

DNA sequence variants of the platelet-derived growth factor A-chain gene

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Summary

Background Platelet-derived growth factor A-chain (PDGF-A) is a potent connective tissue mitogen implicated in lung growth and development. PDGF-A may have a role in asthma through effects on fibroblasts and bronchial smooth muscle cells.

Objective To test the hypothesis that there exist variations in the PDGF-A gene associated with the asthma phenotype.

Methods We screened genomic DNA from normal and asthmatic subjects using single-stranded conformational polymorphism (SSCP) for mutations in the promoter and all seven exons of the gene.

Results Four transition polymorphisms (three novel) were identified: one each in exons 3 and 4 (overall population allele frequencies 0.18 and 0.02, respectively) which did not alter the protein sequence, one in exon 4 (frequency 0.005) which resulted in a valine to isoleucine substitution, and one in intron 5 (frequency 0.5). The intron 5-sequence variant is close to the 3' end of exon 5 but does not appear to affect alternative splicing of PDGF-A exon 6 RNA. The frequencies of the polymorphisms in exons 3 and intron 5 did not differ between the asthmatic and non-asthmatic subjects, but there was a significant frequency difference between Caucasian and African-American subjects for each of these polymorphisms ($P = 0.03$ and 0.003 , respectively).

Conclusion No association was found between the sequence variants in the PDGF-A gene and the development of asthma. However, the allele frequency of some of the sequence variants differed between the Caucasian and African-American subjects.

Keywords: platelet-derived growth factor A-chain, polymorphism, mutation, single-stranded conformational polymorphism analysis, asthma, airway remodelling

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Introduction

Platelet-derived growth factor A-chain (PDGF-A) is a potent connective tissue mitogen which has been implicated in cell growth, tissue development, wound healing and the pathogenesis of several diseases. The human PDGF-A gene is localized to the distal short arm of chromosome 7 (7p21-p22) and spans ~ 24 kilobases. The

gene contains a 5'-flanking region with promoter activity, seven exons and six introns [1–3]. Alternative RNA splicing results in two PDGF-A proteins with different carboxyl-terminals due to the presence or absence of exon 6 in the mature mRNA [1,3–6]. The different carboxyl-terminal regions may alter protein function by changing the affinity for the cell membrane and result in either a cell-bound or secreted protein product.

Although a number of polymorphisms have been detected in the PDGF-A gene [2,7,8], a systematic study of this gene with respect to sequence variants has not been

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Table 1. Oligonucleotide primers and their respective annealing temperatures

	Forward 5'-3'	Reverse 3'-5'	T _m (°C)*
Core promoter (39–58, 306–326)	GGG GCT TTG ATG GAT TTA GC	GAG GAG GAG GAG GAG TAG GCG	55
Exon 1 (1011–1030, 1189–1207)	GAT GGT ACT GAA TTT CGC CG	GAG GAG CCG GCG CAG GGA C	55
Exon 2 (1223–1242, 1343–1362)	GAC CGT GTG GCC TCT GCT TG	AGG GAA GGG GCG CGA TTT AC	60
Exon 3 (1497–1516, 1622–1641)	GCA TTC ACG GTG TTC TCC TT	CTG GAC TCA CCG ATG CTT CT	55
Exon 4 (1749–1768, 1957–76)	CTG TGC CCC CGC CGT TGC AG	GAG GGG AGG GGA GGG CTC AC	64
Exon 5 (2010–2029, 2176–2195)	GGC TCT GTT CTC TCT GGC AG	CGT TCT CCA ACA CCG ATG CC	60
Exon 6 (2299–2318, 2389–2408)	CCC TGC CCT TTT GTT AAC AG	GTG TCC GGC AGA CAG TCC TA	55
Exon 7 (2527–2546, 2672–2691)	GTC TCA GTG TCT GTC TTT CC	CAC AGT TTT TCA CGG AGG AG	55

*T_m = annealing temperature in degrees.

reported. Gene transcription and RNA processing are two important mechanisms involved in the control of gene expression that could be altered as a result of naturally occurring DNA sequence variants. We therefore used single-stranded conformational polymorphism analysis (SSCP) to screen genomic DNA, derived from normal and asthmatic Caucasian and African-American subjects, for sequence variation in the core promoter and all seven exons (including exon–intron boundaries) of the PDGF-A gene. A total of four sequence variants (three novel) were identified. We discuss their structure, frequency, functional implications and phenotype associations.

