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Development of a new, simple and cost-effective diagnostic tool for genetic screening of hereditary colorectal cancer — the DNA microarray assay

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Detection of mutations in families with a hereditary predisposition to colon cancer gives an opportunity to precisely define the high-risk group. 36 patients operated on for colon cancer, with familiar prevalence of this malignancy, were investigated using the DNA microarrays method with the potential detection of 170 mutations in *MLH1*, *MSH2*, *MSH6*, *CHEK2*, and *NOD2* genes. In microarrays analysis of DNA in 9 patients (25% of the investigated group), 6 different mutations were found. The effectiveness of genetic screening using the microarray method is comparable to the effectiveness of other, much more expensive and time-consuming methods.

Key words: colon cancer, HNPCC, DNA microarray

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INTRODUCTION

In25%of cases of patients with colorectal cancer positive familial history is recognized. The most commonly inherited colon cancer predisposition syndrome is hereditary non-polyposis colorectal cancer (HNPCC), also called the Lynch syndrome (LS). This disorder with autosomal dominant inheritance pattern and high penetrance accounts for 2–3% of all CRC diagnoses and is caused by germline mutations in the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6,* and *PMS2* (Wijnen *et al.,* 1993; Hampel *et al*., 2005; Kauff *et al*., 2007; Lu *et al*., 2007).

Detection of mutations in families with a hereditary predisposition to colon cancer gives an opportunity to precisely define the high-risk group by cost-effective carrier screening. The mutation carriers should be subject to regular control examinations, whereas the non-carriers bear only the population risk of the colon cancer, therefore can be considered as a general, risk-free population. Due to several genes and large number of mutations involved (http://www.insight-group.org), genetic testing of HNPCC is challenging, and in practice preceded by pedigree analysis, microsatellite instability assay, and/or immunohistochemistry for MMR proteins, despite the fact that detection of the MMR gene mutation alone is enough to confirm a LS diagnosis. Thus, simple and effective methods for genetic screening are still investigated.

MATERIAL AND METHODS:

Patients. In our pilot study the group of 36 adults after the surgery for colon cancer, with familiar prevalence of this malignancy, were investigated (minimum 2 family members with colorectal/endometrial cancer in 2 generations). In this group, in 6 patients, hereditary non-polyposis colorectal cancer (HNPCC) was recognized based on the Amsterdam criteria (Vasen *et al.,* 1991). All patients received a collection kit for samples and a questionnaire. Genomic DNA was extracted from buccal mucosa samples collected by each patient him/herself and sent back to the genetic laboratory.

Genetic examination. Mutations were detected using the DNA microarrays SNP method in APEX technology (INNO GENE S.A., Poland), with the potential detection of 169 unique mutations in *MLH1*, *MSH2*, *MSH6*, *CHEK2*, and *NOD2* genes (see Table 1 for details).

RESULTS

In the microarray analysis of 9 patients (25% of the investigated group), 6 different mutations were found: 83C>T (1 patient), 1321G>A (1 patient), and 1852_1853delAAinsGC (2 patients) in *MLH1*, IVS2+1G>A (1 patient) in *CHEK2*, 1077-10T>C (2 patients) in *MSH2,* and 3020insC (2 patients) in the *NOD2* gene.

In the group of patients with recognized HNPCC the following mutations: 83C>T (*MLH1*) and 1077-10T>C (MSH2) were found in 2 cases (33%). The cost of a single microarray assay was about 367 EUR/477 USD;

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Abbreviations: HNPCC, hereditary non-polyposis colorectal cancer; MMR, mismatch repair; LS, Lynch syndrome

