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Towards the implementation of population -based genetic hemochromatosis screening in Germany. O.Schöffski[1], J.Schmidtke[2] and M.Stuhrmann[2]. [1] Center of Health Economics, University of Hannover, [2]Institute of Human Genetics, Medical School, Hannover, Germany

Hereditary hemochromatosis (HH) is a very frequent recessive disorder among Caucasians and - in most cases - associated with homozygosity for the single missense mutation Cys282Tyr (G845A) in the HFE gene. The prevalence of HH in Germany is estimated to be between 1 in 205 to 1 in 420 individuals, based on two recent molecular genetic studies on HH patients and healthy controls. Homozygosity for Cys282Tyr was present in 77.8% versus 94.6% of the patient groups and heterozygosity was 12.3% versus 9.5% among controls. Large scale Cys282Tyr mutation analysis is technically easy, reliable and cheap. Early diagnosis and treatment largely prevent iron overload and complications like liver cirrhosis, liver cancer, cardiomyopathy and diabetes mellitus. Population-based genetic screening can identify the vast majority of still asymptomatic HH homozygotes, who could extremely benefit from the knowledge of their genotype. To investigate the economic consequences of a genetic HH screening in Germany, we performed a decision tree analysis with TreeAge (Data). Quantification of screening and treatment costs and the effect on life expectancies was achieved with Excel-calculations (Version 5a). Under the very conservative assumptions of 10% penetrance, carrier frequency of 10%, mean age of onset of complications 54 years, and 90% compliance regarding treatment (phlebotomy), we calculated the present value to be 14.52 DM per tested person versus 3.42 DM per person who will not be tested. The life expectancies for 25years old males will be 48.99843 years (if not tested) versus 48.99970 years (if tested). By dividing the difference of costs by the difference of life expectancy, we calculated the costs for one further year of life to be 8881.70 DM, which is very well acceptable in comparision to the costs of other health care measures. Under less stringent conditions (higher penetrance, higher carrier frequency) the costs decrease substancially. We conclude that genetic HH screening in Germany is feasible under health economic aspects. It is appropriate to implement a pilote screening project as well as to study the remaining uncertainities like the penetrance of the disease, the appropriate age of the test persons and the psychological impact of genetic HH.
(1 US$ ≡ 1.65 DM)

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Prenatal diagnosis of FGFR3 mutations in thanatophoric dysplasia types I and II. E. Spector, A. Hansen-Higa and G. Bellus. Univ. of Colorado SOM.

Thanatophoric dysplasia types I and II (TD1 and TD2) are autosomal dominant lethal skeletal dysplasias. TD1 is more common and is characterized by rhizomelia, platyspondyly and megalencephaly. Straight femora, taller vertebral bodies and severe cloverleaf skull characterize TD2. TD1 is caused by sporadic mutations in the fibroblast growth factor receptor 3 (FGFR3). Eight distinct FGFR3 missense mutation located in exon 7 (C742T: R248C, C746G: S249C), exon 10 (G1108T: G370C, A1111T: S371C, A1118G: Y373C), and exon 19 (T2419G: J807G, T2419A: J807R, A2421T: J807C) have been described. These mutations either create unpaired cysteine residues in the FGFR3 extracellular domain or disrupt the stop codon, leading to translation of an additional 144 amino acids. One common FGFR3 mutation has been found in all patients with TD2 (A1948G: K650E). This mutation is within the intracellular tyrosine kinase domain of the FGFR3 gene.

We report here the results of mutation analysis of FGFR3 exons 7, 10, and 19 in 76 patient samples referred to our laboratory to rule out TD1 and TD2. Clinical information was derived from ultrasound studies, X-rays, and/or obtained at autopsy and ranged from short limbs to a classical description of TD1 or TD2. Genomic DNA was isolated from a variety of tissues including amniotic fluid cells (direct and tissue culture), chorionic villus cells (direct and tissue culture) and fetal tissues (frozen tissue or tissue culture) and analyzed by PCR amplification followed by either restriction enzyme analysis or allele specific oligonucleotide analysis. An identifiable mutation was found in 56.6% of samples analyzed (43). Eight patients were found to have the A1948G: K650E TD2 mutation. Thirty-five patients had TD 1 mutations. The C742T: R248C mutation was found most frequently (20 patients), followed by the A118G: Y373C (8 patients). Four patients possessed the C746G:S249C mutation and 2 patients possessed mutations in the exon 19 stop codon.

