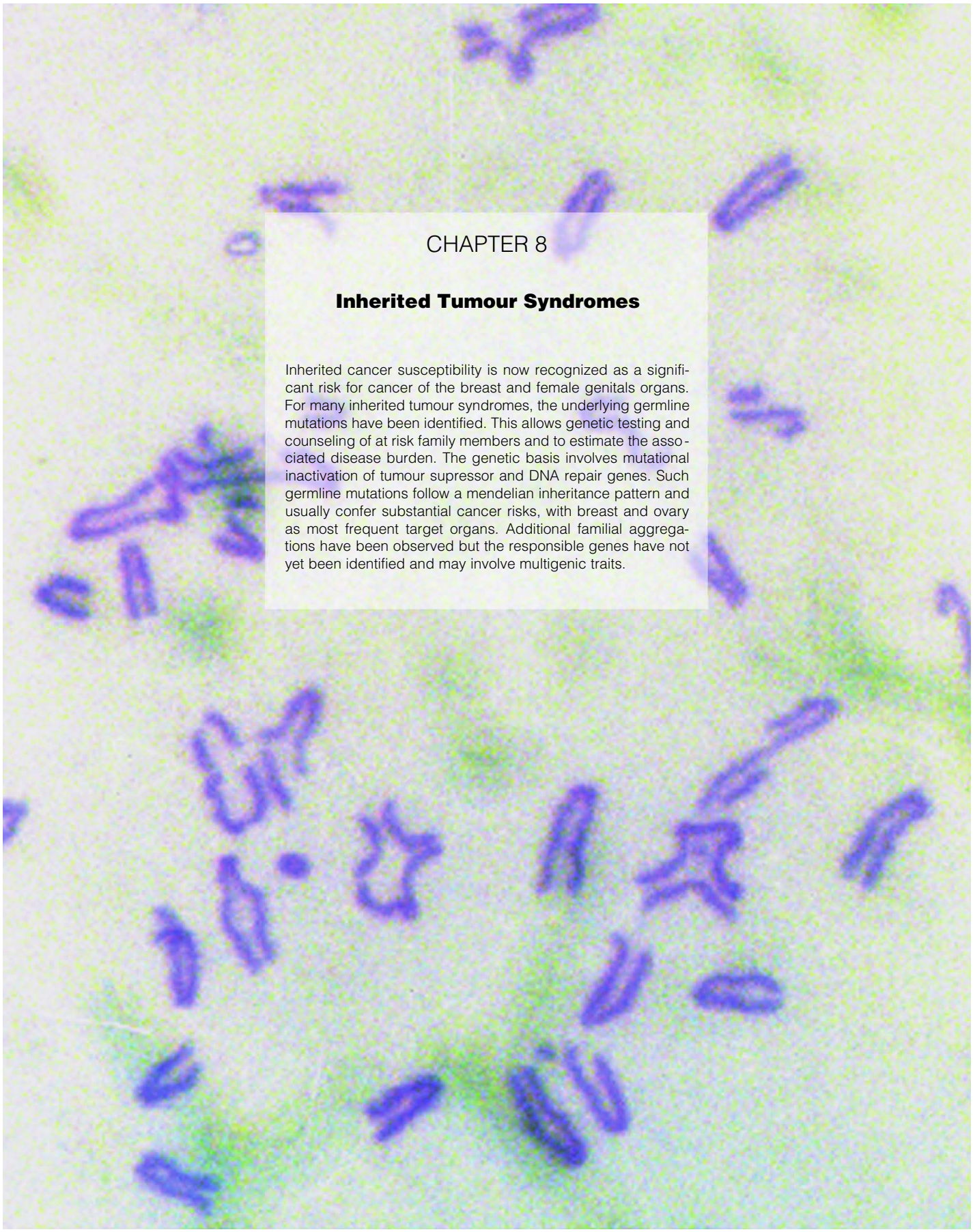

CHAPTER 8

Inherited Tumour Syndromes

Inherited cancer susceptibility is now recognized as a significant risk for cancer of the breast and female genitals organs. For many inherited tumour syndromes, the underlying germline mutations have been identified. This allows genetic testing and counseling of at risk family members and to estimate the associated disease burden. The genetic basis involves mutational inactivation of tumour supressor and DNA repair genes. Such germline mutations follow a mendelian inheritance pattern and usually confer substantial cancer risks, with breast and ovary as most frequent target organs. Additional familial aggregations have been observed but the responsible genes have not yet been identified and may involve multigenic traits.

Familial aggregation of cancers of the breast and female genital organs

D. Goldgar
M.R. Stratton

Evidence of familial aggregation of breast, ovarian, and other tumours of the female genital organs derived from anecdotal observation of large families and from systematic analyses of cancer incidence in relatives of cancer cases. Although there are a number of potential measures of familial aggregation, the most commonly used is the familial relative risk (FRR) or standardized incidence rate (SIR). This is defined as the ratio of the incidence of disease among relatives of an individual with disease compared with the incidence in the population as a whole. The FRR is most often estimated through comparison of family history data between cases and controls, with the resulting odds ratio used as an estimator of the familial risk. Using genealogical resources linked to cancer registries has a number of advantages; the number of cases is usually large compared to case-control studies and, more importantly, all cancers found among relatives are confirmed in the cancer registry.

Breast cancer

Evidence that women with a positive family history of breast cancer are at increased risk for developing the disease has been accumulating for over 50 years; virtually every study has found significantly elevated relative risks to female relatives of breast cancer patients. Most studies have found relative risks between 2 and 3 for first-degree relatives of breast cancer patients selected without regard to age at diagnosis or laterality. A recent review of 74 published studies {2238} calculated familial relative risks of 2.1 (95% CI 2.0, 2.2) for breast cancer in any first degree relative, 2.3 for a sister affected, and 2.0 for an affected mother, and a relative risk of 3.6 if an individual had both a mother and sister affected. For individuals with a first degree relative diagnosed with breast cancer under age 50, the relative risk to develop breast cancer before age 50 was 3.3 (CI 2.8, 3.9).

In a population-based study of familial cancer using the Utah Population Database, Goldgar et al. {1029} studied the incidence of breast and other cancers among 49 202 first-degree relatives of 5559 breast cancer probands diagnosed before age 80. This study estimated a relative risk of 1.8 in first degree relatives of these breast cancer probands. When restricted to early-onset cancer (diagnosed before age 50), the relative risk of breast cancer among first-degree relatives increased to 2.6 and the risk for early-onset breast cancer among these relatives was 3.7 (95% CI. 2.8–4.6). The Swedish family cancer database {715} contains >9.6 million individuals, with data on nearly 700,000 invasive cancers and consists of individuals born in Sweden after 1934 and their parents. Analyzing cancers diagnosed between the years 1958 to 1996, the standardized incidence ratio for breast cancer was 1.85 (95% CI 1.74–1.96) for having an affected mother, 1.98 (1.79–2.18) for having an affected sister, and 2.4 (1.72–3.23) if both mother and sister were affected. Other studies found larger familial effects among relatives of young bilateral probands compared with young probands with unilateral breast cancer {700,1246,2129}.

The issue of relationship of histology to familial breast cancer is less clear {375, 500,1724,2441,2989}. Some studies found that lobular carcinoma is more often associated with a positive family history {791} while others {1566} observed that cases with tubular carcinoma were more frequently associated with a positive family history. Multicentricity was also found to be positively associated with family history {1564}. Occurrence of breast cancer in a male conveys a two to three fold increased risk of breast cancer in female relatives {94,2449}.

Ovarian cancer

In a population-based case-control study of families of 493 ovarian cancer cases and 2465 controls, Schildkraut and Thompson {2557} reported an odds ratio for ovarian cancer in first degree relatives of 3.6 (95% CI 1.8–7.1). A comprehensive study of first-degree relatives of 883 ovarian cancer probands from the Utah Population Database estimated a relative risk of 2.1 (1.0–3.4) for ovarian cancer in the relatives {1029}. Analysis of the Swedish family cancer database {715} found a standardized incidence ratio for ovarian cancer of 2.81 (95% CI 2.21–3.51) for having an affected mother, 1.94 (0.99–3.41) for having an affected sister, and 25.5 (6.6–66.0) if both mother and sister were affected. A meta-analysis of all case-control and cohort studies published before 1998 estimates the risk to first degree relatives at 3.1, with a 95%CI of 2.6-3.7 {2801}.

Endometrial cancer

Gruber and Thompson {1071} in a study of first-degree relatives of 455 cases of primary epithelial carcinoma of the endometrium and 3216 controls, report an odds ratio (OR) of 2.8 (CI 1.9 – 4.2) for having one or more relatives affected with endometrial cancer. In a similar size study (726 cases and 2123 controls) Parrazini et al. {2173} found a smaller effect, with an OR of 1.5 (CI 1.0–2.3). This may partly be explained by the fact that in the former study, cases were restricted to ages 20-54, while in the latter, the median age at diagnosis was 61. A Danish case-control study of 237 cases of endometrial cancer diagnosed under age 50 and 538 population controls reported an OR for family history of 2.1 (1.1–3.8). In contrast to most other sites, the two registry/geneaology based studies of endometrial cancer produced conflicting results, with the Utah study finding a FRR of 1.32 and the Swedish family cancer database reporting a SIR of 2.85. The reason for this discrepancy is unclear, but may to some extent reflect differences in the age distribution of the two populations.

Cervical cancer

In the Utah Population Database {1029}, a FRR to first degree relatives of 999 cervical cancer cases of 1.74 was obtained (95% CI 1.03-2.53) while in the Swedish

family cancer database {715}, a slightly higher risk of 1.93 (1.52-2.42) in mothers of invasive cervical cancer cases and 2.39 (1.59-3.46) in sisters. Unlike many other cancers, there did not appear to be a significant effect of age at diagnosis in familial risk of cervical cancer, although the risks to mothers did depend on the number of affected daughters. In this study, significant familial aggregation was also found for in situ carcinoma of the cervix (FRR 1.79, (1.75-1.84).

Table 8.01
Specific inherited syndromes involving cancers of the breast and female genital organs.

Syndrome	MIM	Gene	Location	Associated sites / tumours
BRCA1 syndrome	113705	*BRCA1*	17q	Breast, ovary, colon, liver, endometrium, cervix, fallopian tube, peritoneum
BRCA2 syndrome	600185	*BRCA2*	13q	Breast (female and male), ovary, fallopian tube, prostate, pancreas, gallbladder, stomach, melanoma
Li-Fraumeni	151623	*TP53*	17p	Breast, sarcoma, brain, adrenal, leukaemia
Cowden	158350	*PTEN*	10q	Skin, thyroid, breast, cerebellum, colon
HNPCC	114500	*MLH1* *MSH2* *MSH6*	3p 2p 2p	Colon, endometrium, small intestine, ovary, ureter/renal pelvis, hepatobiliary tract, brain, skin
Muir Torre	158320	*MLH1* *MSH2*	3p 2p	HNPCC sites plus sebaceous glands
Peutz-Jeghers	175200	*STK11*	19p	Small intestine, ovary, cervix, testis, pancreas, breast
Ataxia Telangiectasia	208900	*ATM*	11q	Breast (heterozygotes)

Multiple cancer sites

In most but not all studies, a familial association between cancers of the breast and ovary have been found, particularly when the breast cancer cases have been diagnosed at a young age. Undoubtedly, the majority of the association between breast and ovarian cancer detected in these population studies is due to the *BRCA1* gene, which is known to be involved in a large proportion of extended kindreds with clearly inherited susceptibility to breast and ovarian cancer. It is likely that some of the discrepant results are linked to the frequency of *BRCA1* deleterious alleles in the respective populations in these studies.

For breast cancer, the most consistent finding has been a small (FRR/SIR ~ 1.2) but highly significant familial association with prostate cancer. Other sites found to be associated in at least two studies with breast cancer in the familial context have been thyroid cancer and other endocrine-related tumours.

For endometrial cancer, there is a familial association with colorectal cancer which is consistently found in a number of studies with statistically significant OR/SIRs ranging from from 1.3 to 1.9. Some, but not all studies have also reported associations with ovarian cancer, particularly among relatives of younger patients.

The strongest and most consistent familial association between cervical and other sites is for lung cancer with statistically significant SIRs of 1.8 and 1.64 found in the Swedish FCDB and the Utah UPDB, respectively. Other cancers with possible associations in both studies are lip/skin (SIR 2.4 and 1.83) and bladder cancer (SIR=1.6), though the latter was not statistically significant in the UPDB study.

In addition to this statistical and observational evidence for the role of genetic factors in the development of these cancers, a number of specific genes have been identified. Of these, the most important in terms of both risk and frequency are the breast cancer susceptibility loci *BRCA1* and *BRCA2*, and the mismatch repair genes *MSH2*, *MLH1*, and *MSH6* in the context of the hereditary non-polyposis colorectal cancer (HNPCC).

Search for additional genes

While some of the familial clustering may be due to shared environmental factors, it seems likely that a number of additional loci remain to be identified for cancers of the breast and female genital tract. Some studies have shown that only about one-fifth of the familial aggregation of breast cancer is attributable to the *BRCA1* and *BRCA2* genes {107,592,2230} and that these genes only explain less than half of all high risk site-specific breast cancer families {898,2631}. Whether the remaining familial aggregation is due to additional moderate to high risk loci or to the combined effects of a number of more common, but lower risk, susceptibility alleles is unknown {2236}. In contrast, it appears that almost all of the familial clustering in ovarian cancer can be ascribed to the effects of the *BRCA1/2* and HNPCC loci {2802}. Although no systematic studies have been done for endometrial cancer, it is also likely that the HNPCC loci account for a substantial fraction of familial aggregation in this cancer as well.

BRCA1 syndrome

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Definition
Inherited tumour syndrome with autosomal dominant trait and markedly increased susceptibility to breast and ovarian tumours, due to germline mutations in the *BRCA1* gene. Additional organ sites include colon, liver, endometrium, cervix, fallopian tube, and peritoneum.

MIM No. 113705 {1835}

Synonyms
Breast cancer 1, early onset breast ovarian cancer syndrome.

Incidence
The prevalence of *BRCA1* mutations in most Caucasian populations is estimated to be 1 in 883 {897}. However, in certain populations, this is higher, e.g. 1% in Ashkenazi Jews {3065}. Using recombination techniques, *BRCA1* mutations have been dated to the early Roman times {1997}. De novo mutations are rare.

Diagnostic criteria
A definitive diagnosis is only possible by genetic testing. *BRCA1* mutations are common in certain populations and in families with numerous early onset breast cancer cases (≥4 cases of breast cancer at <60 years) or in those with ovarian cancer at any age in addition to early onset breast cancer. The chance of a mutation in either *BRCA1* or *BRCA2* is lower (<30%) when only two or three breast cancer cases are present in a family. The main difference between *BRCA1* and *BRCA2* is the increased risk of male breast cancer in *BRCA2*. The American Society of Clinical Oncology (ASCO) guidelines suggest offering testing at a probability of mutation of >10% but many other countries will only offer testing to those with a chance >30% because of the need to concentrate resources.

Breast tumours
Penetrance
Analyses of worldwide data submitted to the Breast Cancer Linkage Consortium (BCLC) have provided general estimates of penetrance {8}. Estimates for specific populations have shown that the Ashkenazim have a lower than average lifetime breast cancer penetrance of about 50-60% {3065}. Population based studies in UK breast cancer patients also revealed a lower penetrance and indicate that the presence of a mutation within a familial breast cancer cluster does confer a higher penetrance {2230}. This may be due to an association with other genes or epidemiological factors that are present in the family. There are also reports of variable penetrance dependent on the position of the mutation within the *BRCA1* gene {2914}.

Clinical features
Breast cancer in *BRCA1* mutation carriers occurs more often at a younger age, typically before age 40 {1687}. It tends to progress directly to invasive disease without a precancerous DCIS component {8,1574}. Accordingly, there appears to be a lower chance of early detection by mammographic screening and a higher proportion of invasive cancers {1025}. There is an almost linear increase in the lifetime risk of contralateral breast cancer from the age of 35 years, reaching a level of 64% by the age of 80 {742}.

Pathology
Certain morphological types of breast cancer, including medullary carcinoma, tubular carcinoma, lobular carcinoma in situ, and invasive lobular carcinoma, have been reported more commonly in patients with a positive family history of breast cancer {191,1566,1684,1724, 2441}.
Patients with *BRCA1* germline mutations have an excess of medullary or atypical medullary carcinoma compared to controls {8,764,1767}. Tumours in *BRCA1* mutation carriers are generally of a higher grade than their sporadic counterparts {8,764,1767}. Ductal carcinoma in situ (DCIS) adjacent to invasive cancer is observed less frequently while the frequency of lobular neoplasia in situ is similar in both groups {8}. However, in a multifactorial analysis of the BCLC database, the only features significantly associated with *BRCA1* were total mitotic count, continuous pushing margins, and lymphocytic infiltrate. All other features, including the diagnosis of medullary and atypical medullary carcinoma, were not found to be significant {1572}.
BRCA1-associated tumours are more likely to be estrogen (ER) and progesterone receptor (PgR) negative {766, 1352,1574,2121}. Data on *ERBB2* are limited but *BRCA1*-linked tumours are more likely to be negative than controls {1352,1574}. *BRCA1*-linked tumours show a higher frequency of *TP53* mutations and p53 expression than sporadic breast cancer {580,581,765,1574}. *BRCA1*-associated tumours show very low expression of Cyclin D1 in both the invasive and in situ components {2122}. The absence of Cyclin D1 in these tumours could be an additional evidence

Table 8.02
Probability of *BRCA1/2* mutation in women with breast/ovarian cancer.

