Genetic Testing and Prevention of Hereditary Cancer at the MMCI – Over 10 Years of Experience

Genetické testování a prevence hereditárních nádorů v MOÚ – více než desetiletá zkušenost

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Summary

Hereditary cancer syndromes are frequently seen in young cancer patients and patients with a positive family history. Genetic testing is important for the identification of high-risk individuals, and for the early introduction of specialized preventive care or prophylactic surgeries. High-risk tumour suppressor genes (*BRCA1* and *BRCA2*) and DNA repair genes (*MLH1*, *MSH2* and *MSH6*) are responsible for a substantial part of hereditary breast, ovarian and colorectal cancer. Other hereditary cancers are seen less frequently, but genetic testing has increased for many other site-specific cancers and complex syndromes. Genetic centres and molecular genetic laboratories are located mostly within university or regional hospitals. Some genetic centres are private. It is highly recommended (Czech Society for Medical Genetics) that all laboratories are accredited according to ISO 15,189 and that genetic testing of hereditary cancer syndromes is indicated by medical geneticists. The indication criteria and prevention strategies were published in Supplement 22 of Clinical Oncology 2009 (in Czech). Preventive care for high-risk individuals is organized by thirteen Oncology Centres, which provide most of the oncology care in the Czech Republic. Genetic testing and preventive care for high-risk individuals and mutation carriers is covered by health insurance. The molecular genetic laboratory at the MMCI provides molecular genetic testing of *BRCA1, BRCA2, CHEK2* for hereditary breast/ovarian cancer, *MLH1, MSH2, MSH6* for Lynch syndrome, TP53 for Li-Fraumeni syndrome, *CDKN2A* for familial malignant melanoma syndrome and CDH1 gene for hereditary diffuse gastric cancer. Other syndromes are tested in specialized laboratories elsewhere. The use of genetic testing is increasing because of more frequent referrals from oncologists and other specialists and the increasing variety of genes tested. However, in some patients the testing is not recommended and other family members are dying because of the late diagnosis of hereditary syndrome. Greater awareness of the importance of genetic testing in oncology is needed.

Key words

hereditary cancer – syndromes – genes – genetic testing – prevention

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Souhrn

Dědičné nádorové syndromy jsou často přítomny u mladých pacientů a pacientů s rodinným výskytem onemocnění. Genetické testování je důležité pro identifikaci rizikového jednice a pro časný začátek specializované preventivní péče nebo pro indikaci profylaktických operací. Vysoce rizikové tumor supresorové geny (*BRCA1* a *BRCA2*) a DNA reparační geny (*MLH1*, *MSH2* a *MSH6*) jsou zodpovědné za významnou část hereditárních nádorů prsu, ovaria a kolorekta. Jiné hereditární nádory se objevují méně často. Genetické testování pro specifické typy dědičných nádorů nebo nádorové syndromy se rozšiřuje. Genetická centra a molekulárně genetické laboratoře jsou většinou součástí univerzitních nebo místních nemocnic, některá centra jsou soukromá. Společnost lékařské genetiky ČLS doporučuje, aby všechny laboratoře měly akreditaci dle ISO 15 189 a dále aby indikace k testování nádorových syndromů prováděli lékařští genetici. Indikační kritéria a preventivní postupy byly publikovány v supplementu 22 Klinické onkologie 2009. Preventivní péče o rizikové jedince je organizována ve třinácti onkologických centrech, která provádějí většinu onkologické péče v ČR. Genetické testování a preventivní péče jsou hrazeny z veřejného zdravotního pojištění. Molekulárně genetická laboratoř Masarykova onkologického ústavu poskytuje testování *BRCA1*, *BRCA2*, *CHEK2* genů pro hereditární syndrom nádorů prsu/ovaria, *MLH1*, *MSH2* a *MSH6* genů pro Lynchův syndrom, *TP53* pro Li-Fraumeni syndrom, *CDKN2A* pro familiární maligní melanom a *CDH1* pro hereditární difuzní karcinom žaludku. Jiné syndromy jsou vyšetřovány ve spolupracujících laboratořích. Využívání genetického testování se zvyšuje kvůli narůstajícímu počtu odeslaných pacientů onkology a jinými specialisty na genetické vyšetření, ale i kvůli zvětšujícímu se spektru testovaných genů. Nicméně stále se u mnoha pacientů na genetické vyšetření zapomíná a jejich příbuzní umírají kvůli pozdní diagnóze hereditárního syndromu. Je důležité větší povědomí lékařů o úloze genetického testování v onkologii.

Klíčová slova

dědičný nádor – syndromy – geny – genetické testování – prevence

Introduction

Breast cancer is a very common malignancy all over the world, especially in developed countries. In the Czech Republic, there were 5 533 women diagnosed with breast cancer in 2005 and the incidence is slowly growing (2,1% annually). The crude incidence in 2005 was 105,4 cases per 100 000 inhabitants [1]. The cumulative risk of breast cancer for Czech women (0–74 years) is 6–7%, which is still less than in other Western European countries. The other frequent malignancy is colorectal cancer with 4 746 and 3 236 newly diagnosed cases in males and females in 2005, respectively (94,9 males and 61,7 females per 100 000 inhabitants), which is the highest incidence rate for males in the world. The cumulative risk (0–74 years) of colorectal cancer is 7,32% for males and 3,6% for females (Cancer Incidence in Five Continents Vol. IX, IARC 2007).

In 1992 and 1993 first DNA repair genes for hereditary nonpolyposis colorectal cancer, *MLH1* and *MSH2* were found [2–5]. In 1994 and 1995, tumor suppressor genes *BRCA1* and *BRCA2*, which are responsible for a high proportion of hereditary breast and ovarian cancer, were discovered [6,7]. Since that time the genetic counseling and testing for these most frequent hereditary cancers could be introduced. Some other genes causing less frequent syndromes were already known before, for example *TP53* for Li-Fraumeni syndrome, *RB1* gene for hereditary retinoblastoma [8–10]. The spectrum of hereditary cancer syndromes, which can be tested, and the location of genetic centers and laboratories within the Czech Republic were published [11–13].

Hereditary breast and ovarian cancer syndrome

Breast cancer may be repeatedly seen in families. There are many genes, which can more or less predispose women to breast cancer (Fig. 1). Mutations in tumor sup-

