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Identification of nine alternatively spliced α2,3-sialyltransferase, *ST3Gal IV*, transcripts and analysis of their expression by RT-PCR and laser-induced fluorescent capillary electrophoresis (LIF-CE) in twenty-one human tissues

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In order to characterise the candidate α2,3-sialyltransferases necessary for biosynthesis of the selectin ligand SLe^x and related antigens we have cloned and sequenced, from peripheral blood leukocytes of single individuals, various transcripts from the human *ST3Gal III, IV and VI* genes. Our clones have revealed a considerable heterogeneity in transcript isoforms. Among our *ST3Gal IV* clones we isolated nine alternatively spliced transcripts covering the coding region of the human *ST3Gal IV* gene (A1, A1 − 12, A1 + 18, A2, A2 − 12, A2 + 18, B, B − 12 and B + 18). Five of these isotranscripts A1 − 12, A1 + 18, A2 − 12, A2 + 18 and B + 18 have not been described before. In order to investigate if the alternatively spliced isotranscripts were specific for human PBL, we analysed the expression by RT-PCR and laser-induced fluorescent capillary electrophoresis (LIF-CE) in twenty other human tissues. We found a tissue specific expression of *ST3Gal IV* A1, A1 − 12, A1 + 18, A2, A2 − 12, A2 + 18 and B + 18 as well as a general expression of *ST3Gal IV* B and B − 12 isotranscripts in all tissues examined.

Keywords: **sialyltransferase, *ST3Gal IV*, transcripts, RT-PCR, capillary electrophoresis**

Introduction

The recruitment of human peripheral blood leukocytes (PBL) to sites of infection and inflammation requires the surface expression of Sialyl Lewis x glycoconjugates (SLe^x) on the white blood cells and of E- and P-selectins on the activated endothelial cells [1–3]. This important phenomenon has been the topic of several reviews [4–7]. In order to characterise the candidate α2,3-sialyltransferases necessary for biosynthesis of SLe^x and related antigens we have cloned and sequenced human *ST3Gal III*, *ST3Gal IV* and *ST3Gal VI* gene transcripts from PBL of single individuals. Our clones have revealed a considerable heterogeneity in transcript isoforms corresponding to alternative splicing. Database studies comparing genomes and Expressed Sequence Tags (ESTs) sequences have recently shown that an unexpected high frequency of genes (38 to 42%) occur as alternatively spliced forms in these databases [8,9]. In a recent report Modrek et al. [8], revealed in a random sample of their database, that 74% of alternative splices actually modifies the protein product, whereas only 22% were alternatively spliced in the 5′-UTR and only 4% in the 3′-UTR. The most abundant category of alternatively spliced genes occurs in cell surface proteins/receptors (29%) including membrane anchored receptors, integral membrane proteins and proteins involved in cell surface adhesion. Twenty-nine per cent of the alternatively spliced genes encoded functions specific to the immune system cell surface receptors. Fourteen per cent of alternatively spliced genes showed tissue specificity for the minor isoforms.

We are specifically studying membrane proteins of the Golgi apparatus. In this paper we report a new and efficient approach to study the alternatively spliced forms of the coding region of *ST3Gal IV* from a single RT-PCR amplification giving both qualitative information and an estimate of the relative expression of *ST3Gal IV* isotranscripts in various tissue samples.

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In 1993, Sasaki et al. published the first sequence of a human Galβ(1-3/1-4) GlcNAc α2,3-sialyltransferase (*ST3Gal IV*). cDNA was cloned from human melanoma cell line WM266-4, and the sequence was denoted ST4 (GeneBank acc. No X74570) [10]. A year later another α2,3-sialyltransferase was cloned from placenta called STZ (acc. No. L23767) [11]. This later report also described three isoforms of the STZ gene; B-long, A-long and short. The B-form has later been shown to consist of four isoforms B1, B2, B3 [12] and BX [13], which all differ in the 5′-UTR of the transcripts. The short form was determined to be an A-form called A2 and the A-long was denoted A1 [12]. When comparing the ST4, which showed to be a type B1 [10], with the sequence of the STZ type B1 a difference in 12 nucleotides in exon 6 was revealed [12]. These 12 nucleotides are deleted in the ST4 sequence. The corresponding amino acids are Arg-Tyr-Ile-Glu-Leu, with an exchange from Ser to Arg of the first amino acid in this sequence. The sequence is located in the putative stem region of the enzyme. Kitagawa et al. reported that, although they screened human placenta cDNA extensively, the B1 $-$ 12 isoform was not detected in this tissue [12]. In order to map and characterise the isotranscripts of *ST3Gal IV* in various human tissues we have utilized the technique of RT-PCR, stretching only over the coding region, and laser-induced fluorescent capillary electrophoresis (LIF-CE) for separation of amplified fragments. As an internal control we choose the housekeeping glycolytic gene glyceraldehyde 3-phosphate dehydrogenase (acc. No. J04038, NM_002046) [14,15].

