Germline PTEN mutations are rare and highly penetrant

Cecilie F Rustad[1], Merete Bjørnslett[2], Ketil R Heimdal[1], Lovise Mæhle[1], Jaran Apold[3], Pål Møller[1]

[1]Section for Inherited Cancer, Department of Medical Genetics, Rikshospitalet-Radiumhospitalet Medical Centre, Oslo, Norway, [2]Section for Molecular Genetics, Department of Medical Genetics, Rikshospitalet-Radiumhospitalet Medical Centre, Oslo, Norway, [3]Centre for Medical Genetics and Molecular Medicine, Institute of Clinical Medicine, University of Bergen, Norway

Corresponding author: Dr. P Møller, Section for Inherited Cancer, Department of Medical Genetics, Rikshospitalet--Radiumhospitalet Medical Center, N-0310 Oslo, Norway, phone +47 22 935 67 5, fax +47 22 935 21 9, Email: pal.moller@klinmed.uio.no

Abstract

Cowden syndrome (multiple hamartoma syndrome, MIM 158350) is an early onset syndrome characterized by multiple hamartomas in the skin, mucous membranes, breast, thyroid and endometrium. Patients with Cowden syndrome have increased risk of breast cancer, thyroid cancer and endometrial cancer. In 1997 germline mutations in PTEN were demonstrated to cause Cowden syndrome. We report the results of diagnostic and predictive testing in all families with Cowden syndrome or suspected Cowden syndrome registered at the Norwegian cancer family clinics. PTEN mutations were found in all six families meeting the clinical criteria for Cowden syndrome, in none of the two families assumed to have Cowden syndrome but not fulfilling the criteria, and in none of the eight families selected in our computerized medical files to have a combination of breast and thyroid cancers. Age-related penetrances for the various neoplasms are given. All families but one were small and de novo mutations were found.

Introduction

In 1963 Lloyd and Dennis described a patient named Rachel Cowden who died of bilateral breast cancer in her thirties [1]. Cowden syndrome is now recognized as an autosomal dominant syndrome characterized by multiple hamartomas originating from all three germ-cell layers. Mucocutaneous lesions including trichilemmomas are seen in 90-100% of patients [2]. There is increased risk of early breast cancer from 14 years of age, and lifetime risk is estimated to 25-50% [3-5]. Goitre and thyroid adenomas are frequently seen and the estimated prevalence of thyroid cancer is 3-7% [3, 6, 7]. We have previously reported endometrial cancer in one Norwegian family with Cowden syndrome [8].

In 1995 the International Cowden Syndrome Consortium was formed and a set of clinical diagnostic criteria were suggested; see Table 1 [2, 9, 10]. In 1997 the susceptibility gene for Cowden syndrome was identified on chromosome 10q23.3 and was found to be PTEN [11, 12]. Germline mutations are found throughout the PTEN gene, the majority in exons 5, 7 and 8 [2, 13]. The frequency of germline PTEN mutations, including mutations in the promoter region, in Cowden syndrome have been reported to approach 85-90% [14]. Genotype/phenotype correlations have been suggested, but have not been confirmed [15-17].

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Table 1. International Cowden Syndrome Consortium operational criteria (version 2000) as given by Charis Eng [2]

Pathognomonic criteria
    Trichilemmomas, facial
    Acral keratoses
    Papillomatous papules
    Mucosal lesions

Major criteria
    Breast carcinoma
    Thyroid carcinoma (non-medullary), especially follicular thyroid carcinoma
    Macrocephaly (megalencephaly) (say >97 percentile)
    Lhermitte-Duclos disease
    Endometrial carcinoma

Minor criteria
    Other thyroid lesions (e.g. adenoma or multinodular goitre)
    Mental retardation
    Gastrointestinal hamartomas
    Fibrocystic disease of the breast
    Lipomas
    Fibromas
    Gastrointestinal tumours (e.g. renal cell carcinoma, uterine fibroids) or malformation

Operational diagnosis in an individual
    1. Mucocutaneous lesions alone if
        a. there are six or more facial papules, of which three or more must be trichilemmoma, or
        b. cutaneous facial papules and oral mucosal papillomatosis, or
        c. oral mucosal papillomatosis and acral keratoses, or
        d. palmo-plantar keratoses, 6 or more
    2. Two major criteria but one must include macrocephaly or LDD
    3. One major and three minor criteria
    4. Four minor criteria

Operational diagnosis in a family where one individual is diagnostic for Cowden syndrome
    1. The pathognomonic criterion/criteria
    2. Any major criterion with or without minor criteria
    3. Two minor criteria

Nelen has estimated the incidence to be between 1 per 200,000 and 1 per 250,000 in the Dutch population [16].

