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ORIGINAL INVESTIGATION

Detection and characterisation of large *SERPINC1* deletions in type I inherited antithrombin deficiency

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Abstract Methods routinely used for investigating the molecular basis of antithrombin (AT) deficiency do not detect large *SERPINC1* rearrangements. Between 2000 and 2008, 86 probands suspected of having AT-inherited type I deficiency were screened for *SERPINC1* mutations in our laboratory. Mutations causally linked to the deficiency were identified by sequencing analysis in 63 probands. We present here results of multiplex ligation-dependent probe amplification (MLPA) analysis performed in 22 of the 23 remaining probands, in whom sequencing had revealed no mutation. Large deletions, present at the heterozygous state, were detected in 10 patients: whole gene deletions in 5 and partial deletions removing either exon 6 ($n = 2$), exons 1–2 ($n = 1$) or exons 5–7 ($n = 2$) in 5 others. Exon 6 partial deletions are a 2,769-bp deletion and a 1,892-bp deletion associated with a 10-bp insertion, both having 5′ and/or 3′ breakpoints located within *Alu* repeat elements. In addition, we identified the 5′ breakpoint of a previously reported deletion of exons 1–2 within an extragenic *Alu* repeat. Distinct mutational mechanisms explaining these *Alu* sequence-related deletions are proposed. Overall, in this series, large deletions detected by MLPA explain almost half of otherwise unexplained type I AT-inherited deficiency cases.

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Introduction

Antithrombin (AT) is a major circulating inhibitor of blood coagulation proteases, and belongs to the serine protease inhibitor (serpin) superfamily (Silverman et al. 2001). AT is a 464 amino acid protein—432 without the signal peptide—encoded by *SERPINC1* (GenBank X68793.1) on chromosome 1q23–25. *SERPINC1* comprises 7 exons encompassing 13.5 kb of genomic DNA. The gene is rich in *Alu* repeat elements, with nine complete and one partial repeats in its intronic regions (Olds et al. 1993). Hereditary AT deficiency (MIM 107300) is an autosomal disorder which increases the risk for recurrent venous thromboembolism (VTE) (Egeberg 1965; Van Boven et al. 1999; Lijfering et al. 2009).

AT has two functional domains, a heparin binding site and a reactive centre. The reactive centre complexes and inactivates coagulation proteases, mainly thrombin and factor Xa (Björk and Olson 1997). To date, more than 200 distinct mutations responsible for AT deficiency have been identified. They reduce the level of the protein (quantitative or type I deficiency), or affect its function [qualitative or type II deficiency, altering the heparin binding site (type IIHBS) or the reactive site (type IIRS)], or even have multiple or pleiotropic effects (Lane et al. 1997). This classification is of clinical importance as type I and type IIRS deficiencies are strong risk factors for thrombosis while heterozygous type IIHBS mutations or other mild variants such as AT Dublin or Cambridge II are at reduced risk of VTE (Daly et al. 1990; Lane et al. 1997, Perry et al. 1998; Lijfering et al. 2009). The mutation pattern of the *SERPINC1* gene is heterogeneous, comprising mostly point mutations and small insertion/deletions. Large rearrangements account for only a small proportion of the mutations reported so far: 17 out of the 220 (7.7%) distinct mutations in the 2008.3 Human Gene Mutation professional Database (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=SERPINC1). Screening for *SERPINC1* mutations is usually performed by direct sequencing analysis, a method that is not able to detect large gene rearrangements. This might have contributed to the small proportion of large rearrangements detected.

Recently, a rapid comparative quantification method, based on multiplex ligation-dependent probe amplification (MLPA) has been developed with proven reliability and sensitivity in detecting deletions or duplications involving one or more exons (Sellner and Taylor 2004; Schouten et al. 2002). Between 2000 and 2008, 86 probands with type I AT-inherited deficiency were studied in our laboratory. However, a significant proportion of these deficiencies (27%, 23/86) remained unexplained after extensive sequencing analysis. The aim of the present work was to search for large *SERPINC1* rearrangements in these unexplained cases by means of MLPA analysis.

