# Letters to the Editor

## 7q11.23 Deletions in Williams Syndrome Arise as a Consequence of Unequal Meiotic Crossover

*To the Editor:*

Williams syndrome (WS) (Williams et al. 1961; Beuren et al. 1962) is a multisystem disorder characterized by mental retardation, a specific neurobehavioral profile, characteristic facies, infantile hypercalcemia, cardiovascular abnormalities, progressive joint limitation, hernias, and soft skin. Recent studies have shown that hemizygosity at the elastin (ELN) gene locus on chromosome 7q is associated with WS (Ewart et al. 1993). Furthermore, two FISH studies using cosmid recombinants containing the 5' or the 3' end of the ELN gene revealed deletion of the entire ELN gene in 90%–96% of classical WS cases (Lowery et al. 1995; Nickerson et al. 1995). However, the size of the 7q11.23 deletions and the mechanism by which these deletions arise are not known.

To begin to understand the complex genetic defect underlying the pathogenesis of WS, we describe in this letter a high-resolution genetic map of the q11 region of chromosome 7 and use several of these linked markers, including a new and highly informative tetranucleotide-repeat polymorphism within the first intron of the ELN gene, for haplotype analysis of 31 WS patients and unaffected family members. This study provides for the first time both an estimate of the size of the 7q11.2 deletions in WS and an insight into the mechanism responsible for the generation of these 7q deletions.

We analyzed 7q deletions in a total of 31 sporadic WS patients, by haplotype analysis. Diagnostic criteria included characteristic facies, mental retardation, and gregarious personality. More than 65% of these patients had supravalvular aortic stenosis (SVAS), as evidenced by echocardiography. Peripheral whole-blood samples were collected with informed consent of the patients and family members, and total genomic DNA was isolated. Patients and family members were genotyped for three ELN gene markers. These included a new tetranucleotide-repeat polymorphism within the first intron (ELNi1), a CA-repeat polymorphism within intron 18 (ELNi18; Foster et al. 1993), and an *Rma*I RFLP within exon 20 of the ELN gene (ELNe20; Tromp et al. 1991). The new ELN gene marker within intron 1 was detected by primers HEI1.3S (5'-GCCCACATGGGCAGA-TTGCT-3') and HEI1.4A (5'-CCCTCATCCACAGAC-AGGTC-3'). PCR-amplification conditions used with these oligomers were as follows: a 5–25-μl reaction contained a final concentration of 1.5 mM MgCl$_2$, 0.2 mM of each dNTP, 1 μM HEI1.3S, 0.8 μM HEI1.4A, 0.2 μM P$^{32}$-HEI1.4A (5' end-labeled), 25 units × AmpliTaq polymerase/ml (Perkin-Elmer), and 5 μg total genomic DNA/ml. Amplification reactions were performed with a thermal cycler (GeneAmp System 9600, Perkin-Elmer), which was programmed for 25 cycles, each cycle of which consisted of a 94°C incubation for 30 s, a 60°C incubation for 30 s, and a 72°C incubation for 30 s. Products were separated on denaturing acrylamide gels and were visualized by autoradiography. In addition to the ELN gene markers, the following polymorphisms were used for haplotype analysis: D7S672 (probe AFM289ve9), D7S653 (probe AFM259zc1), D7S1870 (probe AFMa060xc9), D7S849 (probe 1129AC/TG), D7S675 (probe AFM295yg9), and D7S634 (probe
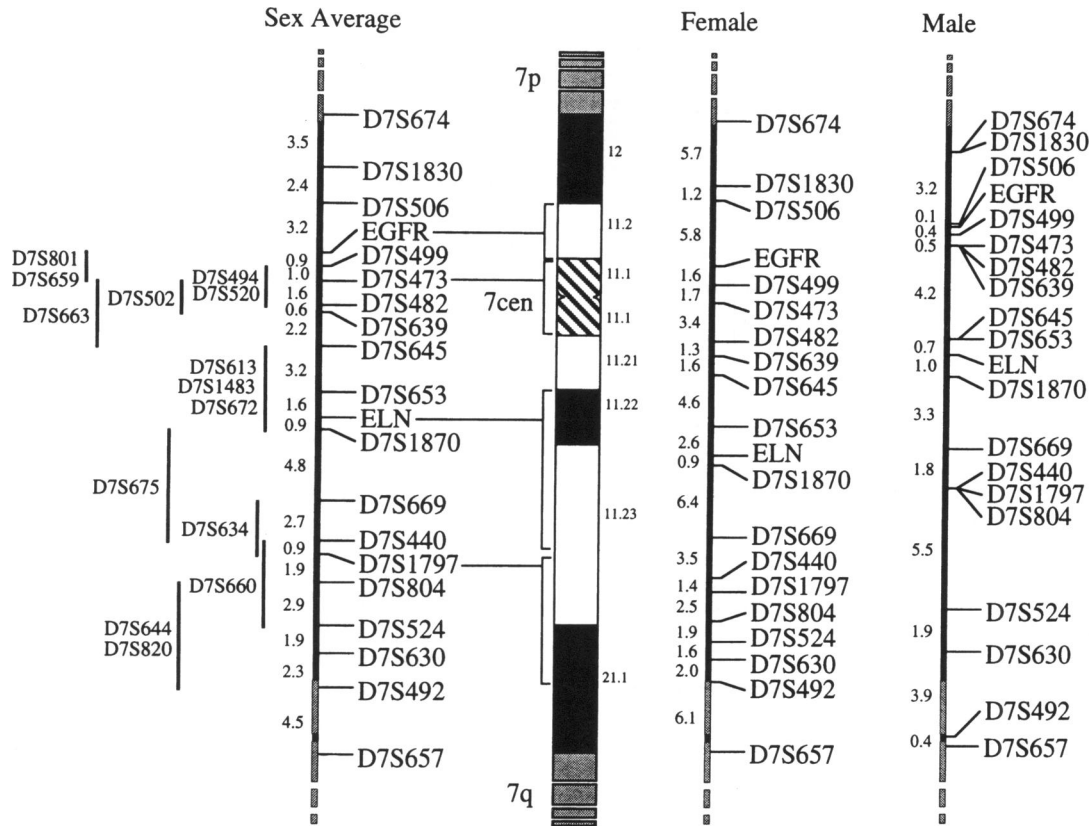
**Figure 1** Microsatellite map of the midsection of chromosome 7 containing the ELN gene. Markers were placed with 1,000:1 odds for order. Nonuniquely placed markers are shown to the left of the sex-average map, associated with unbroken vertical bars to indicate their map intervals. The three maps were prepared with different scale factors and cover 56 cM in the female map, 27 cM in the male, and 43 cM when sex is not considered (the sex-average map). Map distances are in Kosambi centimorgans. Tie-lines to the chromosome 7 ideogram indicate cytogenetic placement of markers on the chromosome. Detailed description of the mapping procedures and further data on the new polymorphism ELNi1 are available through the World Wide Web Genlink resource (http://www.genlink.wustl.edu).