Chance of mutation	Clinical criteria
<10%	Single breast cancer / ovarian cancer case at <40 years in non Ashkenazim
10-30%	2-3 female breast cancers <60 years (no ovarian / male breast cancer)
30%	One female breast cancer <60 and one ovarian cancer
	Female breast cancer <40 in Ashkenazi
>60%	Four cases of female breast cancer at <60 years
	≥2 cases female breast cancer <60 and ovarian cancer any age
	≥2 cases female breast cancer <60 and male breast cancer any age

From R.A. Eeles {749}.

of hormone independence of *BRCA1*-associated breast cancers.

Prognosis and prognostic factors
Studies on the prognosis of breast cancer associated with *BRCA1* range from poorer prognosis, to no difference, to a better prognosis {441}. There is a potential survival bias since at least one patient in each family must have survived in order to have blood taken for gene testing. The most optimal studies are therefore those which have taken this into consideration, either by discounting the proband in a family who has presented for testing {3022} or by testing specific founder mutations in archival tumour tissue material from all cases in a specific population (for example, see Foulkes et al. {904}).

Ovarian tumours

Age distribution and penetrance
About 7-10% of ovarian carcinomas are due to inherited *BRCA1* (or *BRCA2*) mutations; as these are on autosomes, they can be inherited from either the mother or the father. Although ovarian cancer can occur earlier in *BRCA1* (and indeed *BRCA2*) carriers, the presence of an older onset ovarian cancer still can indicate an underlying mutation in either of these genes. The penetrance for ovarian cancer in *BRCA1* mutation carriers is shown in Fig. 8.02; it starts to rise at an earlier age than the curve for *BRCA2*, which starts to rise at about 50 years. The penetrance is 44-60% by age 70. This is markedly higher than the lifetime risk of 1.8% (1 in 55) for sporadic ovarian cancer in women living in developed countries.

Clinical features
In a retrospective cohort study of Jewish subjects, women with advanced-stage ovarian cancer and a *BRCA1* or *BRCA2* founder mutation had a longer survival than women with non-hereditary ovarian cancer (P = 0.004) and a longer median time to recurrence (14 months versus 7 months) (P< 0.001) {329}.
BRCA1/2 heterozygotes had higher response rates to primary therapy compared with patients who had sporadic disease (P = 0.01), and those with advance-stage disease had improved survival compared with patients who had advanced stage sporadic carcinoma {422}.

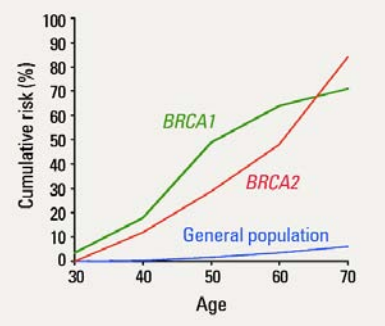

Fig. 8.01 The breast cancer penetrance of *BRCA1* and *BRCA2* from the BCLC data.

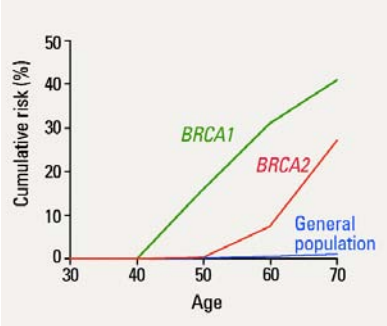

Fig. 8.02 The ovarian cancer penetrance of *BRCA1* and *BRCA2* from the BCLC data.

Pathology
In patients with *BRCA1* germline mutations, epithelial tumours (carcinomas) are the most common histological diagnosis. All subtypes of malignant epithelial ovarian neoplasms have been reported, including the very rare entity of malignant transitional cell carcinoma {3102}. Interobserver variation in typing of ovarian carcinoma is likely to account, at least in part, for the different results reported to date {572,1716,2513}.
Some studies indicate that papillary serous adenocarcinoma is the predominant ovarian cancer that occurs in familial ovarian cancer syndromes {229,2479, 2800} while others report that they occur with similar frequency in *BRCA1/2* mutation carriers and sporadic cases {329, 2239,3102}. The large majority of studies have shown mucinous carcinoma to be under-represented in *BCRA1* mutation carriers {50,229,1974,2239,2479,2800, 3102}.
The frequency of endometrioid and clear cell carcinoma occurring in *BRCA1* mutation carriers is similar to that of sporadic cases {50,229,1353,2239,2479, 2800,3102,3272}.
The current data suggest that germline mutations in *BRCA1/2* genes do not pre-

Table 8.03
Lifetime cancer risks of *BRCA1* carriers.

Cancer site	Relative risk (95% CI)	Cumulative risk by age 70, % (95% CI)
Breast	Age-dependent	87
Ovary	Age-dependent	44
Colon	4.11 (2.36-7.15) {896} 2.03 (1.45-2.85) {2915}[1]	-
Cervix	3.72 (2.26-6.10)	3.57 (3.16-4.04)
Uterus	2.65 (1.69-4.16)	2.47 (2.02-3.04)
Pancreas	2.26 (1.26-4.06)	1.2 (0.9-1.7)
Prostate	3.33 (1.78-6.20) {896} 1.82 (1.01-3.29) {2915}[2]	2.64 (1.95-3.57) (Europe) 7.67 (4.77-12.20) (North America)
All cancers[3] – male	0.95 (0.81-1.12)	16.89 (14.52-19.81)
All cancers[3] – female	2.30 (1.93-2.75)	23.27 (21.73-24.89)

From D. Ford et al. {896} and D. Thompson et al. {2915}.
[1] When considered together with rectal cancer, the relative risk was no longer significantly elevated above 1.0; no excess risk was noted among men.
[2] For men under the age of 65.
[3] All cancers other than nonmelanoma skin cancer, breast cancer, or ovarian cancer.

dispose individuals to the development of borderline neoplasms {1044,1704}. However, occasional invasive {2479, 3272} and borderline {50} mucinous neoplasms have been reported.
Stromal tumours and malignant germ cell ovarian neoplasms appear not to be associated with *BRCA1/2* germline mutations. However, several families in which more than one relative had been diagnosed with a malignant ovarian germ cell tumour have been published {2790}. Single cases of dysgerminoma {3103} and transitional cell ovarian carcinoma {3101} have been observed in *BRCA1* carriers with a family history of breast and ovarian cancer. The development of these lesions may be unrelated to the germline *BRCA1* mutations in these cases.
The first report on *BRCA1*-associated ovarian carcinoma found that overall the tumours were of higher grade and higher stage than their historic age-matched controls {2479}. These findings have been largely reproduced by a number of other groups {50,229,2239,3102,3272}. In contrast, Berchuck et al. {229} found that although the *BRCA1* cases in their study were all of advanced stage (III/IV), they were half as likely to be as poorly differentiated as cases without mutations. Johannsson et al. {1353} did not identify a difference in grade between the ovarian cancers in their *BRCA1* mutation carriers and the control population-based cancer registry group.

Prognosis and prognostic factors
The majority of *BRCA1* ovarian cancers are serous cystadenocarcinomas which have a poor prognosis generally if diagnosed when they have spread outside the ovary. Studies of ovarian cancer occurring in *BRCA1* carriers have reported a somewhat better prognosis {213}, but it is uncertain whether this is because of the bias in carrier detection in this population or whether they are more sensitive to treatment. If the latter were true, this would refer to platinum treatments as these data have been reported prior to the use of taxanes.

Tumours of the fallopian tube

Definition
Hereditary fallopian tube carcinoma arises from epithelium overlying the lamina propria of the endosalpinx in women at high hereditary risk to develop ovarian carcinoma, typically due to loss of the wild-type allele of *BRCA1* or *BRCA2*. The tumour has to fulfill the clinical and histological criteria for tubal carcinoma {1256} as well as clinical genetic criteria shown in Table 8.02.

Incidence
From 1997 to 2002, a total of 15 hereditary breast/ovarian family related tubal tumours have been reported in literature. In 8 cases a *BRCA1* mutation was detected. However, the true incidence of both hereditary and sporadic tubal carcinoma is probably much higher. This is caused by the fact that primary tubal tumours are often mistaken for primary ovarian carcinomas {3150}. Moreover, some primary ovarian carcinomas might actually derive from inclusion cysts lined by tubal epithelial cells included into the ovarian stroma {2247}.

Age distribution
In general the age of onset is younger in hereditary cases when compared to sporadic cases.

Diagnostic criteria
The criteria of Hu et. al. {1256} as modified by Sedlis {2614} and Yoonessi {3185} are applied to differentiate hereditary tubal carcinomas from ovarian- and endometrial-carcinoma. These criteria require that: a) the main tumour is in the fallopian tube and arises from the endosalpinx, b) the histological features resemble a tubal pattern, c) if the tubal wall is involved, the transition between malignant and benign tubal epithelium should be detectable, d) the fallopian tube contains more tumour than the ovary or endometrium.

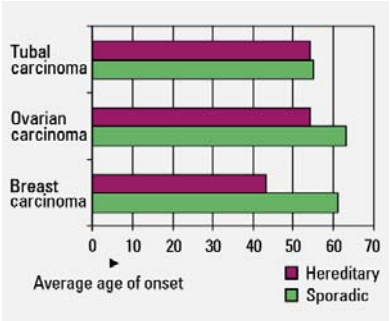

Fig. 8.03 Average age of onset of *BRCA1* and *BRCA2* related carcinomas.

Table 8.04
BRCA1 mutation status in relation to histopathology of 200 malignant ovarian epithelial tumours.

Histologic type	*BRCA1*-negative families	*BRCA1*-positive families
Serous	80 (59%)	44 (67%)
Mucinous	12 (9%)*	0 (0%)*
Endometrioid	10 (7%)	5 (8%)
Clear cell	13 (10%)	3 (4%)
Undifferentiated	10 (7%)	9 (14%)
MMMT[a]	3 (2%)	1 (2%)
Transitional cell	2 (2%)	1 (2%)
Mixed[b]	5 (4%)	2 (3%)
Total	135 (100%)	65 (100%)

Excludes borderline tumours.
Adapted from B.A. Werness et al. {3102}.
[a] Malignant müllerian mixed tumour.
[b] > 10% minor histologic type.
* P = 0.01 for the difference in prevalence between *BRCA1*-positive and *BRCA1*-negative families.

Clinical features
Symptoms and signs. To date, there is no indication that clinical hereditary tubal carcinoma features are different from those of its sporadic counterpart. In addition to occasional abdominal discomfort, the classical triad of symptoms include: (i) prominent watery vaginal discharge, (ii) pelvic pain and (iii) a pelvic mass {158}. Cervical cytology reveals adenocarcinomatous cells in approximately 10% of patients {3185}.

Tumour marker. As in ovarian carcinoma, elevation of serum CA125 levels are found in approximately 80% of cases {1173}.

Imaging. CT/MRI are inconclusive with respect to the differential diagnosis of tubal or ovarian carcinomas. However, these techniques can be helpful in determining the extent of disease. Likewise, ultrasonography can not distinguish tubal from ovarian disease {2720}.

Histopathology and grading
Serous papillary carcinoma is the most common form of hereditary tubal carcinoma.
Grading is of limited value in these tumours and, if used, is based on the papillary architecture, nuclear atypia and mitotic activity. Grade I cancers show papillary growth with well differentiated columnar cells and low mitotic rate. Grade II cancers are papillary with evi-

dent gland formation with intermediately differentiated cells with moderate mitotic activity. Grade III shows solid growth with loss of papillae and a medullary/glandular pattern. The cells are poorly differentiated and the mitotic activity is high.

Immunohistochemistry. Being predominantly of serous papillary type, hereditary tubal carcinomas are positive for cytokeratins 7 and 8, MUC1, CEA, OVTL3, OV632, CA125, and negative or showing only low expression for cytokeratin 20, CEA and vimentin. Also, p53 is often expressed, and cyclins E and A and Ki67 show a varying number of proliferating cells, whereas staining for ERBB2 and cyclin D1 is usually negative. Steroid receptor content varies. In the rare clear cell cancers, p21 is highly expressed.

Seeding and metastasis
Hereditary tubal carcinomas presumably spread like their sporadic counterparts. Empirical data are available to date point to a mode of spread similar to ovarian cancer.

Prognosis
The five-year survival rate of 30% in sporadic cases varies with stage {158,3185}, but not with grade. The survival rate of hereditary tubal carcinomas has yet to be established since only small numbers of patients have been reported and most patients have still not completed their 5-year follow-up.

Other tumours
BRCA1 predominantly predisposes to female breast cancer and ovarian cancer. Unlike *BRCA2*, it is not thought to predispose to male breast cancer. A few families with male breast cancer and a *BRCA1* mutation have been described, but these may be within the numbers expected by chance. A study of causes of mortality by Ford et al. {896} reported an increased risk of colon cancer and prostate cancer. However, a reanalysis {2914} has shown a small pancreatic cancer excess, as is seen in *BRCA2* carriers and an excess of prostate cancer risk only at age <60 years. The excess of colonic cancer was counteracted by a deficit of rectal cancer. See Table 8.03 for details on risk estimates.

Genetics
Chromosomal location and gene structure
The *BRCA1* gene is located on chromosome 17q21 {1109}. The 24 exons of the *BRCA1* gene (22 coding exons; alternative 5'UTR exons, 1a & 1b) span an 81-kb chromosomal region, that has an unusually high density of Alu repetitive DNA (41.5%) {1864,2735}. A partial pseudogene (*BRCA1*Ψ) consisting of a tandem duplication of exons 1a, 1b and 2 lies 44.5kb upstream of *BRCA1* {356,2303}. Exon 11 of *BRCA1* (3.4 kb) encodes 61% of the 1863 amino acid protein. The amino-terminal RING finger domain and the carboxy-terminal BRCT repeats {316} of BRCA1 are highly conserved among vertebrates {2825}, while the rest of the protein bears little homology to other known genes.

Gene expression
Several alternatively spliced transcripts have been described for the *BRCA1* gene, the most prevalent of these lead to in-frame deletions of exon 11 (*BRCA1*-Δ11). Both full length and Δ11 transcripts are ubiquitously expressed. The 100- and 97-kDa Δ11 protein isoforms lack the nuclear localization signal and are cytoplasmic {1864,2904}. However, the full-length 220-kDa protein is predominantly observed in the nucleus. Its expression and phosphorylation is cell-cycle dependent, commencing in G1 and reaching maximal levels by early S-phase. BRCA1 colocalizes with the BRCA2 and Rad51 proteins in discrete foci during S-phase. DNA damage leads to hyperphosphorylation of BRCA1, dispersal of the BRCA1/BRCA2/Rad51 nuclear foci, and their relocalization to PCNA-containing DNA replication structures. In meiotic cells, BRCA1, BRCA2 and Rad51 colocalize on the axial elements of developing synaptonemal complexes {450,2594,2596}. A large protein complex consisting of other tumour suppressor and DNA repair proteins, known as BASC (BRCA1-associated genome surveillance complex) has been identified. Among these, partial colocalization of BRCA1 with Rad50, MRE11 and BLM in nuclear foci analogous to those observed with BRCA2 and Rad51 has been demonstrated {3054}. In addition to its interactions with BRCA2, Rad51, and BASC, the BRCA1 protein has been shown to form complexes with a number of other proteins involved in diverse cellular functions, including DNA repair, transcription, chromatin remodeling, and protein ubiquination (reviewed in {3018}). During mouse embryonic development, *Brca1* exhibits a dynamic expression

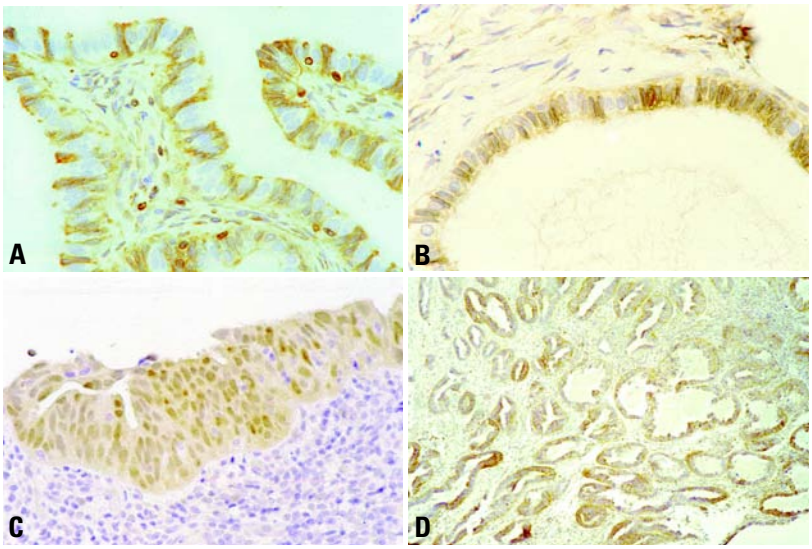

Fig. 8.04 **A** Normal endosalpinx, stained for bcl-2, which is a differentiation marker of serous tubal cells. **B** Tubal cell-lined inclusion cyst in the ovary stained for bcl-2. **C** Dysplastic lesion in a fallopian tube of a *BRCA1* mutation carrier, stained for p53. **D** Serous adenocarcinoma of the fallopian tube stained for bcl-2 (note: not all serous carcinomas are bcl-positive).

