CHAPTER 5

DISCUSSION

5.1 Pathological background of the patients

In this study, pathological features of 31 patients were invasive ductal carcinoma and stage I grouping (6/17) (Appendix A). The study also showed that the stage I grouping was mostly found in 17 Thai breast cancer patients with (2/17) and (6/17) of high and low-grade tumors frequency, respectively. The right breast side (R) was found greater than the left breast side (L) and an average age at diagnosis in the study was 48 years old. Some reports suggested that the *BRCA1*-related breast tumors significantly more often belonged to ductal type (Johannsson *et al*., 1997). Nevertheless, medullary carcinoma, tubular carcinoma, invasive lobular carcinoma and lobular carcinoma *in situ* were reported to be found more commonly in association with a positive family history of breast cancer than other cancer subtypes (Breast Cancer Linkage Consortium, 1997 ; Lakhani, 1999). This work was limited by small numbers of cases, a lack of clinicopathological profile of patients and no strong family history. Hence, it may be difficult to use the histologic and clinical phenotype of an individual patient's breast cancer in this study in predicting whether she has an underlying germ-line mutation in *BRCA1*.

The previous studies indicated that the age onset breast cancer on Thai patients with inherited germ-line *BRCA1* mutation was at younger than 32 years (Patmasiriwat *et al*., 2002). Age was a powerful influence on the risk of cancer. Age-specific incidence rates were shown to rise to maximum around age 50 with a small decline in risk at older ages in Thailand (Chindavijak and Martin, 1999). Generally, most of the Asian population's data available on *BRCA1* mutation was derived from family-based studies and case reports (Liede and Narod, 2002). The age variation at diagnosis of breast cancer depends on the population and criteria studied. The age distribution at diagnosis of breast cancer may be different in each country. In Asian population, the mean age of breast cancer was generally younger than that in North America and Europe (Liede and Narod, 2002). The age-specific rates for

breast cancer have been attributed to the responsiveness of breast tissue to ovarian hormone. Therefore, age-specific incidence rates are actively associated from puberty to menopause (Hulka and Moorman, 2001). Postmenopausal women in the North America and Europe are on average, much heavier than Asian women and have higher levels of endogenous estrogen. The postmenopausal breast cancer patients have been more preferentially found than the premenopausal breast cancer patients. It suggested that postmenopausal breast cancer was relatively less common among Asian population with 10% of young female Asian population over age 50 years compared with 27% of young female North American population (Liede and Narod, 2002).

Recent data showed that female carriers of the *BRCA1* mutation were initially estimated to have an 87% risk of developing breast cancer during their lifetime (Hofmann *et al*., 2000 ; White *et al*., 2002). Thus, the risk of the proband's daughter carrier with *BRCA1* mutation was estimated as above. However, the histological features of proband's daughter was unknown. Histological features of breast tumors was dependent on age at diagnosis (Vaziri *et al*., 2001). Therefore, estimation of risk requires combination of data such as other histopathological features of patients by using predictive markers such as estrogen receptor (ER), progesterone receptor (PR), HER-2, p53 and CA 15-3, morphology and clinical data, for patient harboring mutations in the *BRCA1* and sporadic breast cancers. The information could be regarded as a feature of phenotype triggered by the *BRCA1* germ-line mutation or could help in selecting subjects at high risk of developing the inherited form of the disease, assessing prognosis, guiding therapy and monitoring patients with diagnosed disease (Raicevic-Maravic and Radulovic, 2000 ; Duffy, 2001).

5.2 PCR-SSCP analysis

Amplification of *BRCA1* exons was performed by polymerase chain reaction. The major requirement for PCR-SSCP analysis is the quality and quantity of PCR products and the size of the amplification product that should be 200-400 bp (Iau *et al*., 2001). Most PCR products in this study were about 200-400 bp, although

few were larger than 400 bp. The PCR conditions were optimized and each exon's PCR product was verified by agarose gel electrophoresis. The SSCP analysis was performed under non-denaturing conditions. Each single-stranded DNA molecule adopts a specific conformation at a given temperature. Most changes in the DNA sequence result in a change of DNA conformation (Orita *et al*., 1989). Thus, the single-stranded DNA carrying mutation can be identified by its altered electrophoretic mobility on a non-denaturing polyacrylamide gel. The abnormal PCR bands of the patient were compared to those of wild type bands of normal control individual. The presence of an abnormal band was finally verified by direct sequencing. The SSCP band patterns of each exon in this study were different depending on PCR fragment and size. Theoretically, the simplest band pattern includes 5 bands if both mutated and wild-type sequences are present, one band for each single-stranded DNA sequence and one band for the double-stranded DNA, which in some systems may appear between single-stranded bands (Sheffield *et al*., 1993 ; Welsh *et al*., 1997). In this study, the double-stranded bands apparently run off the gel. The study showed that 9/31 of the DNA fragment mobility shifts were detected with SSCP, but only 1/31 of these contained mutations when confirmed by direct sequencing. The single-stranded band tended to faint, which depended on PCR fragment and size (some reanneal more readily than others) (Humphries *et al*., 1997). This causes a problem for the detection of variants and can lead to misdetection. Therefore, a combination of SSCP analysis with other mutation detection methods (heteroduplex analysis, protein truncation test or denaturing high-performance liquid chromatography) might provide more reliable results than either one alone (Soto and Sukumar, 1992 ; Kozlowski *et al*., 1996 ; Geisler *et al*., 2001).