Table 1. Characterization of mutations investigated in the DNA microarrays test

Gene	Mutation name
MLH1	37delG; 66delG; 69A>T; 74T>C; 83C>T; 85G>T; 104T>G; 131C>T; 137G>T; 161G>A; 161delG; 184C>A; 184C>T; 191A>G; 194G>A; 199G>A; 199G>T; 200G>A; 203T>A; 206G>A; 229T>C; 230G>A; 238T>G; 250A>G; 256C>T; 277A>G; 298C>T; 299G>C; 304G>A; 306G>T; 306+1G>A; 320T>G; 332C>T; 350C>G; 350C>T; 382G>C; 392C>A; 394G>C; 454-1G>A; 464T>G; 479C>T; 544A>G; 546-2A>G; 577T>C; 589-2A>G; 595G>C; 649C>T; 676C>T; 677G>A; 677G>T; 677+3A>G; 731G>A; 739T>C; 778C>T; 790+1G>A; 793C>T; 794G>A; 803A>G; 842C>T; 875T>C; 883A>C; 883_884+2delAGgt; 884-2A>C; 1013A>G; 1038G>C; 1252delGA; 1321G>A; 1409+1G>C; 1421G>A; 1474G>A; 1489dupC; 1490insC; 1517T>C; 1528C>T; 1569G>T; 1625A>T; 1646T>C; 1649T>C; 1652A>C; 1658delCCA; 1672G>T; 1693A>T; 1721T>C; 1731G>A; 1733A>G; 1744C>G; 1756G>C; 1766C>A; 1783delAG; 1808C>G; 1820T>A; 1846delAAG; 1852_1853delAAinsGC; 1852delAAG; 1853A>C; 1853A>G; 1865T>A; 1918C>T; 1937A>G; 1942C>T; 1943C>T; 1958T>G; 1959G>T; 1961C>T; 1963A>G; 1976G>A; 1976G>C; 1984A>C; 1989G>T; 2027T>G; 2040C>A; 2041G>A; 2059C>T; 2103G>C; 2103+1G>A; 2223del11
MSH2	4G>A; 226C>T; 339G>A; 380A>G; 435T>G; 499G>C; 505A>G; 518T>C; 560T>C; 593A>G; 595T>C; 687delA; 806C>T; 862C>T; 892C>T; 942+3A>T; 998G>A; 1077-10T>C; 1077A>T; 1147C>T; 1165C>T; 1216C>T; 1226delAG; 1255C>A; 1373T>G; 1571 G>C; 1654A>C; 1738G>T; 1786delAAT; 1787A>G; 1799C>T; 1861C>T; 1865C>T; 1906G>C; 2064G>A; 2089T>C; 2090G>T; 2131C>T; 2168C>T; 2245G>A; 2251G>A; 500G>A; 2633delAG
MSH6	467C>G; 1186C>G; 1784delT; 1787delT; 3261delC; 3514dupA; 3838C>T
CHEK2	1100delC; IVS2+1G>A
NOD2	3020insC

whereas the cost of detection of one mutation in the examined group was 1468 EUR/1908 USD.

DISCUSSION

Genetic diagnostics may provide efficient and cost-effective tools for testing patients with genetically related colorectal cancer, if all costs are considered (Ladabaum *et al.,* 2011; Wang *et al.,* 2012). In the group of patients with hereditary colon cancer, or suspicion of hereditary symptoms, screening and pre-symptomatic clinical examination of all family members is recommended. It is a very effective method in the secondary prophylaxis of the malignant transformation. In the analysis of mutations, the DNA sequencing of the following 4 MMR genes: *MLH1*, *MSH2*, *MSH6*, and *PMS2,* may be considered as a 'gold standard'. Performance of this strategy is difficult to estimate, and it is not known if laboratory proficiency testing will be an adequate validity measure (Bonis *et al.,* 2007; Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group 2009; Palomaki *et al.,* 2009). Because of the high lifetime, colorectal cancer risk for the Lynch syndrome patients (reaching 80%) (Chung *et al.,* 2003; Brodersen *et al.,* 2004), the effectiveness of screening in this group of patients is well supported (Järvinen *et al.,* 2000; Dove-Edvin *et al.,* 2005). Despite this, there is suboptimal uptake of screening by high-risk individuals (Bleiker *et al.,* 2005; Geary *et al.,* 2007; Rees *et al.,* 2008). The discovery of cancer-causing germline mutations has proved to be highly advantageous in determining patients' lifetime risk status (Lynch *et al.,* 2009). The knowledge about the colorectal cancer risk can determine the patients' and their physicians' decision-making regarding surveillance

and management (Watson *et al.,* 2003). The localization of the mutation also gives an opportunity to predict the clinical follow-up of the disease, as for example the lower risk of extra colonic cancer (such as endometrial cancer) in the type 2 Lynch syndrome (*MLH1*-related) or later onset CRCs and a greater number of endometrial carcinomas in Lynch syndrome-*MSH6* type (Lynch *et al.,* 2010).

The DNA microarray method, based on the DNA hybridization seems to be a simple and effective method for genetic testing. The application of DNA microarrays for fundamental biomedical research has recently been reviewed elsewhere. (Schulze *et al.,* 2001; Smyth *et al.,* 2003; Egeland *et al.,* 2005; Chagovetz *et al.,* 2009). There are very promising indications for using this method in cancer research (Wadlow *et al.,* 2005; Perez-Cabornero *et al.,* 2009; van Roon *et al.,* 2011). The main benefits of the microarray method are: large scale screening (>100 mutations); short turn around time processing (days), low cost (< 500 EUR/test), ease of upgrading the open platform (new mutations). The frequency of the mutation's detection using DNA microarrays seems to be similar when compared to other studies.