AFM203vb6). These markers were detected as described in the Genome Database (Weissenbach et al. 1992; Gyapay et al. 1994; Iles et al. 1994).
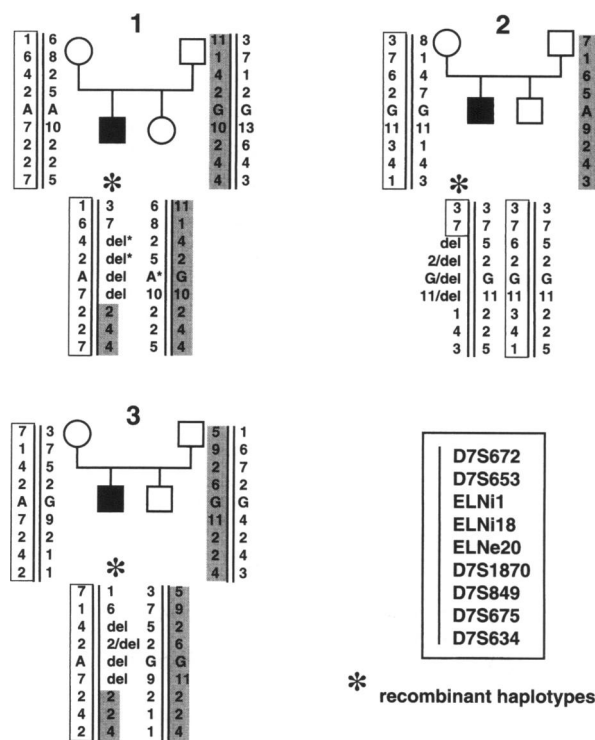
To investigate the size of deletions associated with WS, we generated a high-resolution linkage map of the chromosomal region containing the ELN gene (fig. 1). We could uniquely place polymorphic locus D7S1870 on the 1994 Chromosome 7 Workshop report's microsatellite-marker map (Helms et al. 1995) by adding genotype data for the ELN and D7S1870 loci. D7S1870 was located telomeric to the ELN gene, at a distance of 0.9 cM.