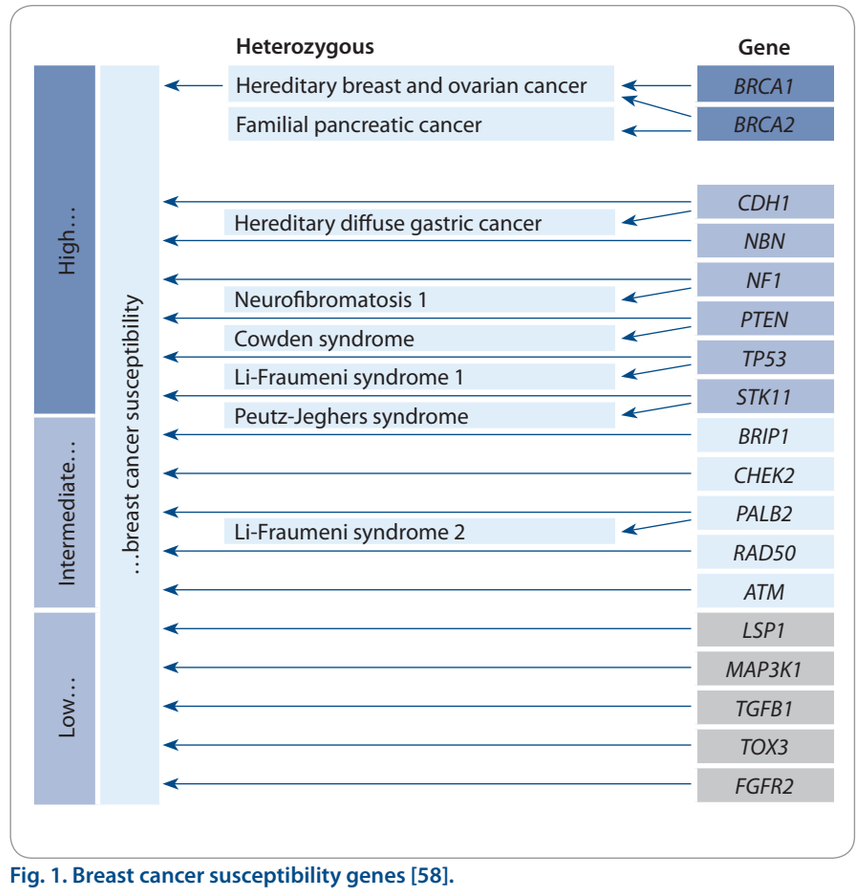

Fig. 1. Breast cancer susceptibility genes [58].

pressor genes *BRCA1* (OMIM#113705, Online Mendelian Inheritance in Man) and *BRCA2* (OMIM#6001856) predispose to breast and ovarian/fallopian tube cancers and some other cancerous diseases (colorectal, prostate, gastric, hepatobiliary, melanoma, pancreatic) and are considered the most frequent cause of hereditary breast or ovarian cancer [14]. The frequency of *BRCA1/2* mutations in general population is estimated to be 1 : 300 to 1 : 800, but in more recent study in Canada the frequency is estimated to be higher, 1 : 140 to 1 : 300 [15]. The frequency of these mutations in the Czech population is not known.

*BRCA1/2*b iology

BRCA1 and *BRCA2* proteins are essential in the homologous recombination (HR) process. This mechanism is central in the repair of DNA double-strand breaks that can lead to chromosome translocation and genomic instability. Double strand DNA damage can be induced by many chemotherapy agents such as topoisomerase inhibitors, alkylating agents and platinum drugs [16].

The main pathway for repair of such lesions is HR in a process that uses a region of DNA with high sequence identity, often the identical sister chromatid, to copy and replace the damaged DNA sequence. HR is conservative and potentially error-free. Cells that lack either *BRCA1* or *BRCA2* are unable to repair DNA double-strand breaks by HR. This defect results in the repair of these DNA lesions by non-conservative, potentially mutagenic mechanisms that in turn favor genomic instability, and these tumors should also develop alterations in genes that control these check-points in order to progress [16].

Triple-Negative and/or Basal-Like Breast Cancers

Breast cancer is a heterogeneous disease with different morphologies, molecular profiles, clinical behavior and response to therapy. A triple-negative breast cancer (TNBC) is a particular type of breast cancer characterized by an absence of estrogen (ER), progesterone (PR) and HER-2 receptors' expression. TNBC comprised about 11 to 17% of Caucasian and up to 30% of black and Hispanic women with breast cancer [17,18].

The triple-negative group of breast cancer is not a homogeneous disease entity, but encompass other molecular subtypes of breast cancer. A substantial fraction (70–80%) of triple-negative tumors displays a basal-like phenotype as defined by gene-expression profiling or immunohistochemical studies (the expression of basal cytokeratins (e. g. cytokeratins 5, 14, and 17), and/or the expression of EGFR). This molecular subtype of TNBC is called triple-negative basal-like breast cancer (TN-BLBC). On the other hand, not all basal-like breast cancer (BLBC) is triple-negative. Up to 20% of BLBC express ER or over express HER2 [18].

There is a link between the *BRCA1* pathway and TNBC. More than 75% of tumors arising in women carrying a germline *BRCA1* mutation have morphologic features and gene expression profiles very similar to those of nonhereditary TNBC and often display a basal-like phenotype. These findings support the hypothesis that loss of *BRCA1* function may play a major role in TNBC development. Clinically, the triple-negative or the basal-like phenotype indicates the possible presence of a germline *BRCA1* mutation. However, the additional usefulness of assays that measure the expression of cytokeratins and other "basal-associated" markers in determining *BRCA1* mutation status remains unclear given the substantial overlap between basal-like and triple-negative cancers [18]. Since not all TNBC harbor mutations in *BRCA1*, it appears that it is not structural mutations alone to be necessary for the development of TNBC. The low *BRCA1* expression could also be the result of gene regulatory mechanisms, such as DNA methylation or an expression of inhibitor of DNA binding 4 (ID4), representing a negative regulator of *BRCA1* [19].

Triple-negative and basal-like breast cancers are usually high-grade, invasive ductal carcinomas, characterized by an unusually attenuated relationship between the size of the primary tumor and the probability of survival. Their rapid growth and frequent occurrence in young women can make mammography detection difficult. They are more likely than other types of breast cancer to metastasize to viscera, particularly to the lungs and brain, and are less likely to metastasize to bones. Multiple studies have indicated that triple-negative and basal-like breast cancers, as a group, are associated with an adverse prognosis. There is a sharp decrease in survival during the first 3 to 5 years after diagnosis, but distant relapse after this time is much less common than among patients with ER-positive cancers. Thus, although as a group triple-negative and basal-like breast cancers are biologically aggressive; many are potentially curable, reflecting their heterogeneity [18,19].

Chemotherapy nevertheless improves the outcome to a greater extent when used in patients with TNBC than when used in patients with the much more common ER-positive subtype. Neoadjuvant studies suggest that there is a subgroup of women with TNBC whose tumors are extremely sensitive to chemotherapy, but there are many women for whom chemotherapy is of uncertain benefit. Currently, there is no preferred standard form of chemotherapy for TNBC, retrospective analyses suggest that the addition of docetaxel or paclitaxel to anthracycline-containing adjuvant regimens may be of greater benefit for the treatment of TNBC [18].

The biology of triple negative breast cancer is studied at MMCI with the support of the grant from Ministry of Health of the CR NS/10357.

Genetic testing

Since 1999 almost three thousand probands (patients) and two thousands of family members were tested at MMCI.

The indication criteria for testing were published [20, 21]:

Sporadic cases:

- sporadic breast or ovarian cancer diagnosed before the age of 40
- sporadic bilateral breast/ovarian cancer before the age of 50
- sporadic medullary breast cancer or triple negative breast cancer (ER, PR and HER2 negative) before 50
- duplication of breast and ovarian cancer at any age

Tab. 1. Detection rate (in %) of pathogenic BRCA mutations in different risk categories of patients [26].