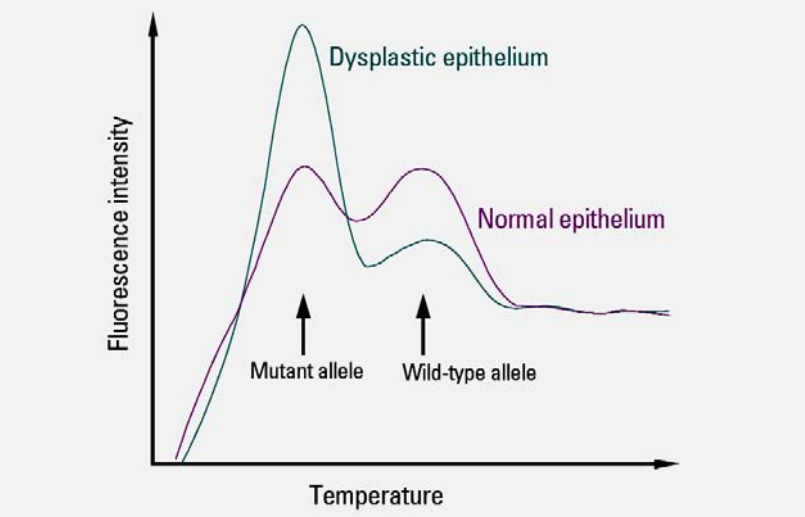

Fig. 8.05 To assess whether wild-type and/or mutated *BRCA1* alleles are lost in dysplastic tubal epithelium of a *BRCA1* mutation carrier, light-cycler polymerase chain reaction (PCR) melting curve analysis is performed. This technique utilizes the properties of probes to anneal less stringent to mutated DNA than to wild-type DNA, resulting in a lower denaturation temperature for mutated DNA. Two peaks, indicating different denaturing temperatures, are detected in non-dysplastic epithelium, indicating the presence of both wild-type and mutated *BRCA1* DNA. One clear peak at the melting temperature for the mutated *BRCA1* DNA in the dysplastic epithelium indicates loss of wild-type *BRCA1* DNA. From J.M. Piek et al. {2246}.

pattern, which parallels *Brca2* expression in that the highest expression levels occur in epithelial tissues undergoing concurrent proliferation and differentiation. In adult mice, *Brca1* and *Brca2* expression is induced during mammary gland ductal proliferation, morphogenesis and differentiation occuring at puberty and again during proliferation of the mammary epithelium during pregnancy {1582,1769,2323}.

Consistent with its role as a tumour-suppressor gene, the wild-type allele of *BRCA1* is lost in the majority of tumours of individuals with inherited mutations, presumably leading to absence of normal protein {560}. In sporadic cancer, BRCA1 protein expression is absent or reduced in the majority of high grade breast carcinomas and sporadic ovarian tumours {2493,3130}. Although few somatic mutations in the *BRCA1* coding sequence have been identified {1846}, somatic inactivation of protein expression may occur through several mechanisms, including gross chromosomal rearrangements – approximately 50% of primary breast tumours show loss of heterozygosity of chromosome 17q21 {559, 1134}, or epigenetic inactivation of expression, such as promoter hypermethylation {426}.

Gene function

The BRCT domain of BRCA1 is a protein-protein interaction module found in proteins involved in DNA repair and cell cycle control {316}. The RING domain mediates the interaction with BARD1 and the dimer displays ubiquitin ligase (E3) activity {159}. The physiologic substrates of this activity remain unknown although the Fanconi anaemia D2 protein is a likely candidate {958}. The integrity of the RING and BRCT domains is indispensable for the functions of BRCA1 as demonstrated by the presence of cancer-associated mutations in these regions.

A number of different mutations have been introduced into mouse *Brca1*, all resulting in embryos with γ-irradiation hypersensitivity and genetic instability. Mice with a conditional mutation of *Brca1* in the mammary gland developed tumorigenesis associated with genetic instability, providing an important link to human disease {3167}. Interestingly, mouse cells lacking Brca1 are deficient in repair of chromosomal double-strand breaks (DSB) by homologous recombination {1931}. Taken together, these results suggest a role for BRCA1 in the DNA damage response.

Expression of wild type but not disease-associated *BRCA1* alleles in *BRCA1*-deficient human cells restores resistance to DNA-damaging agents {2595} and several BRCA1-containing complexes involved in DNA repair have been identified. These include S-phase nuclear foci containing BRCA2 and Rad51 {450}, the hRad50-hMre11-NBS1^{p95} (R/M/N) complex, involved in a wide variety of DNA repair processes {3258}, and the BASC complex which contains ATM, the BLM helicase, mismatch repair proteins MSH2, MSH6, MLH1 and the R/M/N complex {3054}. DNA damaging agents induce BRCA1 hyperphosphorylation, which is likely to modulate the association of BRCA1 with these different protein complexes {2597}. These biochemical approaches corroborate the notion that BRCA1 participates in the cellular response to promote DNA break recognition and repair, as shown in Fig. 8.08.

The involvement of BRCA1 in a variety of DNA repair processes suggests that it may be an upstream effector common to various responses to DNA damage {3018}. In line with the idea of *BRCA1*'s pleiotropic role, it also acts as a negative regulator of cell growth. Ectopic expression of BRCA1 causes cell cycle arrest at G1 via the induction of the cdk inhibitor p21$^{Waf1/CiP1}$ {2745}. Conversely, inhibition of *BRCA1* expression with antisense oligonucleotides results in the accelerated growth of mammary epithelial cell lines {2917}. Also, BRCA1 seems to be required for efficient radiation-induced G2/M and S-phase checkpoints pointing to a broad involvement of BRCA1 in checkpoint control {3166,3178}.

Several lines of evidence suggest that one of the molecular functions of BRCA1 is the regulation of transcription. The BRCA1 C-terminus acts as a transactivation domain and germline mutations found in *BRCA1* abolish this activity {1899}. BRCA1 can be copurified with RNA polymerase II and upon replication blockage, a novel complex containing BRCA1 and BARD1 is formed, suggesting that BRCA1 protein redistributes to different complexes in response to replication stress {476,2593}. BRCA1 also associates and, in some cases, modulates the activity of several proteins involved in the regulation of gene expression such as transcription factors, coactivators, corepressors and chromatin remodeling complexes {297,1247, 1899,3255}. A recent exciting development, of yet unknown physiologic signifi-

cance, was the discovery of direct DNA binding by BRCA1 in vitro which may be important for its function in transcription and DNA repair {2198}.
Putative BRCA1 transcriptional target genes identified so far play a role in some aspect of the DNA damage response. BRCA1 induces the transactivation of $p21^{WAF1/CiP1}$ in p53-dependent and independent manners, insuring a potent cell cycle arrest, reinforcing the connection between cell cycle checkpoint control and transcription regulation {2130,2745}. Experiments using cDNA arrays identified the DNA-damage-responsive gene *GADD45* as a major target of BRCA1-mediated transcription {1138,1727}. These results, coupled with studies showing that disruption of p53 partially rescues embryonic lethality in *Brca1*$^{-/-}$ mouse, link the p53 pathway and BRCA1 function {1108,1710}. Importantly, the majority of tumours derived from *BRCA1*-linked patients or from *Brca1*$^{-/-}$ mice present mutations in p53 {581,3167}.

Mutation spectrum

Germline mutations in *BRCA1* have been detected in 15-20% of clinic-based breast cancer families, and in 40-50% of breast-ovarian cancer families {2657, 3023}. Mutations occur throughout the entire coding region, and hence the mutation spectrum has taught us relatively little about the gene's function. The majority of the mutations are predicted to lead to a prematurely truncated protein when translated. In conjunction with the observed loss of the wildtype allele in tumours arising in mutation carriers {560}, this indicates that inactivation of the gene is an important step in tumorigenesis. Despite the strong variability in mutations detected in families, founder effects have led to some mutations being very prevalent in certain populations of defined geographical or ethnic background. An example is the 185delAG mutation, which is present in approximately 1% of all individuals of Ashkenazi Jewish descent {1151}. As a result, mutation spectra may vary according to ethnic background of the sampled population {2824}. In some populations, specific large interstitial deletions or insertions, which are difficult to detect by conventional PCR-based mutation scanning technologies, have been observed to be particularly frequent. They may comprise between 10 and 20% of the total mutation spectrum {944,1229}.
In recent years, an increasing number of missense changes are being detected in *BRCA1*, of which the clinical significance is uncertain. These already comprise up to 40% of all known sequence changes in *BRCA1*. The Breast Cancer Information Core (BIC) maintains a website providing a central repository for information regarding mutations and polymorphisms {http://research.nhgri.nih.gov/bic/}.

Genotype-phenotype correlations

Initially, the breast and ovarian cancer cancer risks conferred by mutations in *BRCA1* were estimated from *BRCA1*-linked, multiple-case families (see Figs. 8.01 and 8.02) {896,898}. More recently, estimates from specific populations have come up with lower estimates {106,3065}. This could point to 1) the existence of mutation-specific risks (because different populations have different mutation spectra, the overall cancer risks would differ), 2) the existence of genetic variants in other genes, particularly prevalent in certain populations, which might modify the *BRCA1*-related cancer risks, 3) population-specific differences in environmental risk modifiers.

BRCA1 mutation position

One report observed a significant correlation between the location of the mutation in the gene and the ratio of breast to ovarian cancer incidence within each family {974}, suggesting a transition in risk such that mutations in the 3' third of the gene were associated with a lower proportion of ovarian cancer. It wasn't clear, however, whether this was due to higher breast cancer risks, or lower ovarian cancer risks. A much larger study of 356 *BRCA1*-linked families {2914} found the breast cancer risk associated with mutations in the central region to be significantly lower than for other mutations (relative risk, 0.71), and the ovarian cancer risk associated with mutations 3' to nucleotide 4191 to be significantly reduced relative to the rest of the gene (relative risk, 0.81). Recent work suggests that the risk to ovarian cancer might also be influenced by genetic variation in the wildtype *BRCA1* copy in *BRCA1* carriers {1009}.

Genetic risk modifiers

One study showed that the risk for ovarian cancer was 2.11 times greater for *BRCA1* carriers harbouring one or two rare *HRAS1* alleles, compared to carriers with only common alleles (P = 0.015). Susceptibility to breast cancer did not appear to be affected by the presence of rare *HRAS1* alleles {2240}. Likewise, a length-variation of the polyglutamine repeats in the estrogen receptor co-activator *NCOA3* and the androgen receptor influences breast cancer risk in carriers of *BRCA1* and *BRCA2* {2342,2345}. The variant progesterone receptor allele named PROGINS was associated with an odds ratio of 2.4 for ovarian cancer among 214 *BRCA1/2* carriers with no past exposure to oral contraceptives, compared to women without ovarian cancer and with no PROGINS allele {2487}.
These results support the hypothesis that pathways involving endocrine signalling may have a substantial effect on *BRCA1/2*-associated cancer risk. Genetic variation in the genes constituting the DNA repair pathways might also be involved. A C/G polymorphism in the 5' untranslated region of *RAD51* was found to modify both breast and ovarian

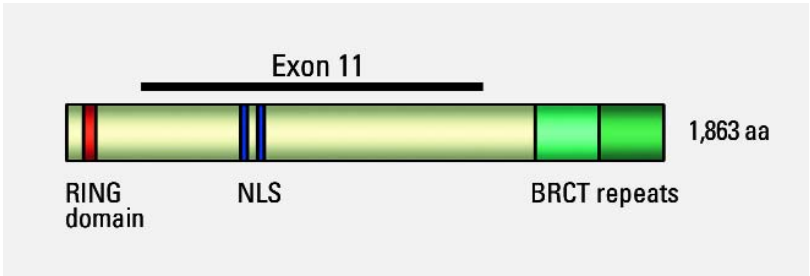

Fig. 8.06 Functional domains in BRCA1. The RING domain contains a C3HC4 motif that interacts with other proteins. NLS = nuclear localization signal. BRCT = BRCA1-related C-terminal. The proportion encoded by exon 11 is indicated.

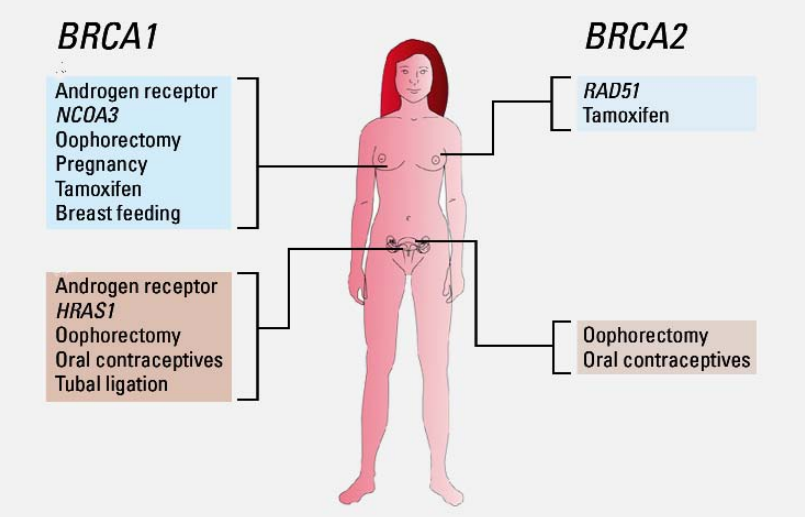

Fig. 8.07 Factors that modify risk of breast or ovarian cancer. Most of these proposed factors are based on results of a single study and require confirmation. From S.A. Narod {1975}.

cancer risk, initially only in carriers of *BRCA2* {1328,1644,3053}.

Hormonal factors as risk modifiers

Oral contraceptives

Because of the observed protective effects of oophorectomy and tamoxifen, it is of concern that supplemental estrogen, in the form of oral contraceptives or hormone replacement therapy, may increase the risk of breast cancer. In the Oxford overview analysis, current use of birth control pills was associated with a relative risk of 1.2 {539}. However, in a recent large American case-control study, no adverse effect was noted {2607}. In a large international case-control study of oral contraceptives and hereditary breast cancer {1977} a mild increase in risk was seen among *BRCA1* carriers (relative risk 1.2) but not among *BRCA2* carriers (relative risk 0.89). The overall result was not significant, but risk increases were found for women who first took a contraceptive before age 30, for women who developed breast cancer before age 40, for women with five or more years of pill use, and for women who first took an oral contraceptive prior to 1975. It appears that short-term use of modern contraceptives poses no increase in risk, but further studies are needed in this regard. No studies have been conducted yet regarding whether or not HRT increases the risk of breast cancer in *BRCA1/2* mutation carriers.

It is important to establish whether oral contraceptives are hazardous to the breast, because their use has been proposed as a preventive measure against ovarian cancer. A protective effect of oral contraceptives on ovarian cancer risk has been observed in three case-control studies of *BRCA1/2* mutation carriers {1976,1979,1980} but there has been one conflicting report {1886}. In a recent study of 232 ovarian cancer cases and 232 controls, oral contraceptive use was associated with a 56% reduction in the risk of ovarian cancer ($p = 0.002$) {1976}. Tubal ligation has been found to be protective against ovarian cancer in the general population {1126} and among *BRCA1* mutation carriers {1980}. An adjusted relative risk of 0.39 was reported for tubal ligation and subsequent ovarian cancer (a risk reduction of 61%). The mechanism of risk reduction is unclear.

Pregnancy

Hormonal levels rise dramatically during pregnancy and two groups found pregnancy to be a risk factor for early breast cancer in *BRCA1/2* mutation carriers. Johannsson et al. reported ten pregnancy-related breast cancers in 37 *BRCA1/2* mutation carriers, versus the expected 3.7 {1351}. Jernstrom et al. reported that the risk of breast cancer increased with each pregnancy in *BRCA1/2* carriers before the age of 40 {1348}. This was found for *BRCA1* and *BRCA2* mutation carriers, but was only significant for the former group. In the general population, pregnancy offers protection against breast cancer after the age of 40, but appears to increase the risk for very early-onset breast cancer {227}. This is consistent with the hypothesis that the ovarian hormones produced during pregnancy are mitogenic, and accelerate the growth of existing tumours. During pregnancy breast differentiation occurs and thereafter the population of susceptible cells is reduced. This may explain why pregnancy prevents breast cancers at a later age. In the general population, only a small proportion of breast cancers occur before age 40, and pregnancy confers an overall advantage. Early-onset breast cancers are typical among *BRCA1* mutation carriers, however, and a high proportion of cancers occur before age 40. A case-control study of breast-feeding and breast cancer in *BRCA1/2* mutation carriers reported a protective effect in women with *BRCA1* mutations, but not with *BRCA2* mutations {1347}. *BRCA1* mutation carriers who breast-fed for more than one year were 40% less likely to have breast cancer than those who breast-fed for a shorter period ($p = 0.01$). The observed protective effect among *BRCA1* carriers was greater than that observed for members of the general population {224}.

Prognosis and preventive options

The overall life expectancy of unaffected women with a *BRCA1* or *BRCA2* mutation clearly is decreased due to their high risk of developing breast cancer and ovarian cancer, in particular at young ages. The overall mortality from breast and ovarian cancer within 10 years of diagnosis of cancer is still significant, 40% and 60% respectively.

Currently the following avenues are being explored to improve the prognosis of women with a *BRCA1* or *BRCA2* mutation, all aiming for either early detection or prevention of breast cancer and/or ovarian cancer: i) regular surveillance, ii) prophylactic surgery, and iii) chemoprevention.

Preventive surveillance

No evidence exists that regular breast surveillance using mammography leads to earlier detection of cancers in mutation carriers {1442}. Preliminary results on

breast surveillance using MRI suggest that there is an increased frequency of early detection of tumours, but definite conclusions cannot yet be made {1875, 2835}. Also, no evidence exists that regular ovarian surveillance detects ovarian cancer at curable stages.

Prophylactic surgery

Prophylactic bilateral mastectomy lowers the risk of breast cancer in mutation carriers by more than 90%, also on the long-term {178,1407}. Prophylactic bilateral salpingo-oophorectomy prevents ovarian cancer, though a minimum long-term risk of 4% of peritoneal cancer remains after this procedure {2344}.