Methods

Patient materials

DNA for initial SSCP screening was obtained from 25 patients with mild–moderate asthma, according to American Thoracic Society (ATS) criteria [9], and 25 normal controls. All asthma subjects received treatment with leukotriene antagonists or placebo, in addition to rescue treatment with inhaled beta-agonists as needed. Additional clinical characteristics of these subjects have been described in detail [10]. The DNA was extracted from Epstein-Barr virus immortalized lymphocytes prepared from peripheral blood according to standard techniques [11]. After mutations were found, and when additional subjects were required to complete association studies, DNA was collected as previously described [12].

Mutation identification

Genomic DNA was screened for mutations using SSCP analysis according to the method of Orita *et al.* [13] with minor modifications. The oligonucleotide primers were designed to screen the core promoter and seven exons, including exon–intron boundaries, and yield a PCR product between 180–240 basepairs (bp) in length. The specific oligonucleotide primers are listed in Table 1 along with their respective annealing temperatures.

The kinase labelling reaction was carried out by mixing 3.0 µL of [γ -³²P]ATP (3000 Ci/mmol, 10 µCi/µL) (DuPont-New England Nuclear, Boston, MA, USA), 2 µL of 10 µM primers each, 9.5 µL dH₂O, 2 µL of 10X T4 polynucleotide kinase buffer (Boehringer Mannheim, Mannheim, Germany) and 1.5 µL of T4 polynucleotide kinase (10 U/µL) (Boehringer Mannheim). The mixture was incubated at 37 °C for 40 min for labelling and then 70 °C for 10 min to inactivate the enzyme. The total PCR reaction volume was 25 µL, containing 100 ng of genomic DNA in the presence of 1 µL of [³²P]-labelled primers reaction mixture, 2.5 µL of dNTPs (2 mM each), 2.5 µL 10X PCR Buffer (containing 1.5 mM MgCl₂) (Promega Corp., Madison, WI, USA), and 0.3 µL Taq DNA polymerase (0.5 U) (Promega Corp.). All PCR reactions were performed using a PTC-100TM programmable thermocycler (MJ Research, Inc., Waltham, MA, USA) with 30 cycles, each consisting of 94 °C for 1 min, 55–64 °C for 1 min (see Table 1) and 72 °C for 1 min. This was followed by a prolonged extension step for 7 min at 72 °C. The labelled PCR products were stored at

–20 °C overnight before denaturation and gel electrophoresis.

Amplification of the G + C-rich regions of the gene (promoter, exon 1 and exon 2) required different PCR reagents. For the promoter and exon 1, a 25- μ L reaction volume was used containing 200 ng of genomic DNA in the presence of 1 μ L of [^{32} P]-labelled primer mixture, 2.5 μ L of dNTPs consisting of 2 mM dATP, 2 mM dCTP, 2 mM dTTP and a 2-mM, 3 : 1 ratio of dGTP to 7-deaza-dGTP, 1.25 μ L of DMSO, 2.5 μ L 10X PCR Buffer (containing 1.5 mM MgCl₂) and 0.3 μ L Taq DNA polymerase. The reaction conditions were identical for exon 2, except that the regular mixture of dNTPs was used without addition of 7-deaza-dGTP.

PCR products were diluted 2 : 1 in stop solution consisting of 95% formamide, 20 mM EDTA, 0.05% (wt/vol) xylene cyanol, 0.05% (wt/vol) bromophenol blue, and denatured at 95 °C for 5 min. Samples were placed on ice for 2 min and loaded onto an MDE non-denaturing acrylamide gel prepared according to the manufacturer's instructions (AT Biochem, Malvern, PA, USA). Electrophoresis was performed at 40 W for 4 h at 4 °C. The gel was vacuum dried and exposed to autoradiograph film for 2 to 12 h.

DNA sequencing

To identify the DNA sequence associated with the altered electrophoresis migration pattern, the PCR products were cloned into the pCR2.1[®] vector according to the TA cloning method (Invitrogen Corp., San Diego, CA, USA) and subjected to automated sequencing (ABI 377S: Applied Biosystems Inc., Foster City, CA, USA) using an M13R primer.