Inclusion criteria and phenotype	Number of families/patients	*BRCA1* mutation % (n)	*BRCA2* mutation % (n)	Overall mutation % (n)
I. HOC + HBOC	120	50,8% (61)	10% (12)	73 (60,8%)
I. HBC	200	20,5% (41)	12% (24)	65 (32,5%)
I. Overall	320	31,9% (102)	11,3% (36)	138 (43,1%)
II. HOC + HBOC	40	55% (22)	2,6% (1)	23 (57,5%)
II. HBC	212	11,8% (25)	11,3% (24)	49 (23,1%)
II. Overall	252	18,7% (47)	10% (25)	72 (28,6%)
I. + II. Familial cases HOC + HBOC	160	51,9% (83)	8,2% (13)	96 (60%)
I. + II. Familial cases HBC	412	16% (66)	11,7% (48)	114 (27,7%)
I. + II. Familial cases – overall	572	26% (149)	10,7% (61)	210 (36,7%)
III. A Bilateral breast cancer patient	29	17,2% (5)	13,8% (4)	9 (31%)
III. B Bilateral ovarian cancer patient	7	14,3% (1)	0	1 (14,3%)
III. C Patient with breast and ovarian cancer	19	57,9% (11)	15,8% (3)	14 (73,7%)
III. Duplex cancer patients – overall	55	30,9% (17)	12,7% (7)	24 (43,6%)
IV. Early onset ovarian cancer patient	19	5,3% (1)	0	1 (5,3%)
IV. Early onset breast cancer patient	121	5,8% (7)	5% (6)	13 (10,7%)
IV. Overall	140	5,7% (8)	4,3% (6)	14 (10%)
V. Male breast cancer	16	18,8% (3)	18,8% (3)	6 (37,5%)
VI. Healthy person in high-risk (I.) family	77	19,5% (15)	6,5% (5)	20 (26%)
VII. Out of criteria families	150	8% (12)	5,3% (8)	20 (13,3)

Abbreviations: HOC – hereditary ovarian cancer syndrome; HBOC – hereditary breast and ovarian cancer syndrome; HBC – hereditary breast cancer only syndrome. I. – Three and more cases; II. Two cases in a family. VI. – families, where no patient with cancer can be tested. Testing is starting in a healthy relative.

- men with breast cancer at any age

Familial cases

- families with two breast or ovarian cancer in close relatives (at least one before 50)
- families with three or more breast or ovarian cancers at any age

By the testing of unselected breast cancer patients, using methods identifying about 80% of detectable mutations, it was estimated that there is about 2,4% frequency of BRCA1/2 mutations [22].

Gene *CHEK2* (OMIM#604373) is considered to be a gene causing a moderate increase of breast cancer risk (2–5×) and may also predispose to some other cancers like prostate, brain, sarcomas, thyroid or lung [23,24]. In some families clinically resembling Li-Fraumeni (or LFS-like) syndrome, mutations in *CHEK2* gene may be found. We were testing predominantly two mutations, del 9–10 (del 5567 bp) and c.1100delC, in some of the breast/ovarian cancer families.

Deletion of exon 9 and 10 (genomic deletion of 5 567 bp) was disclosed in two USA patients having breast or ovarian cancer. Both patients were of Czechoslovakian ancestry. This deletion was subsequently found in 8/631 (1,3%) breast cancer patients in CR and Slovakia, and in no control women. All patients were sharing the same haplotype indicating that this mutation had a single source [25].

Mutation detection rate

According to our testing results, the overall mutation detection rate in 2 100 tested patients was around 26%. The detection rate differed according to the inclusion criteria and the results can be found in the Tab. 1 [26]. The *BRCA1/2* mutations were most frequently found in ovarian or breast/ovarian cancer families (HOC or HBOC) where the frequency of mutation detection was reaching 61% (three or more cases in a family) and 57,5% (two cases). The detection rate was much lower in families with only breast cancer cases, 32,5% with three

and more cases and 23,1% with only two cases. It is very important to offer the genetic testing to all women with bilateral breast cancer bellow 50 and women with the breast and ovarian cancer (detection rate 31% and 73,7%, respectively). In a sporadic early onset breast cancer, the mutation was discovered in about 10% of tested young women. Male breast cancer is frequently hereditary, with mutation found in 37,5% of tested male patients. The occurrence of ovarian cancer in a family is a high predictor of possible heritability.

Testing methods

In 2007 a new methods were implemented in the testing protocol (Tab. 2). First of all the heteroduplex analysis and protein truncation tests were exchanged for more reliable and sensitive method, high resolution melting analysis (HRM), which can detect more variants and missense mutations in both genes with the sensitivity reaching 98% [27]. By using this method new previously undetectable mutations in *BRCA1* gene p.Glu1413X and cryptic splice site c.213–12A>G were found.

Tab. 2. Laboratory methods used for *BRCA1* and *BRCA2* analysis at MMCI.

1999–2006:	heteroduplex analysis at MDE (Cambrex)
2007–until now:	HRM (High Resolution Melting)
Sequencing	
1999–2006:	ALF express II (Pharmacia)
2007–until now:	3130 Genetic Analyser (Applied Biosystems)
2005–until now:	MLPA-*BRCA1* (*BRCA2* tested in 1 000 families, no positive results, not used)

Testing results

BRCA1/2 genes

There is a broad spectrum of mutations found in *BRCA1* and *BRCA2* genes in the Czech patients tested. *BRCA1* mutation was found in 392 families (78 different mutations), *BRCA2* mutation in 159 families (61 different mutations). Altogether pathogenic mutation was found in 551 out of 2 100 families tested (26,2%). Mutations are scattered all over the coding sequences of both genes and many families have their private mutation. Several mutations are seen more frequently in our population, specifically c.5266dupC, c.3700_3704del5 and p.Cys61Gly in *BRCA1* gene and c.7913_7917del5 and c.8537_8538del2 in *BRCA2* gene [26, 28]. Altogether with other 5 frequent mutations they represent about 54,5% of all detected mutations found (Tab. 3). But the testing of these 10 mutations is insufficient in our population and the screening of all coding regions of both genes is necessary.

In our population there is a high frequency of large genomic rearrangements in *BRCA1* gene, which can be detected by MLPA– multiplex ligation-dependent probe amplification (Tab. 4). This method may detect additional mutations in about 6% of patients previously tested negative [29]. No large deletions or duplications in *BRCA2* gene were found in 1 000 patients tested and this method is not used for regular testing in our laboratory.

Tab. 3. Ten most frequent causal mutations found in the *BRCA1* and *BRCA2* gene in the Czech patients tested at MMCI, responsible for 54,5% of all detected mutations. 2 100 families were tested during period 1999-2009, *BRCA1* mutation was found in 392 families (78 different mutations), *BRCA2* mutation was found in 159 families (61 different mutations). Altogether pathogenic mutation was found in 551 families (26,24%).

Gene	Systematic nomenclature	Protein level	Number of families	Mutation frequency
BRCA1	c.5266dupC	p.Gln1756ProfsX74	128	33% (of all *BRCA1*)
	c.3700_3704del5	p.Val1234GlnfsX8	48	
	c.181T > G	p.Cys61Gly	27	
	c.1687C > T	p. Gln563X	15	
	c.213-12A > G	cryptic splice site	11	
	c.68_69del2	p.Glu23ValfsX17	11	
	del. 5-14	g.21716_53298del31583	11	
	del. 21-22	g.77128_80906del3779ins236	10	
***BRCA1* total**	**8 mutations**		**261**	66,6% (of all *BRCA1*)
BRCA2	c.8537_8538del2	p.Glu2846GlyfsX22	22	
	c.7913_7917del5	p.Phe2638X	17	
BRCA2 total	2 mutations		39	24,5% (of all *BRCA2*)
***BRCA1* and *BRCA2* total**	**10 mutations**		**300**	**54,4% (of all *BRCA1* and *BRCA2*)**

Tab. 4. Large genomic rearrangements in *BRCA1* gene.