The incidence of breast cancer in *BRCA1* carriers is maximal in the age group 40 to 55 and then declines slightly thereafter {1978}. This observation suggests that ovarian hormones may have a promoting role in breast carcinogenesis. In support of this, oophorectomy has been found to be protective against breast cancer in *BRCA1/2* mutation carriers in several studies {1976,2504}. Rebbeck et al. compared the breast cancer risk in a historical cohort of *BRCA1* mutation carriers, some of whom had undergone an oophorectomy and some of whom had both ovaries intact {2343,2344}. The estimated relative risk of breast cancer associated with oophorectomy was approximately one-half. This was confirmed in a case-control study {763} and in a prospective follow-up study of 170 women {1413}. Among *BRCA1* mutation carriers; the risk of breast cancer among women who had an oophorectomy was decreased by 61% (odds ratio 0.39; 95% CI 0.20 to 0.75). These studies suggest that oophorectomy might be used as a strategy to decrease the risk of breast cancer among *BRCA1* mutation carriers. However, in young women the procedure is associated with acute and long-term side effects.

Members of a *BRCA1*-linked family are at risk also to develop tubal carcinoma {3271}. Piek et al. studied prophylactically removed fallopian tubes of 12 women with a predisposition for ovarian cancer, in 7 of whom a *BRCA1* mutation was detected {2246}. Six showed dysplasia, including one case of severe dysplasia. Five harboured hyperplastic lesions, and in one woman no histological aberrations were found. Therefore, it is recommended to perform a complete adnexectomy in women harbouring a *BRCA1* mutation. Whether an abdominal hysterectomy should be performed to dissect the intrauterine part of the tube, is still in debate. However, most studies indicate that tubal carcinomas in fact predominantly arise in distal parts of the tube.

The interest of women with a *BRCA1* or *BRCA2* mutation in the various options differs greatly between countries {1425}, and may also change over time when the efficacy of surveillance, chemoprevention, or treatment improves. However, at present in some countries up to 50-60% of unaffected women chose to have prophylactic bilateral mastectomy, and 65% prophylactic bilateral salpingo-oophorectomy {1012,2285}.

Chemoprevention

Tamoxifen is an anti-estrogenic drug that is routinely used in the treatment of estrogen-receptor positive breast cancer that has also been demonstrated to be of value in reducing the risk of primary invasive and pre-malignant breast cancer in high risk women {865,1464,1976} and of contralateral breast cancer in unselected women {10}. Narod, et al. {1976} studied tamoxifen and contralateral breast cancer in a case-control study of *BRCA1* and *BRCA2* mutation carriers. Tamoxifen use was equivalent to a 62% risk reduction in *BRCA1* carriers. A reduction in risk of contralateral cancer was also seen with oophorectomy and chemotherapy. This result implies that the combination of tamoxifen and oophorectomy may be more effective than either treatment alone, and that the two prevention strategies may be complementary. Until more definitive guidlines are established, the interest in participation in chemoprevention trials is likely to remain small {2285}.

Table 8.05
Effects of modifying factors on breast and ovarian cancer risk.

	Breast cancer		Ovarian cancer	
	BRCA1	***BRCA2***	***BRCA1***	***BRCA2***
Genetic factors				
Androgen receptor	↓↑	?	↓↑	?
NCOA3	↑	?	?	?
RAD51	–	↑	?	?
HRAS1	?	?	↑	?
Lifestyle factors				
Oophorectomy	↓	↓?	↓	↓
Mastectomy	↓	↓	–	–
Tubal ligation	–	–	↓	↓?
Pregnancy*	↑	↑?	?	?
Breastfeeding	↓	↑?	?	?
Oral contraceptives	↑?	↑?	↓	↓
Tamoxifen	↓	↓	–	–
Hormone-replacement therapy	?	?	?	?

↑? = suggested increase in cancer risk, but uncertain
↓? = suggested decrease in cancer risk, but uncertain
↑ = significant increase in cancer risk
↓ = significant decrease in cancer risk
? = not studied
– = no modifying effect seen

From S.A. Narod {1975}.
* The pregnancy effect was seen for early-onset (40 years) breast cancer only.

BRCA2 syndrome

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Definition

Inherited tumour syndrome with autosomal dominant trait and markedly increased susceptibility to early onset breast cancer and an additional risk for the development of male breast cancer and, less frequently, pancreatic and ovarian cancer. Occasionally, carriers of a *BRCA2* germline mutation present with skin melanoma, gall bladder and bile duct tumours, and cancer of the fallopian tube.

MIM No. 600185 {1835}

Synonyms

Site specific early onset breast cancer syndrome, breast cancer 2, FANCD1.

Incidence

The *BRCA2* syndrome is generally uncommon (about 1 in 1000 individuals), but in certain populations, it is more prevalent. For example, a specific mutation (6174delT) is present in 1.5% of the Ashkenazim and another (999del5) in 0.6% of Icelanders, due to founder effects {2382,2921}.

Diagnostic criteria

BRCA2 mutations are more often present in families with multiple female breast cancer (>4 cases of early onset at <60 years) and male breast cancer. The risk of ovarian cancer is lower than in *BRCA1* families. The definitive diagnosis relies on the identification of a *BRCA2* germline mutation.

Breast tumours

Penetrance and age distribution

Analyses of the worldwide data submitted to the Breast Cancer Linkage Consortium (BCLC) studies have been used to provide general estimates of penetrance (see Fig. 8.01) {8}. Population based studies of mutations in breast cancer patients from the UK have shown a lower penetrance than the BCLC, indicating that the presence of a mutation within a familial breast cancer cluster does confer a higher penetrance {2230}. This may be due to association with other genes or exposure and lifestyle factors that are present in the family. Specific estimates for different populations have shown that the Ashkenazim have a somewhat lower lifetime breast cancer penetrance of about 50-60% {3065}. There are also reports of variable penetrance, dependent upon mutation position {2914}. There is an increased risk of contralateral breast cancer of about 56% lifetime after a diagnosis of a first breast primary. Breast cancer in *BRCA2* carriers occurs more often at younger ages than in the general population, but at older ages than in *BRCA1* carriers.

Pathology

Although lobular and tubulo-lobular carcinoma has been reported to be associated with *BRCA2* germline mutation in one study {1767}, this has not been confirmed in a larger study and no specific histological type is thought to be associated with *BRCA2* {8,1572}. In a multifactorial analysis, the only factors found to be significant for *BRCA2* were tubule score, fewer mitoses and continuous pushing margins. All other features were not found to be significant {1572}. *BRCA2* tumours are overall higher grade than sporadic cancers {8,43,1767}. Ductal carcinoma in situ (DCIS) is observed less frequently in *BRCA1* cases than in controls, but this is not the case for *BRCA2*. Lobular carcinoma in situ shows no difference between the groups {8}.

Invasive lobular carcinoma clearly does have a familial association and a trend has been identified in familial breast cancer not linked to *BRCA1* or *BRCA2* (i.e. BRCAX) {1571}.

BRCA2 tumours are similar to sporadic cancers in steroid receptor (ER, PgR) expression {766,1574,2121}. Data on ERBB2 are limited but *BRCA1* and *BRCA2* tumours are more likely to be negative than controls {1574}. *BRCA2* tumours do not show a higher frequency

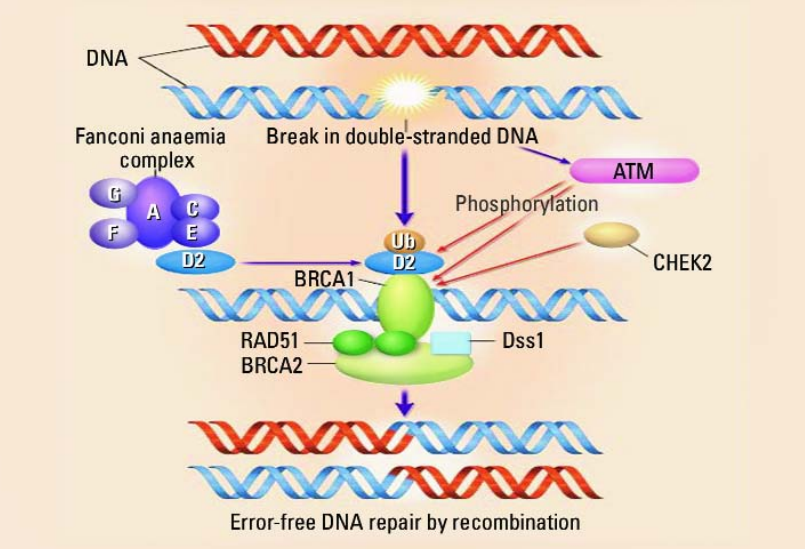

Fig. 8.08 Several genes (*ATM, CHEK2, BRCA1* and *BRCA2*) whose inactivation predisposes people to breast and other cancers participate in the error-free repair of breaks in double-stranded DNA by homologous recombination. Genes for another chromosome instability disorder named Fanconi anaemia have been connected to this DNA repair pathway. Ub denotes mono-ubiquitin. From A.R. Venkitaraman {3019}.

of *TP53* mutation and p53 expression compared to sporadic breast cancer {580,581,1574}.

Prognosis and prognostic factors
Since the breast cancers associated with *BRCA2* mutations are more often estrogen receptor positive and are associated with DCIS, they would be expected to have a better prognosis. The most systematic study to investigate prognosis has analysed the survival of Ashkenazi women with breast cancer who have mutations as tested from paraffin-stored tissue. This is possible because they have a single 6174delT founder mutation. There was no difference in survival between carriers and non-carriers {441}.

Risk modifiers and prevention
The preventive effect of oophorectomy and tamoxifen, mastectomy, and the possible hazard associated with oral contraceptives are similar in both BRCA syndromes have been dealt with in the preceding section on *BRCA1*.

Ovarian tumours
Penetrance and age distribution
About 7-10% of ovarian carcinomas are due to inherited *BRCA1* or *BRCA2* mutations; as these are on autosomes, they can be inherited from either the mother or the father. Although ovarian cancer can occur earlier in *BRCA1* and indeed *BRCA2* carriers, the presence of an older onset ovarian cancer still can indicate an underlying mutation in either of these genes. The penetrance of ovarian cancer in *BRCA2* carriers is shown in Fig. 8.02; the risk of developing ovarian cancer by age 70 in BRCA2 families is approximately 27% {898}. It should be noted that the penetrance curve starts to rise later than for *BRCA1* which could have implications for the timing of prophylactic oophorectomy.

Pathology
Compared with the information on the pathology of *BRCA1*-associated ovarian cancers, little is reported on *BRCA2* mutation-related ovarian tumours. The paucity of information is accounted for by the low incidence of this disease compared with that of *BRCA1*-linked cases {329,973}. Some recent studies indicate that the histological phenotype of these ovarian neoplasms is similar to that of *BRCA1*-associated carcinomas and are predominantly of papillary serous type {329,2239,3272}. A single case of an ovarian malignant mixed müllerian tumour (carcinosarcoma), has been reported as occurring in a *BCRA2* mutation carrier {2748}.
The data on grade are similar to those of *BRCA1* ovarian cancers with an association with higher grade but limited numbers in study and interobserver variation {329,2239,2479,3102,3272} in the scoring of grade should be taken into account when considering the evidence There are no data to support a role of *BRCA2* in borderline ovarian lesions {1044,1704} nor are there germ cell or sex cord stromal tumours.

Prevention by oral contraceptives
Although it has been long known that oral contraceptives can decrease the risk of developing ovarian cancer in the general population {2}, recently there is evidence that this may also be true for hereditary ovarian cancer {1976,1979,1980}. See the preceding section on *BRCA1* syndrome for further details.

Prognosis and prognostic factors
In a retrospective cohort study, women with *BRCA1* or *BRCA2* founder mutation advanced-stage ovarian cancer had a longer survival compared with women with non-hereditary ovarian cancer (P = 0.004) and a longer median time to recurrence (14 months versus 7 months) (P< 0.001) {329}.
Studies of ovarian cancer occurring in *BRCA2* carriers have reported a better prognosis {329}, but it is uncertain whether this is because of the bias in carrier detection in this population or whether they are more sensitive to treatment. If the latter is true, this would be platinum treatments as these data are prior to the use of taxanes.

Tumours of the fallopian tube
Hereditary fallopian tube carcinoma arises from epithelium overlying the lamina propria of the endosalpinx in women at high hereditary risk to develop ovarian carcinoma. Loss of the wild-type breast cancer 1 or 2 gene (*BRCA1/2*) allele is most likely pivotal in carcinogenesis of these tumours. To be unequivocally identified, the tumour has to fulfill the clinical and histological criteria for tubal carcinoma {1256} as well as clinical genetic criteria.

Incidence
From 1997 to 2002, a total of 15 hereditary breast/ovarian family related tubal tumours have been reported in literature. In 4 cases, a *BRCA2* mutation was detected. However, the true incidence of hereditary tubal carcinoma is probably much higher, as is suggested for its sporadic counterpart. This is caused by the fact that primary tubal tumours are often mistaken for primary ovarian carcinomas {3150}. Moreover, some primary ovarian carcinomas might actually derive from inclusion cysts lined by tubal epithelial cells included into the ovarian stroma {2247}.

Age distribution
In general the age of onset is younger in hereditary cases when compared to sporadic cases.

Diagnostic criteria
The criteria of Hu et. al. {1256} as modified by Sedlis {2614} and Yoonessi {3185} are applied to differentiate hereditary tubal carcinomas from ovarian and endometrial carcinoma. These criteria require that: (i) the main tumour is in the fallopian tube and arises from the endosalpinx, (ii) the histological features resemble a tubal pattern, (iii) if the tubal wall is involved, the transition between malignant and benign tubal epithelium should be detectable, (iv) the fallopian tube contains more tumour than the ovary or endometrium.

Clinical features

Symptoms and signs. To date, there is no indication that clinical hereditary tubal carcinoma features are different from those of its sporadic counterpart; abdominal discomfort is more or less common, but an atypical complaint. The classical but rare triad of symptoms include: (i) prominent watery vaginal discharge, (ii) pelvic pain and (iii) pelvic mass {158}. It has been reported that approximately 10% of patients will have adenocarcinomatous cells in cervical cytology {3185}.

Tumour markers. As in ovarian carcinoma elevation of serum CA125 levels can be found in approximately 80% of cases {1173}.

Imaging. CT / MRI are inconclusive with respect to the differential diagnosis of

tubal or ovarian carcinomas. However, these techniques can be helpful in determining the extent of disease. Likewise, ultrasonography can not distinguish tubal from ovarian disease {2720}.

Pathology

Histopathology and grading. Serous papillary carcinoma is the most common form of hereditary tubal carcinoma. Grading is of limited value in these tumours and, if used, is based on the papillary architecture, nuclear atypia and mitotic activity. Grade I cancers show papillary growth with well differentiated columnar cells and low mitotic rate. Grade II cancers are papillary with evident gland formation with intermediately differentiated cells with moderate mitotic activity. Grade III shows solid growth with loss of papillae and a medullary/glandular pattern. The cells are poorly differentiated and the mitotic activity is high.

Immunoprofile. Being predominantly of serous papillary type, hereditary tubal carcinomas are positive for cytokeratins 7 and 8, MUC1, CEA, OVTL3, OV632, CA125, and negative or showing only low expression for cytokeratin 20, CEA and vimentin. Also, p53 is often expressed, and cyclins E and A and Ki67 show a varying number of proliferating cells, whereas staining for HER-2/neu and cyclin D1 is usually negative. Steroid receptor content varies. In the rare clear cell cancers, p21 is highly expressed.

Seeding and metastasis
Hereditary tubal carcinomas presumably spread like their sporadic counterparts. However, only empirical data are available to date, pointing to a mode of spread similar to ovarian cancer.

Survival
The five-year survival rate of 30% in sporadic cases varies with stage {158,3185}, but not with grade. The survival rate of hereditary tubal carcinomas has yet to be established since only small numbers of patients have been reported and most patients have still not completed their 5-year follow-up.

Prophylactic interventions
In one study, 30 women with either a documented deleterious *BRCA1* or *BRCA2* mutation or a suggestive family history underwent prophylactic oophorectomy {1617}. Five of these (17%) were found to have clinically occult malignancy, 3 of which involved a primary fallopian tube malignancy. Three of the five were known *BRCA1* mutation carriers, one had a documented *BRCA2* mutation. Therefore, it is recommended to perform a complete adnexectomy in women carrying a *BRCA1* or a *BRCA2* mutation. Whether an abdominal hysterectomy should be performed to dissect the intra-uterine part of the tube, is still in debate. However, most studies indicate that tubal carcinomas in fact predominantly arise in distal parts of the tube.

Table 8.06
Cancer risks of *BRCA2* carriers.

Cancer site or type	Relative risk (95% CI)	Cumulative Risk By Age 70, % (95% CI)
Breast (female)	Age-dependent	84 (43 – 95)
Breast (male)	150	6.3 (1.4 – 25.6)
Ovary	Age-dependent	27 (0 – 47)
Gall bladder and bile ducts	4.97 (1.50 – 16.5)	-
Prostate	4.65 (3.48 – 6.22)	7.5 (5.7 – 9.3)
Prostate before age 65	7.33 (4.66 – 11.52)	-
Pancreas[1]	3.51 (1.87 – 6.58)	Males: 2.1 (1.2 – 3.0) Females: 1.5 (0.9 – 2.1)
Stomach[1]	2.59 (1.46 - 4.61)	-
Malignant melanoma[1]	2.58 (1.28 – 5.17)	-
All cancers[2]	2.45 (2.15 – 2.78)	-

From D. Ford et al. 1998 {898}, D.F. Easton et al. 1997 {744} and the Breast Cancer Linkage Consortium 1999 {11}.
[1] Relative risks were slightly higher for individuals aged 65 or under.
[2] All cancers other than nonmelanoma skin cancer, breast cancer, or ovarian cancer.

Other tumours

BRCA2 confers an increased risk of ovarian cancer, but not as high as that for BRCA1. Statistically significant increases in risk were observed for a number of other tumour types, including prostate, pancreatic and stomach cancer. The risk for prostate cancer is probably not sufficiently high to cause an appreciable fraction of early-onset prostate cancer cases. The risk for male breast cancer, although the hallmark of *BRCA2* mutations, is based on only four observed cases and hence is very imprecise.

