequencing

Fanconi anemia, George Martin was one of the first scientists to recognize the intimate relationship between genetic instability, cancer and aging by studying the Werner progeria syndrome.

It was originally characterized in in three siblings with a combination of pancytopenia, hyperpigmentation, and multiple congenital anomalies including short stature and urogenital and skeletal manifestations 1. While this classical presentation with hyperpigmentation, short stature and pancytopenia remains the most frequent phenotype, manifestations in some patients are subtle. In addition to congenital anomalies patients with Fanconi anemia are predisposed to hematological malignancies and solid tumors. Up to this point, mutations in 19 genes are known to cause Fanconi anemia, with all sharing a common pathway. These genes have all been shown to be involved in DNA repair pathway for inter-strand cross-links 4. Thus the inability to respond to DNA damage is thought to be the cause of bone marrow failure as well as the propensity for these patients to develop malignancies 3. Not all patients with Fanconi anemia have characteristic physical anomalies resulting often in delayed diagnosis; the mean age of diagnosis in patients without congenital abnormalities is significantly higher than those with congenital abnormalities 1. Virtually all patients develop bone marrow failure by the age of 40, with the median age of bone marrow failure of 7 years of age 1. This bone marrow failure is progressive and all hematopoietic lineages are affected 2. Cancer is highly prevalent in patients with Fanconi anemia, with hematologic cancers being most common. Solid tumors are also of great concern in these patients, especially if they live to middle age. Liver tumors are the next most common, followed by brain, kidney, breast and adrenal gland 1,2. Largely because of this increased risk of malignancy, these patients have a short life expectancy with a median estimated survival of 23 years 1. Thrombocytopenia and macrocytosis are often the first irregularities with development of granulocytopenia, anemia and eventually severe aplasia 2. When Fanconi anemia is expected, the initial functional test includes analysis for an excessive chromosome breakage. This testing can also be done on samples collected by chorionic villus sampling, amniocentesis or percutaneous umbilical blood sampling 2. Definitive diagnosis of Fanconi anemia is identified with increased chromosome breaks per cell with an average range of 1. Congenital and physical anomalies can occasionally be surgically repaired. Because of the risk of bone marrow failure and hematologic malignancy, the best therapy for these patients involves hematopoietic stem cell transplantation. Transplants with HLA matched donors have greatly improved the outcomes of these patients, especially for those transplanted before the age of 10 3. Prior to transplantation, blood transfusions can be used treat anemia and thrombocytopenia. Androgen therapy can often increase reticulocytosis, hemoglobin, white blood cell and platelet counts over the course of months. G-CSF therapy can also increase granulocyte counts though only temporarily, with progression of bone marrow failure and an eventual loss of response usually within a year 3. Because of the improved survival with respect to hematologic manifestations, screening for the development of solid malignancies has become more important as they become increasingly common with survival into adulthood. Management and treatment of these malignancies is complicated by extreme sensitivity to certain chemotherapeutic agents because of the aforementioned defects in DNA repair pathways. Vaccination against HPV can help to prevent development of squamous cell carcinomas of the head and neck and cervix 3. Endocrine abnormalities such as hypothyroidisim, glucose intolerance and growth hormone deficiency are common and should be screened for and treated in a similar fashion as in non-Fanconi anemia patients 2,3,5. Uniparental Disomy Uniparental disomy UPD is defined as the presence of two copies of chromosomes originating from a single parent 6. This can further be broken down into uniparental heterodisomy with two different alleles from a single parent being transmitted, and uniparental isodisomy with two copies of the same allele from the single parent being transmitted 5. UPD is caused by errors in meiotic and mitotic nondisjunction, and can result in aneuploidy or chromosomal rearrangement 5. These types of abnormalities have been shown to be related to the age of the mother at conception. Uniparental isodisomy is characterized

by long regions of homozygosity affecting a single chromosome.. In this particular patient, the UPD16 likely results in Fanconi anemia due to the presence of two identical DNA segments on two chromosome 16 homologs which were inherited from a parent who carries a mutation in the FANCA gene, or other FA associated genes on chromosome 16, thus resulting in homozygous mutation. Fanconi Anemia and its Diagnosis. A DNA repair disorder characterized by accelerated decline of the hematopoietic stem cell compartment and other features of aging. Basic Principles and Practice, Chapter 27, Nelson Textbook of Pediatrics, Chapter , Eggerman, T et al. Trends in Molecular Medicine.