BRCA1 gene	Genomic DNA	The expected minimal effect	Number of families
del. 1a, 1b, 2	Deletion of 36,9 kb	Loss of transcription	3
del. 1–17	?	Loss of transcription	4
del. 5–14	g.21716_53298del31583	Loss of 4/5 of protein, FS	15
del 8	Deletion of 3,5-4 kb	p.Gln148AspfsX51	3
del part. 11–12	g.34845_42405del6561	Loss of 1/2 of protein, FS	2
dupl. 13–14	?	FS	1
del 18–19	g.63651_65590del1940	p.Asp1692AlafsX2	1
del 20	g.68764_75792del7029	p.1732_1759del28, IFD	1
del 20	g.65740_73907del8168	p.1732_1759del28, IFD	1
del 21–22	g.77128_80906del3779ins236	p.1760_1802del43, IFD	11
Deletion of all gene allele	?	Loss of transcription	1

FS – frame shift; IFD – in frame deletion

Variants with unknown clinical significance

In about 13% of tested families only a variant with unknown clinical significance (UV) is found; the functional tests are mostly not available. In that case we offer predictive testing only for research purposes and preventive care is offered to both carriers and non-carriers.

Together with the research team at the Institute of Experimental and Clinical Medicine in Prague some detected *BRCA2* unknown variants located in an important part of the gene (exon 18) and segregating with the disease are examined by functional test which may improve our knowledge of the biological significance of the DNA change [30] with the support of the grant from Ministry of Health of the CR NS/10536–3/2009.

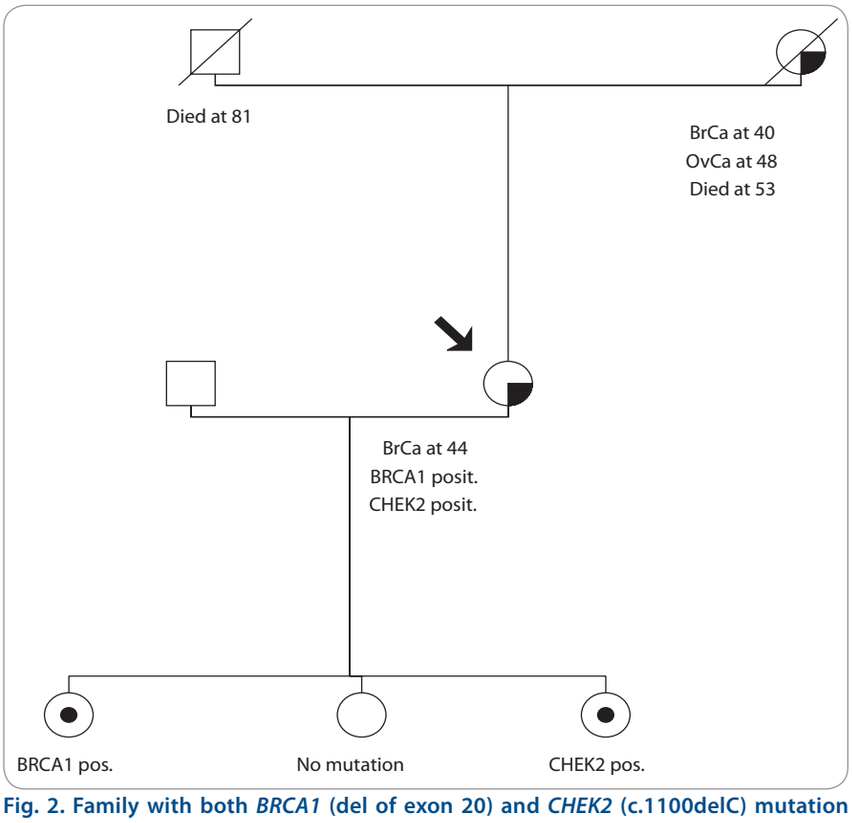

Fig. 2. Family with both *BRCA1* (del of exon 20) and *CHEK2* (c.1100delC) mutation in a proband with breast cancer at 44 years.

CHEK2 gene

CHEK2 mutations were found in 17 *BRCA1*/2 negative families, in eight cases del 9–10, in nine cases c.1100delC. The frequency of *CHEK2* mutation carriers among unselected breast cancer Czech patients was estimated to be about 1,3% for del 9–10 [25] and 0,44% for c.1100delC, with control frequencies 0% and 0,27%, respectively [31]. It is considered to be low frequency gene in our population causing moderate increase of breast cancer risk (2–4×).

CHEK2 mutations were tested only in families, where no mutations in *BRCA1* or *BRCA2* genes were discovered. In one family both *BRCA1* (deletion of exon 20) and *CHEK2* mutations (c.1100delC) in proband were detected, both by MLPA analysis (Fig. 2).

Predictive testing

Predictive testing of known familial mutation was done in 1 796 relatives. Mutation was found and carrier status of *BRCA1*/2 was confirmed in 806 cases, *CHEK2* in 12 cases. In 978 cases predic-

tive testing was negative and carrier status of *BRCA1*/2 was excluded. If predictive testing of *CHEK2* is offered, carriers and non-carriers are recommended to have preventive screening as women with moderate risk of breast cancer [24].

Preventive care

The protocol for the *BRCA1* and *BRCA2* carriers follow-up and for other individuals with high risk of breast cancer was published in 2001 and 2009 [20,21]. The main purpose of these publications was to standardize the prevention within oncology centers and provide to the carriers the most updated preventive procedures. MRI is introduced to the breast cancer screening at the age 25 or earlier, if the youngest breast cancer occurred in the family before the age of 35.

Within four years (2005–2008) the preventive MRI examination of breasts was done in 284 high risk women (488 examinations) and six carcinomas were detected. All tumors were with no positive lymphonodes (N0). Mammography was negative in all cases, ultrasound was negative twice, positive four times in a secondary examination after positive MRI [32,33].

Predictive testing is offered to relatives starting at the age of 18. If the woman is not a carrier of the familial mutation, she is advised to have prevention as a woman with moderate risk of breast cancer (2–3×) and have yearly breast check-up by ultrasound and latter by mammography [34,22].

Prophylactic surgeries are explained to all carriers and information brochures are provided. Oophorectomy is recommended between the ages 35 to 40, prophylactic mastectomy at any age [35–40]. The youngest woman, who decided for preventive mastectomy and reconstruction of both breasts, was a 22 years old woman a carrier of *BRCA1* mutation, whose mother died because of breast cancer at 32, right after she was born.