As for all inherited cancer syndromes, the penetrance of the underlying genetic defects and the full clinical spectrum of their expressions have been difficult to assess without access to genetic testing.

Fifty to sixty percent of patients with Bannayan-Riley-Ruvalcaba syndrome (BRRS, MIM 153480) have been shown to have germline mutations in the PTEN gene [14, 18]. An association between germline PTEN mutations and Proteus syndrome (MIM 176920) has been disputed [19-22].

Our aim was to validate strategies to identify families with PTEN mutations and to estimate prevalences and penetrances of PTEN mutations.

Material and methods

The computerized medical files at the Section for Inherited Cancer, Rikshospitalet-Radiumhospitalet Medical Centre, include more than 40,000 patients belonging to more than 3,000 families. These files were analyzed and all families with a diagnosis of Cowden syndrome, all families suspected to have Cowden syndrome, and all families with a combination of breast and thyroid cancers were identified. All of the five other Norwegian genetic centres contributed their families with Cowden stigmata. The four families previously reported to harbour PTEN mutations were included.

We had no families with suspected Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome or Proteus-like syndrome. Our two patients with Lhermitte-Duclos disease were also classified as having Cowden syndrome and included above.

All families were extended and offered genetic testing according to our genetic health care standards [23]. Because all activity was provided as health service, all information was kept in the medical files and no research registry was created. All activities were according to

Table 2. Clinical signs in all demonstrated and assumed mutation carriers in the six Cowden syndrome families with PTEN mutation. Families ordered by genetic position of mutations

| Mutation(s)‖ | Family | Patient (sex, year of birth) | Phenotype (Age in years at diagnosis) | | | | | | |
			Breast	Thyroid	Endometrium	CNS	Gastrointestinal tract ‡	Skin lesions Histology	Others
c.50delAA Frameshift	A	Pid* 1 (F, 1970)	Bilateral mastectomy (34)	Goitre (23)		Lhermitte-Duclos disease (24) Macrocephaly	Hyperplastic colon polyps without dysplasia (26, 8)		Cavernous haemangioma (18)
		Pid 3 (F, 1999)				Macrocephaly Born with hydrocephalus			Born with neural tube defect
		Pid 4 (F) Dead, not tested	Cancer (46)						
c.68T>A Nonsense	B	Pid 1 (F, 1958)	Cancer (24)	Follicular cancer (13)	Simple hyperplasia (43)	Macrocephaly	Hyperplastis colon polyps (42, 3)	+ (35) skin with trichilemmomal hyperplasia	Liver haemangioma (35)
		Pid 4 (F) Dead, not tested	Bilateral cancer (45)	Goitre (35)				Gastric leiomyoma (45)	
c.328C>T Nonsense	C	Pid 1 (F, 1958)	Cancer (35) Prophylactic contra lateral mastectomy		Atypical hyperplasia (36)	Macrocephaly		+(29) Multiple trichillemmomas periorally	Cavernous haemangioma (17) Lipoma (36)
c.565A>T Nonsense	D	Pid 1 (F, 1950)	Bilateral cancer (30) (31)	Adenoma (31)	Uterine polyp (52)	Macrocephaly	Multiple non classifiable gastric, duodenal, colon and rectal fibrous polyps (30)	+ (44)	Hypersementosis in teeth (32) Osteoma (32) Haemangioma (38) Neurofibroma (45)
		Pid 2 (F, 1972)	Adenoma (29)	Goitre (30)		Macrocephaly	Colon polyps with erosions and teleangiestatic granulation tissue (22, 30)	+ (22) Papules on right ankle and squamous cell hyperplasia and hyperkeratosis	Ethmoidectomy with removal of polyp from maxillary sinus (22) Dysplasia in nevus (22) Von Willebrands disease (30)
		Pid 3 (F, 1974)	Bilateral adenoma (21)	Goitre and follicular adenoma (22)		Macrocephaly			
		Pid 18 (F, 2003)				Macrocephaly			