It was observed that magnesium, template and primer concentrations influenced the formation of specific SSCP patterns. Gel matrix composition, glycerol or other factors have minimal effects on reproducibility of SSCP patterns (Hennessy *et al*., 1998 ; Bitenc and Marc, 2001). The snap chilling was also reported to have no effect on SSCP band mobility. These results suggest that the refolding of ssDNA occurs rapidly and is more stable than previously thought (Hennessy *et al*., 1998). Some reports indicated that temperature was the most important factor influencing the

sensitivity of SSCP analysis (Glavac and Dean, 1993 ; Hayashi and Yandell, 1993 ; Humphries *et al*., 1997). In this study, analysis for the *BRCA1* mutation was conducted at room temperature for electrophoretic separation. It gave satisfactory results, that could be obtained in a single day. The PCR product bands which showed only weak amplification could still be detected, and therefore, small volumes of samples were needed (Bassam *et al*., 1991). The staining artifacts could occur during silver staining of PCR products. Artifacts can be overcome by increasing the length of washing steps after sensitisation and staining (Sanguinetti *et al*., 1994 ; Vari and Bell, 1996). A fixation step before sensitisation and higher formaldehyde concentration increased the contrast of the bands even at high concentration of nucleic acid, producing a shift in the tone of color from black to grey instead of gold to yellow bands (Vari and Bell, 1996). In a reason above, another factor contributing to the ambiguity of this study that the screening methods under study may have not been suitable for detecting the types of mutation that occurred in the gene. Thus, the mutations were missed in the regions of *BRCA1* or may exist in the promoter and intron regions of the *BRCA1* gene and therefore have not been analyzed. For instance, the sensitivity of SSCP analysis is approximately 70 to 95 percent under the assay conditions used (Arnold *et al*., 1999 ; Gross *et al*., 1999 ; Iau *et al*., 2001). Moreover, the identification of large genomic rearrangement (both deletions and duplications) would be missed by conventional PCR-SSCP or direct sequencing. PCR analysis has been shown to give variable amplification of mutant alleles, which could lead to rearrangements being missed if testing is by this method alone (Couch, 1998 ; Robinson *et al*., 2000). Previous report indicated undetected mutations including large genomic deletion in several families of Dutch patients with breast cancer (Petrij-Bosch *et al*., 1997). The semi-quantitative PCR-based fluorescent assay and gene dosage methods are able to detect large rearrangements in *BRCA1*. Gene dosage methods are detection as size changes at the mRNA level like real-time PCR and multiplex PCR methods (Robinson *et al*., 2000 ; Armour *et al*., 2002). The protein truncation test (PTT) would be chosen as a screening method for frameshift or nonsense mutations that result in premature termination of the exon 11 (Geisler *et al*., 2001). The major advantage of SSCP is its ability to detect point mutations that result

in deletions and insertions (Markoff *et al*., 1998 ; Campos *et al*., 2001). Any putative mutations identified by the screening techniques would ultimately be confirmed by direct sequencing.

A survey of the sequences in the patients whose exons exhibiting changes in SSCP analysis were amplified by PCR using *Pfu* DNA polymerase. The fidelity of DNA synthesis by the *Pfu* DNA polymerase was not different *Taq* DNA polymerase in the PCR. Furthermore, there are patterns of the sequence similarities with the prior study. Thus, occurred mutations in patient ID 17 and patient's healthy daughter (ID 18) were not relevant error of *Taq* DNA polymerase during PCR. We are proposing that the small deletion of nucleotide sequences within intron 7 is involvement of the triplet repeat sequence of TTC.GAA. It is causing an increase in the proportion of misaligned primer-template DNA structures available to the polymerase, a slipped strand mispairing model.

Additionally, the sequence data of ID 17 and ID18 may be found gradual signal loss after trinucleotide repeats region. Previous studies with DNA templates that are rich in di, tri and tetranucleotide repeats have suggested hinder sequencing. Many of the artifacts in sequencing are known to be due to reduced stability of the hybrid formed between the template and the nascent strand. There is no single method for sequencing difficult templates with repeat regions. Therefore, applying a combination of available sequencing protocols (such annealing and denaturation times, the type of denaturant in the reaction mixture and the type of sequencing chemistry), clone the PCR product or anchored primers may be effective in overcoming the sequencing difficulties in tandemly repeated DNA templates. (Zhao *et al*., 2000).

5.3 *BRCA1* mutations in Thai breast cancer patients

The mutation screening for the *BRCA1* gene in 31 Thai breast cancer patients has been performed by SSCP analysis and DNA sequencing. Five out of thirty-one patients (16.1%) had a family history and one out of five patients (20%) with family history had *BRCA1* mutation. Twenty-six out of thirty-one patients without family history (83.9%) did not exhibit the *BRCA1* mutation. These results

represented a minimal estimate of the frequency of *BRCA1* mutations in this population. The risk of harboring a mutation may be limited to women with family histories of breast cancer. Thus, the families with a large number of relatives affected with breast cancer and ovarian cancer may be the important factor in assessing *BRCA1* mutation risks. In addition, some study has reported that mutation frequency was greater in those with both breast and ovarian cancer family versus those with breast cancer-only family history (Couch *et al*., 1997). Thus a clear information of family history is necessary. Finally, some of these cases may just sporadic cancer.

The result from the exon-intron 7 boundary analysis showed intronic *BRCA1* mutations in the proband ID 17 and her daughter. The IVS7 + 34_47delTTCTTTTCTTTTTT was found in patient ID 17. The patient's healthy daughter carried two unclassified intronic *BRCA1* mutations (IVS7+34_47delAAGAAAAGAAAAAA and IVS7+50_63delTTCTTTTTTTTTTT) and one unclassified point mutation (IVS7+38T > C). This is in discrepancy with the belief that significant contribution to inherited breast cancer by this gene, which has been proposed to account for approximately 50% of all hereditary cases (Easton *et al*., 1993). However, it gets along with the belief that significant hereditary breast cancer is caused by genetic abnormality, which has been proposed to account for 5-10% of all breast cancer (Appendix B). Nevertheless, the identified intronic mutations may be associated with an increased likelihood of cancer, but we cannot consider them to be definite mutations on the basis of sequence information alone. Thus, analysis of abnormality of RNA splicing will be necessary to confirm the detected mutation.