Genetics

Chromosomal location and gene structure
BRCA2 is located on chromosome 13q12.3. It consists of 27 exons, of which exon 11 is remarkably large (4.9 kb). The open reading frame is 10,254 basepairs, encoding a protein of 3,418 aminoacids that has no significant similarity to any known protein. Exon 11 encodes a structural motif consisting of eight 'BRC' repeats, through which BRCA2 controls the function of RAD51, a recombinase enzyme, in pathways for DNA repair by homologous recombination.

Gene expression
A wide range of human tissues express *BRCA2* mRNA, in a pattern very similar to that of *BRCA1*, but the highest levels were observed in breast and thymus, with slightly lower levels in lung, ovary, and spleen {2891}. In normal cells, BRCA2 is a nuclear protein, preferentially expressed during the late-G1/early-S phase of the cell cycle {258,480,3012}. In mice, *Brca1* and *Brca2* are coordinately upregulated during ductal proliferation, morphogenesis and differentiation of breast epithelial cells occurring at puberty, pregnancy and lactation {1582,1769,

2323}. Both proteins co-exist with RAD51 in subnuclear foci during S phase, which redistribute following DNA damage {450,2193}.
Exon 12 of the messenger is alternatively spliced, and there is some suggestion that this splice variant is expressed at higher levels in about a third of sporadic breast tumour when compared to normal epithelial cells {266}. In sporadic breast tumours, *BRCA2* mRNA-expression was higher than that in normal surrounding tissues in 20% of the cases, and lower in 11% {267}. In agreement with this, no hypermethylation of the *BRCA2* promotor region has been detected in breast and ovarian cancer {541}.

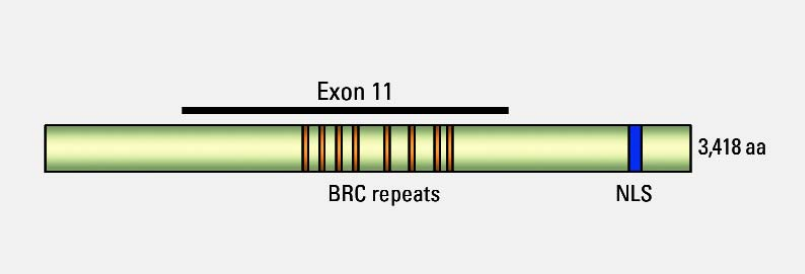

Fig. 8.09 Functional domains in *BRCA2*. There are 8 BRC repeats in the central region of the protein which interact with RAD51. NLS = nuclear localization signal. The proportion encoded by exon 11 is indicated.

Gene function

Loss, or mutational inactivation, of the single wild-type allele in heterozygous carriers of mutations in the *BRCA2* gene is a key step in tumourigenesis. The mechanism by which the encoded protein contributes to disease progression is not yet completely understood but is thought to be related, at least in part, to the proposed role of BRCA2 in the repair of damaged DNA.
BRCA2 encodes a very large (3,418 amino acids in humans) protein that is expressed during S phase of the cell cycle when it is present in the cell nucleus. Although the amino acid sequence of the BRCA2 protein presents few direct clues as to its normal cellular role, some functional domains have been defined. The C-terminal region of BRCA2 contains a functional nuclear localization sequence; many pathogenic truncating mutations in human *BRCA2* are proximal to this domain and would therefore be predicted to encode cytoplasmic proteins. The central part of the protein encoded by the large exon 11 contains eight copies of a novel sequence (the BRC repeat) that has been shown to be capable of binding RAD51 protein. RAD51 is a key protein involved in double-strand DNA break repair and homologous recombination and the interaction with BRCA2 was the first evidence implicating the protein in these processes.
BRCA2-deficient cells and tumours characteristically accumulate aberrations in chromosome structure {3018}. These lesions include breaks involving one of the two sister chromatids, as well as tri-radial and quadri-radial chromosomes typical of Bloom syndrome and Fanconi anaemia. Thus, BRCA2 deficiency may be similar in its pathogenesis to other genetic diseases in which unstable chromosome structure is linked to cancer predisposition.
Chromatid-type breaks, tri-radial and quadri-radial chromosomes are thought to arise from defects in the repair of DNA double-strand breaks (DSBs) during the S phase of cell cycle. During S phase, DSB repair proceeds preferentially through mechanisms involving homologous recombination. These mechanisms enable error-free repair of broken DNA, taking advantage of the availability of the replicated sister chromatid as a substrate for recombination reactions. In BRCA2-deficient cells, DSB repair by homologous recombination is defective. However, alternative – but error-prone - mechanisms for DSB repair such as end-joining or strand-annealing are still present. The end result is that DSBs in BRCA2-deficient cells are mis-repaired, giving rise to mutations and chromosomal rearrangements including translocations or deletions. The resulting genetic instability is believed to potentiate the acquisition of mutations that transform a normal cell into a cancer cell. Thus, BRCA2 works as a tumour suppressor indirectly through its 'caretaker' role in protecting chromosomal stability.
BRCA2 is essential for homologous recombination because it controls the intra-cellular transport and activity of RAD51. In BRCA2-deficient cells, RAD51 fails to efficiently enter the nucleus. After exposure of BRCA2-deficient cells to DNA damaging agents, RAD51 fails to localize

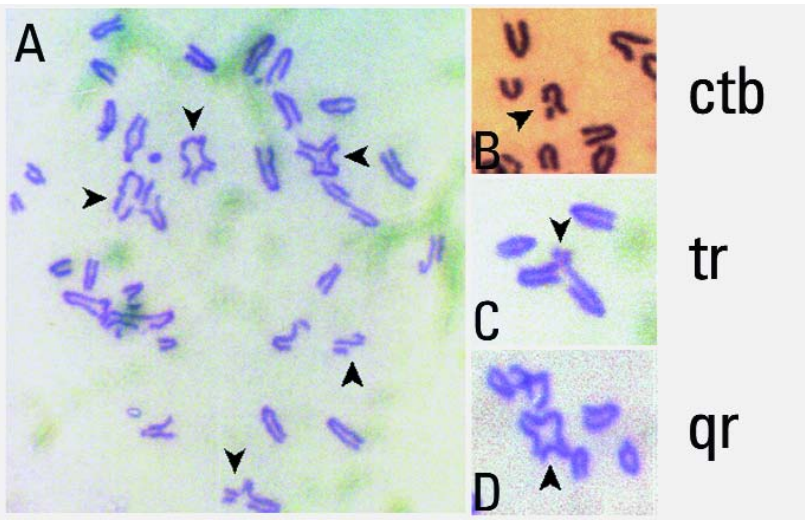

Fig. 8.10 Aberrations in chromosome structure reminiscent of Bloom syndrome and Fanconi anaemia accumulate during the division of BRCA2-deficient cells in culture. Enlargements of characteristic aberrations are shown in the panels on the right hand-side (ctb, chromatid break, tr, tri-radial and qr, quadri-radial). Reproduced from K.J. Patel et al. {2193}.

in typical nuclear foci that may represent sites for DNA damage processing. Moreover, BRCA2 controls the assembly of RAD51 into a nucleoprotein filament that coats DNA, a critical intermediate structure in recombination reactions.

Unexpected and potentially informative insight into the role of *BRCA1/2* genes in DNA repair in humans in vivo has come from recent studies on Fanconi anaemia (FA), a complex disorder characterized by congenital abnormalities, progressive bone marrow failure and cancer susceptibility. FA is a recessively inherited disorder which can result from mutation in at least 8 individual genes. It has recently been suggested that one of the previously unidentified FA genes, FANCD1, is in fact BRCA2 {1251}. The cellular consequences of homozygosity for *BRCA2* mutation, including spontaneous chromosome instability and hypersensitivity to DNA cross-linking agents, are rather similar to those observed in cells derived from FA patients. This is not the only link between FA and breast cancer susceptibility genes. Another FA gene product, FANCD2, can interact and co-localize with BRCA1 {958}. Thus it seems that the pathways disrupted in FA and breast cancer susceptibility are intimately connected. Only a small proportion of FA, which in itself is rare, is caused by *BRCA2* mutation but the importance of this finding is that it connects together two previously different bodies of work on DNA repair.

A current simplified model on how *BRCA2* and several other genes involved in breast cancer predisposition act coordinately to repair DNA damage is indicated in Fig. 8.08. ATM and CHEK2 protein kinases signal the presence of double-stranded DNA breaks and phosphorylate (red arrows) a number of downstream effector proteins, including BRCA1. This induces their migration to sites where DNA is repaired. BRCA2 carries the DNA-recombination enzyme RAD51 to the same sites, guided there by the DNA-binding structures formed between its C-terminal domain and Dss1 protein. A complex of Fanconi anaemia proteins – termed A, C, D2, E, F, and G – triggers the ubiquitination of the D2 protein alone and its colocalization with BRCA1.

Other roles for BRCA2 have been suggested in chromatin remodelling and gene transcription {1442}. Such functions – which remain very poorly characterized – may help to explain why cancer predisposition associated with *BRCA2* mutations should be specific to tissues such as the breast and ovary. However, notwithstanding these other potential functions, it seems likely that loss of BRCA2 function engenders genomic instability leading to oncogene activation and tumour suppressor loss that culminates in tumourigenic progression. A major challenge for future work will be to understand how this basic pathogenic mechanism plays out in the complex tissue environments of the breast, ovary or prostate, giving rise to site-specific epithelial malignancies.

Mutation spectrum

Germline mutations in *BRCA2* have been detected in 5-10% of clinic-based breast cancer families, and in similar frequencies of breast-ovarian cancer families {2657,3023}. Somatic mutations in sporadic breast and ovarian tumours are extremely rare. Mutations occur throughout the entire coding region, and hence the mutation spectrum did not provide immediate clues to functional gene domains. The majority of the mutations are predicted to lead to a prematurely truncated protein when translated. In conjunction with the observed loss of the wildtype allele in tumours arising in mutation carriers {560}, this indicates the importance of gene inactivation for tumourigenesis to occur. Despite the strong variability in mutations detected in families, founder effects have led to some mutations being very prevalent in certain populations of defined geographical or ethnic background. Examples are the 999del5 mutation, which is present in approximately 0.6% of all Icelandic individuals {2920}, and the 6174delT mutation found in an equal proportion of Ashkenazi Jews {2083}. As a result, mutation spectra may vary according to ethnic background of the sampled population {2824}. In recent years, an increasing number of missense changes are being detected in *BRCA2* of which the clinical significance is uncertain in the absence of a functional assay. These already comprise up to 50% of all known sequence changes in *BRCA2*. Although many of them are expected to be rare neutral polymorphisms, some might be associated with elevated levels of breast cancer risk. An example is the arginine for histidine substitution at codon 372 {1167}. Many known deleterious *BRCA1* and *BRCA2* mutations affect splicing, and these typically lie near intron/exon boundaries. However, there are also potential internal exonic mutations that disrupt functional exonic splicing enhancer (ESE) sequences, resulting in exon skipping. A T2722R mutation segregated with affected individuals in a family with breast cancer and disrupted 3 potential ESE sites {816}. The mutation caused deleterious protein truncation and suggested a potentially useful method for determining the clinical significance of a subset of the many unclassified variants of *BRCA1* and *BRCA2*. As more functional and structural information on the BRCA1 and BRCA2 proteins accumulates, our understanding of genetic variation in these genes will improve. The Breast Cancer Information Core (BIC) maintains a website providing a central repository for information regarding mutations and polymorphisms (http://research.nhgri.nih.gov/bic/).

Genotype-phenotype correlations

Evidence is accumulating that the risks conferred by pathogenic *BRCA2* mutations are dependent on the position of the mutation in the gene, genetic variation in other genes, and environmental or lifestyle factors.

BRCA2 mutation position

Truncating mutations in families with the highest risk of ovarian cancer relative to breast cancer are clustered in a region of approximately 3.3 kb in exon 11 {972}. This region of *BRCA2*, bounded by nucleotides 3035 and 6629, was dubbed the 'ovarian cancer cluster region,' or OCCR. Notably, this region coincides with the BRC repeats that are critical for the functional interaction with the RAD51 protein. A much larger study of 164 families confirmed that OCCR mutations are associated with a lower risk of breast cancer and with a higher risk of ovarian cancer {2913}. The extent of risk modification is too moderate, however, to be used in genetic counseling.

Genetic risk-modifiers

A length-variation of the polyglutamine repeats in the estrogen receptor co-activator NCOA3 influences breast cancer risk in carriers of *BRCA1* and *BRCA2* {2345}. Although it should be noted that most of the carriers in these studies are *BRCA1* carriers, and there was insuffi-

cient power to determine the effect in *BRCA2* carriers alone. Similarly, the variant progesterone receptor allele named PROGINS was associated with an odds ratio of 2.4 for ovarian cancer among 214 *BRCA1/2* carriers with no past exposure to oral contraceptives, compared to women without ovarian cancer and with no PROGINS allele {2487}. A C/G polymorphism in the 5' untranslated region of *RAD51* was found to modify both breast and ovarian cancer risk in carriers of *BRCA2* {1644,3053}. These results support the hypothesis that genetic variation in the genes constituting endocrine signalling and DNA repair pathways may modify *BRCA2*-associated cancer risk.

Hormonal risk modifiers

As in the BRCA1 syndrome, the breast cancer risk of BRCA2 carriers is influenced by hormonal factors, including oral contraceptives and pregnancy (see page 56).

Prognosis and prevention

Life expectancy and preventive strategies are similar to those discussed for BRCA1 carriers (see page 56).

Li-Fraumeni syndrome

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M. Olivier

Definition

Li-Fraumeni syndrome (LFS) is an inherited neoplastic disease with autosomal dominant trait. It is characterized by multiple primary neoplasms in children and young adults, with a predominance of soft tissue sarcomas, osteosarcomas, breast cancer, and an increased incidence of brain tumours, leukaemia and adrenocortical carcinoma. The majority of Li-Fraumeni cases is caused by a *TP53* germline mutation.

MIM Nos. {1835}

Li-Fraumeni syndrome	151623
TP53 mutations (germline and sporadic)	191170
CHEK2 mutations	604373

Synonym

Sarcoma family syndrome of Li and Fraumeni.

Incidence

From 1990 to 1998, 143 families with a *TP53* germline mutations were reported {2086}. The IARC Database {www.iarc.fr/p53/germline.html} currently contains 223 families {2104a}.

Diagnostic criteria

The criteria used to identify an affected individual in a Li-Fraumeni family are: (i) occurrence of sarcoma before the age of 45 and (ii) at least one first degree relative with any tumour before age 45 and (iii) a second (or first) degree relative with cancer before age 45 or a sarcoma at any age {273,957,1650}.

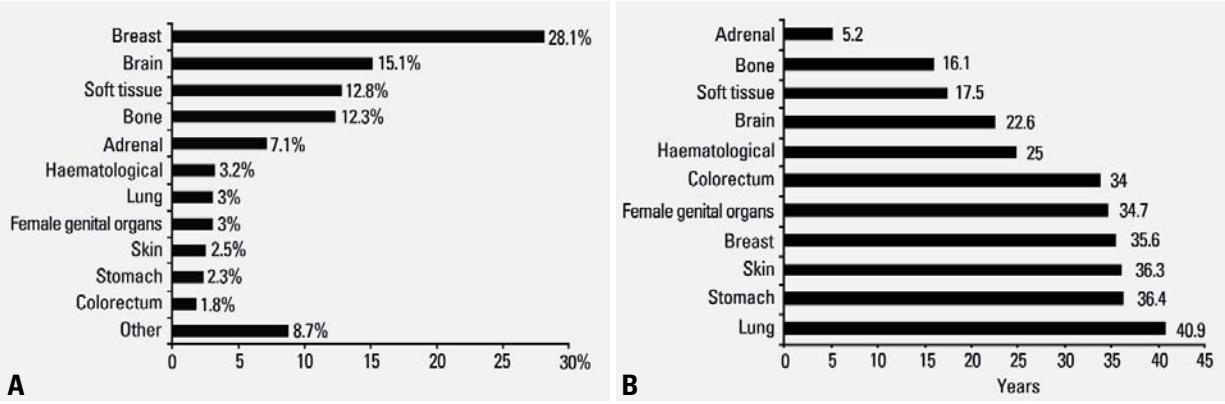

Fig. 8.11 **A** A fraction of tumours in families with a *TP53* germline mutation. **B** Mean age of patients with tumours caused by a *TP53* germline mutation, according to organ site.

Breast tumours

Frequency

Breast cancers are the most frequent neoplasms developed in families with a *TP53* germline mutation. Thirty-seven % of these families are defined as Li-Fraumeni syndrome and 30% as Li-Fraumeni-like syndrome. In the 219 families with a *TP53* germline mutation reported in 1990–2001 (IARC *TP53* database: www.iarc.fr/p53), a total of 562 tumours developed in individuals with a confirmed *TP53* germline mutation. Of these, 158 (28%) were breast tumours. Eighty-three (38%) families with a *TP53* mutation had at least one family member with a breast tumour. Among the families in which at least one case of breast cancer developed, the mean number of breast tumours per family was 1.9.

Age and sex distribution

Breast cancers associated with a *TP53* germline mutation develop earlier than their sporadic counterparts, with a mean age of 35+10 years (range 14-67 years old). The mean age of women with Li-Fraumeni-like syndrome (LFL) is approximately 8 years higher {2104a} However, breast cancers associated with *TP53* germline mutations never developed in young children, suggesting that hormonal stimulation of the mammary glands constitutes an important co-factor.

Sporadic breast tumours occur approximately 100 times more frequently in females than in males {1475}, and none occurred in males among the 158 reported breast cancer with *TP53* germline mutations.