Materials and methods

Cloning and sequencing

Total RNA was prepared from blood samples (5 ml of EDTA anticoagulated blood) of single healthy individuals using the Qiagene Rneasy midikit (Qiagene Ltd., UK). cDNA was synthesized using Promega Reverse transcription system with poly dT-primers (Promega Corp. Madison, USA). Cloning primers were designed to cover the coding region of the *ST3Gal IV* A and B isoforms respectively and not to distinguish primarily between differences in the 5′-UTRs (acc. No. X74570, L23767 [10,11]), (Figure 1, Table 1). Thus, a sense primer for amplifying both the type A1 and A2 isoforms (STIV-10s) was designed using exon 4. For amplifying only the A2 isoforms the splice junction between exons 4 and 6 was used as in primer STIV-11s [16]. The sense primer specific for the B isoforms (STIV-4s) was complementary to a sequence in exon 3. The anti sense primer STIV-4as was common for both A and B types and was complementary to a sequence in exon 14. All primers were purchased from Scandinavian Gene Synthesis AB (Köping, Sweden).

In the PCR reaction for cloning *ST3Gal IV* type A the final concentrations were 1.0 μM of STIV-10s and STIV-4as, 150 μM dNTP (Amersham Pharmacia Biotech AB, Uppsala, Sweden), 1 mM $MgCl_2$, 50 mM KCl, 10 mM Trizma base and 1 unit of Taq DNA polymerase (Boeringer Mannheim, GmbH, Germany). In the PCR reaction for cloning *ST3Gal IV* type A2 and B the final concentrations were 0.6 μM of STIV-11s or STIV-4s and STIV-4as, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Trizma base and 1 unit of Taq DNA polymerase (Boeringer Mannheim). Ten μl of cDNA was used as template. The PCR-program was 85°C 10 min, 95°C 15 sec, 60°C 15 sec, 68°C 3 min for 30 cycles and finally 68°C for 10 min.

The amplified fragments were purified on Agarose gel (GIBCO BRL, Life Technologies, Paisley, Scotland) and recovered using GenElute Minus EtBr Spin Columns (Sigma, S:t Louis, MO, USA). Fragments were ligated into the pTAg cloning vector pCR2.1 and transformed into INVαF′ One Shot competent cells (Invitrogene BV, Groningen, the Netherlands).

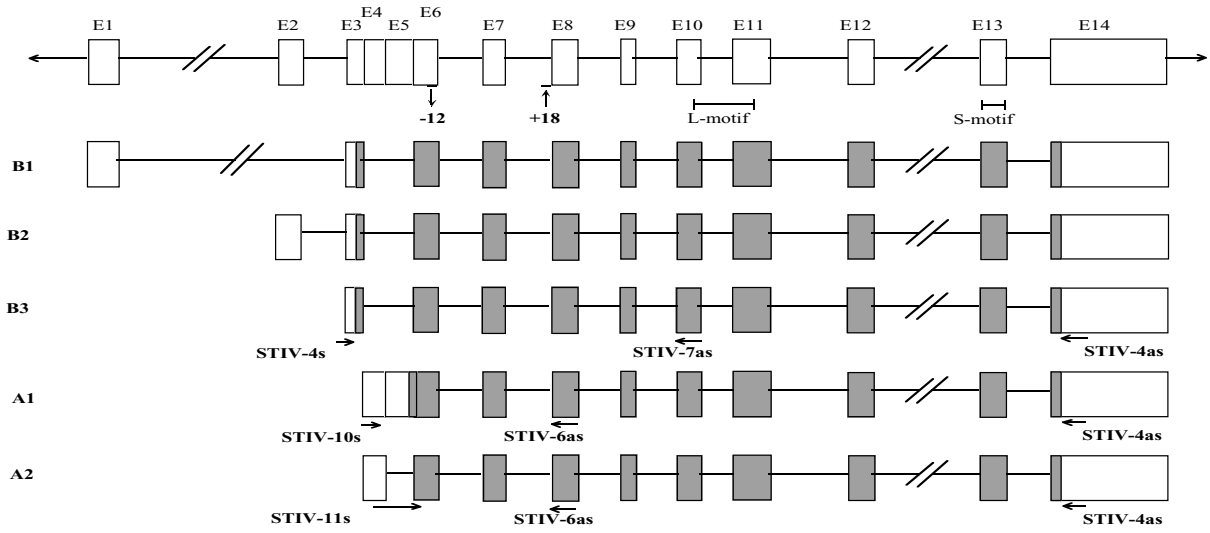

Figure 1. Genomic map of the *ST3Gal IV* gene also showing the corresponding A1, A2, B1, B2 and B3 transcripts. Open boxes denote 5′- and 3′-untranslated sequences and shaded boxes represent coding sequences according to Kitagawa *et al.* [12]. Amplifying primers are marked by arrows and aligned under the corresponding transcripts to indicate the products obtained. The positions of the $-$12 and $+$18 nucleotide differences are marked by bars and vertical arrows.