The 31 WS patients and unaffected family members were genotyped with the three ELN gene markers and the polymorphic loci located proximal (D7S672 and D7S653) and distal (D7S1870, D7S849, D7S675, and D7S634) to the ELN gene locus, as determined by our high-resolution genetic map (fig. 1). D7S849 could not be placed on the map, because of the moderate informativity of the marker (heterozygosity .56). However, the position of D7S849 relative to other markers used in

this study could be established on the basis of the fact that it segregated with marker alleles distal to the ELN gene (fig. 2).

Genotype data were subsequently used to generate haplotypes to study the inheritance of deleted and undeleted markers. The ELN gene markers detected hemizygosity in 25 informative WS patients (table 1). Similarly, one allele represented by D7S1870 was found to be deleted in 27 informative WS patients (table 1). A simultaneous deletion of the ELN gene locus and D7S1870 was observed in 23 patients informative for markers in both loci. Our genetic map locates D7S1870 0.9 cM distal to ELN.

Another distal locus, D7S849, was found to be hemizygous in 4 (13%) of the 31 patients, suggesting size heterogeneity of the WS deletion. Proximal loci D7S672 and D7S653 and distal loci D7S675 and D7S634, on the other hand, were found to be heterozygous in all informative WS patients, indicating that the location of these markers falls outside the region deleted in WS (table 1). On the basis of genetic mapping of these unde-

**1**

**2**

**3**

D7S672
D7S653
ELNi1
ELNi18
ELNe20
D7S1870
D7S849
D7S675
D7S634

\* recombinant haplotypes

**Figure 2** Haplotype analysis of WS families. Patients and family members were genotyped for markers indicated in the boxed list, in proximal to distal order. The order of the ELN markers is arbitrary, since the orientation of the ELN gene on the chromosome is unknown. The three representative families (families 1–3) of patients 1124, 1093, and 1420, respectively, are shown. One maternal chromosome is identified by an empty box, and one paternal chromosome is indicated by shading. del = allele deleted; \* = allele identity inferred; and n/del = uninformative (n = any allele number).

leted markers, the proximal locus D7S653 (1.6 cM) and the distal locus D7S675 (0–7.5 cM) were located nearest to the chromosomal region deleted in WS patients. Taken together, mapping and genotype data indicate that the deletion in WS patients involves a genetic distance of 0.9–2.5 cM on the sex-average map (the distance between markers D7S653 and D7S1870).

Deletions were located on the paternal chromosome in 12 patients (41%) and on the maternal chromosome in 17 patients (59%). $\chi^2$ Analysis revealed no statistically significant difference ($P < .1$) between the maternal and paternal distribution of the WS deletions. Clearly, deletions in WS patients can occur on chromosome 7 derived from either parent.

We identified 12 families that were simultaneously informative for proximal, deleted, and distal markers and that had at least one unaffected sibling of the WS patient, to allow for the determination of chromosomal phase. We observed that WS patients in all of these 12 families not only were deleted for at least one marker in the ELN gene region but also had an apparent recombination between proximal and distal markers flanking

the deletion (fig. 2). In contrast, homologue chromosomes in the same WS patients did not show any recombination event between these proximal and distal markers. These data strongly implicate meiotic recombination as an underlying mechanism of the generation of deletions causing WS.

In conclusion, our genotype data demonstrate the simultaneous deletion of ELN and D7S1870 in 23 WS patients. Furthermore, we have shown by genetic mapping that D7S1870 is located distal to the ELN gene and that the genetic distance between ELN and D7S1870 is 0.9 cM. These mapping and haplotype studies together indicate that a large portion of genomic DNA distal to the ELN gene that spans 0.9–2.5 cM is missing in most WS patients with SVAS. Although physical distances corresponding to a given genetic distance vary depending on the genomic region studied (Chumakov et al. 1995), our results are consistent with deletions spanning a region of 1–2.5 Mb including the ELN gene in WS patients. Clearly, therefore, deletions at 7q11.2 in WS patients are sufficiently large to affect several genes, including the ELN gene. WS therefore may indeed be a contiguous gene–deletion syndrome. In confirmation of these results, the gene for replication factor C subunit 2, RFC2, has most recently been shown to be deleted in WS patients (Peoples et al. 1996).