Pathology

Of the 158 breast tumours recorded, the majority (146 cases, 92%) have not been classified histologically, but recorded as just breast cancers. Histologically classified cases included carcinoma in situ (4 cases), adenocarcinoma (1 case), Paget disease (2 cases), malignant phyllodes tumour (2 cases), comedocarcinoma (1 case), spindle cell sarcoma (1 case), and stromal sarcoma (1 case).

Prognosis and prognostic factors

The breast cancers that occur in LFS are of younger onset and so may have a poorer prognosis due to this early age at diagnosis. In mice, there is relative radioresistance in *p53* mutants, however, radioresistance due to germline mutation has not been convincingly shown in man.

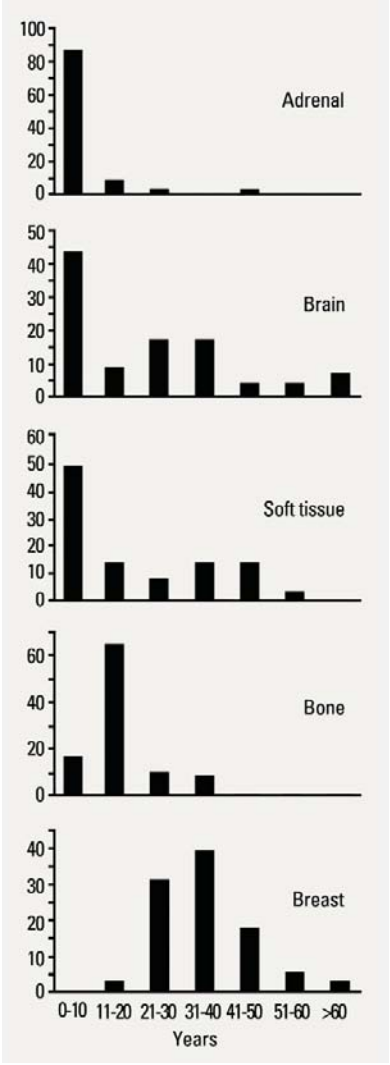

Fig. 8.12 Age distribution of patients with tumours caused by a *TP53* germline mutation.

Other tumours

Frequency

Following breast cancer, brain tumours and sarcomas (osteosarcomas and soft tissue sarcomas) are the next most frequent manifestations. The sporadic counterparts of these tumours also show somatic *TP53* mutations, suggesting that in these neoplasms, *TP53* mutations are capable of initiating the process of malignant transformation {1475,2087}.

Age distribution

In general, tumours associated with a *TP53* germline mutation develop earlier than their sporadic counterparts, but there are marked organ-specific differences. As with sporadic brain tumours, the age of patients with nervous system neoplasms associated with *TP53* germline mutations shows a bimodal distribution. The first peak of incidence (representing medulloblastomas and related primitive neuroectodermal tumours) is in children, and the second (mainly astrocytic brain tumours) in the third and fourth decades of life {2087}. Adrenocortical carcinomas associated with a *TP53* germline mutation develop almost exclusively in children, in contrast to sporadic adrenocortical carcinomas, which have a broad age distribution with a peak beyond age 40 {1475}.

Genetics – *TP53*

Chromosomal location

The *TP53* gene encompasses 20 kilobases on chromosome 17p13.1. *TP53* belongs to a family of growth suppressors that also comprises two other members, *TP73* and *TP63*. Whereas the two latter genes are mostly involved in the regulation of differentiation and development, *TP53* plays specialized functions as a tumour suppressor {1643}.

Gene structure

The gene contains 11 exons, the first one non-coding. The first intron is particularly large (10 kilobases). The coding sequence is concentrated over 1.3 kilobases. *TP53* is ubiquitously expressed, mostly as a single mRNA species (although rare alternatively spliced variants have been reported). The promoter does not contain a classical TATA box but shows binding elements for several common transcription factors, including c-Jun and NF-kappaB {1107}.

Gene expression

The p53 protein is constitutively expressed in most cell types but, in normal circumstances, does not accumulate to significant level due to rapid degradation by the proteasome machinery. In response to various types of cellular stress, the p53 protein undergoes a number of post-translational modifications that release p53 from the negative control of MDM2, a protein that binds to p53 and mediates its degradation.

These modifications result in the intranuclear accumulation of p53 and in its activation as a transcription factors. Two major signaling pathways can trigger *TP53* activation. The first, and best characterized, is the pathway of response to DNA damage, including large kinases of the phosphoinositol-3 kinase family such as *ATM* (ataxia telangiectasia mutated) and the cell-cycle regulatory kinase *CHEK2*. Both of these kinases phosphorylate p53 in the extreme N-terminus (serines 15, 20 and 37), within the region that binds to MDM2. The second is activated in response to the constitutive stimulation of growth-promoting signaling cascades. The central regulator in this pathway is p14ARF, the alternative product of the locus encoding the cyclin-kinase inhibitor *p16/CDKN2a*. p14ARF expression is activated by E2F transcription factors, and binds to MDM2, thus neutralizing its capacity to induce p53 degradation. This pathway may be part of a normal feedback control loop in which p53 is activated as a cell-cycle brake in cells exposed to hyperproliferative stimuli {2267}.

Gene function

After accumulation, the p53 protein acts as a transcriptional regulator for a panel of genes that differ according to the nature of the stimulus, its intensity and the cell type considered. Broadly speaking, the genes controlled by p53 fall into three main categories, including cell-cycle regulatory genes (*WAF1, GADD45, 14-3-3S, CYCLING*), pro-apoptotic genes (*FAS/APO1/CD95, KILLER/DR5, AIF1, PUMA, BAX*) and genes involved in DNA repair (O^6*MGMT, MLH2*). The p53 protein also binds to compoments of the transcription, replication and repair machineries and may exert additional controls on DNA stability through the modulation of these mechanisms. Collectively, the p53 target genes mediate two type of cellular responses: cell-cycle arrest, followed by DNA repair in cells exposed to light forms of genotoxic stress, and apoptosis, in cells exposed to levels of damage that cannot be efficiently repaired. Both responses contribute to the transient or permanent suppression of cells that contain damaged, potentially oncogenic DNA. In the mouse, inactivation of *Tp53* by homologous recombination does not prevent normal growth but results in a strong predisposition to early, multiple cancers, illustrating the crucial role of this gene as a tumour suppressor {714}.

Mutation spectrum

The *TP53* gene is frequently mutated in most forms of sporadic cancers, with prevalences that range from a few percents in cervical cancers and in malignant melanomas to over 50% in invasive carcinomas of the aero-digestive tract. Over 75% of the mutations are single base substitutions (missense or nonsense), clustering in exons 5 to 8 that encode the DNA-binding domain of the protein. Codons 175, 245, 248, 273 and 282 are major mutation hotspots in almost all types of cancers. Together, these codons contain over 25% of all known *TP53* mutations. Other codons are mutation hotspots in only specific tumour types, such as codon 249 in hepatocellular carcinoma and codon 157 in bronchial cancer. Mutation patterns can differ significantly between between different types cancers or between geographic areas for the same cancer type (as for example hepatocellular carcinoma). These observations have led to the concept that mutation patterns may reveal clues on the cellular or environmental mechanisms that have caused the mutations {1107}. In sporadic breast cancers, *TP53* is mutated in about 25% of the cases. However, several studies have reported accumulation of the p53 protein without mutation in up to 30-40% of invasive ductal carcinoma in situ. The mutation pattern is similar to that of many other cancers and does not provide information on possible mutagenic events. There is limited evidence that the mutation prevalence is higher in *BRCA1* mutation carriers.

Germline *TP53* mutations have been identified in 223 families. Of these families, 83 match the strict LFS criteria, 67 correspond to the extended, LFL definition, 37 have a family history of cancer that does not fit within LFS or LFL definitions and 36 have germline mutations without documented familial history of cancer (IARC *TP53* mutation database, www.iarc.fr/p53). The codon distribution of germline *TP53* mutations show the same mutational hotspots as somatic mutations {1475}. The distribution of inherited mutations that predisposes to breast cancer are scattered along exons 5 to 8 with relative "hotspots" at codons 245, 248 and 273, which are also commonly mutated in somatic breast cancer. In contrast, a total of 16 breast cancers have been detected in 5 families with a germline mutation at codon 133, a position which is not a common mutation hotspot in somatic breast cancer. It remains to be established whether this mutant has particular functional properties that predispose to breast cancer.

Genotype-phenotype correlations

Brain tumours appear to be associated with missense TP53 mutations in the

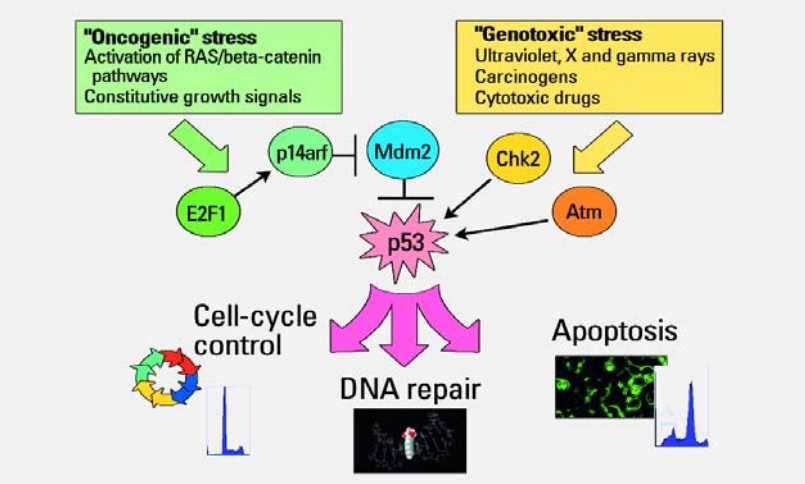

Fig. 8.13 The p53 signaling pathway. In normal cells the p53 protein is kept in a latent state by MDM2. Oncogenic and genotoxic stresses release p53 from the negative control of MDM2, resulting in p53 accumulation and activation. Active p53 acts as transcription factor for genes involved cell cycle control, DNA repair and apoptosis, thus exerting a broad range of antiproliferative effects.

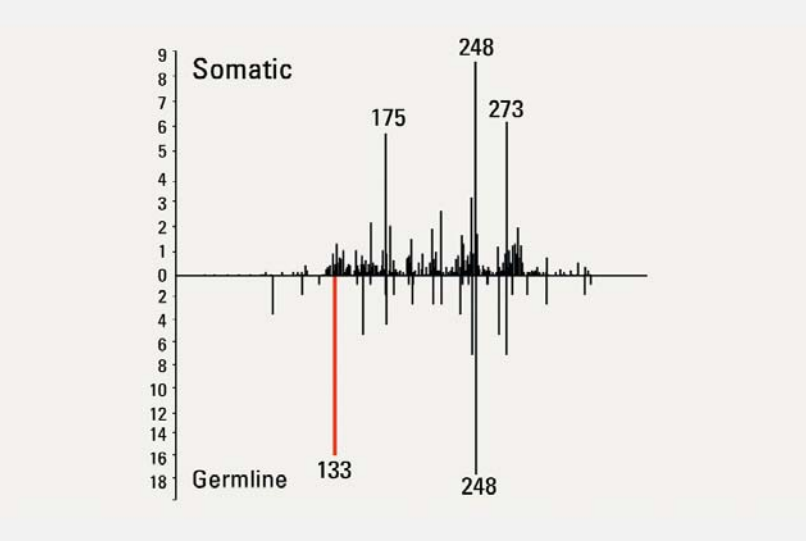

Fig. 8.14 Codon distribution of somatic (top) or germline (bottom) *TP53* mutations associated with breast cancers. Hotspot mutations are indicated. Mutation at codon 133 has been reported in 5 Li-Fraumeni families with breast cancers, but is not a frequent site for somatic mutation in breast cancer in the general population. Compiled from: IARC *TP53* database, www.iarc.fr/p53.

DNA-binding loop that contacts the minor groove, while early onset brain tumours were associated with mutations likely to result in absence of protein or loss of function {2104a}. Adrenocortical carcinomas were associated with missense mutations in the loops opposing the protein-DNA contact surface {2104a}.

Genetics – *CHEK2*

Chromosomal location

CHEK2 is on chromosome 22q12.1.

Gene structure

CHEK2 has 14 exons and there are several homologous loci, which encompass exons 10-14 of the gene, scattered throughout the genome. These gene fragment copies can present problems when analysing *CHEK2* for germline mutations in genomic DNA, and it is important to ensure that the correct copy is being amplified {2742}. This problem can be overcome by amplifying exons 10-14 by the use of a long range PCR using primers located in the non-duplicated region of the gene {2741}. The individual exons can then be subsequently amplified using the product of the long range PCR as a template.

Gene expression

CHEK2 is expressed in nonproliferating and terminally differentiated cells. It is homogenously expressed in renewing cell populations such as epidermis, esophagus, rectum, bladder, stomach, intestine and colon, and heterogenously in conditionally renewing tissues such as lung, breast kidney, salivary, thyroid, parathyroid, adrenal glands, pancreas, prostate, epididymis, sweat glands, endometruim, stromal mesenchymal cells, blood vessels, lymphoid tissues, smooth and cardiac muscle tissues and preipheral nerves. It is absent or cytoplasmic in static tissues such as muscle and brain. CHEK2 remains expressed and can be activated in all phases of the cell cycle in response to DNA damage {1714}.

Gene function

Human *CHEK2* is a homolog of the yeast G2-checkpoint kinases *CDS1* and *RAD53* {1791}. In response to DNA damage, CHEK2 propagates the checkpoint signal along several pathways, which eventually causes cell-cycle arrest in G1, S and G2/M phases {449,820}; activation of DNA repair {1609}, and in apoptotic cell death {1315}. Four of the downstream checkpoint effectors that are established as substrates of CHEK2 in vivo include p53, BRCA1 and Cdc25A and Cdc25C.

Mutation spectrum

Recently, heterozygous germline mutations in *CHEK2* have been identified in three of a subset of individuals with the dominantly inherited Li-Fraumeni syndrome which do not harbour *TP53* mutations {209}. However, one of these was found to be in a pseudogene copy of the *CHEK2* gene. Another one appeared to be neutral polymorphism in the Finnish population. The third was a protein-truncating mutation, 1100delC in exon 10, which abolishes the kinase function of CHEK2. The possibility that this gene is only contributing to the breast cancer cases within LFS families rather than LFS *per se* has been raised {2740}.

The frequency of 1100delC has been estimated in healthy control populations, and was found to vary between 0.3% and 1.7% {1840,2084,2984}. This would also suggest that the 1100delC is a polymorphism, rather than a disease-causing mutation. Yet among unselected patients with breast cancer, its prevalence was found to be approximately 1.5-fold higher than in controls. Significantly elevated frequencies were found among patients with a positive family history and among patients with bilateral breast cancer {2984}. The strongest enrichment of 1100delC carriers (approximately 5-fold) was found among familial breast cancer patients in whom the presence of *BRCA1* or *BRCA2* mutations were excluded {1840,2984}. However, in families with the 1100delC mutation, it appears to cosegregate poorly with breast cancer. The results suggest that *CHEK2*1100delC* is a low risk breast cancer susceptibility allele which may make a significant contribution to familial clustering of breast cancer, including families with smaller numbers of affected cases. As it is enriched among multiple-case families, but unable to explain all breast cancer in families with at least one carrier case, it may interact with other, as yet unknown breast cancer susceptibility alleles.

Search for additional LFS genes

The paucity of large LFS kindreds makes classical linkage methodology difficult. A candidate approach is therefore being used. Candidate genes are those involved in cell cycle pathways, those commonly mutated in multiple tumour types and the breast cancer genes, as this site is commonly affected in LFS kindreds. Using these approaches, the genes *P16* and *PTEN* {379} have been analysed and no germline mutations found.

Cowden syndrome

C. Eng

Definition

Cowden syndrome (CS) is an autosomal dominant disorder caused by germline mutaions of the *PTEN* gene. It is characterized by multiple hamartomas involving organs derived from all three germ cell layers and a high risk of breast, uterine and non-medullary thyroid cancer. The classic hamartoma is the trichilemmoma and is pathognomonic for CS.

MIM No. 158350 {1835}

Synonyms

Cowden disease, multiple hamartoma syndrome.

Incidence

The single most comprehensive clinical epidemiologic study before the CS susceptibility gene was identified estimated the prevalence to be 1:1 000 000 {1990,2776}. Once the gene was identified {1654}, a molecular-based estimate of prevalence in the same population was 1:300 000 {1989}. Because of the difficulty in recognizing this syndrome, prevalence figures are likely underestimated.

Diagnostic criteria

Because of the variable and broad expression of CS and the lack of uniform diagnostic criteria prior to 1996, the International Cowden Consortium {1990} compiled operational diagnostic criteria for CS, based on the published literature and their own clinical experience {785}. These criteria have been recently revised in light of new data, and have been adopted by the US-based National Comprehensive Cancer Network Practice Guidelines {786,1299}. Trichilemommas and papillomatous papules are particularly important to recognize. CS usually presents by the late 20's. It has variable expression and, probably, an age-related penetrance although the exact penetrance is unknown. By the third decade, 99% of affected individuals would have developed the mucocutaneous stigmata although any of the features could be present already. Because the clinical literature on CS consists mostly of reports of the most florid and unusual families or case reports by subspecialists interested in their respective organ systems, the spectrum of component signs is unknown.

Despite this, the most commonly reported manifestations are mucocutaneous lesions, thyroid abnormalities, fibrocystic disease and carcinoma of the breast, gastrointestinal hamartomas, multiple, early-onset uterine leiomyoma, macrocephaly (specifically, megencephaly) and mental retardation {1133, 1693,1748,2776}. Recent data have suggested that endometrial carcinoma should be a component cancer of CS {657,786,1772}. What its frequency is in mutation carriers is as yet unknown.