Table 1. *ST3Gal IV* and *GAPDH* primers used for fragment analysis[1]

Primer	*Isoform*	*Sequence*	*Annealing positions/Isoform*[2]
FAM-STIV-4s	All B	5′-6-FAM-GGAATCCTGCTGCCTGCTGAGAA-3′	−25 – −3/B
FAM-STIV-10s	A1 and A2	5′-6-FAM-CTTCATCTTGAAGGACAGTGG-3′	−170 – −149/A1
FAM-STIV-11s	A2	5′-6-FAM-TTGAAGGACAGTGGGCTGGAAGCTC-3′	−31– −7/A2
HEX-STIV-4as	All A and B	5′-HEX-AGGCAACCGACAGGCTACAG-3′	1022–1003/B
HEX-STIV-7as	All A and B	5′-HEX-AGTGAGCTGTTCCGCAGCCGGTGC-3′	389–366/B
HEX-STIV-6as	All A and B	5′-HEX-AAATAATCCTCAAGCCGCAGG-3′	215–195/B
FAM-GAPDH-s	Int. control	5′-6-FAM-AGTCCACTGGCGTCTTCAC-3′	290–311
GAPDH-as	Int. control	5′-GAGGTCCACCACCCTGTTG-3′	981–963
2-GAPDH-as	Int. control	5′-GCCACAGTTTCCCGGAGG-3′	589–572

[1]Identical primers without fluorochromes were used for cloning of *ST3Gal IV* transcripts.
[2]Annealing positions for *ST3Gal IV* refers to [10,11] and for *GAPDH* [14,15].

Selected clones were all sequenced in both directions using Dye Terminator FS kit on Applied Biosystems 373A and 377 DNA sequencers or Big Dye Terminator kit on ABI PRISM 310 instrument (P-E Applied Biosystems, Foster City, CA, USA). Sequence analyses and alignments were performed using the Lasergene software package (DNASTAR Inc., Madison, WI, USA).

PCR and RT-PCR amplification with fluorescent primers

Total RNA-samples from a panel of tissues were purchased from Clontech (Cat no K4005-Z, Clontech, Palo Alto, CA, USA). The panel contained RNA samples from placenta, fetal brain, fetal liver, adrenal gland, lung, testis, skeletal muscle, brain, spleen, liver, uterus, thymus, salivary gland, trachea, heart, kidney, bone marrow, spinal cord, prostate and colon. Total RNA from peripheral blood leukocytes was prepared as described above.

Sense primers for all the *ST3Gal IV* A and B isoforms were labelled in the 5′-end with the fluorescence dye 6-carboxyfluorescein (6-FAM) without any other change in the primers used for cloning. The antisense primers were similarly labelled with the hexachlorinated analogue to 6-FAM (HEX) (Scandinavian Gene Synthesis AB) (Table 1). The glyceraldehyd-3-phosphate-dehydrogenase (GAPDH) gene transcript (acc. No. J04038, NM_002046) was used as an internal positive control, with a 6-FAM labelled GAPDH sense primer and an unlabelled antisense primer giving raise to a fragment of 692 bp. In order to amplify shorter fragment (<500 bp) we kept the sense primers as above and designed new antisense primers (Table 1). For *ST3Gal IV* type B primer HEX-STIV-7as was used and for the *ST3Gal IV* type A the HEX-STIV-6as was used throughout this study although any of these antisense primers may be used for fragment analysis of these transcripts. An alternative GAPDH antisense primer was also used to give a cDNA fragment of 300 bp length. For each tissue 0.1 μg RNA was used in the RT-step. The PCR reactions were performed as for cloning for *ST3Gal IV* type A2 and B. For *ST3Gal IV* type A1 there was no dNTP added in the PCR reaction while otherwise it was performed as for cloning. In all PCR reactions for analysing tissue expression of the *ST3Gal IV* gene we added 0.04 μM of each GAPDH primer. The standard PCR-program was 85°C 10 min, 95°C 15 sec, 60°C 15 sec and 68°C 3 min for 25 cycles.

Capillary electrophoresis

Electrophoretic analyses were performed on an ABI PRISM 310 instrument equipped with ABI PRISM GeneScan Analysis Software package (P-E Applied Biosystems).

For longer fragment analysis (>500 bp)

The amplified products were diluted 1:10 in distilled water. One μl of the dilution was mixed with 0.5 μl TAMRA-labelled GS-2500 size standard (P-E Applied Biosystems) and 12 μl of distilled water. A 1 ml Hamilton syringe and a 47 cm × 50 μm capillary with 3% GeneScan polymer and 1X Genetic Analyser Buffer with EDTA were used (P-E Applied Biosystems). The injection time was set at 5 sec at 15 kV and electrophoreses were run at 42°C at 2 kV with 90 min detection time. Virtual filter set C, which records the fluorescent light intensities in four windows centered around 530, 542, 567 and 590 nm, was used according to the manufacturers protocol to provide maximal separation of the different dyes and keeping a low signal to noise ratio.