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Table 2. Clinical signs in all demonstrated and assumed mutation carriers in the six Cowden syndrome families with PTEN mutation. Families ordered by genetic position of mutations

Mutation(s)‖	Family	Patient (sex, year of birth)	Phenotype (Age in years at diagnosis)						
			Breast	Thyroid	Endometrium	CNS	Gastrointestinal tract ‡	Skin lesions Histology	Others
c.1008C>G Nonsense	E	Pid 1 (F, 1946)	Cancer (45)	Goitre (21) and papillary cancer (45)		Lhermitte-Duclos disease (51) Macrocephaly		+ (52) Squamous cell hyperplasia on hands	Lipoma (5) Parathyroid adenoma (45) Haemangioma (54)
		Pid 12 (M, 1975)		Goitre (14) Goitre and thyroiditis (31) Thyroidectomy (31)		Macrocephaly			
c.1028T>A, c.1039T>C Missense, missense†	F	Pid 1 (F, 1962)	Atypical hyperplasia and adenoma (33) Bilateral mastectomy (44)	Goitre (33)	Cancer (31)	Macrocephaly	Multiple hyperplastic duodenal and colon polyps (30)		
		Pid 2 (F, 1948)	Adenoma (30)	Goitre (31) Follicular adenoma (40)	Cancer (47)	Macrocephaly	Hyperplastic gastric, small intestine, colon and rectal polyps (37)	+ (36) Fibroepithelial polyps in the axilla	
		Pid 3 (F, 1967)	Adenoma (33)	Follicular cancer (11	Polyps (29)	Macrocephaly	Not examined		Haemangioma (9 months) Cephalic haematoma (1) Chronic cheilitis (5) Ectopic breast tissue (16)
		Pid 4 (F) Dead, not tested	Bilateral cancer (56)	Adenoma (49)	Nodular cystic hyperplasia (50)				Malignant schwannoma in peripheral nerves (33)
		Pid 5 (F, 1965)	Bilateral adenoma (38)	Goitre(38)	Polyp (39)	Macrocephaly	Hyperplastic colon and rectal polyps (39)	+ (38) Dermatofibroma and fibroepithelial polyp	Naevi with cell changes (39)

Table 2. Clinical signs in all demonstrated and assumed mutation carriers in the six Cowden syndrome families with PTEN mutation. Families ordered by genetic position of mutations

Mutation(s)‖	Family	Patient (sex, year of birth)	Phenotype (Age in years at diagnosis)						
			Breast	Thyroid	Endometrium	CNS	Gastrointestinal tract ‡	Skin lesions Histology	Others
		Pid 7 (M, 1950)		Goitre (31)	Pineal gland tumour (42)	Macrocephaly	Multiple colon polyps seeming like ganglioneuroma and Peutz Jeghers polyps (42) Colectomized (42) Gastric, duodenal and small intestine polyps (53) Inflammatory polyp and multiple polyps perianally (53)		Epilepsy (42)
		Pid 8 (F, 1952)	Bilateral cancer (43, 53)	Goitre (28)	Polyp and simple hyperplasia (50)	Macrocephaly	Hyperplastic colon polyp and juvenile hamartomata type colon polyp (49, 2)	Removed epidermal cyst (52)	Liver haemangioma (51) Kidney cancer (52)
		Pid 9 (F, 1955)	Small cysts bilaterally (50)	Goitre (25)	Myoma (39) Uterus removed (45) Polyp isthmus uteri (48)	Macrocephaly	Hyperplastic colon and rectal polyps with focal fibrosis (48, 7)	+ (48)	Lipoma (36)
		Pid 32 (F, 1973)				Macrocephaly			Polydactylia foot (5)
		Pid 42 (M, 1989)				Macrocephaly			Haemangioma (11)

‖ reference sequence NM_000314.1; * pid – patient identification number within the family; † all affected carried both mutations; ‡ age at first detected polyp and total number of polyps up until last date of data collection are given. If the exact number is not given in the medical charts, polyps are interpreted as meaning a few.

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Norwegian legislation. All diagnoses were confirmed in the medical files after written informed consent from each patient if alive or from their relatives if dead. All family members were offered genetic counselling. All genetic testing was subjected to written informed consent.