Patients and methods

Patients

Between 2000 and 2008, DNA samples from 86 probands with a suspicion of type I AT-inherited deficiency were sent to our laboratory for *SERPINC1* analysis. These patients had been previously subjected to thrombophilia screening in specialized clinical haematology laboratories in France or Germany because they had presented at least one unexplained episode of deep venous thrombosis. Forty patients lived in the Paris area, 45 others were from other regions of France (East, 11; North, 7; South, 17; West, 10) and the last one was from Germany. A written informed consent authorizing *SERPINC1* analysis was obtained from every subject, according to the Declaration of Helsinki. Type I AT deficiency is defined by AT antigen and cofactor activity decreased to a similar extent, usually about 50% (normal range, 80–120%). Relatives were investigated whenever available. The clinical characteristics of the subjects, focusing on history of thrombosis and presence or absence of other genetic or acquired risk factors for thrombosis, were recorded in each centre and the absence of causes of acquired AT deficiency was verified.

Sequencing and MLPA *SERPINC1* analyses

Sequencing analysis of the seven exons and the intron–exon boundaries was performed on both strands as previously described (Picard et al. 2003). Every mutation was confirmed independently by analysis of a second PCR product. MLPA AT gene analysis was performed using the SALSA MPLA P227 Serpin C1 kit (MRC Holland, Amsterdam, Netherlands) according to the manufacturer's instructions. This kit detects deletions/duplications of all exons of *SERPINC1* using specifically designed probes. After a normalization step, peak area of each exon is compared to values obtained with a DNA control sample.

Briefly, after a denaturation step of 5 min at 98°C, DNA (75 ng) was submitted to a hybridization step with the SALSA probemix of 16 h at 60°C followed by a ligation step of 15 min at 54°C. Multiplex amplification of the ligation products was performed as follows: 35 cycles (95°C 30 s, 60°C 30 s, 72°C 60 s) then 72°C, 20 min. Reaction products were quantified on an ABI prism 3730 DNA analyzer using the 500LIZ size standard (both from Applied Biosystems, Foster City, USA). Data were

analysed using the GeneMapper software (Applied Biosystems, Foster City, USA). DNA samples from normal individuals were included in each run (at least 1 for 7 patients). Expression of the results included two steps of normalization: first, for each sample, the peak area of each specific fragment was normalized relative to the sum of the peak areas of the control fragments. Second, the normalized peak areas were divided by the mean of specific peak areas obtained for the normal DNAs. A 30–60% decrease in the relative peak area of one specific amplification product indicated the presence of a single copy of the corresponding exon. The observed deletions were always confirmed on a second run.

Nucleotide and amino acid numbering and changes are designated according to den Dunnen and Antonarakis (2001) using the *SERPINC1* cDNA GenBank X68793.1 sequence as a reference. For cDNA numbering, #1 is the A of the ATG translation initiation codon; for amino acid numbering, codon #1 is the ATG initiation codon.