For shorter fragment analysis (<500 bp)

The amplified products were diluted 1:10 in distilled water. One μl of the dilution was mixed with 0.5 μl TAMRA-labelled GS-500 size standard (P-E Applied Biosystems) and 12 μl deionised formamide and denatured at 95°C for 5 min and chilled on ice. A 1 ml Kloehn micro syringe and a 47 cm × 50 μm capillary with Performance Optimised Polymer 4 (POP-4) and 1X Genetic Analyser Buffer with EDTA was used (P-E Applied Biosystems). The injection time was set at 5 sec at 15 kV and the electrophoreses run at 60°C at 15 kV with 30 min detection time using virtual filter set C.

Statistics of the fragment analysis

The resolution indices were calculated according to the triangulation method and the formula $R_s = 2(t_B - t_A)/W_A + W_B$. The t_A is the elution time for peak A, t_B is the elution time for peak B. W_A is the base width of peak A and W_B is the base width of peak B. Reproducibility was tested for within series by aliquoting one standard RT-PCR amplification of total RNA from PBL, to 14 tubes run subsequently, and for between series, 3 series ($n = 25$) of standard RT-PCR amplifications of total RNA from PBL were analysed. Within series and between series values for elution times (min), estimated sizes (bp), peak height (fluorescence intensity) and peak area (total fluorescence) were calculated and expressed as means and coefficients of variation (CV%). The formula used for calculation of standard deviation between series (SD_{btw}) were $SD^2_{total} = SD^2_{within} + SD^2_{between}$.

To estimate the relative expression of the *ST3Gal IV* isotranscripts, the peak area for the individual transcripts were calculated related to the peak area for the internal standard, GAPDH, and expressed as percentage of GAPDH.

Results

Cloning and characterisation of *ST3Gal IV* transcripts

We have, from human PBL, cloned and sequenced 8 of the 9 isotranscripts covering the coding region of the human *ST3Gal IV* gene (A1, A1 − 12, A2, A2 − 12, A2 + 18, B, B − 12 and B + 18). Their structural differences are shown in Figure 2 together with the A1 + 18 isotranscript cloned from human placenta. Five of these, i.e. A1 − 12 (acc. No AF516604), A1 + 18 (acc. No. AF525084), A2 − 12 (acc. No AF516603), A2 + 18 (acc. No AF516602) and B + 18 (acc. No AY040826)