Table 8.07
International Cowden Syndrome Consortium Operational Criteria for the Diagnosis of Cowden Syndrome (Ver. 2000)*.

Pathognomonic criteria	Mucocutanous lesions: Trichilemmomas, facial Acral keratoses Papillomatous papules Mucosal lesions
Major criteria	Breast carcinoma Thyroid carcinoma (non-medullary), esp. follicular thyroid carcinoma Macrocephaly (Megalencephaly) (say, >97%ile) Lhermitte-Duclos disease (LDD) Endometrial carcinoma
Minor criteria	Other thyroid lesions (e.g. adenoma or multinodular goiter) Mental retardation (say, IQ < 75) GI hamartomas Fibrocystic disease of the breast Lipomas Fibromas GU tumours (e.g. renal cell carcinoma, uterine fibroids) or malformation
Operational diagnosis in an individual	1. Mucocutanous lesions alone if: a) there are 6 or more facial papules, of which 3 or more must be trichilemmoma, or b) cutaneous facial papules and oral mucosal papillomatosis, or c) oral mucosal papillomatosis and acral keratoses, or d) palmoplantar keratoses, 6 or more 2. Two major criteria but one must include macrocephaly or LDD 3. One major and 3 minor criteria 4. Four minor criteria
Operational diagnosis in a family where one individual is diagnostic for Cowden	1. The pathognomonic criterion/ia 2. Any one major criterion with or without minor criteria 3. Two minor criteria

*Operational diagnostic criteria are reviewed and revised on a continuous basis as new clinical and genetic information becomes available. The 1995 version and 2000 version have been accepted by the US-based National Comprehensive Cancer Network High Risk/Genetics Panel.

Breast tumours

Age distribution and penetrance

Invasive carcinomas of the breast have been diagnosed as early as the age of 14 years and as late as in the 60's {1693}. However, the majority of CS-related breast cancers occur after the age of 30-35 years {786,788}. A single population-based clinical study, without the benefit of genetic analysis, suggested that benign breast disease can occur in two-thirds of affected women while CS females have a 25-50% lifetime risk of developing invasive breast cancer {786,2776}. Male breast cancer can occur in CS as well but the frequency is unknown {817,1771}.

Clinical features

It is believed that the clinical presentation of breast cancer in CS is no different from that of the general population. However, no formal data is currently available.

Pathology

Like other inherited cancer syndromes, multifocality and bilateral involvement is the rule. With regard to the individual cancers, even of the breast and thyroid, as of mid 1997, there has yet to be a systematic study published. There exists, however, one study which has attempted to look at benign and malignant breast pathology in CS patients. Although these are preliminary studies, without true matched controls, it is, to date, the only study that examines breast pathology in a series of CS cases. Breast histopathology from 59 cases belonging to 19 CS women was systematically analysed {2578}. Thirty-five specimens had some form of malignant pathology. Of these, 31 (90%) had ductal adenocarcinoma, one tubular carcinoma and one lobular carcinoma-in-situ. Sixteen of the 31 had both invasive and in situ (DCIS) components of ductal carcinoma while 12 had DCIS only and two only invasive adenocarcinoma. Interestingly, it was noted that 19 of these carcinomas appeared to have arisen in the midst of densely fibrotic hamartomatous tissue.

Benign breast disease is more common than malignant, with the former believed to occur in 75% of affected females. Fibrocystic disease of the breast, breast hamartomas, and fibroadenomas are commonly seen.

Uterine tumours

Age distribution and penetrance

Since endometrial carcinomas have only recently been suggested to be a minor component of CS {786}, it is unknown what the true frequency is among mutation carriers or what the age distribution is. Anecdotal cases suggest that the frequency could be 6-10% in affected women.

Benign tumours of the uterus are common in CS. Uterine leiomyomas are believed to occur in almost half of affected women {1693}. They are usually multi-focal and occur at a young age, even in the 20's. Other benign uterine pathologies such as polyps and hyperplasias have been found in CS patients but are of unknown frequency.

Clinical features

There have been no systematic studies of uterine tumours in CS. Clinical observation and anecdotal reports suggest that the leiomyomas can become quite symptomatic, presenting with bleeding and pain. It is unclear if the clinical presentation of the endometrial carcinomas is different from that of sporadic cases.

Pathology

There have been no systematic studies of uterine tumours in CS although it is believed that the histopathology is no different from that of typical sporadic cases.

Prognosis and prognostic factors

Whether the prognosis differs from sporadic cases is unknown.

Thyroid tumours

Age of distribution and penetrance

Apart from breast cancer, the other major component cancer in CS is nonmedullary thyroid cancer. Nonmedullary thyroid carcinomas occur at a frequency of 3-10% of affected individuals, regardless of sex, in non-systematic clinical series {1693,2776}. It is unclear, however, whether the age of onset is earlier than that of sporadic cases.

Benign thyroid disease occurs in approximately 70% of affected individuals. Component features include multinodular goitre and follicular adenomas. These benign tumours can occur at any age and can even manifest in teenagers.

Clinical features

Many of the benign tumours in CS individuals remain asymptomatic. However, the most common presenting sign or symptom would be a neck mass. Like many inherited syndromes, CS thyroid lesions can be multifocal and bilobar.

Pathology

No systematic studies have been performed to examine the thyroid in CS. However, clinical observations and clinical reports suggest that the histology of the nonmedullary thyroid carcinoma is predominantly of the follicular type {786, 2776}.

Other tumours

Dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos disease) is the major manifestation in the central nervous system. Peripheral lesions include verrucous skin changes, cobblestone-like papules, fibromas of the oral mucosa, multiple facial trichilemmomas and hamartomatous polyps of the colon.

Genetics

Chromosomal location and mode of transmission

CS is an autosomal dominant disorder, with age related penetrance and variable expression {787}. The CS susceptibility gene, *PTEN*, resides on 10q23.3 {1651, 1654,1990}.

Gene structure

PTEN/MMAC1/TEP1 is comprised of 9 exons spanning 120-150 kb of genomic distance {1649,1651,1654,2777}. It is believed that intron 1 occupies much of this (approximately 100 kb). *PTEN* encodes a transcript of 1.2 kb.

Gene expression

PTEN is expressed almost ubiquitously in the adult human. In normal human embryonic and foetal development, PTEN protein is expressed ubiquitously as well, although levels might change throughout development {1008}. *PTEN* is very highly expressed in the developing central nervous system as well as neural crest and its derivatives, e.g. enteric ganglia {1008}.

Gene function

PTEN encodes a dual specificity lipid and protein phosphatase [reviewed in {3043}]. It is the major 3-phosphatase

acting in the phosphoinositol-3-kinase (PI3K)/Akt apoptotic pathway {1730, 2774}. To date, virtually all naturally occurring missense mutations tested abrogate both lipid and protein phosphatase activity, and one mutant, G129E, affects only lipid phosphatase activity [reviewed in {3043}]. Overexpression of PTEN results, for the most part, in phosphatase-dependent cell cycle arrest at G1 and/or apoptosis, depending on cell type [reviewed in {3043}]. There is also growing evidence that PTEN can mediate growth arrest independent of the PI3K/Akt pathway and perhaps independent of the lipid phosphatase activity {3096-3098} [reviewed in {3042}].

Murine models null for Pten result in early embryonic death {688,2268,2817}. Hemizygous knock-out of Pten result in various neoplasias, and the spectra are different depending on the particular model. While the neoplasias are reminiscent of the component tumours found in the human syndrome, none of the three models are similar to CS.

Mutation spectrum

As with most other tumour suppressor genes, the mutations found in *PTEN* are scattered throughout all 9 exons. They comprise loss-of-function mutations including missense, nonsense, frameshift and splice site mutations {309, 1771}. Approximately 30-40% of germline *PTEN* mutations are found in exon 5, although exon 5 represents 20% of the coding sequence. Further, approximately 65% of all mutations can be found in one of exons 5, 7 or 8 {309, 1771}.

Although *PTEN* is the major susceptiblity gene for CS, one CS family, without *PTEN* mutations, was found to have a germline mutation in *BMPR1A*, which is one of the susceptibility genes for juvenile polyposis syndrome {1250,3262}. Whether *BMPR1A* is a minor CS susceptiblity gene or whether this family with CS features actually has occult juvenile polyposis is as yet unknown.

Genotype-phenotype correlations

Approximately 70-80% of CS cases, as strictly defined by the Consortium critieria, have a germline *PTEN* mutation {1654,1771}. If the diagnostic criteria are relaxed, then mutation frequencies drop to 10-50% {1723,1991,2959}. A formal study which ascertained 64 unrelated CS-like cases revealed a mutation frequency of 2% if the criteria are not met, even if the diagnosis is made short of one criterion {1772}.

A single research centre study involving 37 unrelated CS families, ascertained according to the strict diagnostic criteria of the Consortium, revealed a mutation frequency of 80% {1771}. Exploratory genotype-phenotype analyses revealed that the presence of a germline mutation was associated with a familial risk of developing malignant breast disease {1771}. Further, missense mutations and/or mutations 5' of the phosphatase core motif seem to be associated with a surrogate for disease severity (multiorgan involvement). One other small study comprising 13 families, with 8 *PTEN* mutation positive, could not find any genotype-phenotype associations {1989}. However, it should be noted that this small sample size is not suitable for statistical analyses and no conclusions should be drawn.

Previously thought to be clinically distinct, Bannayan-Riley-Ruvalcaba syndrome (BRR, MIM 153480), which is characterized by macrocephaly, lipomatosis, haemangiomatosis and speckled penis, is likely allelic to CS {1773}. Approximately 60% of BRR families and isolated cases combined carry a germline *PTEN* mutation {1774}. Interestingly, there were 11 cases classified as true CS-BRR overlap families in this cohort, and 10 of the 11 had a PTEN mutation. The overlapping mutation spectrum, the existence of true overlap families and the genotype-phenotype associations which suggest that the presence of germline *PTEN* mutation is associated with cancer strongly suggest that CS and BRR are allelic and are along a single spectrum at the molecular level. The aggregate term of PTEN hamartoma tumour syndrome (PHTS) has been suggested {1774}.

Recently, the clinical spectrum of PHTS has expanded to include subsets of Proteus syndrome and Proteus-like (non-CS, non-BRR) syndromes {3260}. Germline *PTEN* mutations in one case of macrocephaly and autism and hydrocephaly associated with VATER association have been reported {625,2341}.

Hereditary non-polyposis colon cancer (HNPCC)

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Definition

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant disorder characterized by the development of colorectal cancer, endometrial cancer and other cancers due to inherited mutations in one of the DNA mismatch repair (MMR) genes {1725}.

MIM Nos. {1835}

Familial nonpolyposis colon cancer, type 1	120435
Familial nonpolyposis colon cancer, type 2	120436

Synonyms

Lynch syndrome, hereditary colorectal endometrial cancer syndrome {3007}, hereditary defective mismatch repair syndrome {595}.

Incidence

Approximately 2-5% of all cases of colorectal cancer are due to HNPCC {12}. The estimated frequency of carriers of a DNA mismatch repair gene mutation in the general population is one in 1000.

Diagnostic criteria

The International Collaborative Group on HNPCC (ICG-HNPCC) proposed a set of diagnostic criteria (Revised Amsterdam Criteria) to provide uniformity in clinical studies {3010}. These criteria identify families that are very likely to represent HNPCC. Other widely used criteria are the Bethesda Criteria that can be used to identify families suspected of HNPCC that need testing for microsatellite instability {2398}.

Endometrial tumours

Predisposed individuals from HNPCC families have a high risk (30-80%) of developing colorectal cancer. The most frequent extracolonic cancer is endometrial cancer. The lifetime risk of developing this cancer is 30-60% by age 70 {14,731,3009,3071}. HNPCC-associated endometrial cancer is diagnosed approx. 10 years earlier than in the general population. The mean age at diagnosis is 50 years. Patients with colorectal cancer associated with HNPCC have a better prognosis than patients with common sporadic colorectal cancer {2526,3070}. In contrast, a recent study showed that the survival of endometrial cancer associated with HNPCC does not differ significantly from endometrial cancer in the general population {305}.

Pathology of endometrial tumours

In patients from families with proven germline mutations in the MMR genes, *MLH1*, *MSH2*, *MSH6*, or from (suspected) HNPCC families, the majority of endometrial tumours were reported to be of the endometrioid type with diverse grading and staging {650,2174}. Certain histopathologic features such as mucinous differentiation, solid-cribriform growth pattern, high grade and possible necrosis might suggest that a tumour is due to a mismatch repair defect {1481, 2174,2206}.
Loss of MLH1 protein expression occurs in endometrial cancer associated with HNPCC {235,650,1276, 1768,2174,2264} but also in 15-30 % of sporadic cancers with somatic inactivation of MLH1 {2518,2772}.
Abrogation of MSH2 and/or MSH6 protein expression, especially at a young age seems to be a more specific indicator for HNPCC {235, 650, 2174,2264}. Already in the hyperplastic precursor lesions such loss of expression can be encountered {235, 650}.

Other cancers

Many other cancers have been reported in HNPCC {13,14,3009,3010}. The frequency of specific cancers depends on the prevalence of the cancer in the background population {2178}. Cancer of the stomach for example is frequently observed in families from Finland and Japan, both countries with a high prevalence of stomach cancer in population. The ages at diagnosis of most cancers reported are earlier than their sporadic counterparts.

Table 8.08
Revised Amsterdam Criteria.

There should be at least three relatives with colorectal cancer (CRC) or with an HNPCC-associated cancer: cancer of the endometrium, small bowel, ureter or renal pelvis.

- one relative should be a first degree relative of the other two,
- at least two successive generations should be affected,
- at least one tumour should be diagnosed before age 50,
- familial adenomatous polyposis should be excluded in the CRC case if any,
- tumours should be verified by histopathological examination.

Table 8.09
Bethesda Criteria.

1. Individuals with two HNPCC-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers (endometrial, ovarian, gastric, hepatobiliary, small bowel cancer or transitional cell carcinoma of the renal pelvis or ureter)
2. Individuals with colorectal cancer and a first degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or colorectal adenoma; one of the cancers diagnosed at age <45 y, and the adenoma diagnosed at age <40 y
3. Individuals with colorectal cancer or endometrial cancer diagnosed at age <45 y
4. Individuals with right-sided colorectal cancer with an undifferentiated pattern on histopathology diagnosed at age <45 y
5. Individuals with signet-ring-cell-type colorectal cancer diagnosed at age <45 y
6. Individuals with adenomas diagnosed at age <40

Genetics of *MLH1*, *MSH2*, *MSH6*

Chromosomal location and structure

HNPCC is associated with germline mutations in five genes with verified or putative DNA mismatch repair function, viz. *MSH2* (MutS homologue 2), *MLH1* (MutL homologue 1), *PMS2* (Postmeiotic segregation 2), *MSH6* (MutS homologue 6), and possibly *MLH3* (MutL homologue 3). Structural characteristics of these genes are given in Table 8.11. Endometrial cancer appears to be part of the syndrome in families with mutations in any one of these genes, but is particularly associated with *MSH2* and *MSH6* germline mutations {236,3011,3114}.

Table 8.11
Characteristics of HNPCC-associated human DNA mismatch repair genes.

Gene	Chromosomal location	Length of cDNA (kb)	Number of exons	Genomic size (kb)	References
MSH2	2p21	2.8	16	73	{1495,1680 2213,2563}
MLH1	3p21-p23	2.3	19	58-100	{353,1121,1494 1666,1679,2167}
PMS2	7p22	2.6	15	16	{2008,2010}
MSH6	2p21	4.2	10	20	{30,2009. 2163,2563}
MLH3	14q24.3	4.3	12	37	{1674}

Gene product

HNPCC genes show ubiquitous, nuclear expression in adult human tissues, and the expression is particularly prominent in the epithelium of the digestive tract as well as in testis and ovary {860,1602, 3132}. These genes are also expressed in normal endometrium, and loss of protein expression is an early change in endometrial tumorigenesis. Studies of *MSH2* or *MLH1* mutation carriers have shown that these proteins may be lost already in atypical hyperplasia (precursor lesion of endometrial cancer) or even in endometrial hyperplasia without atypia in several months before the diagnosis of endometrial cancer, suggesting that immunohistochemical analysis of MSH2 and MLH1 proteins may be useful for pre-screening purposes in HNPCC patients {235,1277}.

Gene function

The protein products of HNPCC genes are key players in the correction of mismatches that arise during DNA replication {1496}. Two different MutS-related heterodimeric complexes are responsible for mismatch recognition: MSH2-MSH3 and MSH2-MSH6. While the presence of MSH2 in the complex is mandatory, MSH3 can replace MSH6 in the correction of insertion-deletion mismatches, but not single-base mispairs. Following mismatch binding, a heterodimeric complex of MutL-related proteins, MLH1-PMS2 or MLH1-MLH3, is recruited, and this larger complex, together with numerous other proteins, accomplishes mismatch repair. The observed functional redundancy in the DNA mismatch repair protein family may help explain why mutations in *MSH2* and *MLH1* are prevalent in HNPCC families, while those in *MSH6*, *PMS2* and *MLH3* are less frequent (and *MSH3* mutations completely absent), although alternative hypotheses (e.g. based on the differential participation of the DNA mismatch repair proteins in apoptosis signaling {863}) have also been proposed.

It is not known why some female HNPCC patients develop endometrial cancer, while others develop colon cancer. Comparison of these two tumour types originating from identical germline mutation carriers suggests the existence of some important tissue-specific differences that may indicate different pathogenetic mechanisms. For example, acquired loss of *MSH2* and *MSH6* appears to characterize endometrial, but not colon carcinomas developing in patients with inherited mutations of *MLH1* {2589}. Moreover, the general MSI patterns and target genes for MSI seem different in endometrial and colorectal cancers from HNPCC patients {1527}. Early inactivation of *PTEN* characterizes most endometrial cancers from HNPCC patients {3261} and tumorigenesis mediated by PTEN inactivation is accelerated by mismatch repair deficiency {3052}. Apart from biosynthetic errors, the DNA mismatch repair proteins also recognize and eliminate various types of endogenous and exogenous DNA damage, and differential exposure to such agents or variable capacity to correct lesions induced by them may also play a role in the organ-specific cancer susceptibility in HNPCC {655}.