Characterisation of *SERPINC1* deletions

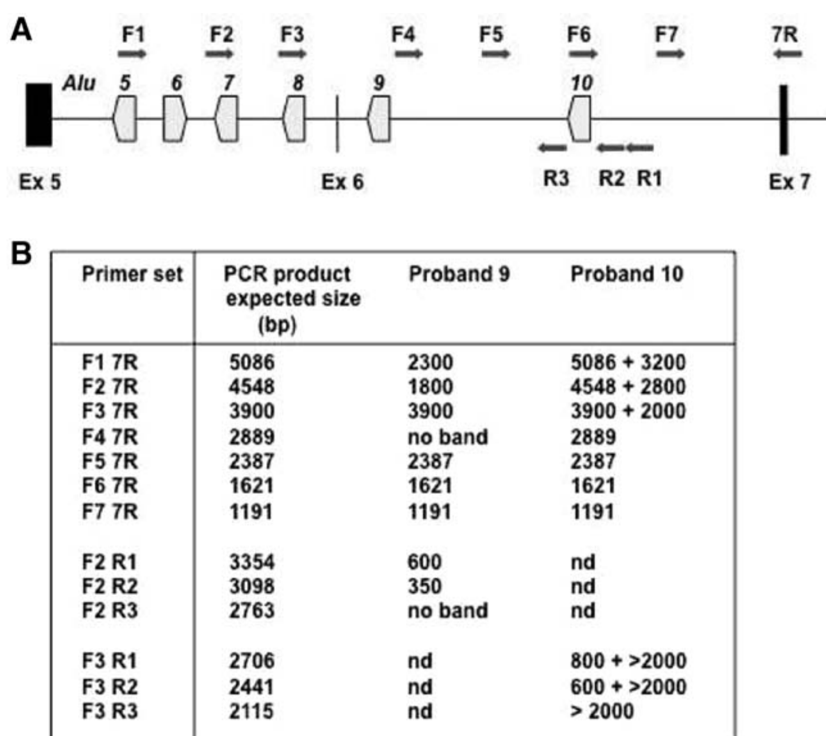
For partial gene deletions, we carried out several series of long-range PCRs in order to localize the deletion breakpoints. The primers were designed using the Primer3Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) in order to span the deleted sequence as follows: F1 5′-acgtcaccatacctggtaatttctgtattc, F2 5′-tttttgagacgaagtcttgctcttgtcc, F3 5′-caccacacctggctaa tttttgtattttc, F4 5′-caacctagggagaagttttaagaaaatgga, F5 5′-gt gcatggaaccctgtgttagagtaatag, F6 5′-tttgtattttttagtagagacg gggtttcac, F7 5′-tgaatatcctctggtaaggactccagttag, 7R 5′-ct cttataaaaaccaggaaaggcc-tgttgg, R1 5′-gagagttaaaaccctgag gactcaatttc, R2 5′-caactcctgctatcatttgaggcaattaaa, R3 5′-aaa atctcattaggagacccgttcaacac, Seq2 5′-gaagtgctgggattacaggtg (Fig. 1). We only describe the experimental conditions which led to successful characterisation of exon 6 deletions. Long-range PCRs using the Expand Long Template PCR system were performed as recommended by the manufacturer (Roche, Mannheim, Germany). Briefly, each reaction contained 200–500 ng DNA, 300 nM primers, 500 µM dNTP, and 0.75 µl of enzyme mix in buffer 3. Cycling was performed as recommended. A control wild-type DNA was amplified in each experiment. Amplification products were tested on 2 or 0.7% agarose gels. Sequencing was performed as previously described using F2 or Seq2 and R1 primers (Picard et al. 2003). Alignment of sequences was performed with NCBI Blast2 software using the *Alu* consensus sequences described by Britten et al. (1989) and the GenBank sequence NT_004487 (*Homo sapiens* chromosome 1 genomic contig reference assembly).

Results

SERPINC1 analysis in 86 probands with type I AT deficiency

In 63 out of 86 probands, the AT deficiency was explained by the presence of already described point mutations (missense or nonsense mutations in 38 and 17 subjects respectively, short deletion or insertion in 8 subjects). Four other probands were heterozygous for already described point mutations explaining borderline AT levels (Cambridge II c.1246G>T, $n = 1$; Dublin c.89T>A, $n = 2$) or type IIHBS deficiency (c.440C>T, $n = 1$), but not consistent with a type I deficiency. In the 19 other probands, no point mutation was found. After sequencing, DNA remained available for 22 among these 23 unresolved cases. MLPA screening performed in the 22 patients demonstrated the presence of large heterozygous deletions in 10 patients: entire gene deletion in probands 1–5 and partial gene deletions involving exons 5–7 in probands 7 and 8, exons 1 and 2 in proband 6 and exon 6 in probands 9 and 10, respectively.

Table 1 summarizes results of *SERPINC1* analysis as well as clinical characteristics and plasma AT levels observed in these 10 probands and in the 10 family members studied. No other genetic or acquired biologic risk factors for thrombosis were identified in these subjects. One family (#1) originated from Germany, whilst the other subjects were from different regions of France. A family history of thrombosis, defined by VTE in at least one first-degree relative, was present in 8 cases. Excepted for proband 8, every proband had clearly low AT antigen and activity level (range 40–60 and 42–60%, respectively, normal values 80–120%) consistent with a typical type I AT deficiency. This was confirmed on at least two different measurements. However, in proband 8, AT heparin cofactor activity was much lower than AT antigen level (20 and 56%, respectively), suggesting the presence of 2 mutations, one responsible for a type I and the other responsible for a type II HBS deficiency. This is consistent with the observation that proband 8 is a compound heterozygote who carries both the c.440C>T substitution changing an amino acid located in the heparin binding site (p.147Thr>Ile), and a three exon-deletion (Picard et al. 2006). We noticed that three other probands are also compound heterozygotes, although they had a typical type I deficiency AT phenotype. These subjects carry both a large deletion and a point mutation: the Cambridge II mutation (c.1246G>T, p.416Ala>Ser) in proband 10; the Dublin mutation (c.89T>A, p.30Val>Glu) in probands 1 and 4. The point mutation in the coding sequence and the large gene deletion are located on distinct alleles in at least 3 cases, as indicated by the presence of a complete gene