Our haplotype data demonstrate a high frequency of recombination between markers flanking the deletion in WS patients, suggesting that the large, 0.9–2.5-cM de novo deletions that we have reported are the result of unequal crossing-over during meiosis. The high frequency of such de novo deletions suggests that genomic regions flanking the ELN locus are particularly susceptible to meiotic mispairing.

Unequal crossover events produce both deletions and duplications. Therefore, in addition to the 0.9-cM deletions that we have observed in WS patients, it is probable that a duplication abnormality also exists, involving a genomic region of 0.9–2.5 cM including the ELN gene. Individuals carrying such hypothetical duplications on 7q are likely to be born at the same frequency as are WS patients. Such paired microdeletion and duplication abnormalities are not unprecedented among human genetic diseases. For example, two dominantly inherited neurodegenerative diseases (hereditary neuropathy with liability to pressure palsies and Charcot-Marie-Tooth disease type 1A) are caused by the deletion and duplication, respectively, of the same 1.5-Mb region on chromosome 17p (for review, see Suter and Patel 1994; Lupski et al. 1996). Similarly, WS and a hypothetical duplication abnormality with a similar or unrelated phenotype may be another example of such paired deletion-duplication mutational events.

## Table 1

Genotypes of 31 WS Patients, for Polymorphic Markers within and Flanking the Elastin Gene, as Determined by Haplotype Analysis of the Respective Families

| Patient | D7S672 | D7S653 | ELNi1 | ELNi18 | D7S1870 | D7S849 | D7S675 | D7S634 | Recombination[a] |
|---|---|---|---|---|---|---|---|---|---|
| 1124 | 1-3 | 6-7 | 4-del | 2-del | 7-del | 2-? | 2-4 | 7-4 | Present |
| 1151 | 3-3 | 5-7 | 6-del | 4-? | 13-del | 3-del | 2-4 | 5-6 | ? |
| 1081 | 1-12 | 6-1 | del-3 | ?-2 | del-12 | 2-5 | 3-3 | 6-3 | ? |
| 1093 | ?-3 | ?-7 | del-5 | ?-2 | ?-11 | 1-2 | 4-2 | 3-5 | Present |
| 1089 | 5-1 | 8-2 | ?-4 | ?-2 | del-11 | ?-2 | 2-4 | 4-2 | ? |
| 1138 | 9-3 | 4-7 | ?-5 | ?-2 | ?-10 | ?-5 | 3-5 | 5-5 | ? |
| 1420 | 7-1 | 1-6 | 4-del | 2-? | 7-del | 2-? | 4-2 | 2-4 | Present |
| 1172 | n.d. | 5-6 | del-5 | ?-2 | del-10 | 2-5 | 4-6 | 4-5 | ? |
| 1A | 6-3 | 7-1 | del-2 | ?-5 | ?-10 | 5-2 | 5-5 | 5-5 | ? |
| 2A | 3-8 | 7-1 | 7-del | 2-? | 10-del | 5-3 | 3-4 | 5-5 | ? |
| 3A | 7-13 | 1-? | 6-del | 4-del | 10-del | 2-4 | 4-2 | 4-5 | ? |
| 5A | 11-12 | 5-1 | del-6 | ?-2 | del-12 | 2-1 | 5-4 | 3-4 | ? |
| 6A | ?-3 | ?-7 | del-4 | del-2 | del-7 | 2-8 | 3-4 | 4-5 | Present |
| 7A | 8-8 | 1-4 | del-5 | ?-2 | del-8 | ?-2 | ?-2 | 6-3 | Present |
| 8A | 7-2 | ?-1 | ?-5 | del-4 | del-13 | ?-2 | 5-2 | 5-4 | ? |
| 9A | ?-3 | ?-7 | ?-4 | del-2 | del-12 | 2-5 | n.d. | 4-6 | ? |
| 10A | 3-? | 7-? | 5-del | 2-? | 10-del | 5-? | 3-4 | 4-4 | ? |
| 11A | 3-? | 7-? | 4-? | 2-? | 7-del | 2-? | 5-4 | 3-6 | ? |
| 12A | 5-3 | ?-7 | del-4 | ?-2 | del-6 | del-6 | 2-4 | 3-3 | ? |
| 13A | ?-3 | ?-7 | del-4 | ?-2 | del-9 | 6-2 | 2-2 | 3-4 | Present |
| 14A | 4-3 | 7-6 | 6-del | 2-? | 9-del | 4-del | 1-4 | 4-3 | Present |
| 16A | 3-3 | 8-5 | del-8 | del-7 | del-10 | ?-2 | ?-4 | 3-5 | Present |
| 19A | 2-2 | 1-7 | ?-4 | ?-2 | del-11 | 4-5 | 2-2 | 5-5 | ? |
| 20A | 8-12 | 1-? | 4-del | 5-del | 10-del | 2-del | 4-3 | 4-5 | Present |
| 21A | 3-? | 7-? | 5-? | 2-? | 8-del | 2-? | 2-? | 1-3 | ? |
| 22A | 3-3 | 7-5 | del-3 | del-5 | del-10 | 6-2 | 5-4 | 3-4 | Present |
| 25A | 11-1 | 1-? | 5-del | 6-del | 7-del | 5-? | 3-4 | 3-2 | Present |
| 26A | 8-6 | 1-? | 4-del | 2-del | 10-del | 2-5 | 3-4 | 4-4 | Present |
| 28A | 8-5 | ?-1 | ?-5 | del-2 | del-7 | 2-5 | 4-4 | 5-3 | ? |
| 29A | 3-11 | 7-1 | ?-6 | ?-4 | ?-10 | 7-2 | 4-3 | 5-6 | Present |
| 33A | 3-4 | ?-7 | del-7 | del-4 | del-10 | ?-5 | 4-5 | 1-6 | ? |