Table 8.10
Extracolonic cancer in 144 HNPCC families known at the Dutch HNPCC Registry.

Cancer site	Number	Mean age (yrs)	Range (yrs)
Endometrium	87	49	24-78
Stomach	26	51	23-82
Ureter/pyelum	24	55	37-72
Small bowel	22	51	25-69
Ovarian cancer	28	48	19-75
Brain	18	42	2-78

Gene mutations

The International Collaborative Group on HNPCC maintains a database for HNPCC-associated mutations and polymorphisms (http://www.nfdht.nl). To date (May 2002), there are 155 different *MSH2* mutations (comprising 39% of all mutations) and 200 (50%) *MLH1* mutations reported to the database, together with 30 (8%), 5 (1%) and 10 (3%) mutations in *MSH6*, *PMS2*, and *MLH3*, respectively. Most *MSH2* and *MLH1* mutations are truncating {2214}. However, 30-40% of *MLH1* and *MSH6* mutations are of the missense type (leading only to an amino acid substitution), which constitutes a diagnostic problem concerning their pathogenicity. Besides commonly used theoretical predictions (evolutionary conservation status of the amino acid, conservativeness of the amino acid change, occurrence of the variant in the normal population, co-segregation with disease phenotype) functional tests may be nec-

essary in the evaluation of the pathogenicity of missense changes.

Microsatellite instability

Microsatellite instability (MSI) is the hallmark of tumours that arise in carriers of *MLH1*, *MSH2*, of *MSH6* mutations. Overall, MSI is detected in approximately 15% of all colorectal cancers. It is measured as alterations in the length of simple repetitive genomic sequences, usually dinucleotide repeats, or mononucleotide runs. As these repeats have a tendency to form mismatches during DNA replication, a mismatch repair defect is expected to increase their mutation frequency. Because the definition of instability applied has been variable, in 1998 an international working group recommended the use of five markers to assess MSI {306}. Tumours are characterized as having high-frequency MSI (MSI-H) if two or more of the five markers show instability (i.e. have insertion/deletion mutations), or as having low-frequency MSI (MSI-L) if only one of the five markers shows instability. The distinction between microsatellite stable (MSS) and low frequency MSI (MSI-L) can only be accomplished if a greater number of markers is utilized. MSI analysis, in conjunction with immunohistochemistry, can greatly improve the efficacy of the molecular screening for HNPCC {650,2516}.

In one study, all 12 endometrial carcinomas from carriers of *MLH1* and *MSH2* germline mutations demonstrated an MSI-high phenotype involving all types of repeat markers, while this was found in only 4 out of 11 (36%) endometrial carcinomas from *MSH6* mutation carriers {650}. In another study, MSI-patterns in endometrial cancers differed from those in colorectal cancers, even though the patients had identical predisposing mutations in the MMR genes *MLH1* or *MSH2* {1527}. In endometrial cancers, the pattern was more heterogeneous and involved a lower proportion of unstable markers per tumour and shorter allelic shifts for BAT markers. These results might point to gene-specific and/or organ-specific differences that may be important determinants of the HNPCC tumour spectrum.

Mutation spectrum

Hereditary non polyposis colorectal cancer (HNPCC) is caused by germline mutations in one of 5 DNA mismatch repair genes (MMR): *MSH2* {864}, *MLH1* {353}, *PMS1* {2010}, *PMS2* {2010}, and *MSH6* (formerly GTBP) {53,1884}. Other genes like *EXO1* {3161}, *MLH3* {1674,3162} and *TGFbRII* {1705} have been reported to possibly cause HNPCC-like syndromes, although no definitive evidence has been delivered yet, both in terms of pathogenicity and/or cosegregation with the disease of the alleged germline mutations in affected families.

To date, more than 300 different predisposing mutations have been identified, most in *MSH2* and *MLH1* and in families complying with the clinical Amsterdam criteria (AMS+) {2214}. Many HNPCC families, however, do not fully comply with these criteria, and in most of these cases the disease-causing mutations are yet unknown. Mutations in *MSH6* have been found in atypical HNPCC families (see below).

In general, MMR mutations are scattered along the coding sequence of *MSH2* and *MLH1* and predict either the truncation of the corresponding protein products, or a subtler amino acid substitutions. These mutations appear evenly distributed throughout the coding regions of the main MMR genes, with some clustering in *MSH2* exon 12 {2214} and *MLH1* exon 16 {3115}. While most of the *MSH2* mutations consist of frameshift or nonsense changes, *MLH1* is mainly affected by frameshift or missense alterations. Most of the mutations found to date are unique, with a few common recurring ones {2214}. Genomic deletions have also been found at both loci {442,2070, 3116}. *MSH2* deletions appear to be a very frequent cause of HNPCC, contributing for up to a quarter of the families selected by Amsterdam criteria {3116}. *MLH1* deletions are less frequent than in *MSH2* {1793,2070}. Southern analysis and/or other PCR-based methods to detect larger rearrangements at the genomic level {443} should be routinely employed when approaching the mutation analyses of these major mismatch repair genes.

Genotype-phenotype correlations

The combination of clinical (number and type of tumours, age of onset, clinical course of the disease, etc.) and genetic (different mismatch repair genes, truncating and missense mutations) heterogeneity in HNPCC represents an ideal opportunity to attempt the establishment of genotype-phenotype correlations. Unfortunately, and notwithstanding the large number of mutations and clinical data collected to date, no clear-cut correlations have been observed between specific MMR gene mutations and their clinical outcome. For example, the identification of identical mutations both in HNPCC and in Muir-Torre or Turcot syndrome does not support the existence of consistent genotype-phenotype correlations {179,1115,1494}.

The most reliable correlation found to date is the association between clear-cut pathogenic mutations at *MSH2*, *MLH1* and *MSH6*, and the resulting spectrum of colorectal and extracolonic tumours. HNPCC kindreds due to *MSH2* or *MLH1* germline mutations are characterized by high penetrance and early onset of colorectal and endometrial cancer. The diagnostic criteria, Amsterdam I and II, established by the International Collaborative Group on HNPCC {3008, 3010} well serve the purpose of selecting families with a high likelihood to carry *MSH2* and *MLH1* mutations {3117}. In addition to the fulfillment of the above criteria, other factors represent valid predictors of the presence of germline *MSH2* and *MLH1* mutations in HNPCC families. These include 1. young age at diagnosis of colorectal cancer, and 2. the occurrence of at least one patient with an extra-colonic cancer, such as those of the endometrium, small intestine, brain, and stomach, within an AMS+ HNPCC kindred. The frequency of mutations identified in these families increased to about 70% {3117}. Moreover, the occurrence of at least one patient with multiple synchronous or metachronous colorectal cancers, and the combined occurrence of colorectal cancer with endometrial cancer in one patient are very good predictors of *MSH2* or *MLH1* mutations {3117}.

The first reports on *MSH6* germline mutations already indicated that the clinical phenotype differed from the "classical" HNPCC caused by *MSH2* and *MLH1* mutations {53,1884}. More recently, *MSH6* germline mutations have been demonstrated in a considerable number of the atypical HNPCC families, i.e. not complying with the Amsterdam criteria (ACI and II) {1497,3039,3114,3160}. In general, the penetrance of colorectal

cancer seemed to be reduced while endometrial cancer seems to represent a more important clinical manifestation among female *MSH6* mutation carriers. Also, the mean age of onset of colorectal and endometrial cancer appeared to be delayed in families with *MSH6* germline mutations {3011,3039,3114}. Notably, MSI analysis of tumours from *MSH6* mutation carriers suggests a reduced penetrance of the MSI-H phenotype and preferential instability at mononucleotide repeats {650,1497,3114,3160}.
An additional *MSH6*-associated clinical phenotype is the papillary transitional cell carcinoma of the ureter and renal pelvis, observed in approx. 10% of the carriers from an extended *MSH6* kindred {3039}. Notably, the lifetime cumulative risk of this tumour type in *MLH1* or *MSH2* mutation carriers is only 2.6% {2673}.

Ataxia telangiectasia syndrome

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Definition
Ataxia telangiectasia syndrome (A-T) is a rare, progressive neurological disorder that manifests at the toddler stage. The disease is characterized by cerebellar degeneration (ataxia), dilated blood vessels in the eyes and skin (telangiectasia), immunodeficiency, chromosomal instability, increased sensitivity to ionizing radiation and a predisposition to cancer, in particular leukaemias and lymphomas. Germline mutations in the *ATM* gene (ataxia telangiectasia mutated), homozygous or compound heterozygous, are the cause of this autosomal recessive disorder. Heterozygous carriers are phenotypically unaffected but exhibit an increased risk to develop breast cancer and often display a variety of age related disorders which may result in reduced life expectancy {2808}.

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Synonyms
Louis-Bar Syndrome, A-T complementation group A (ATA), group C (ATC), group D (ATD) and group E (ATE). The different complementation groups are all linked to the *ATM* gene.

Incidence
The rare A-T disease occurs in both genders and world wide among all races. The disease has an estimated incidence of one per 40,000 to one per 300,000 live births. Approximately 0.2-1% of the general population has been estimated to be heterozygous carriers of a type of germline mutation in the *ATM* gene that in homozygous state causes the A-T syndrome.

Tumours in A-T patients
Individuals with A-T have a 50 to 150 fold excess risk of cancer, with approximately 70% being lymphomas and T cell leukaemias. In younger patients, an acute lymphoblastic leukaemia is most often of T-cell origin, although the pre-B common ALL of childhood has also been seen in A-T patients. When leukaemia develops in older A-T patients it is usually an aggressive T-cell leukaemia (T-PLL, T cell prolymphocytic leukaemia). Lymphomas are usually B cell types. A wide range of solid tumours makes up the remainder of the tumours seen in A-T patients and includes cancers of the breast, stomach, ovary and melanoma.
The presence of missense mutations in A-T patients has been associated with a milder clinical phenotype and altered cancer predisposition. In two British A-T families a T>G tranversion at base pair 7271 was found to be associated with a milder clinical phenotype, lower radiosensitivity but an increased risk of breast cancer. This increased risk was observed in both the homozygote and heterozygotes carriers of this modification (RR 12.7 p=0.0025) {2775}. This sequence alteration has subsequently been found in multiple-case breast cancer families. The expression and activity analyses of the ATM protein in heterozygous cell lines carrying this sequence change indicated that this mutation was dominant negative {462}.

Breast cancer in *ATM* heterozygotes
Heterozygous carriers of *ATM* mutations have a higher mortality rate and an earlier age at death from cancer and ischemic heart disease than non-carriers {2808}. A-T heterozygotes have been reported to have a 3 to 8 fold increased risk of breast cancer. The association between *ATM* heterozygosity and breast cancer risk was initially found among blood relatives of A-T patients {2820}, and in almost every study of A-T relatives since an increased breast cancer risk has been detected {318,741,981,1291, 1334,2105,2257,2819}. Paradoxically, in the years following the cloning of the *ATM* gene {2546}, several studies investigating large breast cancer cohorts failed to find an increased incidence of *ATM* mutations of the type found in A-T patients, and a controversy arose regarding the role of *ATM* in breast cancer susceptibility {194,281,884}. However, a number of recent studies, analysing the frequency of all type of *ATM* mutations, did confirm previous findings of an elevated breast cancer risk in *ATM* mutation carriers {129,351, 462,2592,2775}.

Age distribution and penetrance
Most of the studies finding an increased risk of breast cancer in A-T relatives point to an early onset of the disease. The penetrance has been difficult to estimate since most of the studies are small, and different mutations may have different effects. In several studies of A-T relatives, the elevated risk is restricted to obligate carriers (mothers), and is not increased in other relatives according to their probability of being a mutation carrier. This may point to an interaction with environmental and/or other genetic factors contributing to the elevated breast cancer risk.

Clinical and pathological features
No typical clinical or pathological features are so far known for *ATM* heterozygous breast cancer patients, other than early age at onset (before age 50) and frequent bilateral occurrence {351}.

Response to therapy and prognosis
ATM heterozygotes with breast cancer do not seem to exhibit acute radiation sensitivity as A-T patients do, and excessive toxicity has not been observed after radiotherapy {115,2331,3088}. It has however been speculated whether *ATM* heterozygous breast cancer patients have an increased risk of developing a second breast cancer after radiation treatment, and large multi-center studies are ongoing to answer this question.
There are only few studies evaluating the prognosis of A-T carriers with breast cancer, pointing to a long-term survival. This may be due to their tumours being more susceptible to cell killing by ionizing radiation than tumour cells in non-carriers {2809}.

ATM expression in breast cancer
Normal breast tissue shows a distinct pattern of ATM expression, the protein being found in the nucleus of the ductal epithelial cells and to a lesser extent in the surrounding myoepithelial cells. Decreased ATM expression is often observed in breast carcinomas {102, 1384} and *ATM* mRNA levels have also been found to be lower in invasive breast carcinomas than in normal tissues or benign lesions {3041}.
Significant loss of heterozygosity in sporadic breast tumours across chromosome 11q22-23 where the *ATM* gene is located has been reported {1118,1439, 1553,1754,2375}.

Table 8.12
Proposed *ATM* genotype / phenotype relationships {971}.

Genotype	Phenotype
$ATM^{wt/wt}$	Normal
$ATM^{trun/trun}$	Ataxia telangiectasia, High cancer risk
$ATM^{trun/mis}$	Ataxia telangiectasia, Variant A-T?, High cancer risk?
$ATM^{mis/mis}$	Ataxia telangiectasia? Variant A-T?, High cancer risk?
$ATM^{trun/wt}$	A-T relatives, Elevated breast cancer risk, Increased age related disorders?
$ATM^{mis/wt}$	Few A-T relatives, Moderate breast cancer risk? Increased age related disorders?

Genetics

Chromosomal location
The *ATM* gene is located on human chromosome 11q22-23.

Gene structure
The *ATM* gene has 66 exons scanning 150 kilobase of genomic DNA and is expressed in a wide range of tissues as an approximately 12-kilobase messenger RNA encoding a 350 kD serine/threonine protein kinase. The initiation codon falls within exon 4. The last exon is 3.8kb and contains the stop codon and a 3'-untranslated region of about 3600 nucleotides {2983}.

Gene expression
The major 13 kb *ATM* transcript is observed in every tissue tested to date. Northern blots and RT-PCR products from various tissues failed to disclose any evidence of alternative forms within the coding region. However the first four exons, which fall within the 5'-untranslated region (UTR), undergo extensive alternative splicing. Differential polyadenylation results in 3'UTRs of varying lengths. These structural features suggest that *ATM* expression might be subject to complex post-transcriptional regulation {2547}.

Gene function
The ATM protein plays a central role in sensing and signalling the presence of DNA double-strand breaks (DSBs) formed in cells as a result of normal DNA metabolism (e.g. meiotic or V(D)J recombination) or damage caused by external agents. The kinase domain in the carboxy-terminal region of the protein contains the signature motifs of phosphatidylinositol 3-kinases. ATM's kinase activity is itself enhanced in response to DNA double-strand breaks resulting in a phosphorylation cascade activating many proteins each of which in turn affects a specific signalling pathway. These substrates include the protein products of a number of well characterized tumour-suppressor genes including *TP53*, *BRCA1* and *CHEK2* which play important roles in triggering cell cycle arrest, DNA repair or apoptosis (reviewed in Shiloh et al. {2660}). Additional DSB-induced responses that are ATM dependent include the activation of transcription factors such as AP-1, p73 and NFKB, and deacetylation of chromatin proteins (reviewed in Barzilai et al. {189}).

Mutation spectrum in A-T patients
Since the *ATM* gene was cloned {2546} more than 300 different A-T disease-causing mutations have been reported. The profile of these has revealed that most are unique and uniformly distributed along the length of the gene, no mutational hotspots have been detected. The majority of A-T patients are compound heterozygotes having two different *ATM* mutations and patients homozygous for the same *ATM* mutation are rare. The predominate type of mutation found in the *ATM* gene in A-T patients results in a truncated and unstable ATM protein. Some A-T patients have a milder phenotype (variant A-T) that may be related to the presence of missense mutations or mutations producing an ATM protein retaining some normal function {1825,2592}.

Genotype-phenotype correlations
Gatti et al. {971}, in distinguishing between truncating mutations where no

ATM protein is detected and missense substitutions where mutant protein of variable stability is observed, have suggested that this mutant protein could produce a dominant negative effect in heterozygotes, resulting in an altered phenotype and an increased breast cancer susceptibility. The expected phenotypes that might arise from having two types of A-T carriers in the general population are shown in Table 8.12.

The genotype $ATM^{trun/trun}$ with two truncating mutations causes the classical A-T disorder. The genotype $ATM^{mis/mis}$ with two missense mutations is also found in some children with the classical form of the disease, in particular when these are located within the ATM kinase domain (for instance Belzen et al. {2987}, Angele et al. {101}) but may also be associated with a variant A-T phenotype with some neurological features and cancer susceptibility. Two types of *ATM* heterozygotes exist and the phenotypes differ, i.e. those with truncating mutations that make no protein and those with missense mutations that make reduced amount or partly defective protein {115,462,971, 1825,2331,2592,2775,2809,3088}, and these two groups may have different breast cancer risks. If this proposed model is correct it necessitates a re-analyses of the epidemiological data stratifying for the two types of heterozygotes. The literature to date suggests that germline *ATM* missense mutations are more frequent than the 0.2-1% frequency of A-T causing mutations and hence contribute to a larger fraction of breast cancer patients {351,462,2592}.