Primer set	PCR product expected size (bp)	Proband 9	Proband 10
F1 7R	5086	2300	5086 + 3200
F2 7R	4548	1800	4548 + 2800
F3 7R	3900	3900	3900 + 2000
F4 7R	2889	no band	2889
F5 7R	2387	2387	2387
F6 7R	1621	1621	1621
F7 7R	1191	1191	1191
F2 R1	3354	600	nd
F2 R2	3098	350	nd
F2 R3	2763	no band	nd
F3 R1	2706	nd	800 + >2000
F3 R2	2441	nd	600 + >2000
F3 R3	2115	nd	> 2000

Fig. 1 Mapping of exon 6 deletions in 2 probands. **a** shows the *SERPINC1* region encompassing exons 5–7. *Alu* repeats 5–10 and locations of the PCR primers used to amplify across the deletion sites are also indicated. Size of the amplification products is indicated in **b**

deletion in subjects 1 and 4 and by results of the family study for proband 10.

Characterisation of two novel exon 6 deletions

Long-range PCR was used to characterise large *SERPINC1* deletions: this approach was successful only for the exon 6 deletions (Fig. 1).

For proband 9, F1-7R and F2-7R PCR fragments were about 2.8 kb shorter than expected and no normal size PCR product was amplified. This suggested a deletion of approximately 2.8 kb including exon 6, which was confirmed by the results of amplifications performed with primer F2 coupled with R1, R2 or R3 (Fig. 1b). Again, PCR products of the expected sizes were not observed, which is possibly explained by the position of the primers within *Alu* sequences. Sequencing the 600 bp F2-R1 amplification product with F2 and R1 demonstrated a 2,769-bp deletion (Fig. 2). The 5′ breakpoint is located in intron 5, in a stretch of 13 nucleotides located 844–832 nt before the first nucleotide of exon 6, in the right arm of the *Alu* 7 sequence. The 3′ breakpoint is located in intron 6, in an identical sequence of 13 nucleotides located 1,512–1,524 nt before the first nucleotide of exon 7, in the right arm of the *Alu* 10 repeat element. The exact breakpoints could not be ascertained because of the presence of an identical 13-bp sequence at both ends. This deletion is named c. [1154-(844_832)_1219-(1524_1512)del].

For proband 10, shorter PCR fragments were obtained using primer 7R coupled with F1, F2 or F3 or primer F3 coupled with R1 or R2 (Fig. 1). Analysis of proband 10 father's DNA showed the same migration pattern. However, only the normal size-PCR products were obtained using the mother and the sister's DNA. This suggested a deletion of about 1.8 kb in proband 10 and her father. For both individuals, the F3-R1 amplification product was sequenced in both directions using Seq2 and R1. A deletion of 1,892 bp was identified, beginning in intron 5, 10 nt before exon 6 first nucleotide and ending in intron 6 in the right arm of *Alu* 10 repeat element, 1,219 nt before exon 7 first nucleotide. In addition, an insertion of 10 bases (ATAGAGGTCA) was observed (Fig. 3). We noticed that a sequence being a perfect inverted repeat of these 10 inserted bases was present at nt 1,176–1,185 at the beginning of exon 6. This genotype is designated c.[1154-10_1219-1566delins ATAGAGGTCA]+[1246G>T].

Characterisation of the 5′ breakpoint of a previously reported 5′ untranslated region-exon 2 deletion

Fernandez-Rachubinski et al. previously described a patient who carried a large *SERPINC1* deletion encompassing exon 1 and 2 and a large region 5′ to the coding sequence (Fernandez-Rachubinski et al. 1992). The 3′ deletion breakpoint was located in intron 2, but the position of the 5′ breakpoint remained unknown despite extensive

Table 1 *SERPINC1* mutations, clinical and plasma phenotypes in the 10 families with type I AT deficiency