It appears to be similar to the finding of Patmasiriwat *et al*. (2002) in some part of this study, such as there are some populations that have a very low or undetectable *BRCA1* involvement in breast cancer. Additionally, 20 to 30% of breast cancer with family histories have *BRCA1* and *BRCA2* mutations, similar extent as reported in Japan, China and Taiwan. Therefore, the occurrence of breast cancer associated with mutation in *BRCA1* varies and depends on the population studied and family selection criteria should be stringent (at least two breast cancer cases diagnosed at any age among first degree relatives). *BRCA1* mutations found in this study were different from those reported by Patmasiriwat *et al*. (2002). The result

implied that the frequency of the occurrence of *BRCA1* mutation in patients with strong family history background were high.

The results showed a relative low number of *BRCA1* genetic changes in Thai breast cancer patients compared to those in Westerner. The low frequency of *BRCA1* Asian mutations have been reported in other populations such as Japanese, Korean and Taiwanese (Liede and Narod, 2002). The unexpectedly low frequency of *BRCA1* mutation found in this work could be due to the presence of mutations in either non-coding regions or regulatory regions of the gene, the involvement of other cancer susceptibility genes such as *BRCA2, HER-2 or p53* (Easton, 1999 ; White *et al*., 2002). These observations illustrate the difficulty in predictions for the presence or absence of *BRCA1* mutations on the basis of a woman's family history.

The occurrence of deletional mutation, IVS7 + 34_47del TTCTTTTCTTTTTT, was found in patient ID 17. The deletion was in the noncoding intervening sequence at the +34 to +47 relative to exon 7 of the *BRCA1* gene. Surprisingly, this type of mutation has not previously been reported in the BIC database (Breast Cancer Information Core, 2004). It was notable that the region of deletional mutation in patient ID 17 was nearby the triplet repeat sequence (TRS) of TTC·GAA as shown in Fig. 5.1. The black frame in Fig. 5.1 showed the seven TTC·GAA repeats in normal nucleotide sequence, compared to six TTC repeats in patient ID 17 as shown in Fig. 5.2.

Generally, the *BRCA1* gene contains very high densities of repetitive elements (47%), including 42% of *Alu* sequences and 5% of non-*Alu* repeats. These repetitive sequences might contribute to genetic instability (Welcsh and King, 2001). The triplet repeat sequences have been found to be a cause of inherited human disease as genetic neurodegenerative disease. The expansions or deletions of the TRS were responsible for disease (Monckton and Caskey, 1995 ; Sinden, 1999 ; Wells *et al*., 2005). The triplex repeat disease genes and triplex repeat of *BRCA1* gene were located within exons and introns as summarized in Fig. 5.3 and 5.4, respectively. Disorders of trinucleotide repeat expansion and deletion were summarized in Table 5.1. The structure of TTC·GAA repeat has been proposed to adopt a non-B DNA structure and form the intramolecular triplex DNA based on Y·R·Y triplexes

(pyrimidine · purine· pyrimidine) as shown in Fig. 5.5 and Table 5.2 (Bidichandani *et al*., 1998 ; Gacy and McMurray 1998 ; McMurray, 1999 ; Sinden, 1999 ; Cavadini *et al*., 2000 ; Sinden *et al*., 2002). So far, the TRS associated diseases have not yet been mentioned in the *BRCA1* gene. Thus, deletion of one TTC repeat in this study probably represented the novel type of deletional mutation in the *BRCA1* gene. Schematic diagram of the repeat tract in triplex repeats of the *BRCA1* gene was proposed in Fig. 5.6.

It was implied that a small deletion of nucleotide sequences within intron 7 of the *BRCA1* gene is somehow attributed to the involvement of the triplet repeat sequence of TTC·GAA. The molecular defect of deletional mutation may have been proposed by replication slippage during DNA replication. After synthesis of the first copy of the repeat, the template strand could slip and misalign with the second copy of the repeat as the result of the intervening sequence deletion (Trinh and Sinden, 1991 ; Xu and Solomon, 1996). The continued DNA synthesis will lead to the deletion of one direct repeat. The deletion has arisen as a result of forward slippage and generated a looped structure as shown in Fig. 5.2. and Fig. 5.7. The number of unpaired bases was resulted from strand slippage during replication (Umar *et al*., 1994). Each loop could preferentially overlap or interact mutually between complementary bases (Fig. 5.8B and Fig. 5.8C). Addtionally, the intramolecular triplex structure of TTC·GAA repeat could act as effective blocks to DNA replication both *in vitro* and *in vivo* (Sinden, 1999), leading to an increased genetic instability together with mutation occurrence found in the patient ID 17. In addition, intramolecular triplex deletion might invoke penetrance and severity of the disease increases, whereas the age of onset decreases.

Considering the pedigree (Fig. 4.22), patient ID 17 had a deceased sister (II-1) who had been diagnosed with breast cancer in her 56s. She has three daughters and one son. It was of great interest to further investigate whether the proband's unaffected daughter (III-2) is a *BRCA1* mutation carrier. The daughter is 25 years of age. It is hypothesized that loss of inactivated one allele of proband was transmitted to proband's unaffected daughter concerning the inheritable predisposition gene implicated in the family. The changes in genotype-phenotype was initiated in

proband's daughter. Surprisingly, the DNA sequence showed that the heterozygous T to C (IVS7+38T > C) located in nucleotide position 38 downstream of exon 7. Furthermore, the intronic nucleotide deletion, IVS7+50_63delTTCTTTTTTTTTTT on the sense strand and IVS7+34_47delAAGAAAAGAAAAAA on the antisense strand were found in proband's unaffected daughter. The proposed looped structure of this mutation was shown in Fig. 5.8A. This type of novel mutation has not yet been reported in BIC. As a result, three remarkable features between the proband's unaffected daughter and the proband have been shown; the IVS7+34_47del AAGAAAAGAAAAAA on the antisense strand, one deleted TTC repeat and position of occurred mutation was near triplet repeat sequences. It was implied that one allele of the *BRCA1* gene defect detected in the proband was transmitted to the proband's unaffected daughter. The proband's unaffected daughter has not yet shown the clinical phenotypes but genotype was changing in the *BRCA1* gene. It is very interesting to note that the deletion breakpoint TA exists between the two looped structures. The deletion breakpoints were mostly found in the AT-rich region and involved in both cancer and inherited diseases (Abeysinghe *et al*., 2003). The deletion occurrence was in consistence with the deletion breakpoint in close proximity to triplex repeat structure that was capable of forming slipped structure or looped structure (Sinden and Trinh, 1991 ; Moore *et al*, 1999 ; Sinden *et al*., 2002 ; Chen *et al*., 2005). In other word, the molecular defect of this type of mutation was enhanced by the intramolecular triplex structure as proposed in Fig. 5.6 (Sinden *et al*., 2002). This genomic rearrangement or alternative DNA structures could be generated by a mutagenic mechanism involving slipped mispairing during DNA replication (Mitas, 1997 ; Bacolla and Wells, 2004 ; Krasilnikova and Mirkin, 2004).