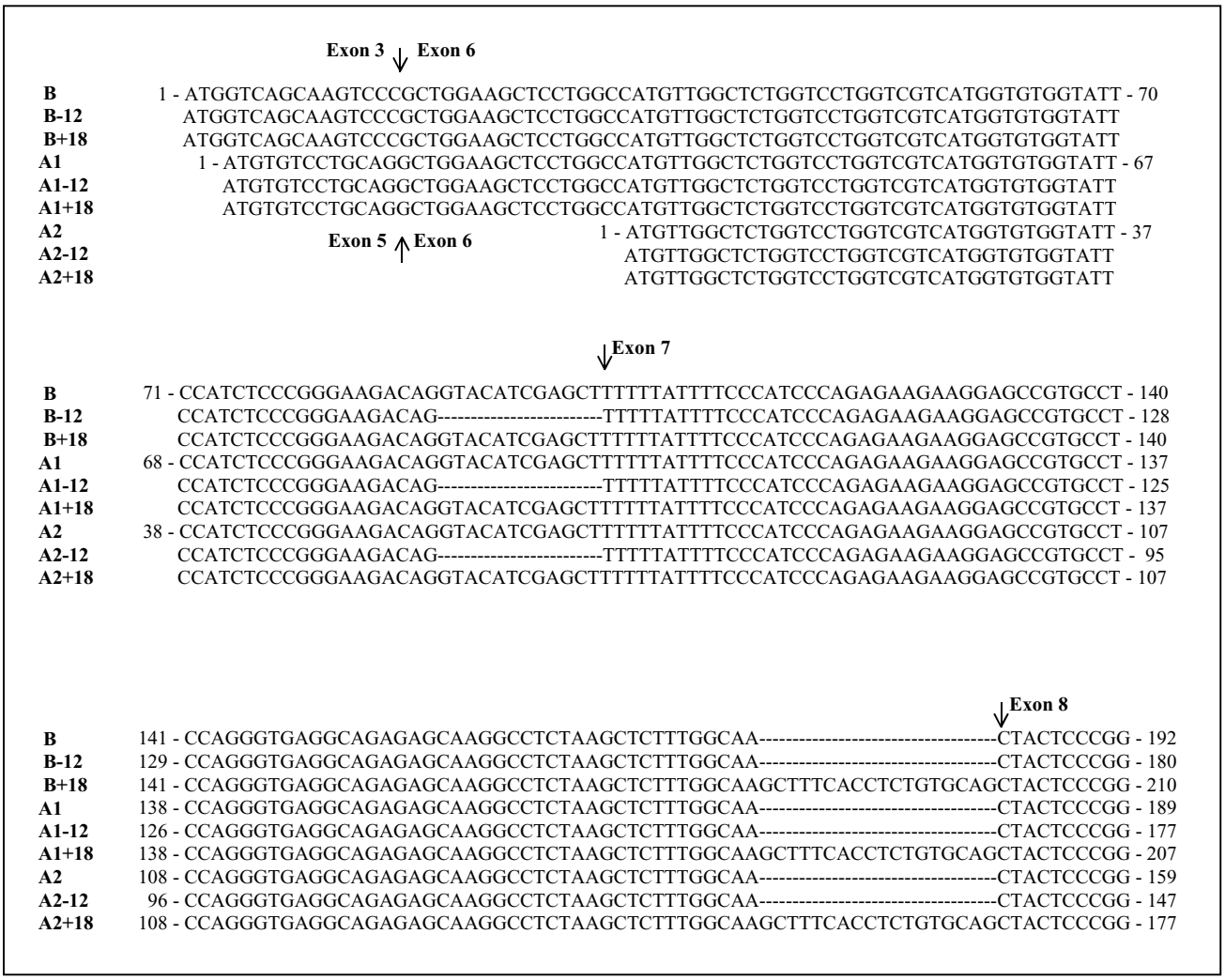

Figure 2. Nucleotide sequences of 9 isotranscripts covering the first 5′-ATG down to exon 8 of the coding sequence of *ST3Gal IV* gene (B, B − 12, B + 18, A1, A1 − 12, A1 + 18, A2, A2 − 12 and A2 + 18) isolated from human PBL and human placenta (A1 + 18). Sequence numbers are given according to [11].

have not been described before. The differences between B and B − 12, A1 and A1 − 12, A2 and A2 − 12 isoforms respectively are the same 12 nucleotides located at the 3′- end of exon 6. These nucleotides correspond to amino acids -Arg-Tyr-Ile-Glu-Leu- located in the putative stem region of the enzyme. The differences between B and B + 18, A1 and A1 + 18 and A2 and A2 + 18 respectively are 18 identical nucleotides in the 3′-end of the intron E7/E8 which correspond to amino acids -Lys-Leu-Ser-Pro-Leu-Cys-Ser- also located in the putative stem region of the enzyme. The alternative splice sites both follow the GT-AG consensus rule for splicing. The sequence data revealed two silent C → T point mutations (Tyr → Tyr) corresponding to nucleotides 252 and 423 in the *ST3Gal IV* B isoform, nucleotides 249 and 420 in the *ST3Gal IV* A1 isoform, and nucleotides 219 and 390 in the *ST3Gal IV* A2 isoform. These mutations were found (homo- or heterozygously) in the three single individuals so far characterised and in all 9 isotranscripts from the *ST3Gal IV* gene. Apart from these silent mutations in exons 8 and 10, respectively, there was complete agreement in nucleotide sequences downstream from exon 8 to exon 14 in all the isotranscripts.

Capillary electrophoresis of *ST3Gal IV* isotranscripts: Longer fragments

Figure 3A shows an electrophoregram of a mixture of longer fluorescent fragments of the *ST3Gal IV* type B isotranscripts obtained from three isolated clones (B − 12, B and B + 18). Figure 3B shows one standard RT-PCR amplification of total RNA from PBL. Due to a lower resolution index and the relative dominance of B over B − 12 and B + 18 isotranscripts the different fragments are not well resolved. Thus, quantification using longer fragment analysis was not possible.

Capillary electrophoresis of *ST3Gal IV* isotranscripts: Shorter fragments

Figure 4A shows an electrophoregram of a mixture of shorter fluorescent fragments of the *ST3Gal IV* type B isotranscripts obtained from the three clones B − 12, B and B + 18. The relative distribution of *ST3GaI IV* type B transcripts obtained by one standard RT-PCR amplification of total RNA from PBL of one single individual is shown in Figure 4B. This pattern, with a relative dominance of B over B − 12 and a very minor occurrence of B + 18 isotranscript, was consistently found in all tissue samples (Table 2). Corresponding analyses were performed with primers for A1 and A2 and for A2 specifically, which showed similar patterns with a relative dominance of the A1 and A2 over the A1 − 12 and A2 − 12 isotranscripts in all tissue samples. The A1 + 18 and A2 + 18 isotranscripts were only detected in placenta (Table 2). Since placenta was the only tissue showing expression of A1 + 18 we cloned and sequenced this isotranscript from placenta (Figure 2).