Family		Sex	Age (years)	Age at first VT (years)	Thromboses	VT: at risk situation	AT heparin cofactor activity (%)	AT antigen (%)	*SERPINC1* gene mutations
1	P	F	40	25	LLDVT + stroke	Postpartum	51	49	EX1_EX7del+c.89T>A
	D	F	20		0		50	52	EX1_EX7del
2	P	F	51	27	LLDVT	Pregnancy	45	50	EX1_EX7del
	D	F	24		0		Normal	Normal	No mutation
	D	F	16		0		Normal	Normal	No mutation
3	P	F	34	26	LLDVT	Cockett	54	60	EX1_EX7del
4	P	F	25	17	LLDVT + PE	OC	43	58	EX1_EX7del+c.89T>A
	D	F	Dead	10 days	Cerebral VT	IUFGR	30		EX1_EX7del
5	P	F	47	37	LLDVT		52	52	EX1_EX7del
	S	F	54		0		52	48	EX1_EX7del
6	P	M	26	<22	LLDVT	0	60	61	EX1_EX2del
7	P	M	62	28	LLDVT	0	50	46	EX5_EX7del
	B	M	69	<50	LLDVT	0	51	40	EX5_EX7del
	N	M	37		0		48	48	EX5_EX7del
8	P	F	37	30	LLDVT		20	56	EX5_EX7del+c.440C>T
9	P	M	56	55	LLDVT	Long travel	42	45	EX6del
10	P	F	36	18	LLDVT + PE	0	43	49	EX6del+c.1246G>T
	F	M	62		0		44	48	EX6del
	S	F	35		0		94		No mutation
	Mo	F	64		0		86		c.1246G>T

Normal range, 80–120%; *B* brother, *D* daughter, *F* female, *M* male, *Mo* mother, *N* nephew, *P* proband, *S* sister, *LL* lower limb, *OC* oral contraceptive, *PE* pulmonary embolism, *DVT* deep venous thrombosis, treatment; *IUFGR* intrauterine foetal growth restriction

analysis of a 684-bp sequence upstream of the coding region. We used the sequence reported by these authors to re-analyse their data using current human genome data.

We compared the original 684-bp region to the GenBank sequence including 20 kb upstream *SERPINC1* (NT_004487). A highly homologous sequence (99% identity) was identified starting 4,718 bp before nt 1, strongly suggesting that the 5′ breakpoint could be located in a GCC sequence 4,716–4,718 bases upstream from *SERPINC1* nt1. This would lead to a 9,480-bp deletion (Fig. 4). This genotype is designated c. 1-(4716_4718)_409-(776_778)del.

Since *SERPINC1* is rich in *Alu* repeats, we conducted a BLAST search for other *Alu* sequences within the region 5 kb upstream the gene and we identified four complete repeats: *Alu* −1 (nt −2629_−2359) and *Alu* −3 (nt −3,566_−3317) oriented in the reverse direction, and *Alu* −2 (nt −2967_−2694) and *Alu* −4 (nt −4755_−4504) oriented in the forward direction. These 4 repeats are clustered in a 2.5-kb region and account for 21% of the sequence 5 kb upstream *SERPINC1*. The 5′ breakpoint of this large deletion is located within the left arm of *Alu* −4 repeat element.

Discussion

Among 86 probands with a suspicion of AT type I inherited deficiency, 63 (73%) subjects carried a heterozygous point mutation responsible for the deficiency. MLPA identified large *SERPINC1* deletion in 10 (12%, 10/85) subjects, accounting for about half (45%, 10/22) the deficiencies unexplained by sequencing. In 12 (14%, 12/85) subjects, AT deficiency remains unexplained. AT plasma levels observed in these subjects were not significantly different from those of patients with an identified genetic defect (not shown). In fact, MLPA is not able to identify large rearrangements that do not change exon dosage, such as inversion, balanced rearrangements or chromosome rearrangements. Moreover, mutations in promoter or intronic regions might also account for the failure of the analyses performed. To our knowledge, no mutation in the *SERPINC1* promoter has been reported yet. This region of the gene remains to be investigated. Furthermore, insertions caused by LINE-1 endonuclease-dependent retrotransposition are not readily detectable by either direct sequencing or any quantitative PCR techniques (Chen et al. 2008).