We sequenced all nine exons, their flanking areas and 1500 basepairs upstream of ATG (the promoter region) in the PTEN gene in all patients examined.

Kaplan-Meier survival estimates were calculated using the computer program Systat 10©. The data were stored in Oracle©, pedigrees were displayed in Cyrillic© and the application to run the database as an electronic patient file system was programmed in dB+©.

Results

We identified six families (family A, B, C, D, E and F) which fulfilled the International Cowden Syndrome Consortium Criteria; for details see Table 2. Four of them had been identified prior to the present study (B, C, D, F) [8]. Two families (G and H) had been clinically assumed to have Cowden syndrome earlier on, but did not fulfil the diagnostic criteria. Eight families had a combination of breast cancer and thyroid cancer.

Mutations in the coding sequence of PTEN were identified in all living affected members of the six families fulfilling the International Cowden Syndrome Consortium Criteria. All together 56 persons were subjected to genetic testing, out of whom 19 were identified as mutation carriers. None of the 37 healthy relatives in these families had mutations. In the two families clinically assumed to have Cowden syndrome, but not fulfilling the clinical criteria, no PTEN mutation was identified; for details see Table 3. In the eight families selected by a combination of breast and thyroid cancer no PTEN mutation was identified. Different cancer types were seen in these families; for details see Table 3.

Of the tested families, five families had a frameshift or nonsense mutation and one family had two missense mutations located in exon 9 located on the same chromosome. The detected mutations were located in exon 1 (c.50delAA and c.68T>A), exon 5 (c.328C>T), exon 6 (c.565A>T), exon 8 (c.1008C>G) and exon 9 (c.1028T>A and c.1039T>C) (reference sequence: NM_000314.1). No mutations were found in regulatory regions.

Except for one family (F), the mutation carrying families were small. One family had a demonstrated de novo mutation (D) and two families most likely had de novo mutations (C and E). We did not observe skipped generations.

The mothers of three of our probands had had breast cancer at age 45 (bilateral breast cancer), 46 and 56 (bilateral breast cancer) years old. Since they all were deceased and our attempts to perform mutation analysis from paraffin embedded tissue from these patients were unsuccessful, they were considered to be mutation carriers by state.

All mutation carriers were examined and all had clinical signs. The youngest mutation carrier was born with a demonstrated clinical sign (macrocephaly, D-18). Most patients had more than one clinical sign; for details see Table 2. Twelve (63.2%) patients had goitre and/or adenoma of the thyroid gland, ten (52.6%) patients had polyps of the gastrointestinal tract, seven (36.8%) patients had benign tumours of the breast, seven (36.8%) patients had endometrial polyps and/or hyperplasia; for details see Table 3. Fig. 1 shows age-related distribution of first clinical sign, as estimated by the Kaplan-Meier algorithm.

One patient (A-1) had multiple small colonic polyps interpreted as not having any dysplasia at age 33. Two years later she still had multiple small polyps less than 5 millimetres in diameter, now histologically described as lymphoid infiltrations and interpreted as an inflammatory condition. Mutation analysis of the APC gene showed APC 7542 G>A, G2502S and APC 1496 C>T, Y486Y, interpreted as normal variation. MYH testing was normal.

Macrocephaly is defined as a head circumference above the 97th percentile [2]. We used centile charts for Norwegian boys and girls aged 0-17 and for all patients above 17 years of age we used the centile for age 17. All our patients that were alive were clinically assessed to have macrocephaly. Macrocephaly was confirmed by exact measurement in 15 patients and judged to be clinically present in the remaining four.

Cancer appeared from age 11. The oldest mutation carrier without diagnosed neoplasia was 49 years old. She had, however, been hysterectomized at age 45. Five (26.3%) patients had breast cancer, age range from 24 to 45 years. Three (15.8%) patients had thyroid cancer, age range 11-45 years. Three (15.8%) patients had tumours of the brain (two had a gangliocytoma at age 24 and 51 and one had a tumour of the pineal gland at age 42). Two (10.5%) patients had endometrial cancer at 31 and 47 years of age. One (5.3%) patient had kidney cancer at 52 years of age. For details, see Table 3. Results of Kaplan-Meier analysis for age of onset of cancer are given in Fig. 2.