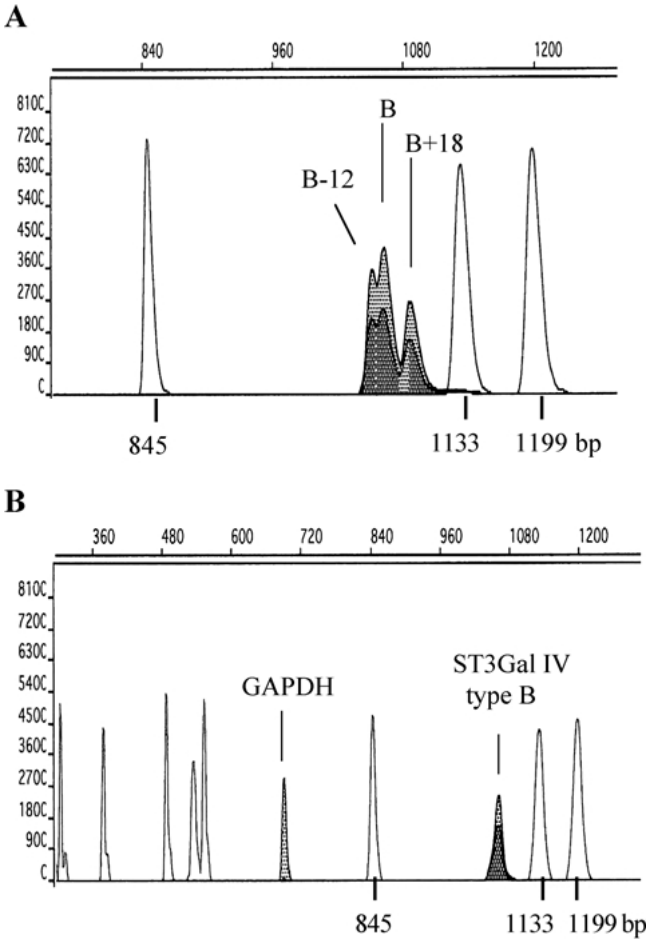

Figure 3. Electrophoregrams of longer fragments of the *ST3Gal IV* type B isotranscripts obtained from (A) three isolated clones (B, B − 12, B + 18) and (B) from a standard RT-PCR amplification of total RNA from human PBL.

Statistics of the LIF-CE fragment analyses

Longer fragment analysis

The resolution index (R_s), calculated for the size marker peaks of the GS-2500 standard at 1133 bp and 1199 bp, was 2.5. The variations in peak areas for size markers 845 bp, 1133 bp and 1199 bp were between 7.4% and 7.5% (CV_{within}) and 8.9% to 9.1% (CV_{total}). For the 692 bp GAPDH fragment the size was estimated by the software to be 691.99 ± 0.12 bp (CV_{total} 0.02%). For this fragment the variation in peak area was 7.0% (CV_{within}) and 12.9 % (CV_{total}).

The true lengths of the amplified *ST3Gal IV* fragments were 1189 bp (A1 − 12), 1201 bp (A1), 1219 bp (A1 + 18), 1032 bp (A2), 1020 bp (A2 − 12), 1050 bp (A2 + 18), 1047 bp (B − 12), 1059 bp (B) and 1077 bp (B + 18) respectively. The length estimated by the software for corresponding fragments was 1199 bp (A1 − 12), 1211 bp (A1), 1036 bp (A2), 1025 bp (A2 − 12), 1059 bp (A2 + 18) 1053 bp (B − 12), 1065 bp (B) and 1089 bp (B + 18).

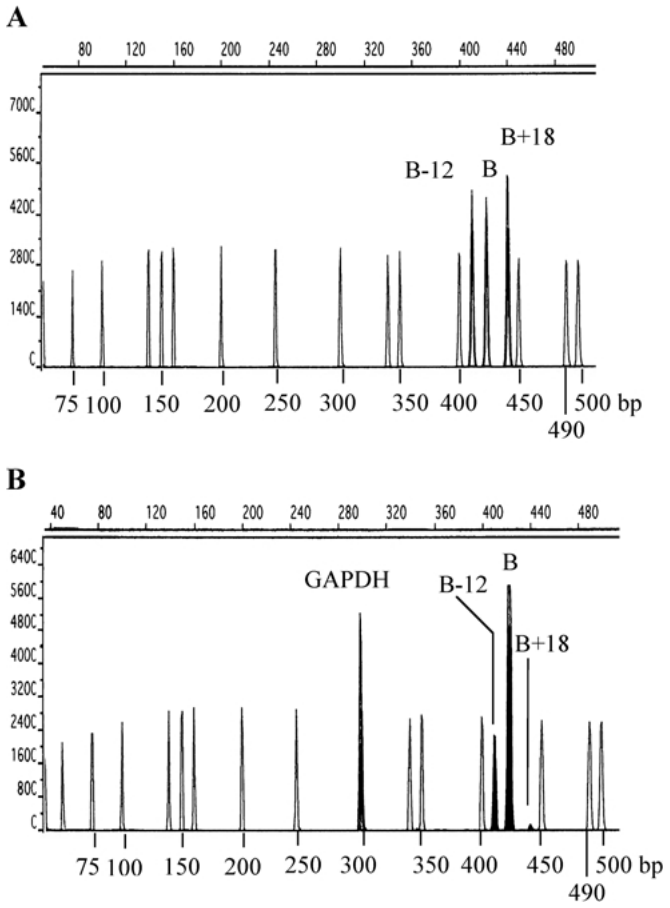

Figure 4. Electrophoregrams of shorter fluorescent fragments of the *ST3Gal IV* type B isotranscripts obtained from (A) three isolated clones (B, B − 12, B + 18) and (B) from a standard RT-PCR amplification of total RNA from human PBL.