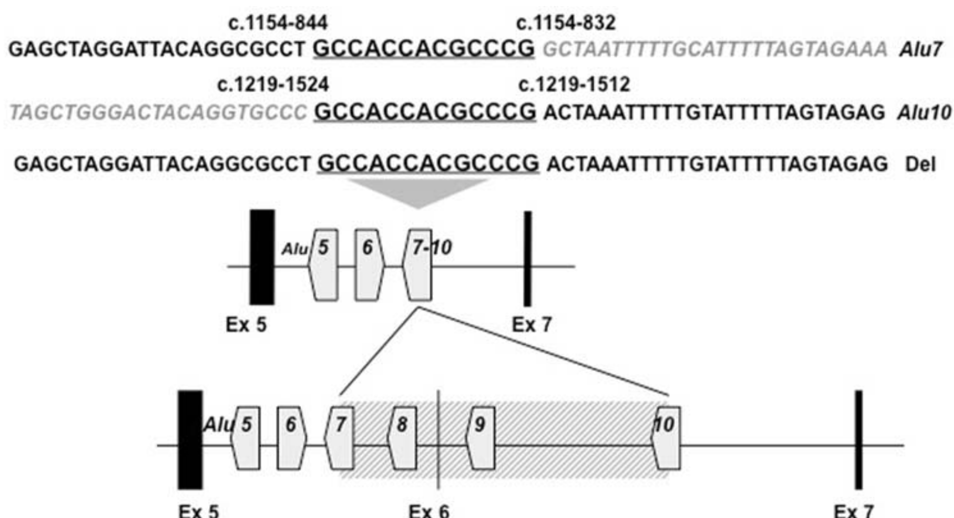


Fig. 2 Diagram of the 2,769 bp deletion including exon 6 in proband 9. The sequences of normal *Alu* 7 and normal *Alu* 10 are aligned with the deleted sequence. The identical 13-bp sequence located within *Alu* 7 and *Alu* 10 repeats is underlined. The structure of the abnormal allele in the region of the deletion is shown below

Co-segregation of large deletions with type I AT deficiency could be observed in five families, arguing for these mutations being responsible for low AT level. Unexpectedly, four probands are heterozygous for both a large gene deletion and a type II missense mutation. These three type II variants, (a type IIHBS, the Dublin and the Cambridge II variants) are mild risk factors for thrombosis, which is consistent with the "usual" character of the clinical manifestations observed in these compound heterozygotes (Daly et al. 1990; Lane et al. 1997; Perry et al. 1998; Picard et al. 2007).

Five complete and five partial gene deletions were identified. The two exon 6 deletions were fully characterised at the DNA sequence level. One is a 1,892-bp deletion, examination of the sequence surrounding the breakpoints reveals a short inverted 8-bp repeat next to the 3′ breakpoint in *Alu* 10 and 23 nucleotides downstream the 5′ breakpoint, in exon 6. This mutation can be readily