Chapter 7 : Cytogenetics Case - Case

To explore the potential of flow cytometry in the prenatal exclusion or confirmation of Fanconi anemia (FA). Indications for prenatal diagnosis were (1) FA-negative family history, but suspicious.

This is a review of the genetic, molecular and clinical aspects of FA as well as of the future prospects. Introduction Fanconi Anemia FA is a rare genetic disorder that is mainly inherited in an autosomal recessive pattern and is rarely X-linked. It was first described in by the Swiss pediatrician Guido Fanconi who described three affected siblings with congenital abnormalities and progressive marrow failure at the age of years [1]. It is the most frequent inherited instability syndrome, characterized by bone marrow failure, hypersensitivity to cross-linking agents and high risk for Acute Myeloid Leukemia AML. Age of diagnosis ranges from birth to 49 years and the male-to-female ratio is 1. The incidence rate is about 1 in , births in the USA; however the frequency is higher among specific populations due to founder effects [4]. Chromosome instability is caused by defects in FA proteins which participate in several DNA repair pathways, including homologous recombination, DNA mismatch repair, nucleotide excision repair and translesion DNA synthesis [5,6]. Clinical Features of FA Clinical features of FA patients include various congenital abnormalities, mainly skin and upper limbs disorders, Table 1 , progressive bone marrow failure and increased risk for squamous cell cancer []. Aplastic anemia usually occurs at a median age of years. The relative risk of AML as compared to the general population is much higher and the median age in reported cases is 13 years, with a range from birth to 50 years of age. Absence of marrow dysfunction, however, does not rule out the diagnosis of FA []. Marrow cellularity is best evaluated by bone marrow biopsy and the bone marrow often appears dysplastic, but the relationship between clonal cytogenetic abnormalities and progression to leukemia is not always clear. The most common cytogenetic abnormalities observed in FA patients involve chromosomes 1,3,4 or 7, but there is a striking association between chromosome 3q26q29 amplifications partial trisomies and tetrasomies and rapid progression to MDS or AML [17]. Compared to the general population, the risk is approximately fold higher for all solid tumors, but hundred- to thousand-fold higher for cancers of the head and neck, esophagus, liver, vulva, and cervix []. The mechanisms by which defects in the FA complex increase susceptibility to specific solid tumors are not completely elucidated. Genomic instability caused by subsequent somatic events occurring relatively early in the life of FA patients may explain the high rate of solid tumors in FA. It is also noteworthy that there are associations between oral androgens and liver tumors, as well as certain Human Papilloma Viruses HPVs and gynecologic, head and neck cancers []. The ubiquitinated ID complex is translocated to the chromatin at DNA damage foci at both sites and the surrounding areas [59]. Downstream proteins have an important role in this repair mechanism, connecting the FA-pathway with DSB repair [48,49]. Diagnosis of FA Early identification of FA allows differential diagnosis from other disorders, precludes inappropriate management of hematologic disease such as AA, MDS, AML and permits appropriate consideration of possible treatment or supportive care [4,7]. Cytogenetic analysis The first diagnostic test used is the chromosomal breakage test. If there is a founder effect in the population tested leading to a limited number of specific mutations there can be a targeted genetic diagnosis. This relatively inexpensive assay may be useful for the differential diagnosis of FA, but FA cases with significant lymphocyte mosaicism may be missed [52]. In addition the FA complementation group cannot be identified by this approach. Next Generation Sequencing NGS Since FA patients need special clinical management, diagnosis should be firmly established, to exclude conditions with overlapping phenotypes. All pathogenic and novel variants, as well as variants of unknown significance as determined by bioinformatics, are confirmed by PCR and Sanger sequencing [69]. Multiplexed next-generation sequencing, based on massively parallel sequencing, is an effective molecular diagnostic approach for FA. The procedure, performed on genomic DNA, reduces the turnaround time, number of assays, and cost for a reliable detection of the disease-causing mutation. To increase the efficiency of the molecular diagnosis, genes involved in other bone marrow failure syndromes e.