Shorter fragment analysis

The resolution index (R_s), calculated for the size marker peaks at 490 and 500 bases was found to be 3.4. The variations in peak areas for size markers at 350 and 450 bases were between 2.4% and 4.6% (CV_{within}) and 9.6% and 9.7% (CV_{total}). For the 300 bases GAPDH fragment the size was estimated by the software to be 298.53 ± 0.07 bases (CV_{total} 0.02%). For this fragment the variation in peak area was 2.3% (CV_{within}) and 12.9 % (CV_{total}).

The true lengths of the amplified *ST3Gal IV* fragments were 382 bases (A1 − 12), 394 bases (A1), 412 bases (A1 + 18), 225 bases (A2), 213 bases (A2 − 12), 243 bases (A2 + 18), 414 bases (B − 12), 426 bases (B) and 444 bases (B + 18) respectively. The length estimated by the software for corresponding fragments was 381 bases (A1 − 12), 393 bases (A1), 410 bases (A1 + 18), 224 bases (A2), 212 bases (A2 − 12), 242 bases (A2 + 18) 411 bases (B − 12), 423 bases (B) and 441 bases (B + 18).

Tissue-specific distribution and expression of the *ST3Gal IV*- transcripts

The different tissues showed a noticeable difference in *ST3Gal IV* expression with the highest expressions of *ST3Gal IV* type B isotranscripts in adrenal glands and placenta and the lowest in bone marrow and liver (Table 2). In adrenal glands the B isotranscript dominated completely with much lower expression of B − 12, B + 18, A1, A2 and A2 − 12 isotranscripts. In placenta all 9 isotranscripts could be detected. There was high expression (>50% of GAPDH) of the B, B-12, A1 and A2 isoforms and low expression (<10% of GAPDH) of the other isotranscripts. Trachea, testis, PBL and colon showed moderate and high expression of the B-12 and B isotranscripts respectively. In these tissues there was a highly differential expression of the A1 and A2 isotranscripts. In trachea, there were no A1 − 12, A1, A1 + 18 or A2 + 18 but only A2 − 12 and A2 isotranscripts detected. In colon on the other hand, the only isotranscripts detected besides the B isotranscripts were A1 and A2. In fact colon showed, next to placenta, the highest expression of A1 in any tissue examined. The A1 isotranscripts were found only in six of the examined tissues. The A1 − 12 and A1 + 18 isotranscripts were detected only in placenta. The A2 transcripts were found in thirteen of the twenty-one tissues. The A2 − 12 and A2 transcripts showed the same relative expression as the B − 12 and the B transcripts but at much lower amounts. The A2 − 12 transcript was expressed only in five tissues and for skeletal muscle this was the only A isotranscript detected.

Discussion

Northern blot analyses have earlier been used to show that the human *ST3Gal IV* gene has a tissue specific expression [11,12,17] and our results show good agreement with those reports. Northern blot gives rough qualitative information on mRNA sizes, may handle many samples at a time, gives relative abundance of transcript levels and gives information on alternatively spliced variants from one single gene or from multigene family members depending on the hybridisation stringency. The major limitations are the technique's high sensitivity for RNA degradation, which severely compromises the results, the relatively low resolution of agarose gel electrophoresis, and the lack of sensitivity ($\sim 10^6$ copies of mRNAs). The RT-PCR-CE technique, as we have exemplified here, gave us the possibility to separate and identify 9 isotranscripts of the *ST3Gal IV* gene, with minor differences in the coding regions, and to estimate the relative expression of these isotranscripts in many tissues in one run. The method based on RT-PCR combined with capillary electrophoresis (RT-PCR-CE) makes it possible to detect even very low amounts of transcripts (in theory only one copy). Calibration curves have been shown linear as low as at 0.002 to 0.02 amol (10^{-18}) of each DNA fragment [18,19] corresponding to 10 to 500 molecules [20,21]. In this paper we have shown excellent resolution indexes at 3.4 and 2.5 for fragments at 490 and 500 bases and 1133 and 1199 bp, respectively.

Table 2. Tissue expression of *ST3Gal IV* isotranscripts relative to GAPDH (100%)

	Isotranscript expression vs GAPDH (%)											
	B transcripts				*A1 transcripts*				*A2 transcripts*			
Tissue	*B − 12*	*B*	*B + 18*	*All B*	*A1 − 12*	*A1*	*A1 + 18*	*All A1*	*A2 − 12*	*A2*	*A2 + 18*	*All A2*
Adrenal gland	31	223	2	256	–	5	–	5	1	4	–	5
Placenta	54	213	3	270	6	51	1	58	10	59	1	70
Trachea	22	179	–	201	–		–	–	1	4	–	5
Testis	23	171	–	194	–	2	–	2	1	6	–	7
PBL	22	149	2	173	–	5	–	5	–	7	–	7
Colon	16	146	1	163	–	10	–	10	–	3	–	3
Spinal cord	12	115	1	128	–	–	–	–	–	1	–	1
Fetal brain	20	97	1	118	–	–	–	–	–	1	–	1
Uterus	13	69	–	82	–	–	–	–	–	1	–	1
Brain	12	65	–	77	–	–	–	–	–	1	–	1
Fetal liver	11	50	1	62	–	–	–	–	–	–	–	–
Heart	6	47	–	53	–	–	–	–	–	1	–	1
Kidney	7	46	2	55	–	–	–	–	–	–	–	–
Salivary gland	7	40	–	47	–	–		–	–	–	–	–
Spleen	7	37	–	44	–	–	–	–	–	2	–	2
Thymus	5	31	–	36	–	–	–	–	–	–	–	–
Lung	5	26	–	31	–	–	–	–	–	1	–	1
Skeletal muscle	4	23	–	27	–	–	–	–	<1	–	–	<1
Prostate	3	24	–	27	–	–	–	–	–	–	–	–
Bone marrow	2	20	–	22	–	1	–	1	–	–	–	–
Liver	3	15	–	18	–	–	–	–	–	–	–	–