Frequency determination

Allele frequencies for the polymorphisms in exon 3 and mutation A in exon 4 were determined by SSCP; the frequency of mutation B in exon 4 was determined by SSCP and confirmed by RFLP with *Sfa*NI (New England Biolabs Inc., Beverly, MA, USA) when required. Allele frequency for the polymorphism in intron 5 was determined by RFLP with *Bbv*I enzyme (New England Biolabs) and confirmed with *Bbs*I enzyme (New England Biolabs). Reaction conditions for the enzyme digests consisted of 10 μ L of PCR product, 2.5 μ L of 10X enzyme buffer (New England Biolabs), and 3–8 U of enzyme in a total reaction mixture of 25 μ L which was incubated for 2–12 h at 37 °C. After digestion, the reaction mixture was denatured at 65 °C for 20 min and run on a 1.5–2% agarose gel for visualization.

Detection of alternative RNA splicing

Monocytes were purified from whole blood drawn from

individuals determined to be homozygous-wild type (WT) at intron 5 or individuals homozygous-mutant (M) at intron 5 using a previously described method [14]. Monocytes were grown in tissue culture in RPMI for 4 h. Total RNA was extracted using the Trizol[®] (Life Technologies, Inc., Gaithersburg, MD, USA) method according to the manufacturer's directions. Samples were aliquoted and stored at –80 °C.

RT-PCR was performed using two sets of primers designed to amplify the exon 4–6 boundary (forward: caagaccaggacggctcatt; reverse: gggttttaaccttttctttccg), exon 5–6 boundary (forward: ccaaggtggaatacgtcagg; reverse: same as for exon 4–6), or exon 5–7 (forward: same as for exon 5–6, reverse: aaagggtgcggctcctcct). RT reactions were performed with 2 μ g total RNA, 1 μ g random hexamers (Life Technologies Inc.), 50 nmoles dNTP, 25 U rRNasin[®] ribonuclease inhibitor (Promega), 200 U M-MLV RT (Promega) in 25 μ L total volume consisting of 50 mM Tris-HCL (pH 8.3), 75 mM KCl, 3 mM MgCl₂, and 10 mM DTT at 37 °C for 60 min. PCR reactions were performed with 4 μ L 1 : 5 diluted RT reactions, 40 nmoles dNTP, 20 pmoles each primer, 2 U Taq polymerase (Promega) in 50 μ L total volume consisting of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton[®] X-100 and 1.5 mM MgCl₂. The same RT reaction mixture was analysed by each primer set for comparisons. Depending on the primer set, the annealing temperature and number of PCR cycles varied between 55 °C and 58 °C and 27–30, respectively. PCR products were separated on a 1.5–2% agarose gel, photographed and quantified with Gel-Pro Analyser; densitometry software (Media Cybernetics, Silver Spring, MD, USA).

Statistical analysis

Computations were performed using StatView[®] 4.5 software for MacIntosh (Abacus Concepts Inc., Berkeley, CA, USA). Goodness of fit for chi-square (χ^2 -test) was used for comparison of deviation in appropriately designed contingency tables. Results are expressed as fractions and considered statistically significant at the $P < 0.05$ level.

Results

Coding region mutations

SSCP identified alternative conformers at three loci in the open reading frame. Sequence analysis revealed all three to be single nucleotide polymorphisms (SNPs) (Table 2). In exon 3 there was a single transition consisting of a silent T to C change at bp 468 (from ATG start codon [1]) ('mutation 3', Fig. 1). There were two mutations in exon 4: a transition consisting of a C to T change at base 830

Table 2. Polymorphisms in exon 3, exon 4, intron 5 and effects on protein sequence

Nucleotide sequence		Effect on protein sequence
Exon 3		
Wild type	GGGTCCATGCCAC	No change in protein sequence
Polymorphism	GGGTCCA <u>C</u> GCCAC	
Exon 4		
Wild type	CGC GTC CAC	No change in protein sequence Valine–isoleucine
Mutation A	CGT GTC CAC	
Mutation B	CGC <u>A</u> TC CAC	
Intron 5: 12 base pairs 5' to coding region		
Wild type	GAGTGGCTGCCTTCGTCGC	Intronic region
Polymorphism	GAGTGGCTG <u>T</u> CTTCGTCGC	

(‘mutation 4A’) and a transition consisting of a G to A at base 831 (‘mutation 4B’, Fig. 1). The locus affected by mutation 4A is silent (i.e. arginine to arginine), while the locus affected by mutation 4B results in the substitution of isoleucine for valine.

The allele frequencies of the identified sequence variants are listed in Table 3. At the locus identified in exon 3, the frequency of the minor allele was 0.15 among the 88 Caucasian asthmatic subjects, 0.22 among the 93 Caucasian non-asthmatic subjects, 0.21 among the 74 African-American asthmatic subjects, and 0.14 among the 90 African-American non-asthmatic subjects. The allele frequency of this mutation is significantly greater among Caucasian non-asthmatic subjects than African-American non-asthmatic subjects ($P = 0.03$), but there is no significant difference between asthmatic and non-asthmatic subjects.