Surgical prevention of breast cancer in BRCA carriers – 10 years of experience

The carriers of *BRCA1*/2 mutation are consulted by geneticists at MMCI and the information about possibilities of

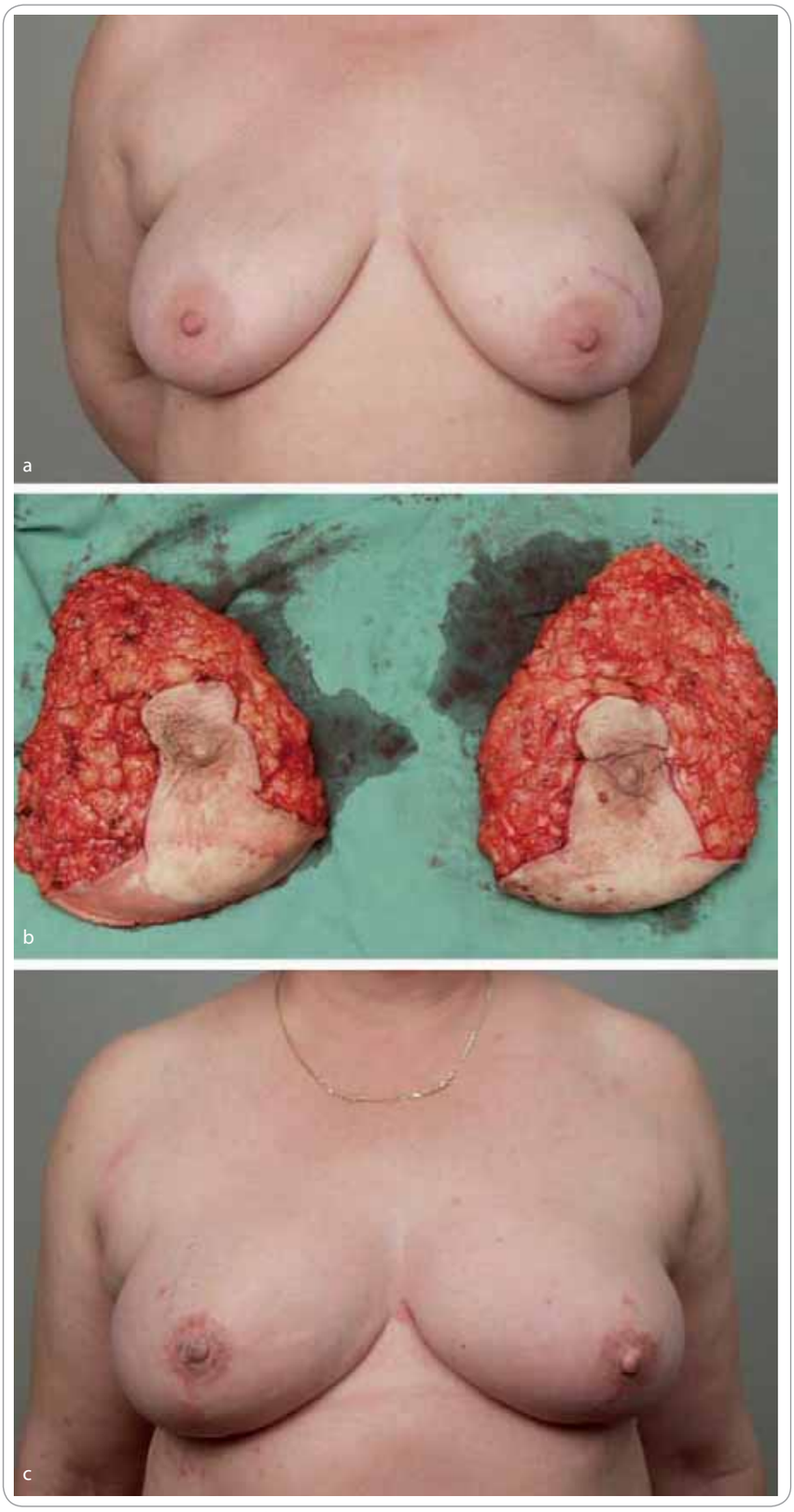

Fig. 3. A. Healthy carrier of *BRCA1* mutation, before surgery, scars after biopsies; B. Skin sparing mastectomies; C. The result of bilateral reconstruction with DIEP, reconstruction of nipples and tattoo.

Tab. 5. Different types of cancer and potential lifetime risks of the disease in Lynch syndrome.

Colorectal (men)	28–75%
Colorectal (women)	24–52%
Endometrial	27–71%
Ovarian	3–13%
Gastric	2–13%
Urological	1–12%
Small intestine	4–7%
Brain	1–4%
Hepatobiliary tract	2%

prophylactic mastectomy, the reconstruction methods and the importance for cancer prevention are provided to all of them. As a consequence of these consultations 84 women, carriers of *BRCA1* or *BRCA2* mutation, underwent prophylactic mastectomy with immediate reconstruction. From those 84 women forty were healthy carriers without any previous surgery for breast cancer. Eighty prophylactic mastectomies were performed, 46 skin sparing and 34 subcutaneous mastectomies. The other women were patients treated before for unilateral (40 patients) or bilateral (4 patients) breast cancer. Among those patients 34 prophylactic skin sparing mastectomies and 6 prophylactic subcutaneous mastectomies on healthy breasts were performed. On the other previously treated breast mastectomy was completed in 15 cases and the scar excision and reconstruction in 25 cases.

I all 83 cases the reconstruction was performed in one time with prophylactic mastectomy. In 63 patients 126 DIEP with own tissue reconstruction was used, in 20 patients the reconstruction with the use of silicon implants was done (Fig. 3). One woman decided for the delay of the reconstruction after the prophylactic surgery.

According to previous investigation [41] 88% of patients were evaluating the result of prophylactic mastectomy and tissue reconstruction as nice and satisfactory, 96% would be willing to undergo the surgery again on the basis of their own experience.

The efficacy of the prophylactic mastectomy with reconstruction and the psychological significance is the issue of the grant project of the Ministry of Health of the Czech Republic NS/10401-3.

Hereditary nonpolyposis colorectal cancer, Lynch syndrome

Highly penetrant DNA repair genes *MLH1* (OMIM #120436), *MSH2* (OMIM #609309), and *MSH6* (OMIM#600678) are responsible for the majority of hereditary colorectal cancer in families with several cases of colorectal and/or endometrial cancer. These three genes are tested when there is a very young patient with colorectal cancer (bellow 40), or a family with at least two cases of colorectal cancer in close relatives, one younger than 50. Predictive testing is offered to all relatives at risk starting at the age of 18 [42,43]).

In all patients with colorectal cancer bellow 50 MLH1, MSH2, MSH6 and PMS2 proteins are tested at MMCI by immunohistochemistry in the tumor. In case of a pathology result, the genetic counseling is recommended in the pathology report. But oncologists, gastroenterologists or other physicians refer most of the patients.

In 310 tested families (at 2nd MF of CHU, Prague or MMCI) the pathogenic mutation was found in 39 (12,6%). In 24 families the mutation was in *MLH1* gene (14 different mutations), in 13 families in *MSH2* gene (10 mutations) and in 2 families in *MSH6* gene (2 mutations). The most frequent mutation was *MLH1*/c.1489dup.C, which was seen in 8 families. By the use of MLPA four different intragenic rearrangements were found in five families, three large deletions causing loss of at least half of the coding regions of the gene (*MLH1*, del 1–13, *MSH2* del 1–8 and 9–16), and one large duplication. No large rearrangement was found in *MSH6* gene. In 29 families variants of unknown clinical significance (UVs) were found. If the predictive testing is offered in these families with UV, it is for segregation analysis and research purposes. In those families both carriers and non-carriers of UVs are advised to have colonoscopy every 2–3 years together with other preventions.