However, the mutation analysis of *BRCA1* present herein would be inadequate to provide the molecular defect of the *BRCA1*. Thus, further insight into the structure and function of the *BRCA1* gene and its protein product will facilitate the development of more comprehensive mutation-detection strategies and improve the interpretation of alterations in the sequence of the gene.

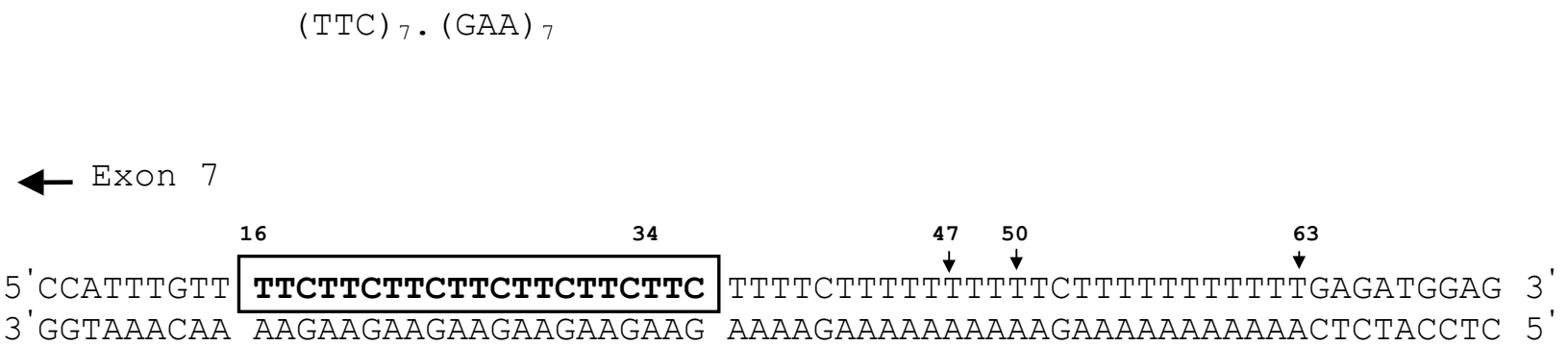


Fig. 5.1 Deduced normal nucleotide sequence containing the $(TTC)_7.(GAA)_7$ repeat tract in intron 7 of the *BRCA1* gene.

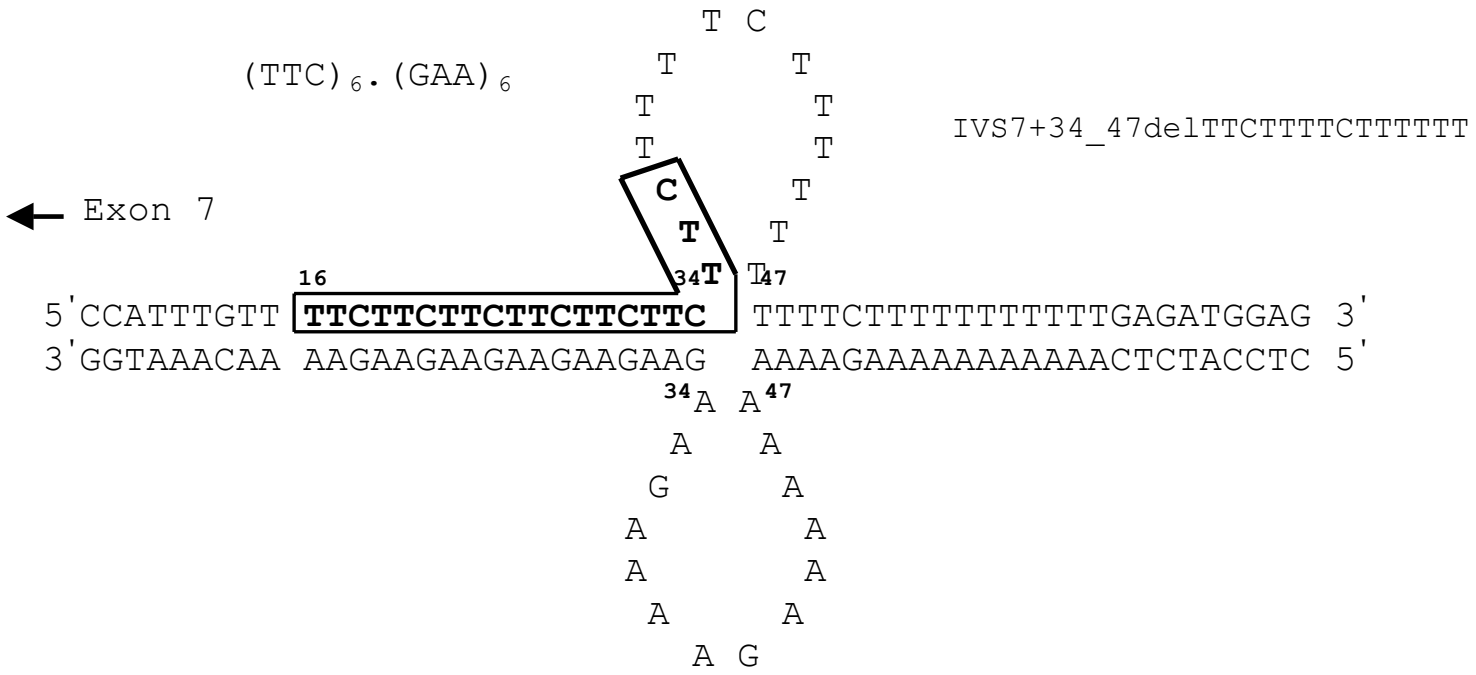


Fig. 5.2 Proposed looped structure involved in IVS7+34_47delTTCTTTTCTTTTTT. One deleted TTC is shown in the black frame.