Mutation 4A was rare, with only two heterozygote Caucasian asthmatic subjects identified among 20 asthmatic and 25 non-asthmatic subjects, yielding an allele frequency of ~ 0.02 . Mutation 4B was very rare, with only one heterozygote Caucasian asthmatic subject among 20 asthmatic and 84 non-asthmatic subjects, yielding an allele frequency of ~ 0.0048 . Given the rare occurrences of these mutations, comparison of allele frequencies between asthmatic and non-asthmatic subjects was not pursued further.

No mutations were identified by SSCP for exons 1, 2, 6 and 7.

Non-coding region mutations

The promoter of PDGF-A is highly G + C-rich and difficult to reliably amplify by PCR. With this caveat, no consistent mutations were identified by SSCP in the core promoter.

In intron 5 close to the 3'-end of exon 5, there was a

transition consisting of a C to T change at bp 1063 (‘mutation 5’, Fig. 1). Allele frequencies of the identified sequence variants are listed in Table 3. Allele frequencies were significantly different between Caucasian (0.48) and African-American subjects (0.60), $P = 0.003$, but there was no significant difference between asthmatic and non-asthmatic subjects.

Effect of mutation 5 on alternative splicing

Mutation 5 is 12 base pairs from the 3'-end of exon 5, in a region of potential importance in the generation of alternative mRNA splice variants. To investigate the possibility that this mutation is a factor in regulating the known variant RNA splicing of exon 6 [1,3–6], we measured the distribution of RNA products derived from monocytes obtained from individuals of known variant genotype. Figure 2 shows a representative experiment comparing RNA from monocytes of subjects homozygous for wild type (WT) allele with RNA from monocytes of subjects homozygous for the mutant 5 (M) allele. Two alternatively spliced RNA species are detected with primers for exon 5–7. No obvious difference was detected in the distribution of the two RNA products between genotypes, suggesting that this mutation does not overtly affect the regulation of PDGF-A RNA splicing.

Discussion

PDGF-A chain released from inflammatory cells in the airways of asthmatics has been implicated by others in the process of airway constriction, through its effects on the deposition of connective tissue matrix and the stimulation of smooth muscle cell and fibroblast proliferation [15]. Given the suspected role of PDGF-A in the development of altered airway anatomy [16] and the heterogeneity of the