The fragments studied are usually around a few hundred bp, but the technique is valid for single or double stranded fragments in the range of 20 to 2000 bp [20,22,23]. The reproducibility of the technique in terms of retention times is excellent with a coefficient of variation of 1–2% [22] or as we have shown here for fragment sizes with CV's = 0.02%. Including all the steps in the RT-PCR-CE procedures the total variation for peak areas reported is between 15 and 25% [23,24] which also fit very well with our data.

As shown in this paper RT-PCR-CE has the resolution, sensitivity, speed and high throughput capacity ideal for quantitative and qualitative analysis of specific gene transcripts in biological tissues. Surprisingly, very few publications are found in the literature where this technique has been systematically employed [25] and nothing has, to our knowledge, so far been published for glycosyltransferase genes. We used only a standard instrumentation, and employed 2 differently labelled fluorescence primers yielding double-labelled fragments with minor differences in size. In most cases the differences in alternatively spliced genes correspond to larger deletions and/or insertions than reported here [9]. In those cases, the resolution for our longer fragment analyses will be quite enough.

The presence of various isoforms in human PBL has not been systematically addressed although Gassen et al. reported the expression of some of these isoforms in leukemic cell lines [16]. In order to characterise the candidate α2,3-sialyltransferases necessary for biosynthesis of SLe^x, the minimal structural requirement for E-, P- and L-selectin ligands, and related antigens we have cloned and sequenced, from peripheral blood leukocytes of single individuals, various transcripts from the human *ST3Gal III*, *IV and VI* genes. The coding region of *ST3Gal IV* revealed 9 human A1, A2 and B isotranscripts without regarding differences in the 5′-UTRs (Figures 1 and 2). Our studies of *ST3Gal IV* have confirmed a 12 nt deletion (Figure 2) which matches the splice site sequence between exons 6 and 7 of both A and B isoforms [10–12]. In addition, we have found a novel insertion of 18 nt that matches the splice site sequence between exons 7 and 8 of A1, A2 and B isoforms (Figure 2). The transcripts result from splices according to the GT-AG consensus rule for splicing [26,27]. The 12 and 18 nt differences in the isoforms we report in this study, correspond to 4 and 6 amino acids respectively within the putative stem region of the sialyltransferase. This part of the enzyme is considered of relevance for the localisation and retention within the Golgi system [28,29]. A recent report has nicely confirmed that the cytoplasmic, transmembrane and stem regions of glycosyltransferases also specifies their functional localisation and stability in the Golgi [30]. These data implies that the isoforms reported here might be located differently and have different functionality within the Golgi. The 18 nt insertion corresponds to a Lys-Leu-Ser-Pro-Leu-Cys-Ser sequence and deletes a potential N-glycosylation sites of the enzyme. A specific loss of

one of two N-glycosylation sites in a secreted form of α2,6-sialyltransferase, *ST6Gal I* (ST_{tyr}), has been shown to generate proteins that were not cleaved and secreted from Golgi in transfected COS-1 cells [31]. The deletion of a potential N-glycosylation site of *ST3Gal IV* might have a similar effect on the trafficking or/and activity of this transferase although this must be proved by further studies.

In order to investigate if the 12 nt deletion and the +18 nt sequence was specific for human PBL, we examined a panel of tissues. We found expression of *ST3Gal IV* B − 12 and B isotranscripts in all tissues. For the three B isoforms examined, we always found the same pattern with a dominance of B and a minor expression of the B − 12 and B + 18 transcripts. This relative distribution was also true for the tissues expressing A1 and A2 isotranscripts. The +18 transcript generally showed a very low expression and the B + 18 isotranscript was only found in 8 of the 21 tissues examined. The A1 + 18 and A2 + 18 isotranscripts were only detected in placenta. Noteworthy, in skeletal muscle the only A isoform detected was the A2 − 12. Modrek et al. [8] revealed that 14% of alternatively spliced genes showed tissue specificity for the minor isoforms. This is nicely demonstrated here by the expression of A1 − 12, A1, A1 + 18, A2 − 12, A2, A2 + 18 and B + 18 in different tissues and the general expression of B − 12 and B isotranscripts in all tissues examined.

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