Differences in the frequency of the mutation detection rate are observed between HNPCC cohorts, depending on the inclusion criteria and the investigated population. In Spaepen *et al.* (Spaepen *et al.,* 2006), study of patients with HNPCC, pathogenic mutations were found in 11% — 25 out of 225 investigated patients. In colorectal cancer patients without preselection and regardless of family history, 38 pathogenic mutations among 870 participants (4%) were found (Barnetson *et al.,* 2006). In 281 patients diagnosed with CRC before the age of 50 years or with CRC and at least one additional HNPCC-associated cancer, 25 pathogenic mutations (8.9%) were detected (Niessen *et al.,* 2006). In 93 unrelated Taiwanese families that fulfilled the Amsterdam criteria II 38 pathogenic mutations in the *MSH2* or *MLH1* genes were identified in 61 families (Tang *et al.,* 2009). In another study of 76 Chinese probands from HNPCC families the overall mutation rate was 33%, and 22 different mutations were found in the *MLH1* and *MSH2* genes (Fu *et al.,* 2008). The mutation detection rate with our DNA microarray assay was 25% in patients with familial history (14% if only MMR mutations were considered), and 33% in HNPCC patients, which is similar to those presented in other studies.

In all HNPCC families with mutations detected, the recognition of high-risk carriers is easy, and can decrease the number of investigated persons by about 50% and reduce the cost, as well as psychological stress of unaffected family members.

The effectiveness of genetic screening using the microarray method is similar to the effectiveness of other approaches, that are much more expensive and time-consuming. The method needs to be validated in further studies among larger group of patients; however, we believe that the assay can be widely used as a simple, accepted, and cost-effective method in colorectal cancer screening programs.

Conflicts of Interest Statement

Kaszuba M, Sikorski A, Wojciechowicz J — collaboration with INNO GENE SA Poland. Banasiewicz T — honoraria for the lecture about DNA microarray analysis (paid by INNO GENE SA Poland). There are no more conflicts of interest of the other authors.

REFERENCES

Barnetson RA, Tenesa A, Farrington SM, Nicholl ID, Cetnarskyj R, Porteous ME, Campbell H, Dunlop MG (2006) Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. *N Engl J Med* **354**: 2751–2763.

Bleiker EM, Menko FH, Taal BG, Kluijt I, Wever LD, Gerritsma MA, Vasen HF, Aaronson NK (2005) Screening behavior of individuals at high risk for colorectal cancer. *Gastroenterology* **128**: 280–287.

Bonis PA, Trikalinos TA, Chung M, Chew P, Ip S, DeVine DA, Lau J (2007) Hereditary nonpolyposis colorectal cancer: diagnostic strategies and their implications. *Evid Rep Technol Assess* **150**: 1–180.

Brodersen NH, Sutton S, Goff S, Hodgson SV, Thomas HJ (2004) Anticipated reactions to genetic testing for hereditary non-polyposis colorectal cancer susceptibility. *Clin Genet* **66**: 437–444.

Chagovetz A, Blair S (2009) Real-time DNA microarrays: reality check. *Biochemical Society Transactions* **471**: 475-477.

Chung DC, Rustgi AK (2003) The hereditary nonpolyposis colorectal cancer syndrome: genetics and clinical implications. *Ann Intern Med* **138**: 560–570.

Dove-Edwin I, Sasieni P, Adams J, Thomas HJ (2005) Prevention of colorectal cancer by colonoscopic surveillance in individuals with a family history of colorectal cancer: 16 year, prospective, follow-up study. *BMJ* **331**: 1–6.

Egeland RD, Southern EM (2005) Electrochemically directed synthesis of oligonucleotides for DNA microarray fabrication. *Nucleic Acids Res* **33**: e125.

Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (2009) Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med* **11**: 35–41.

Fu L, Sheng JQ, Sun ZQ, Han M, Huang JS, Mu H, Han WL, Niu HL, Li AQ, Wu ZT, Li SR (2008) Mutation of hMLH1 and hMSH2 genes in hereditary nonpolyposis colorectal cancer: analysis of 76 probands. *Zhonghua Yi Xue Za Zhi* **88**: 1983–1985 (in Chinese).

Geary J, Thomas HJ, Mackay J, Dorkins H, Barwell J, Hodgson SV (2007) The management of families affected by hereditary non-polyposis colorectal cancer (HNPCC). *Fam Cancer* **6**: 13–19.

Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J, Panescu J, Fix D, Lockman J, Comeras I, de la Chapelle A (2005) Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* **352**: 1851–1860.

Järvinen HJ, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomäki P, de la Chapelle A, Mecklin JP (2000) Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* **118**: 829–834.

Kauff ND (2007) How should women with early-onset endometrial cancer be evaluated for lynch syndrome? *J Clin Oncol* **25**: 5143–5146.