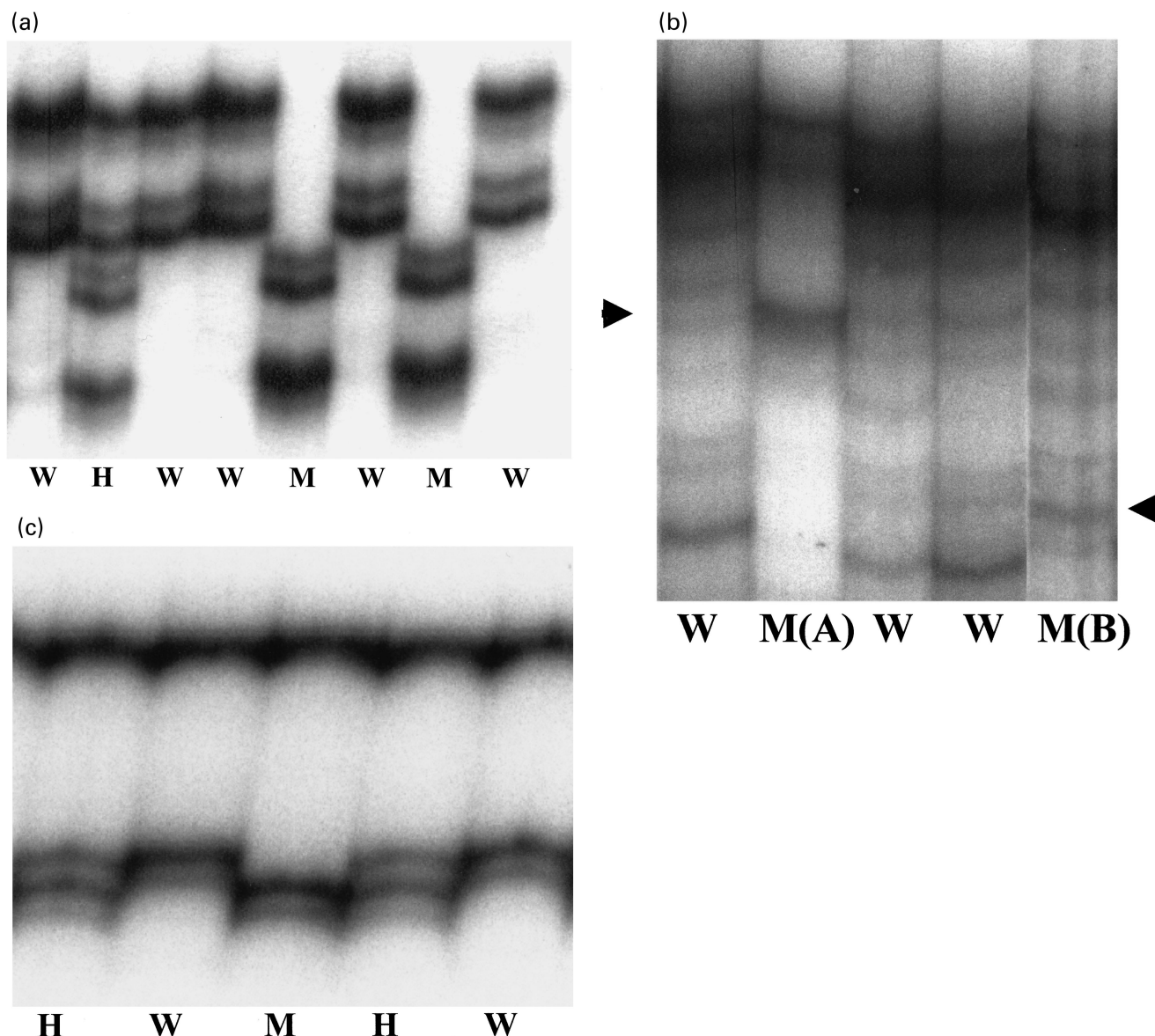


Fig. 1. SSCP bands for exon 3, exon 4 and intron 5. (a) Exon 3: eight lanes are shown with wild type (W), heterozygote (H), wild type, wild type, mutant (M) and wild type, mutant (M) and wild type, respectively. (b) Exon 4: mutation 4A is shown with surrounding wild type subjects on the left portion of the figure, and mutation 4B is shown with surrounding wild type subjects on the right portion of the figure. Arrows highlight the mutant bands. (c) Intron 5: five lanes are shown with heterozygote, wild type, mutant, heterozygote and wild type bands, respectively.

asthma phenotype in the population, we hypothesized the potential for sequence variability in the PDGF-A gene associated with the development of asthma. We detected and identified four transition mutations in the PDGF-A gene, one of which resulted in an alteration of protein structure. An intronic sequence variant near a site of known RNA diversity was not associated with the presence of this splice variant. Our data indicate that sequence variants in the PDGF-A gene are unlikely to be a factor in the development of asthma.

Previous PDGFA polymorphisms have been reported, though to our knowledge none have been associated with alterations in gene expression or protein function. Bonthron *et al.* [2] described a minisatellite spanning the splice donor site in IVS4 that results in partial duplication of exon 4, with an infrequent length polymorphism of a microsatellite (CCTCC)₁₈ (beginning about 13 bp downstream of the exon 4 splice donor site) within the minisatellite. Ferns *et al.* [8] identified a *StyI* RFLP through Southern blotting with a cDNA clone, and Bonthron *et al.* [7] more recently reported

Table 3. Allele frequencies and Hardy–Weinberg equilibrium *P*-values

	Diagnosis	Race	WT	Het	Mut	Allele frequency	HW
Mutation 3	Normal	Cauc	58	28	7	WT 0.78/Mut 0.22	0.18*
	Asthma	Cauc	63	23	2	WT 0.85/Mut 0.15	0.95
	Normal	AA	65	25	0	WT 0.86/Mut 0.14	0.12*
	Asthma	AA	45	27	2	WT 0.79/Mut 0.21	0.38
Mutation 4A	Normal	Cauc	25	0	0	WT 1.00/Mut 0.00	0.99
	Asthma	Cauc	18	2	0	WT 0.98/Mut 0.02	0.81
Mutation 4B	Normal	Cauc	84	0	0	WT 1.00/Mut 0.00	1.00
	Asthma	Cauc	19	1	0	WT 0.99/Mut 0.0048	0.91
Mutation 5	Normal	Cauc	24	37	22	WT 0.51/Mut 0.49	0.32**
	Asthma	Cauc	26	37	19	WT 0.54/Mut 0.46	0.41
	Normal	AA	11	19	22	WT 0.39/Mut 0.61	0.09**
	Asthma	AA	10	31	22	WT 0.40/Mut 0.60	0.87