NOTE.—Marker loci are listed in proximal to distal order, from left to right. Genotype data are represented with the identification number of the maternal allele on the left and with the paternal allele on the right. del = marker allele deleted; ? = uninformative; and n.d. = no data.
[a] Between the proximal and distal markers on the deletion chromosome.

ZSOLT URBÁN,[1] CYNTHIA HELMS,[2] GYÖRGY FEKETE,[3] KATALIN CSISZÁR,[1] DAMIEN BONNET,[4] ARNOLD MUNNICH,[4] HELEN DONIS-KELLER,[2] AND CHARLES D. BOYD[1]

[1]Department of Surgery, University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, New Brunswick; [2]Department of Surgery, Washington University School of Medicine, St. Louis; [3]The Second Department of Pediatrics, Semmelweis University of Medicine, Budapest; and [4]Institut Necker—IFREM Hopital des Enfants-Malades, Paris

## Acknowledgments

## References

Beuren AJ, Apitz J, Harmjanz D (1962) Supravalvular aortic stenosis is associated with mental retardation and a certain facial appearance. Circulation 26:1235–1240

Chumakov IM, Rigault P, Le Gall I, Bellanne-Chantelot C, Billault A, Guillou S, Soularue P, et al (1995) A YAC contig map of the human genome. Nature Suppl 377:175–297

Ewart AK, Morris CA, Atkinson DL, Jin W, Sternes K, Spalone P, Stock AD, et al (1993) Hemizygosity at the elastin locus

in a developmental disorder, Williams syndrome. Nat Genet 5:11–16

Foster K, Ferrel L, King-Underwood L, Povey S, Attwood J, Rennik R, Humphries SE, et al (1993) Description of a dinucleotide repeat polymorphism in the human elastin gene and its use to confirm assignment of the gene to chromosome 7. Ann Hum Genet 57:87–96

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–1994 Généthon human genetic linkage map. Nat Genet 7:246–339

Helms C, Dutchik J, Lee M, Lacy M, Veile R, Burgess A, Hing A, et al (1995) 182 microsatellite loci placed on the chromosome 7 map. (Abstract and fig 3 in: L-C Tsui, H Donis-Keller, K-H Grzeschik [1994] Report of the Second International Workshop on Human Chromosome 7 Mapping 1994. Cytogenet Cell Genet 71:1–31)

Iles DE, Lehmann-Horn F, Scherer SW, Tsui L-C, Weghuis DE, Suijkerbuijk RF, Heytens L, et al (1994) Localization of the gene encoding the $\alpha_2/\delta$-subunits of the L type voltage dependent calcium channel to chromosome 7q and analysis of the segregation of flanking markers in malignant hyperthermia susceptible families. Hum Mol Genet 3:969–975

Lowery MC, Morris CA, Ewart A, Brothman LJ, Zhu XL, Leonard CO, Carey JC, et al (1995) Strong correlation of elastin deletions, detected by FISH, with Williams syndrome: evaluation of 235 patients. Am J Hum Genet 57:49–53

Lupski JR, Roth JR, Weinstock GM (1996) Chromosomal duplications in bacteria, fruit flies, and humans. Am J Hum Genet 58:21–27