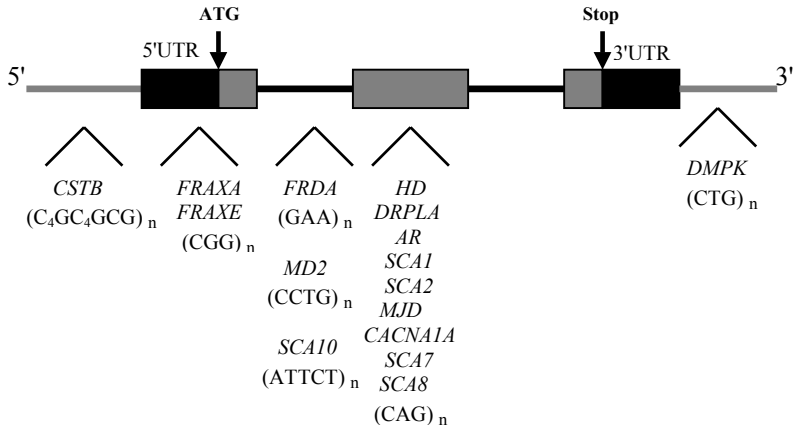


Fig. 5.3 Location of repeat tract in triplex repeat disease genes. An idealized corresponding gene is shown with the gray shaded area representing the coding region. The transcription start and stop are indicated. The dark shaded area represents the 5' upstream and 3' downstream untranslated region (UTR). Intervening sequences (IVS) are shown as black lines and the flanking region as the gray shaded lines (Sinden *et al*., 2002).

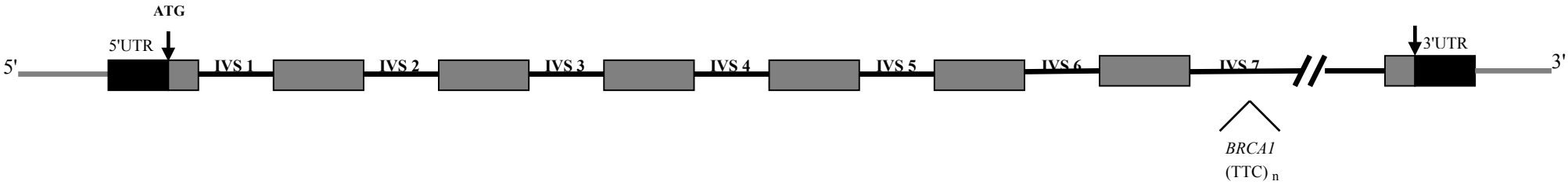


Fig. 5.4 Location of repeat tract in triplex repeat of *BRCA1* gene. An idealized gene is shown with the gray shaded area representing the coding region. The transcription start and stop are indicated. The dark shaded area represents the 5' upstream and 3' downstream untranslated region (UTR). Intervening sequences (IVS) are shown as black lines and the flanking region as the gray shaded lines.

Table 5.1 Examples of disorders of trinucleotide repeat expansions and deletion (modified from Antonarakis and Cooper 2003)

Disorder	Inheritance	Gene[a]	Chromosome	OMIM ID No[b]	Repeat	Normal	Mutant	Repeat location
Fragile X syndrome	XLD	*FMR1*	Xq27.3	309550	CGG	6-52	60-200 premutation, 230-1000 full mutation	5′ UTR
Fragile E Mental retardation	XLD	*FMR2*	Xq28	309548	GCC	7-35	130-150 premutation, 230-750 full mutation	5′ UTR
MD	AD	*DMPK*	19q13	160900	CTG	5-37	50-3000	3′ UTR
SBMA	XLR	*AR*	Xq13-21	313700	CAG	11-33	38-66	Coding
HD	AD	*HD*	4p16.3	143100	CAG	6-39	36-121	Coding
DRPLA	AD	*DRPLA*	12p13.31	125370	CAG	6-35	51-88	Coding
SCA1	AD	*SCA1*	6p23	601556	CAG	6-39	41-81	Coding
SCA2	AD	*SCA2*	12q24.1	601517	CAG	14-31	35-64	Coding
SCA3	AD	*MJD*	14q32.1	109150	CAG	12-41	40-84	Coding
SCA6/EA2	AD	*CACNA1A*	19p13	601011	CAG	7-18	20-23 (SCA6), 21-27 (EA2)	Coding
SCA7	AD	*SCA7*	3p12-13	164500	CAG	7-17	38-130	Coding
Friedreich ataxia	AR	*FRDA*	9q13-21.1	229300	GAA	6-34	80 premutation, 112-1700 full mutation	Intron 1
PME1	AR	*CSTB*	21q22.3	601145	CCCCGCCCCGCG	2-3	35-80	5′ flanking
Synpolydactyly	AD	*HOXD13*	2q31-q32	142989	$(GCG)_n(GCT)_n(GCA)_n$	15	22-29	Coding
Breast cancer	AD	*BRCA1*	17q21	113705	TTC	7	6	Intron 7

HD: Huntington disease; SCA: spinocerebellar ataxia; MD: myotonic dystrophy; SBMA: spinobulbar muscular atrophy; DRPLA: Dentatorubropallidoluysian atrophy; EA2: episodic ataxia type 2; PME: progressive myoclonus epilepsy; OPMD: Oculopharygeal muscular dystrophy; XLD: X-linked dominant; AD: autosomal dominant; XLR: X -linked recessive; AR: autosomal recessive; FraX: fragile X; ND: not determined; UTR: untranslated region; BRCA1: Breast cancer susceptibility gene1

[a]For full names of genes and links to further information see the Human Gene Nomenclature Database Search Engine (http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl).