*Normal Cauc vs. Normal AA, $\chi^2 P = 0.03$. **Total Cauc vs. Total AA, $\chi^2 P = 0.003$. Cauc = Caucasian, AA = African-American, WT = wild type, Het = heterozygote, Mut = mutant, HW = Hardy–Weinberg equilibrium *P*-value.

identity of this *StyI* RFLP with a newly defined IVS3 minisatellite, 220 bp upstream of exon 4. These authors also reported another C/T transition 489 bp downstream from the IVS4 minisatellite. These previously identified polymorphisms have not been associated with disease, and because they are unlikely to have functional consequences, attempts to associate them with asthma were not undertaken in this study. Instead, a comprehensive and systematic screen of the important regions of the PDGF-A gene for mutations was undertaken.

In this study SSCP was used to screen genomic DNA from normal and asthmatic subjects for mutations in the PDGF-A gene promoter and seven exons. We screened 100 chromosomes, giving us 95% power to find sequence variants occurring with a frequency of 0.03 and 99% power to find variants occurring with a frequency of 0.05 [17]. However, it is possible that with this number of samples and with the limitations of SSCP (70–80% sensitivity [18]) there may still exist undetected polymorphisms in the wider population, but their allele frequency is likely to be low.

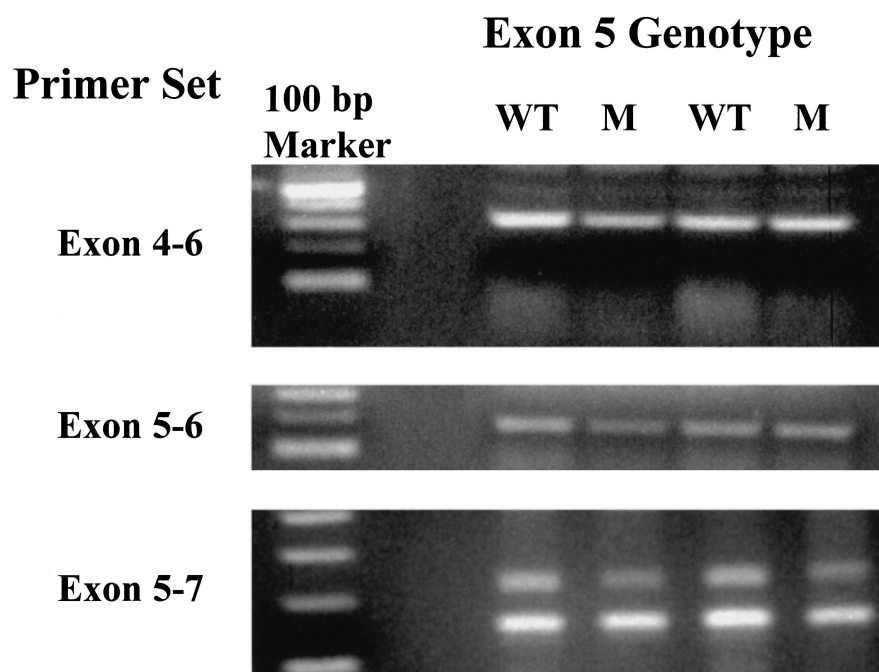


Fig. 2. RNA splice variants from monocytes of subjects WT and M for the mutant 5 allele. Two alternatively spliced RNA species are detected with primers for exon 5–7, with no obvious difference in distribution of the two RNAs.

We found four mutations in PDGF-A, only one of which had been previously reported: one located in exon 3; two located in exon 4; and one located in intron 5. Mutation 3 is relatively common, with an allele frequency greater among non-asthmatic Caucasians than African-Americans, but does not alter amino acid sequence. The mutation we describe in exon 3 is consistent with variation in the reported sequences of PDGF-A in the GENBANK database, as reported by Bonthron *et al.* [7], and our reported allele frequency at this site is similar to that reported by those authors in their population. Mutation 4A is rare and also does not alter amino acid sequence. Mutation 4B is very rare and results in an amino acid substitution from valine to isoleucine. Valine and isoleucine are closely related lipophilic amino acids; therefore, this mutation is unlikely to have significant impact on tertiary protein structure or function.