Discussion

Cowden syndrome is rarely found in families attending our cancer genetics clinic. In the national

Table 3. Number of patients with benign neoplasms and different cancer types, and the age range

	PTEN mutation positive families fulfilling the Cowden syndrome criteria (6 families, only demonstrated mutation carriers included)		Assumed Cowden syndrome families but not fulfilling the criteria, no PTEN mutation detected (2 families)		Breast- and thyroid-cancer kindreds, no PTEN mutation detected (8 families)	
	Number of patients	Median/Mean age (range) years	Number of patients	Median/Mean age (range) years	Number of patients	Median/Mean age (range) years
Benign neoplasms						
Goitre and/or adenoma	12	29/27.25 (14-38)			1**	34
Polyps of GI tract	10	38/36.5 (22-49)				
Haemangioma	8	26.5/28.1 (0.75-54)				
Mucocutaneous lesions	9	36/36.6 (22-52)	1	59		
Endometrial polyps/simple hyperplasia	7	43/42.4 (29-52)				
Benign tumours of the breast	7	33/33.4 (21-50)			1**	25
Lipoma	3	36/27.3 (10-36)				
Cancers						
Breast	5*	35/35.4 (24-45)	4	42.5/46.5 (25-76)	12	50.5/52 (40-65)
Thyroid	3	13/23 (11-45)	5	50/49.2 (24-84)	11	45/46.4 (22-83)
Brain	3†	42/39 (24-51)				
Endometrial	2	39/39 (31-47)			1	67
Kidney	1	52	1	27		
Testicular			1	62		
Mediastinal			1	54		
Ovarian					2	51.5/51.5 (49-54)
Colon					2	57/57 (49-65)
Prostate					1	58
Histiocytoma					1	45
Malignant melanoma					2	57.5/57.7 (56-59)
Cervical					1	34
Adrenal					1	56

*two patients had bilateral disease, scored for age at first cancer; † two patients had a gangliocytoma (Lhermitte-Duclos disease), one had a tumour of the pineal gland; ** same patient.*

survey presented here, PTEN mutations were found in all families fully meeting the Cowden syndrome criteria, but in no Cowden syndrome-like families not meeting the criteria. Penetrance of PTEN mutations was high and expressions were Cowden syndrome stigmata in early infancy or childhood and cancer in adolescence or early adulthood [24, 25]. In addition, the families were small with demonstrated de novo mutations, indicating low fitness before modern treatment and low population burden of mutation carriers.

Ten years ago we reported the well defined Cowden syndrome families we knew at the time at Rikshospitalet-Radiumhospitalet Medical Centre [8]. The national survey presented here revealed just two additional families, both fulfilling the clinical criteria for Cowden syndrome. Obviously, the numbers are too small to arrive at conclusions with respect to clinical manifestations in the mutation carriers. On the other hand, we had a large sample of cancer families initially not ascertained through Cowden criteria – the families may be representative for families selected this way. On the other hand, we did not
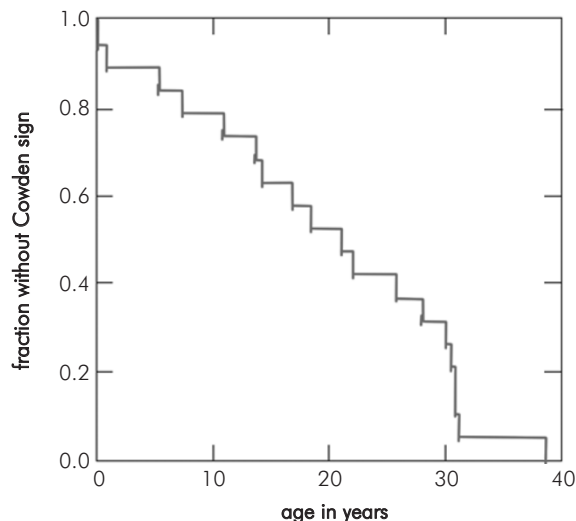
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Fig. 1. Age at onset of first clinical sign estimated by Kaplan-Meier algorithm for demonstrated mutation carriers. See Table 2 for details of clinical signs
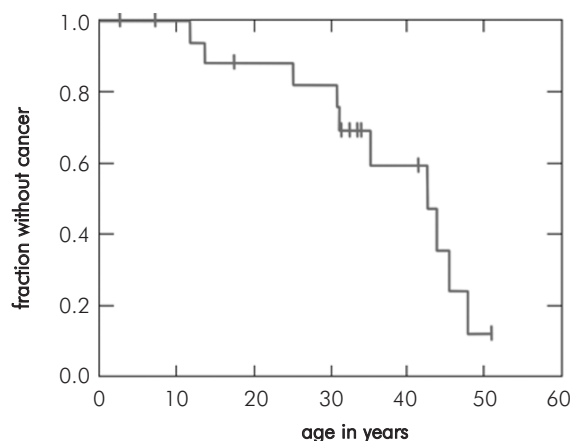