[b]See OMIM (http://www.ncbi.nlm.nih.gov/Omim/)

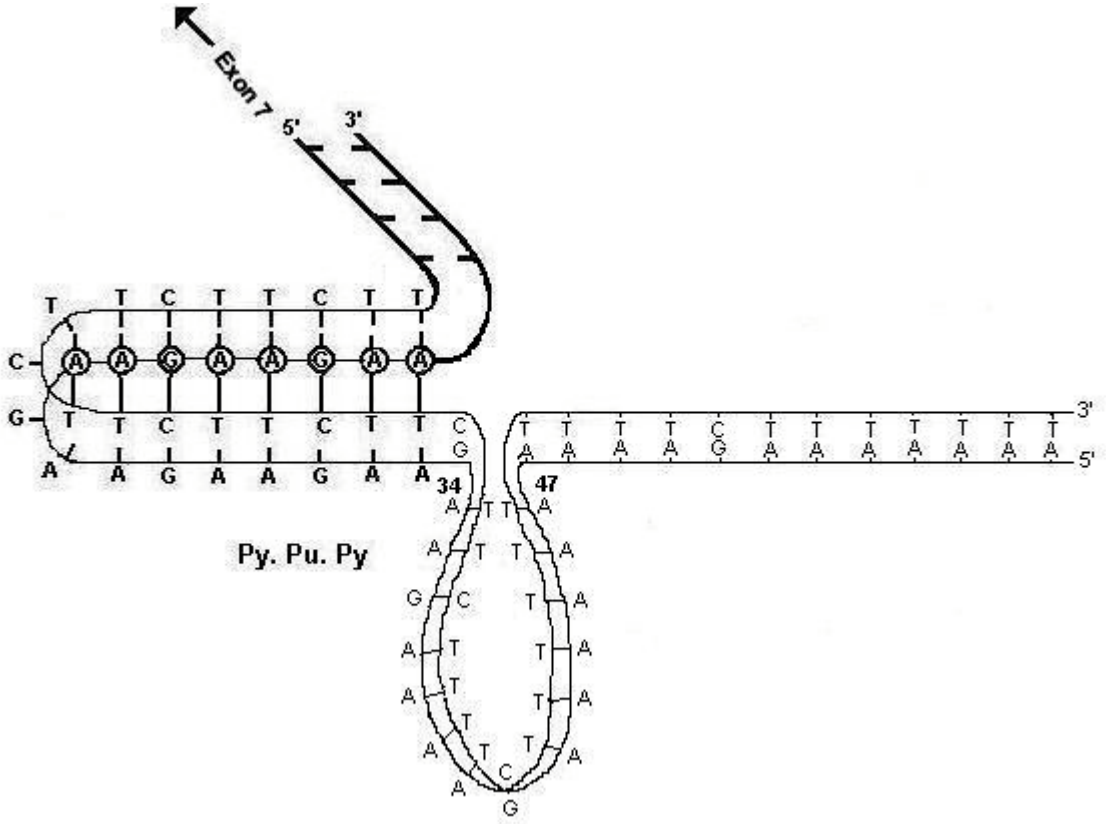


Fig. 5.6 Proposed intramolecular triplex DNA structures and looped structure for IVS7+34_47 delTTCTTTTCTTTTTT of the patient ID 17.