Mutation 5 is relatively common, with an allele frequency greater among African-Americans compared with Caucasians. The location of mutation 5 close to the 3'-exon-intron boundary of exon 5 suggests that it might be involved in exon 6 RNA splicing. These findings are intriguing because the PDGF-A RNA is characterized by alternative splicing of exon 6 [1,3-6]. The mutation we describe in intron 5 is located 12 base pairs 3' to the donor splice site. While this mutation is not in an area of extensive sequence homology, this particular base pair site is conserved between human and mouse species [19]. Differential splicing may result in gene products with different functions and expression in tissues. Alternative splicing may affect the biology of PDGF-A because exon 6 encodes a cell retention signal, such that the splice product missing exon 6 is excreted and does not remain bound to the cell [20-28]. To test the hypothesis that mutation 5 affects RNA splicing, we examined mRNA from monocytes of genotyped individuals for variation in the quantity of the different RNA species. Our study indicates that mutation 5 is not associated with dramatic changes in PDGF-A RNA splicing, and both splice variants may be produced by cells harbouring two wild type or two mutant alleles.

Although we failed to establish an association with the diagnosis of asthma, we did find a difference in the frequency of two PDGF-A sequence variants between the Caucasian and African-American subjects. Previous authors have identified medically important metabolic traits and pharmacogenetic effects based on ethnic-specific polymorphisms [29]. While it is not clear that our reported polymorphisms have an impact on gene expression or protein function, it is intriguing to speculate whether there may exist differences in PDGF-A-linked disease susceptibility between Caucasians and African-Americans. Excess production of PDGF-A with resultant fibroblast and smooth

muscle cell proliferation and connective tissue elaboration has been investigated in a number of diseases, including idiopathic pulmonary fibrosis [30-32], bronchiolitis obliterans organizing pneumonia [33], and arteriosclerosis [28,34]. Our data increase the number of available PDGF-A polymorphisms to facilitate future disease-association studies.

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References

- 1 Bonthron DT, Morton CC, Orkin SH, Collins T. Platelet-derived growth factor A chain: gene structure, chromosomal location, and basis for alternative mRNA splicing. *Proc Natl Acad Sci USA* 1988; 85 (5):1492-6.
- 2 Bonthron D, Collins T, Grzeschik K-H, Van Roy N, Speleman F. Platelet-derived growth factor A chain: confirmation of localization of PDGFA to chromosome 7p22 and description of an unusual minisatellite. *Genomics* 1992; 13:257-63.
- 3 Betsholtz C, Johnsson A, Heldin C-H et al. cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumour cell lines. *Nature* 1986; 320:695-9.
- 4 Wise RJ, Orkin SH, Collins T. Aberrant expression of platelet-derived growth factor A-chain cDNAs due to cryptic splicing of RNA transcripts in COS-1 cells. *Nucl Acids Res* 1989; 17 (16):6591-601.
- 5 Rorsman F, Bywater M, Knott TJ, Scott J, Betsholtz C. Structural characterization of the human platelet-derived growth factor A-chain cDNA and gene: alternative exon usage predicts two different precursor proteins. *Mol Cell Biol* 1988; 8:571-7.
- 6 Andersson M, Ostman A, Westermark B, Heldin CH. Characterization of the retention motif in the C-terminal part of the long splice form of platelet-derived growth factor A-chain. *J Biol Chem* 1994; 269:926-30.
- 7 Bonthron DT, Smith SJ, Campbell R. Complex patterns of intragenic polymorphisms at the PDGFA locus. *Hum Genet* 1999; 105 (5):452-9.
- 8 Ferns GA, Ross R. *StyI* RFLP of the human platelet derived growth factor (PDGF) A-chain gene. *Nucl Acids Res* 1990; 18 (1):208.
- 9 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Resp Dis* 1987; 136:225-44.
- 10 In KH, Asano K, Beier D et al. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *J Clin Invest* 1997; 99 (5):1130-7.
- 11 Sugden B, Mark W. Clonal transformation of adult human leukocytes by Epstein-Barr virus. *J Virology* 1977; 23 (3):503-8.