Fig. 2. Age at onset of first cancer estimated by Kaplan-Meier algorithm for demonstrated mutation carriers

search for Cowden syndrome without cancer. We described that in familial cancer all mutation carrying families had additional Cowden syndrome stigmata. We have not examined whether or not Cowden syndrome-like families without cancer may have PTEN mutations. We have previously reported on frequent cancer syndromes in Norway; we have a large number of cancer kindreds in our computerized database, and we found no additional families when searching for Cowden syndrome associated cancers. We consider the low prevalence and the expressions found representative for Cowden families with cancer.

All families (100%, 95% CI 37-100%) which fulfilled the Cowden syndrome criteria carried PTEN mutations, which is in keeping with the previously reported estimate of 85-90%.[14] Our observed number of mutation carriers gives an observed prevalence of 1 per 242,063, which is within Nelen's estimate of between 1 per 200,000 and 1 per 250,000 [16]. We found only six families with mutations, which were considered too few for meaningful considerations with respect to where in the gene the mutations were located.

Twelve (63.2%) patients had benign thyroid manifestations, which is a little less than three quarters reported by Merg [7]. The youngest age of onset for thyroid cancer was 11 years, which indicates that PTEN mutation carriers contract thyroid cancer both more frequently and at a younger age than the general population. Seven (36.8%) patients had benign breast lesions, compared to earlier reports of 50-60% [3, 6]. Five out of 19 (26.3%) had breast cancer at 24-45 years, which is within 20-30% and age range as previously reported [4]. Two (10.5%) patients had endometrial cancer compared to earlier reports of 6% [3, 7]. Ten (52.6%) patients had gastrointestinal polyps, which is close to earlier reports [7]. None of our mutation carriers had colon cancer. According to Lynch and de la Chapelle a firm association between Cowden syndrome and colorectal cancer has yet to be identified, and according to Eng colorectal cancers are not components of Cowden syndrome [26, 27]. One (5.3%) of our mutation carriers had renal cell carcinoma, supporting the notion that renal cell carcinoma should be added to the operational criteria for Cowden syndrome [28]. Thus, our findings add empirical support to established conclusions which have been questioned because of low numbers reported.

Nine of 19 (47%) mutation carriers were members of one kindred. We consider this a finding corresponding with low and variable fitness in mutation carriers. If so, the uneven distribution of mutation carriers in families was a finding and possibly not a statistical problem reflecting random variation in low numbers.

In sum, our results were that Cowden syndrome families had PTEN mutations, while Cowden-like families and breast and thyroid cancer kindreds did not. The number of Cowden syndrome-like families was not more than what might be expected by chance alone. All examined mutation carriers had clinical signs, and cancer started to occur before the age of 16.

In Cowden syndrome families, genetic testing to identify individuals at risk of contracting cancer cannot wait until the kindreds are old enough to give informed consent. We find it reasonable to offer genetic testing

in infancy/early childhood. Mutation carriers may be offered thyroid screening from childhood, and breast cancer screening may be considered from adolescence (with MRI, to avoid radiation exposure by mammography in puberty). Our material is not large enough to conclude on which age to start breast cancer screening, but the youngest patient with breast cancer described internationally was 14 years old [3]. We feel that we should try to do our best when it comes to screening, and therefore we recommend that one may consider breast cancer screening from adolescence.

Non-mutation carriers and members of Cowden syndrome-like families may not be at risk for cancer in early age, and may need no special health care in infancy or adolescence. Thorough clinical genetic work-up of families and access to genetic testing will discriminate between those who need health service from childhood, and those who do not.

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