Ladabaum U, Wang G, Terdiman J, Blanco A, Kuppermann M, Boland CR, Ford J, Elkin E, Phillips KA (2011) Strategies to identify the Lynch syndrome among patients with colorectal cancer: a cost-effectiveness analysis. *Ann Intern Med* **155**: 69–79.

Lu KH, Schorge JO, Rodabaugh KJ, Daniels MS, Sun CC, Soliman PT, White KG, Luthra R, Gershenson DM, Broaddus RR (2007) Prospective determination of prevalence of lynch syndrome in young women with endometrial cancer. *J Clin Oncol* **25**: 5158–5164.

Lynch HT, Jascur T, Lanspa S, Boland CR (2010) Making sense of missense in Lynch syndrome: the clinical perspective. *Cancer Prev Res (Phila)* **3**: 1371–1374.

Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR (2009) Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. *Clin Genet* **76**: 1–18.

Niessen RC, Berends MJ, Wu Y, Sijmons RH, Hollema H, Ligtenberg MJ, de Walle HE, de Vries EG, Karrenbeld A, Buys CH, van der Zee AG, Hofstra RM, Kleibeuker JH (2006) Identification of mismatch repair gene mutations in young patients with colorectal cancer and in patients with multiple tumours associated with hereditary non-polyposis colorectal cancer. *Gut* **55**: 1781–1788.

Palomaki GE, McClain MR, Melillo S, Hampel HL, Thibodeau SN (2009) EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. *Genet Med* **11**: 42–65.

Perez-Cabornero L, Velasco E, Infante M, Sanz D, Lastra E, Hernández L, Miner C, Duran M (2009) A new strategy to screen MMR genes in Lynch Syndrome: HA-CAE, MLPA and RT-PCR. *Eur J Cancer* **45**: 1485–1493.

Rees G, Martin PR, Macrae FA (2008) Screening participation in individuals with a family history of colorectal cancer: a review. *Eur J Cancer Care* **17**: 221–232.

Schulze A, Downward J (2001) Navigating gene expression using microarrays — a technology review. *Nature Cell Biol* **3**: E190–E195.

Smyth GK, Yang YH, Speed T (2003) Statistical issues in cDNA microarray data analysis; functional genomics: methods and protocols. *Methods Mol Biol* **224**: 111–136.

Spaepen M, Vankeirsbilck B, Van Opstal S, Tejpar S, Van Cutsem E, Geboes K, Legius E, Matthijs G (2006) Germline mutations of the hMLH1 and hMSH2 mismatch repair genes in Belgian hereditary nonpolyposis colon cancer (HNPCC) patients. *Fam Cancer* **5**: 179–89.

Tang R, Hsiung C, Wang JY, Lai CH, Chien HT, Chiu LL, Liu CT, Chen HH, Wang HM, Chen SX, Hsieh LL (2009) TCOG HNPCC Consortium.: Germ line MLH1 and MSH2 mutations in Taiwanese Lynch syndrome families: characterization of a founder genomic mutation in the MLH1 gene. *Clin Genet* **75**: 334–345.

van Roon EH, de Miranda NF, van Nieuwenhuizen MP, de Meijer EJ, van Puijenbroek M, Yan PS, Huang TH, van Wezel T, Morreau H, Boer JM (2011) Tumour-specific methylation of PTPRG intron 1 locus in sporadic and Lynch syndrome colorectal cancer. *Eur J Hum Genet* **19**: 307–312.

Vasen HF, Mecklin JP, Khan PM, Lynch HT (1991) The international collaborative group on hereditary non-polyposis colorectal cancer (ICG-HNPCC). *Dis Colon Rectum* **34**: 424–425.

Wadlow R, Ramaswamy S (2005) DNA microarrays in clinical cancer research. *Curr Mol Med* **5**: 111–120.

Wang VW, Koh PK, Chow WL, Lim JF (2012) Predictive genetic testing of first degree relatives of mutation carriers is a cost-effective strategy in preventing hereditary non-polyposis colorectal cancer in Singapore. *Fam Cancer* **11**: 279–289.

Watson P, Narod SA, Fodde R, Wagner A, Lynch JF, Tinley ST, Snyder CL, Coronel SA, Riley B, Kinarsky Y, Lynch HT (2003) Carrier risk status changes resulting from mutation testing in hereditary nonpolyposis colorectal cancer and hereditary breast-ovarian cancer. *J Hum Genet* **40**: 591–596.

Wijnen JT, Vasen HF, Khan PM, Zwinderman AH, van der Klift H, Mulder A, Tops C, Møller P, Fodde R (1998) Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. *N Engl J Med* **339**: 511–518.