Nickerson E, Greenberg F, Keating MT, McCaskill C, Shaffer LG (1995) Deletions of the elastin gene at 7q11.23 occur in ~90% of patients with Williams syndrome. Am J Hum Genet 56:1156–1161

Peoples R, Perez-Jurado L, Wang Y-K, Kaplan P, Francke U (1996) The gene for replication factor C subunit 2 (RFC2) is within the 7q11.23 Williams syndrome deletion. Am J Hum Genet 58:1370–1373

Suter U, Patel PI (1994) Genetic basis of inherited peripheral neuropathies. Hum Mutat 3:95–102

Tromp G, Christiano AM, Goldstein M, Indik Z, Rosenbloom J, Deak SB, Prockop DJ, et al (1991) A to G polymorphism in the elastin gene. Nucleic Acids Res 19:4314

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, et al (1992) A second generation linkage map of the human genome. Nature 359:794–801

Williams JCP, Berratt-Boyers BG, Lowe JB (1961) Supravalvular aortic stenosis. Ciculation 24:1311–1318

## A Somatic Truncating Mutation in BRCA2 in a Sporadic Breast Tumor

*To the Editor:*

Recently, a second susceptibility gene for hereditary breast and ovarian cancer, BRCA2, was cloned (Woos-

ter et al. 1995; Tavtigian et al. 1996). The subsequent identification of heterozygous germ-line mutations confirmed its role as a predisposing factor in a subset of familial breast and ovarian cancer families (Wooster et al. 1995; Lancaster et al. 1996; Miki et al. 1996; Tavtigian et al. 1996; Teng et al. 1996). The possible involvement of BRCA2 in the sporadic forms of breast and ovarian tumors was addressed in three recent reports analyzing the gene for somatic mutations in 212 primary breast cancers and 55 ovarian cancers (Lancaster et al. 1996; Miki et al. 1996; Teng et al. 1996). Although several alterations were identified, all except two changes were shown to represent germ-line mutations. Moreover, the two somatic BRCA2 alterations were found to be missense mutations resulting in a Asp3095-Glu change (Lancaster et al. 1996) in one case and in a His2415Asn change (Miki et al. 1996) in the other. Given the questionable effect of missense mutations on protein function, the role of BRCA2 in the carcinogenesis of sporadic breast tumors remains unclear.

In order to identify in primary breast cancers the somatic BRCA2 mutations with clear functional consequences, we have analyzed the three large exons—10, 11, and 27—of BRCA2, together constituting 65% of the coding region of the gene (Tavtigian et al. 1996), in 69 unselected samples of frozen breast tumor sections, using the protein-truncation test (PTT) (Roest et al. 1993). Here we report a first case of a truncating somatic mutation in BRCA2 in a primary ductal breast carcinoma with demonstrated loss of heterozygosity (LOH).

PTT was utilized to identify protein-termination mutations in exons 10, 11, and 27 of BRCA2, using genomic DNA and oligonucleotide primers PTT10 f/r (5′-CTT-AATACGACTCACTATAGGGAGACCACCATGTAT-ACTTTAACAGGATTTGGAA-3′/5′-ACACAGAAGG-AATCGTCATC-3′), the overlapping primer sets PTT11A f/r (5′-CTTAATACGACTCACTATAGGGAGACCACC-ATGTTTTTATGTTTAGGTTTATTGC-3′/5′-TGCATT-CCTCAGAAGTGGTC-3′), PTT11B f/r (5′-CTTAAT-ACGACTCACTATAGGGAGACCACCATGAAACCA-AGCTACATATTGCAG-3′/5′-TAATTTCCTACATAA-TCTGCAG-3′), PTT11C f/r (5′-CTTA ATACGACT-CACTATAGGGAGACCACCATGTGGCTTAGAGAA-GGAATATTTG-3′/5′-AAAATAGTGATTGGCAAC-ACG3′), and the primer set PTT27 f/r (5′-CTTAATACG-ACTCACTATAGGGAGACCACCATGACGTTTTCA-TTTTTTTATCA-3′/5′-ATAATTTATTGTCGCC-TTTGC-3′). SDS-PAGE analysis of translated PCR product PTT11A r/f revealed a truncated protein of ~35 kD in breast tumor tissue but not in blood lymphocytes of patient Sp27 (fig. 1a). The genetic identity of tumor and lymphocyte DNA in patient Sp27 was verified by informative markers at D11S524 and D11S554 (data not shown) and was also confirmed at D13S260 and D13S171 (fig. 1b). Sequencing of the variant PTT allele in the approxi-