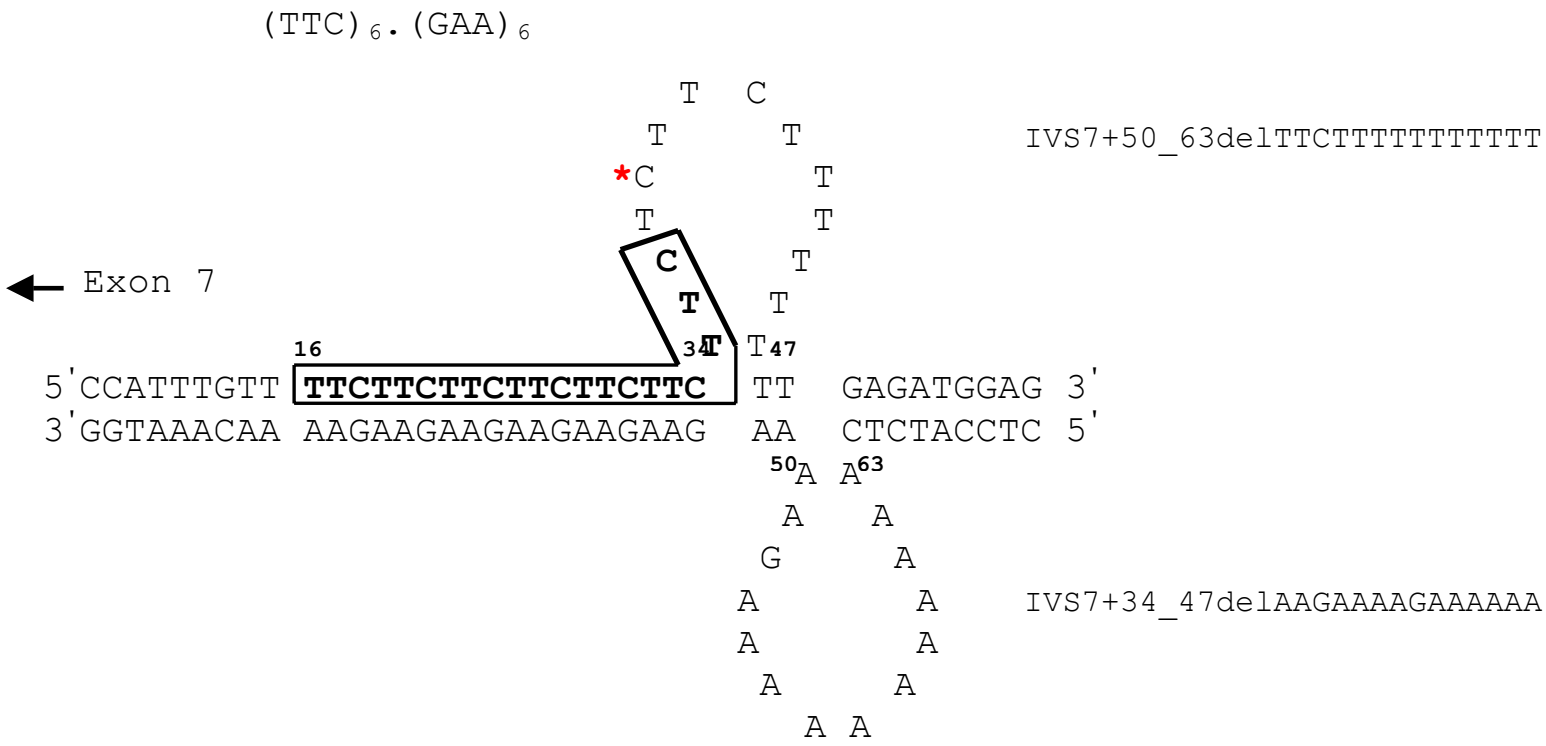


Fig. 5.7 Proposed looped structure involved in IVS7+50_63delTTCTTTTTTTTTT in the sense strand and in IVS7+34_47 delAAGAAAAGAAAAAA in the antisense strand of the proband's daughter. The point mutation IVS7+38T > C is shown with the asterisk.

Table 5.2 DNA repeats, symmetry elements and alternative DNA structures (Sinden *et al*., 2002)

Repeat	Symmetry elements[a]	Alternative DNA structures[b]
$(GT)_n \bullet (AC)_n$	DR	Z-DNA
$(GC)_n \bullet (GC)_n$	DR, IR	Hairpins (ss), Z-DNA
$(AT)_n \bullet (AT)_n$	DR, IR	Hairpins (ss), cruciforms
$(CTG)_n \bullet (CAG)_n$	DR, QP	Flexible helix, mismatched hairpins (ss), slipped strand DNA
$(CGG)_n \bullet (CCG)_n$	DR, QP	Flexible helix, mismatched hairpins (ss), slipped strand DNA, quadruplex DNA
$(GAA)_n \bullet (TTC)_n$	DR, MR	Intramolecular triplex DNA, slipped strand DNA
$(GGGGTT)_n$	DR	Quadruplex DNA (ss)

[a]DR, Direct Repeat; IR, Invert Repeat; QP, Quasipalindrome (imperfect inverted repeat); MR, Mirror Repeat

[b]Structures are those formed in double stranded DNA, unless indicated for single stranded DNA (ss).

A

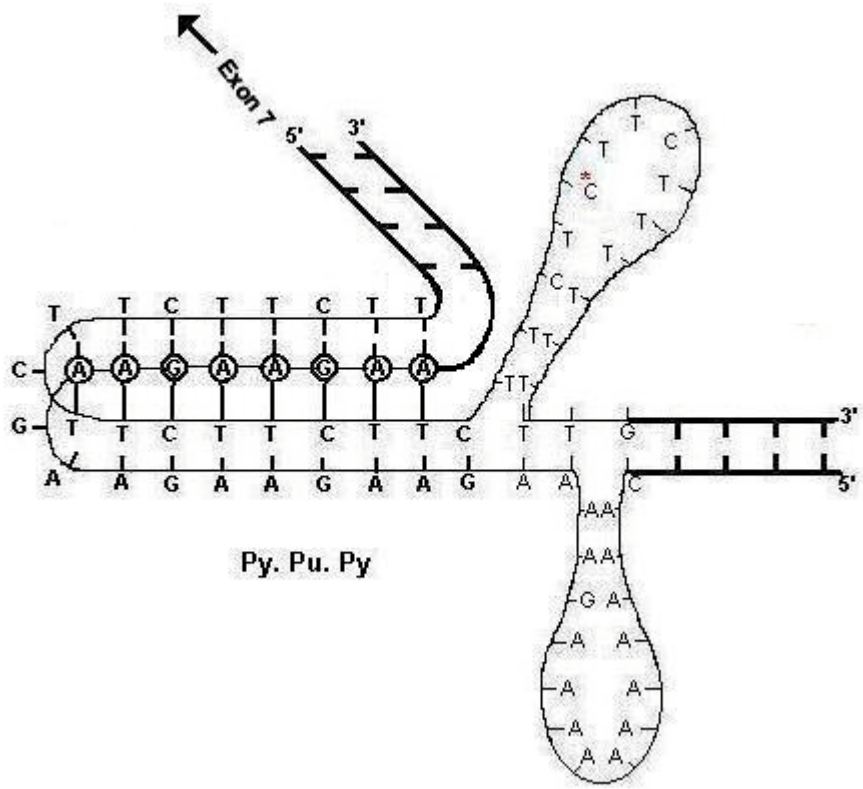
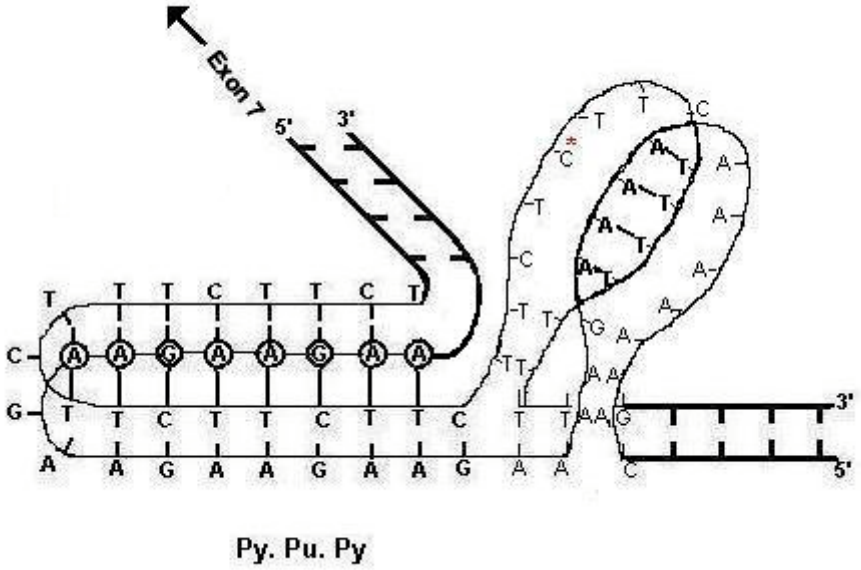
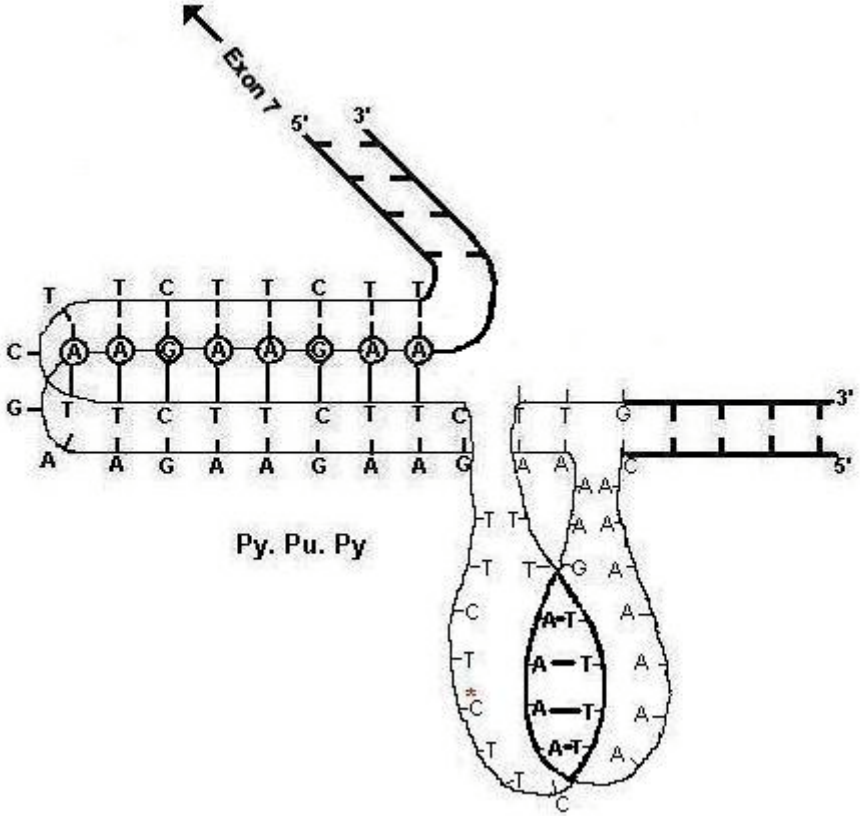