Prevention

The lifetime risk of different cancers in carriers of pathogenic mutations is described in Tab. 5. The complex prevention is offered mostly in the oncology centers and the oncologist is seeing the carrier with Lynch syndrome regularly checking all the results of examinations they have to go through. The oncologist and a specialized nurse are keeping these individuals within the prevention system by telephone calls and invitations for visits. The colonoscopy is starting at the age of 20 (or earlier if very young family members had colorectal cancer). The whole spectrum of examinations was published [44]. Early prevention may be very successful in people with Lynch syndrome. Unfortunately we are still counseling families, where the Lynch syndrome was clinically detectable, but no clinician was referring the patient for testing early enough.

Li-Fraumeni syndrome – LFS

Li-Fraumeni syndrome is caused in many families by *TP53* (OMIM #191170) germline mutations. LFS is considered to be one of the most severe hereditary cancer syndromes where cancer may occur in young individuals and spectrum of cancers is very broad, mostly adrenocortical cancer, breast cancer, leukemia, brain tumors, sarcomas [45–47]. The prevention of cancer is very complicated and predictive testing is not offered to children until the age of 18. Children who have a parent with LFS should be followed regularly by oncologist or informed pediatrician. There are several reasons for not providing predictive testing to healthy children, predominantly because the prevention of cancers related to LFS is not satisfactory.

Genetic testing

Genetic testing is done by direct sequencing of all coding exons of *TP53* gene and by MLPA for large deletions and duplications. So far 85 families with certain probability of having LFS were tested (50 in MMCI, 35 in Prague 2nd MF CHU) and in 8 of them *TP53* mutation was detected. In 7 families LFS was caused by 6 different missense mutations (p.Gly245Ser, p.Arg248Trp, p.Ile254Val, p.Arg267Gln, p.Cys275Phe, p.Glu286Lys,) and one splice site mutation c.375G>A. In one family large deletion encompassing

exon 2–12 was discovered. The proband is a patient with malignant melanoma at 24, bilateral breast cancer at 31, her daughter had brain tumor at 3, her brother had lymphoma at 18, his daughter histiocytoma, her father liposarcoma at 39, her grandmother bilateral breast cancer and died at 46 (Fig. 4). She is followed regularly as a clinical LFS. She had both breasts completely removed and reconstructed by implants. She was offered to have yearly PET examination but refused.

In seven families with clinical suspicion to LFS germline mutation in *CHEK2* gene was found, del 9–10 three times, p.Thr387Asn, p.Ile157Thr four times, but no c.1100delC mutation.

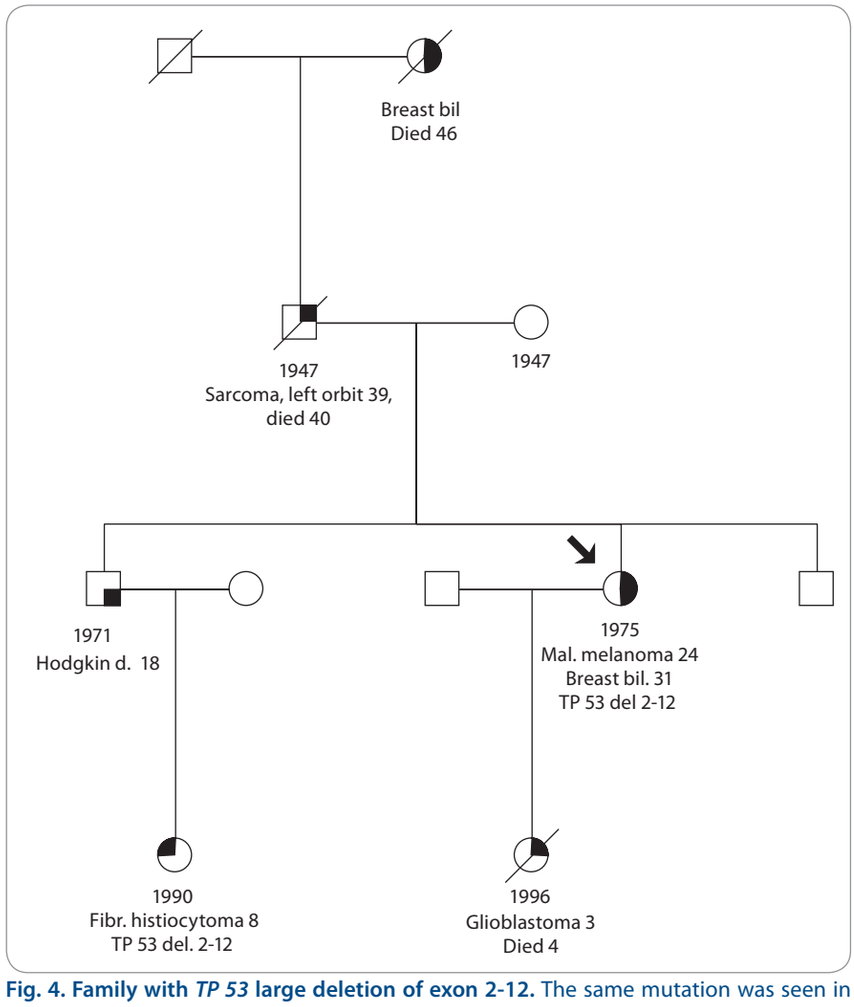

Fig. 4. Family with *TP 53* large deletion of exon 2-12. The same mutation was seen in a proband and her niece.

Prevention

The prevention should be complex, including ultrasound of breasts, stomach, MRI of breasts and brain, colonoscopy, gastroscopy, regular gynecological exam with transvaginal ultrasound, tumor markers, blood and urine analysis etc. [48]. Since 2007 the regular examination by positron emission tomography PET/LD CT (the whole body and brain) is used at MMCI yearly not only for follow-up of cancer patients with LFS, but also for healthy adult carriers starting at 18. In one patient there was a gastric cancer diagnosed early by PET examination (Fig. 5). It was estimated by radiologists that the radiation exposure is around 7mSv from ^{18}F-fluoro-deoxy-glucose (FDG) and 1 mSv from LD CT (for comparison the yearly exposure limit for medical professionals is 50 mSv and there is no limit for patient exposure). The use of PET/CT may be of a great importance for early detection of many cancers in different body sites, but the use of other detection methods without radiation exposure is preferred.

Familial (atypical multiple mole) melanoma syndrome FAMMM

In some families the risk of melanoma is very high, melanomas occur in family members at a young age, with or without multiple moles. Germline mutations in *CDKN2A* (OMIM# 600160) gene may be responsible for some of the familial melanoma cases. So far we have tested 34 families with early onset or familial occurrence of melanomas and discovered pathogenic mutation, c.15–20del6insC, in one family. Both the mother and daughter had malignant melanoma at the age of 31 and 38, respectively. The mothers' sister is also a carrier and is healthy at the age of 65 without any sign of melanoma or multiple moles. Her daughter is a healthy carrier too at the age of 41 (Fig. 6). There may be a high variability of clinical symptoms within a family. In some families higher risk of breast or pancreatic cancer can be seen in *CDKN2A* carriers. In this syndrome the primary prevention is very important and all of the carriers should be well informed. Clinical examinations should start early, from the age of 10, be done regularly with the fotodocumentation of risk moles [49].