If lymphocyte analysis is inconclusive because of revertant mosaicism, fibroblasts are obtained since they do not show genetic reversion in vivo [70,71]. Prenatal Diagnosis and Genetic Counseling Indications for prenatal testing include targeted prenatal testing when the molecular defect is known, ultrasonographic findings, or families with a prior affected child when no information is available on the molecular defects. Molecular testing The disease-causing mutations in families with an affected child must be identified before prenatal testing. Analysis of DNA extracted from fetal cells, obtained by Chorionic Villus Sampling CVS at 10 to 12 weeks of gestation or amniocentesis at weeks of gestation, reveals the existence or not of the molecular defect. Fetal ultrasound evaluation Ultrasound examination can be used to evaluate fetal anomalies consistent with FA. However, this is not a diagnostic test for FA and some characteristic congenital anomalies may not be detectable by ultrasound examination [74]. Preimplantation Genetic Diagnosis PGD PGD may be performed in families where the paternal and maternal mutations have been identified and embryos selected do not have FA or are heterozygotes. PGD is an alternative prenatal diagnosis technique that offers the possibility not only to avoid the termination of a high risk pregnancy, but also to select embryos with particular genetic parameters that benefit an affected member of the family [77]. Genetic counseling of families with FA depends on the FA subtype and should include family and pregnancy histories, clarify the mode of inheritance and explain the genetic testing process [78]. It must provide information about current research opportunities, support groups and future reproductive options. Family members must also be informed about the possibility of developing ovarian and breast cancer if they are carriers of molecular defects that are located in the high risk FANC genes [79]. In case of novel molecular defects, further investigation is required in order to elucidate the consequences in DNA expression, since new approaches for treatment of FA patients may arise [78]. Treatment There are short-term and long-term protocols for treatment of FA patients depending on the symptoms. Since the main disorder is the progressive dysfunction of bone marrow, treatment includes: Approximately half of FA patients respond well to androgens, which stimulate the production of red blood cells, platelets and sometimes white cell production. It is essential that use of androgens is considered in the context of an eventual bone marrow transplant, as their use may adversely affect the ultimate success of a transplant [80]. Growth factors G-CSF stimulate the production of white blood cells [80]. This is the only long-term cure for the blood defects in FA and FA was the first disease for which cord blood transplantation was introduced [81]. This treatment has many risks for FA patients because of their extreme sensitivity to radiation and chemotherapy [80,82]. New Insights FA presents great heterogeneity including serious congenital and hematological abnormalities and researchers worldwide try to develop diagnostic tests, effective treatments and possibly a cure for this disease. New insights for diagnosis of FA are: Fanconi anemia antibody project: The technique mainly used in this research protocol is western blot [83]. MicroRNAs miRNAs regulate gene expression post-transcriptionally, are involved in biological processes, such as cell proliferation, differentiation and apoptosis and are deregulated in cancer. Specific miRNAs are deregulated and play a role in the hematopoietic dysfunction of FA patients [84]. Studies of microRNAs may lead to the discovery of novel factors that are potential targets for chemo sensitization. Endogenous aldehydes are an important source of genotoxicity in the human hematopoietic system, and the FA pathway counteracts them [86]. Some modifier genes or environmental factors might affect levels of aldehydes or other genotoxic substances. ALDH2 encodes an aldehyde dehydrogenase that participates in the oxidative pathway of alcohol metabolism. Over the last two decades enormous efforts in the clinical and genetic field have changed the character of FA from a life-limiting disease to a chronic condition of highly variable severity. Methods of in vitro gene therapy might complement these approaches, mainly for FA patients with molecular defects in the commonly mutated FANC genes [88,89]. Gene therapy in this form will principally address hematological problems associated with FA [90]. In addition, patient and family support groups have an essential role in this context.