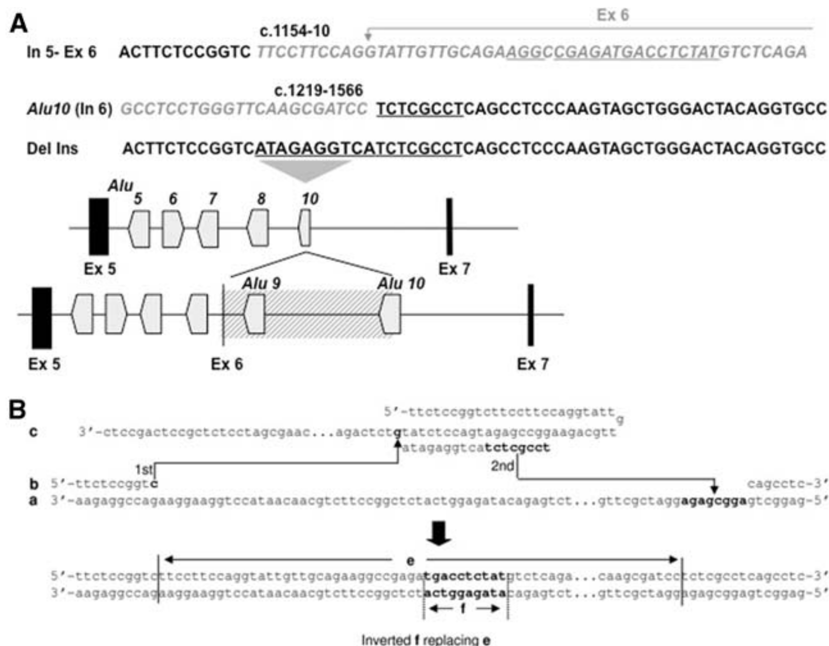


Fig. 3 **a** Diagram of the 1,892-bp deletion including exon 6 and 10-bp insertion observed in proband 10 and her father. The sequences of normal intron 5-exon 6 boundary and normal *Alu* 10 are aligned with the deleted sequence. The 10 bp inserted sequence is ATAGAGGTCA. Inverted repeats are *underlined*. The structure of the abnormal allele in the region of the deletion is shown below. **b** Scheme for generating the mutation. *Top panel* the microhomologies mediated the two steps of replication slippage are *in bold*. *a* Leading strand template. *b* Nascent primer strand. *c* Lagging strand template. See Chen et al. (2005b) for a detailed description of the SRS model. *Lower panel* illustration of the mutant allele. The relationship between the deleted sequence (*e*) and the 'donor' (inverted) sequence (*f*) is indicated

explained by the serial replication slippage (SRS) model (Chen et al. 2005a, b; Férec et al. 2006). During replication, the newly synthesized primer strand could have dissociated from its template strand (designed as the leading strand template for ease of discussion) and annealed to an illegitimate template located on the lagging strand through a 1 bp microhomology. The functional significance of a 1 bp microhomology in mediating replication slippage has been described previously (Sheen et al. 2007; Chauvin et al. 2009). Having copied a short sequence tract from the lagging strand template, the primer strand annealed back to its original template strand in a misaligned configuration; instead of returning to its initial dissociation site, it aligned to a downstream site through a 8 bp microhomology (Fig. 3b, top panel). These two steps of replication slippage, followed by resumption of normal DNA replication, resulted in the observed complex rearrangement allele (Fig. 3b, lower panel).

The second exon 6 deletion removes 2,769 bp. This deletion may have occurred through *Alu*-mediated recombination since both breakpoints are found in an identical 13-bp sequence (i.e. GCCACCACGCCCG) located in the right tail of *Alu* 7 and *Alu* 10 repeats, respectively. Positions 2–12 of this 13-sequence tract are identical to positions 1–11 of a 17-sequence tract (i.e. CCACCA CGCCCAGCTAA) that was involved in an *Alu*-mediated *HFE* deletion (Le Gac et al. 2008). The overlapping 11-bp sequence (i.e. CCACCACGCCC) contains a truncated version of the χ-element, TGGTGG (or CCACCA on the complementary strand), previously reported as a mutational ''super-hotspot'' common to microdeletions, microinsertions, indels (Ball et al. 2005) and gene conversion mutations (Chuzhanova et al. 2009). The aforementioned *Alu* 7 repeat in intron 5 and the *Alu* 10 repeat in intron 6 of *SERPINC1* were also reported to mediate a 2,761-bp deletion, in which the crossover region occurred within the left arms of the involved *Alu* sequences (Olds et al. 1993).

Both novel exon 6 deletions predict the removal of the exon together with flanking intronic sequences, and induce a frameshift after codon 384 leading to a stop codon at position 388, consistent with a quantitative deficiency phenotype. Indeed, several point mutations affecting the C-terminal region of AT after amino acid 384 were previously identified in patients with type I AT deficiency (Lane et al. 1997) indicating that integrity of the C-terminal sequence is necessary for correct synthesis and secretion of the protein. In addition, type I AT deficiency is associated with each of the two other exon 6 deletions already reported (Olds et al. 1993; Lee et al. 2008).

A review of the literature indicates that 10 whole gene and 14 partial large (>100 bp) *SERPINC1* deletions and one duplication have been already observed (Bock and Prochownik 1987; Olds et al. 1993; Emmerich et al. 1994; Lane et al. 1997; Jochmans et al. 1998; Beauchamp et al. 2000; Pavlova et al. 2006; Lee et al. 2008). None of the whole gene but five of the partial deletions were fully characterised (Fig. 5). We propose that a sixth already described deletion of exon 1 and 2 spans 9,480 bp, with a 5′ breakpoint located 4.7 kb upstream *SERPINC1* in an Alu repeat element (Fernandez-Rachubinski et al. 1992). The presence of a 3 bp microhomology (i.e. GCC) at the junction sequence (Fig. 4) can be explained by the simple mechanism of non homologous DNA end joining (Lieber 2008) or the canonical model of replication slippage (Chen et al. 2005c) or microhomology-mediated break-induced replication (Chauvin et al. 2009 and references therein).