Hereditary diffuse gastric cancer

Diffuse gastric cancer is not so frequently seen as intestinal type. In young patients with diffuse gastric cancer or patients with a positive family history of gastric cancer the genetic testing of *CDH1* (OMIM #192090) gene should be offered.

Eighteen young patients with sporadic diffuse gastric cancer or patients with other family members with gastric cancer were tested at MMCI and the pathogenic mutation was not found yet. Only a variant of unknown significance was detected in one family.

It is expected that about 30% of patients with diffuse gastric cancer and positive family history may have a germline mutation in *CDH1* gene but it is very rare in sporadic cases.

In carriers the risk of gastric cancer is very high, reaching 67% and 83% in

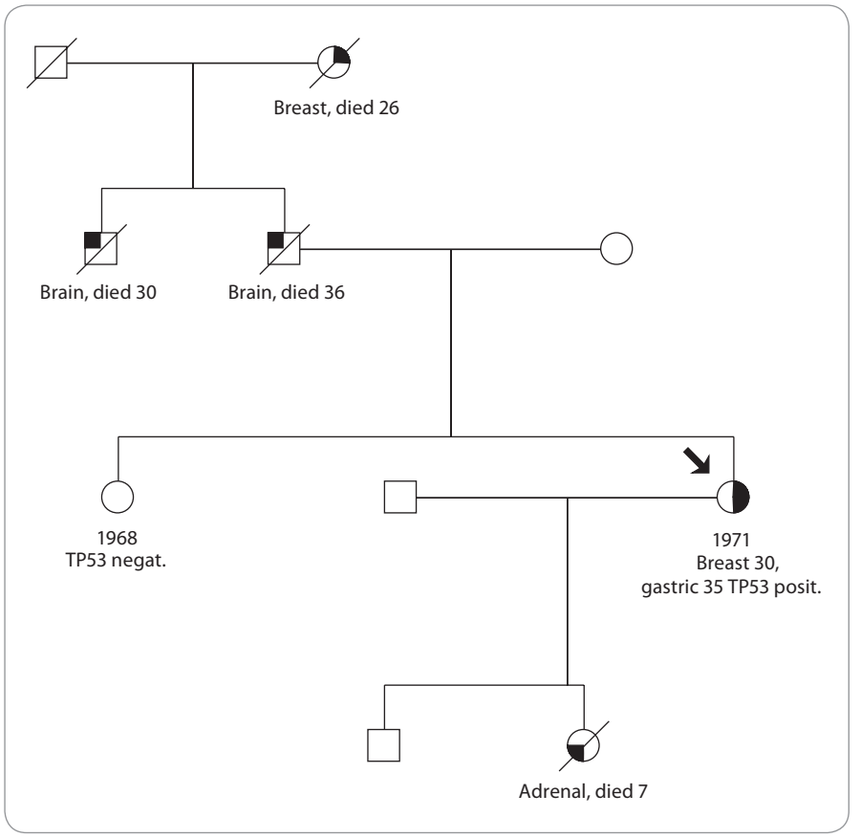

Fig. 5. Family with Li-Fraumeni syndrome, missense mutation in *TP53* p.Cys275Phe.
By the regular use of PET gastric cancer was diagnosed at the early stage.

men and women respectively at the age of 80 [50].

Gastric cancer screening is problematic with the need of chromoendoscopic methods. In some cases prophylactic gastrectomy should be offered. In women carriers of *CDH1* mutation the risk of lobular carcinoma of breast may be increased. Gastric cancer can be frequently seen also as part of the Lynch syndrome, Li-Fraumeni syndrome and in *BRCA2* carriers.

Differential diagnoses of polyposes

Familial adenomatous polyposis coli (FAP) is a severe syndrome in many cases with thousands of polyps in colon, small intestine but also in stomach. The situation may be complicated by desmoids, benign tumors that are frequently growing very progressively threatening the patient's life. According to the mutation location at the *APC* gene (OMIM# 175100) the type of polyposis, severity and possible complications can be predicted (genotype-phenotype correlation) [51,52]. In many cases the polyposis is not seen in any parent and the mutation in *APC* gene occurs de novo (in a germ cell).

So far 35 patients with polyposis were tested (1st MF CHU Prague) and the mutation in *APC* gene was found in 27 patients.

In many cases the pathology report may help us to differentiate between FAP and other polyposis syndromes. In case of hamartomas other syndromes should be tested. Hamartomas are seen in Cowden syndrome (*PTEN* gene OMIM #601728), juvenile polyposis (*SMAD4/BMPR1A* genes, OMIM *#600993/6012999*) or Peutz-Jeghers syndrome (*STK11* gene, OMIM *#602216*) [53,54]. Genetic testing may diagnose the right syndrome and specify the potential risk of cancerous diseases and other complications. Biallelic recessive germline mutations in *MYH/MUTYH* gene (OMIM #604933) are causing mild polyposis in latter age [55]. This type of genetic predisposition is only rarely seen in the Czech population.

Other syndromes are tested in specialized laboratories like neurofibromatosis 1/2, MEN1/2, von-Hippel Lindau syndrome, MEN1/2, tuberous sclerosis, Gorlin syndrome, Birt-Hogg Dubé syndrome and other [50].

Discussion

Genetic testing is highly recommended to patients and families with a possibility of inherited predisposition to cancer. The developments of positional cloning enabled the discoveries of several cancer predisposition genes for common diseases, especially for breast, ovarian and colorectal cancer. This was a great success of cancer genetics, which provided a lot of information on biology of hereditary cancer.

In 1997 genetic clinic was established at MMCI and since that time more than 6 000 probands and their relatives were counseled. Molecular genetic laboratory at MMCI covers the need for genetic testing of substantial part of oncology patients and their relatives not only from Brno, but also from other parts of the Czech Republic. Methods used for mutation detection improved moving from heteroduplex analysis, protein truncation test to high resolution melting analysis, MLPA and much more reliable sequencing. The laboratory completes international quality control tests (EMQN) yearly for *BRCA1*/2 genes, *MLH1*, *MSH2* and *TP53* with an excellent result, which is an important quality assurance of the laboratory work.

The clinical usefulness and limitations of genetic testing depend on many factors. The clinical utility may be characterized as an additional value, which can be used by the patient and doctors in the management of cancer. In some cancer syndromes the additional value does not have to be high enough in order to justify the testing itself.