Test results are available approximately 7 days from receipt of the sample in the laboratory. Information provided by DNA analysis of the FGFR3 gene provides important information to patients.

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PCR-based molecular diagnosis of Prader-Willi and Angelman syndromes using restriction analysis after bisulfite treatment. Potential for quantitative estimation. M.Velinov, N. Zhong, W.T.Brown, E.Jenkins, NYS Institute for Basic Research, Staten Island, NY

Assays to estimate the DNA methylaion in the Prader-Willi (PWS)/Angelman syndrome (AS) region on chromosome 15q are widely used and are recommended for the initial screening of individuals with suspected PWS and AS. (ASHG/ACMG Test and Technology Transfer Committee. A.J.H.G., 58:1085, 1996). The Southern blotting-based methylation tests currently used can identify 100% of the patients with PWS and about 75% of the patients with AS. Recently, a methylation specific PCR (mPCR) technique for PWS/AS diagnosis was developed in order to increase the rapidity of the test and to minimize the amount of the genomic DNA necessary for testing. (Kubota T. et al. Nature Genetics, 16:16, 1997).
We have developed a PCR-based method to detect the DNA mehylation at the proximal 15q region, using restriction analysis after bisulfite treatment of total genomic DNA. Unlike the mPCR test, our method uses only one set of primers to amplify the two subpopulations of fragments from the methylated and non-methylated portion of the genomic DNA. We thus avoid the need to adjust the relative primer amounts required for the mPCR method and we have been able to overcome the potential problems that may occur while using the mPCR technique related to the non-specific inhibition of the PCR process. In addition our protocol, unlike the mPCR method, can be further developed to quantitatively assess the methylated versus non-methylated DNA ratios (Xiong Z. et al. Nucl.Ac.Res., 25:2532-2534, 1997). Such quantitative estimation may be used to detect mosaic PWS/AS patients, as well as patients with duplications of the proximal region of chromosome 15q.
We are currently using this protocol to test control and proven PWS and AS specimens in order to establish the reliability of the method for clinical application.

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Epidermal growth factor's role on human hepatocellular carcinoma transplanted into nude mice. Xiangyang Zhao, S.L. Zhu and T.X. Wang. General Surgery Unit 2, Shanghai Tiedao Hospital, Shanghai Tiedao University, Shanghai, China.

Based on the results of in vivo and in vitro studies, EGF appears to be a hormonally regulated growth factor. In general, hormones have multiple modulatory activity according to concentration, and the hypothesis that a high concentration of EGF is a negative growth factor has been proved in human carcinoma of esophagus and breast in vivo and in vitro studies. Some recent original papers shows that EGF can induce apoptosis in various cancer cells by the mechanism of activation of STAT protein.

This work was focused on clarifying the dose-dependent effects of epidermal growth factor on the growth, cell cycle, and ultra-structure of hepatocellular carcinoma. Human HCC SMMC-7721 was adopted to transplanted to the subcutaneous tissue of nude mice. 10 days After transplantaion, Human EGF(2ug and 10ug respectively) was injected locally into the subcutaneous tissue surrounding the tumor. After 4 weeks treatment, the tumor growth was evaluated by tumor volume, histologic examination; flow cytometric analysis of a cell cycle was performed after DNA staining with Propidium iodide (PI), and a morphological observation by electron microscope was done to qualify programmed cell death. The examination results showed a growth -inhibitory effect noted with 10ug of EGF, while not with 2ug of EGF. In the treated tumor, more apoptotic cells were counted, meanwhile cell cycle change characterized as the increase of S-phase fraction and the decrease of G01 and G2/M phase fraction. In conclusion, EGF shows its multiple modulatory activity in human HCC in vivo, and it can change the cell cycle and induce the apoptosis of HCC cells in uncertain path.

We present the probably mechanism, that EGF induced apoptosis is closely related to great change of cell cycle fraction after the treatment of EGF.