- 12 Drazen JM, Yandava CN, Dube L et al. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nat Genet* 1999; 22:168–70.
- 13 Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 1989; 5:874–9.
- 14 Allam M, Martinet N, Gallati H, Martinet Y. Differential expression of PDGF A-and B-chain genes and production of AA and AB dimers by activated human blood monocytes. *Biochimie* 1992; 74:1097–101.
- 15 Bonner JC, Badgett A, Lindroos PM, Coin PG. Basic fibroblast growth factor induces expression of the PDGF receptor- α on human bronchial smooth muscle cells. *Am J Physiol* 1996; 271:L880–L888.
- 16 Zhang S, Smartt H, Holgate ST, Roche WR. Growth factors secreted by bronchial epithelial cells control myofibroblast proliferation: an in vitro co-culture model of airway remodeling in asthma. *Lab Invest* 1999; 79:395–405.
- 17 Lai E, Riley J, Purvis I, Roses A. A 4-Mb high-density single nucleotide polymorphism-based map around human APOE. *Genomics* 1998; 54:31–8.
- 18 Nataraj AJ, Olivos-Glander I, Kusakawa N, Highsmith WE. Single strand conformational polymorphism and heteroduplex analysis for gel-based mutation detection. *Electrophoresis* 1999; 20:1177–85.
- 19 Rorsman F, Betsholtz C. Characterization of the mouse PDGF A-chain gene. Evolutionary conservation of gene structure, nucleotide sequence and alternative splicing. *Growth Factors* 1992; 6:303–13.
- 20 Shimokado K, Raines EW, Madtes DK et al. A significant part of macrophage-derived growth factor consists of at least two forms of PDGF. *Cell* 1985; 43:277–86.
- 21 LaRochelle WJ, Giese N, May-Siroff M, Robbins KC, Aaronson SA. Molecular localization of the transforming and secretory properties of PDGF A and PDGF B. *Science* 1990; 248:1541–4.
- 22 LaRochelle WJ, May-Siroff M, Robbins KC, Aaronson SA. A novel mechanism regulating growth factor association with the cell surface: identification of a PDGF retention domain. *Genes Dev* 1991; 5:1191–9.
- 23 Ostman A, Andersson M, Betsholtz C, Westermark B, Heldin CH. Identification of a cell retention signal in the B-chain of platelet-derived growth factor and in the long splice version of the A-chain. *Cell Regul* 1991; 2:503–12.
- 24 Khachigian LM, Chesterman CN. Structural basis for the extracellular retention of PDGF A-chain using a synthetic peptide corresponding to exon 6. *Peptides* 1994; 15:133–7.
- 25 Maher DW, Lee BA, Donoghue DJ. The alternatively spliced exon of the platelet-derived growth factor A chain encodes a nuclear targeting signal. *Mol Cell Biol* 1998; 9 (9):2251–3.
- 26 Raines EW, Ross R. Compartmentalization of PDGF on extracellular binding sites dependent on exon-6-encoded sequences. *J Cell Biol* 1992; 116:533–43.
- 27 Matoskova B, Rorsman F, Svensson V, Betsholtz C. Alternative splicing of the platelet-derived growth factor A-chain transcript occurs in normal as well as tumor cells and is conserved among mammalian species. *Mol Cell Biol* 1989; 9:3148–50.
- 28 Zhao X-M, Frist WH, Yeoh T-K, Miller GG. Confirmation of alternatively spliced platelet-derived growth factor-A chain and correlation with expression of PDGF receptor- α in human cardiac allografts. *Transplantation* 1995; 59 (4):605–11.
- 29 Weber WW. Populations and genetic polymorphisms. *Mol Diagnosis* 1999; 4:299–307.
- 30 Martinet Y, Rom WN, Grotendorst GR, Martin GR, Crystal RG. Exaggerated spontaneous release of platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. *N Engl J Med* 1987; 317:202–9.
- 31 Antoniadis HN, Bravo MA, Avila RE et al. Platelet-derived growth factor in idiopathic pulmonary fibrosis. *J Clin Invest* 1990; 86:1055–64.
- 32 Nagaoka I, Trapnell BC, Crystal RG. Upregulation of platelet-derived growth factor-A and -B gene expression in alveolar macrophages of individuals with idiopathic pulmonary fibrosis. *J Clin Invest* 1990; 85:2023–7.
- 33 Aubert J-D, Pare PD, Hogg JC, Hayashi S. Platelet-derived growth factor in bronchiolitis obliterans-organizing pneumonia. *Am J Respir Crit Care Med* 1997; 155:676–81.
- 34 Hachida M, Zhang X, Lu H et al. Association between the degree of platelet-derived growth factor A-chain mRNA expression and coronary arteriosclerosis in the transplanted heart. *Heart Vessels* 1998; 13:24–9.