So far the additional value of *BRCA1* and *BRCA2* genetic testing for hereditary breast and ovarian cancer, or *MLH1*, *MSH2* and *MSH6* for Lynch syndrome is considered to be very high and the testing should be highly recommended in all families which fulfill the testing crite-

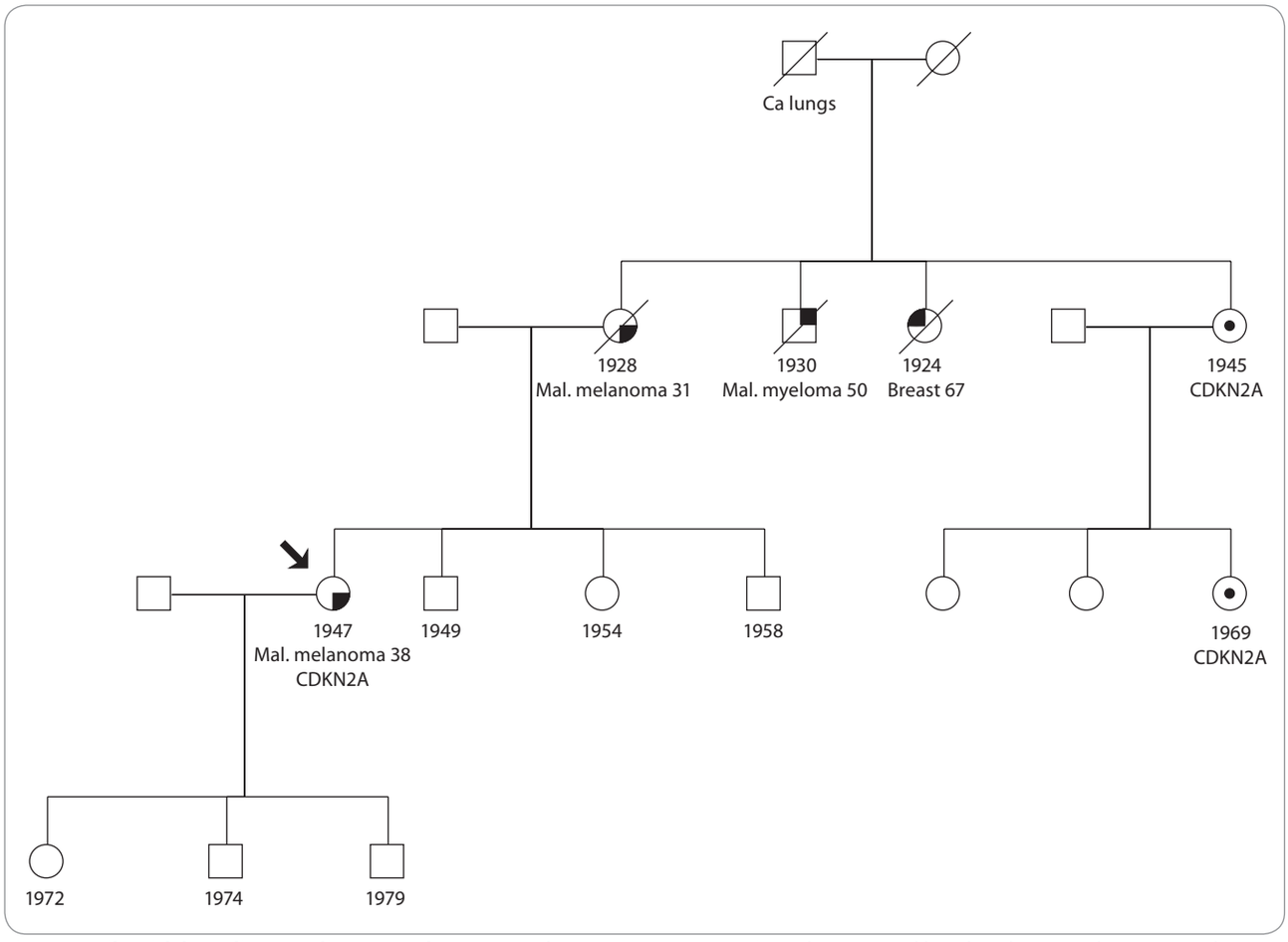

Fig. 6. Family with hereditary malignant melanoma syndrome, *CDKN2A* mutation. The aunt and her daughter are also carriers, the aunt is healthy at the age of 65. The penetrance of the mutation may be variable within the family.

ria. Clinical geneticist should counsel all tested individuals before and after the testing. In those families' not only healthy carriers but also patients should be managed differently for example by the use of more radical surgical therapy.

Genetic testing of germline mutations is important mostly for prevention of disease but not for the evaluation of prognosis or the response to treatment. Some clinical studies are evaluating new options of treatment in hereditary cancer.

The use of cisplatin, carboplatin and targeted agents to treat triple-negative breast cancers carrying dysfunction of *BRCA1* or *BRCA2* pathways is currently being assessed in clinical trials. At this time, the most interesting clinical target in triple-negative breast cancer is the enzyme poly(adenosine diphosphate-ribose) polymerase (PARP), which is involved in base-excision repair after DNA damage [18].

PARP1 is an enzyme that has an important function in the repair of DNA single-strand breaks (SSB) as a part of the base excision repair pathway [18,19]. In this pathway, PARP1 binds to the exposed ends of the corrupted DNA strand and recruits essential enzymes needed to repair SSBs. When PARP1 is inhibited, the base excision repair pathway fails, which leads to accumulation of SSBs. In a replicating cell entering the S-phase, replication is arrested at a SSB site, leading to a DNA double-strand break (DSB). In the absence of *BRCA1*, DSBs cannot be repaired by homologous recombination, and cells activate an alternative repair pathway termed non-homologous (see above). Thus, in *BRCA1*–deficient cells, the damage executed by PARP inhibitors leads to accumulation of structural DNA lesions, which results in genomic instability and finally apoptotic cell death. Since *BRCA2* operates in the same pathway like *BRCA1*, deficiency of this protein renders the cell vulnerable to PARP inhibitors as well [19].

PARP inhibitors (olaparib, iniparib) have recently shown very encouraging clinical activity in early trials of tumors arising in BRCA mutation carriers and in sporadic triple-negative cancers [18,19]. One of these inhibitors, iniparib (BSI-201), was recently used in a randomized phase 2 trial involving patients with triple-negative cancer. When the inhibitor was added to a chemotherapy combination of gemcitabine and carboplatin, there were significant improvements in the rate of tumor regression (48 % vs 16%, P = 0.002), median progression-free sur-

vival (6.9 months vs 3.3 months; hazard ratio, 0.34; P < 0.001), and median overall survival (9.2 months vs 5.7 months; hazard ratio, 0.35; P < 0.001). An updated analysis showed a median overall survival rate of 12.2 months versus 7.2 months (hazard ratio, 0.5; P = 0.005). PARP inhibitors and other targeted agents are now at the forefront of clinical research on the treatment of triple-negative breast cancer [18].

Rare highly penetrant genes are the cause of the predisposition to cancer in a small but a significant proportion of cases. The polygenic inheritance may be more frequently characteristic for the cancer heritability. Genome wide association studies are discovering multiple germline variants in susceptibility loci for different cancer types. The effect of these variants on cancer prediction is mostly low, not exceeding 1,5, and the biological role of them is usually unknown. Over 100 low-penetrance cancer susceptibility loci causing mild increase of cancer risk have been identified [56]. These common variants may explain only about 8% of breast, 20% of prostate and 6% of colorectal cancer predisposition. The role of less frequent (frequencies less than 10%) low-penetrance variants starts to be investigated. Other genetic variants such as large insertions, deletions, copy number variations, translocations and inversions should are also explored. The use of new technologies like whole genome/exome sequencing will help in discovering more moderate or high-risk predisposition loci [57].

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