Fig. 5.8 Proposed intramolecular triplex DNA structures and looped structure formed for the IVS7+50_63delTTCTTTTTTTTTT and IVS7+34_47 delAAGAAAAGAAAAAA of the proband's daughter (A-C). Loop overlap or interact mutually between complementary bases. Upper side (B). Lower side (C).

B



C



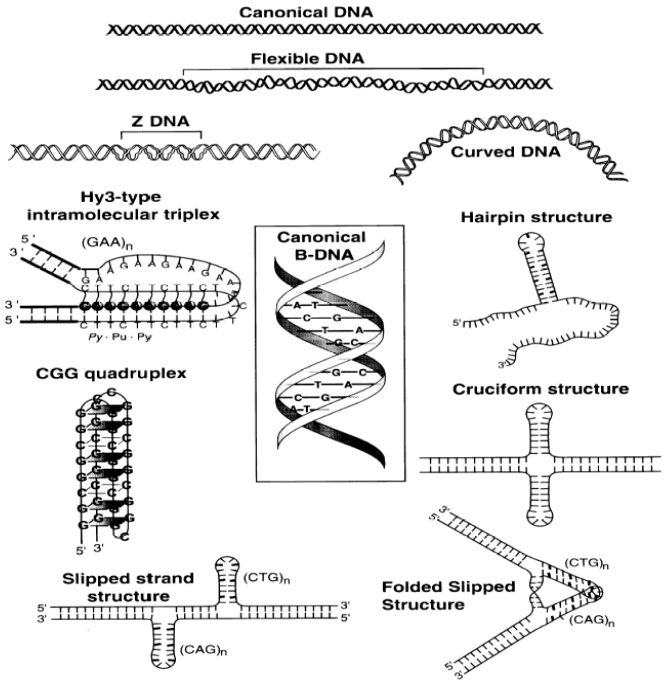


Fig. 5.5 Non-B DNA conformations involved in genomic rearrangements. A canonical B-form helix is shown in the inset. A representation of this helix is also shown at the top of the figure. The next two representations, Z-DNA and stable curved DNA, occur in $(CG)_n$•$(CG)_n$ and $(GT)_n$•$(AC)_n$ runs and DNA containing phased A4-5 T4-5 tracts (and other sequences), respectively, but have not been identified in triplet repeats. Proceeding clockwise, hairpin structures form in most disease-relevant triplet repeat single strands (to some degree). Cruciform structures form in perfect inverted repeats and have not been identified in triplet repeat sequences. Folded slipped strand structures and simple slipped strand structures form in $(CTG)_n$•$(CAG)_n$ and $(CGG)_n$•$(CCG)_n$ repeats. Quadruplex structures have been identified in oligonucleotides containing CGG and TGG repeats. In these structures the guanine are held together by G•G Hoogsteen bonds. A Hy3-type intramolecular triplex (as well as bi-triplex structures) can form in the $(GAA)_n$• $(TTC)_n$ repeat (Sinden *et al*., 2002).