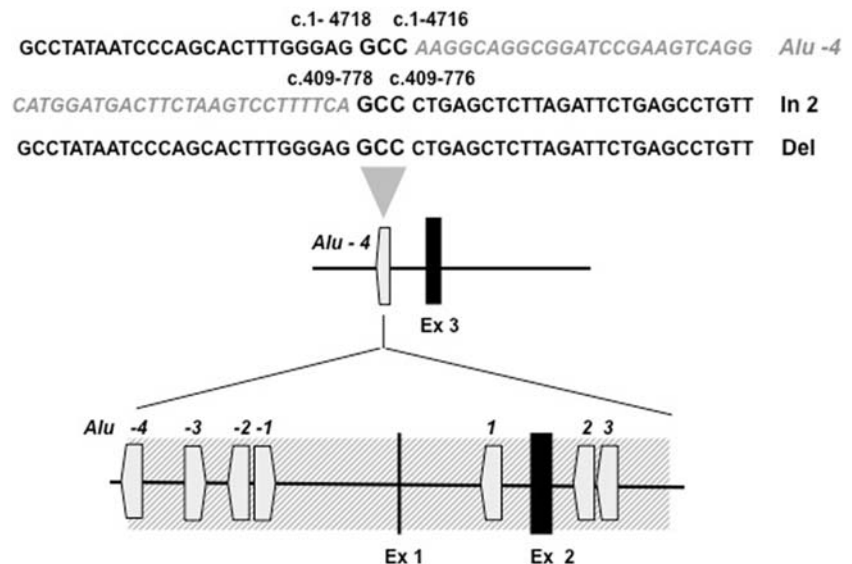


Fig. 4 Diagram of the exons 1–2 deletion partially characterised by Fernandez-Rachubinski et al. (1992) and re-annotated in this study. The sequences of normal *Alu* −4 and intron 2 are aligned with the deleted sequence. The structure of the abnormal allele in the region of the deletion is shown below

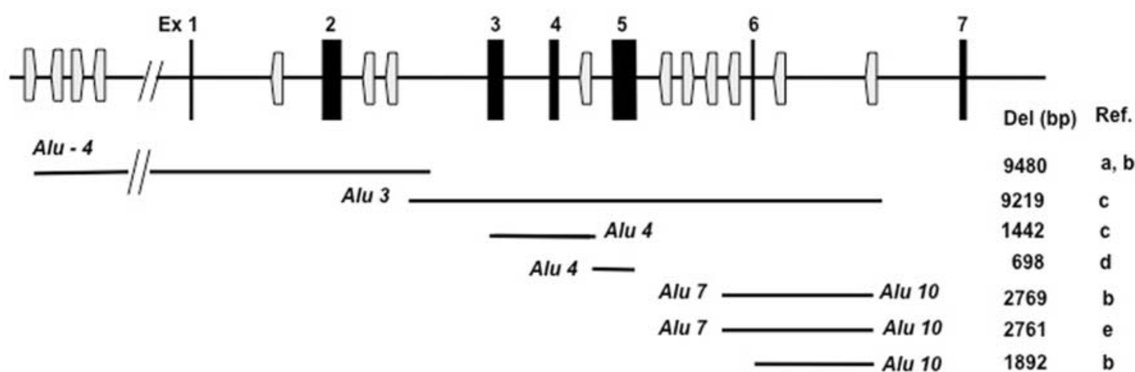


Fig. 5 Map of the characterised large (>150 bp) partial deletions of *SERPINC1*, highlighting the major role of *Alu* repeat elements

Alu repeat elements occur in *SERPINC1* at a higher frequency (22% of the intron sequence) than in the genome as a whole (5%). This high proportion of *Alu* repeats, as previously suggested by Olds et al. (1993), clearly plays an important role in the generation of large *SERPINC1* deletions causing AT type I deficiency (Fig. 5). In this regard, it is worthy of highlighting that we identified a cluster of 4 *Alu* repeats 5 kb upstream *SERPINC1*, which may have been involved in some of the not yet fully characterised *SERPINC1* deletions.

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