SHORT REPORT

Revised exon–intron structure of human *JAK3* locus

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Jak3, a member of the Janus tyrosine kinase family is an intracellular kinase functionally coupled to cytokine receptors that share a common γ_c chain (γ_c). Defects in the γ_c or Jak3 result in T⁺B⁺ severe combined immunodeficiency (SCID). In order to clarify discrepancies between earlier reported genomic organisations of human *JAK3*, the present study was undertaken to redefine its whole exon–intron structure. The genomic structure of human *JAK3* consists of 23 exons and 22 introns, and shows strong homology with the organisation of the murine *JAK3* locus. The exon–intron sequences provided in this report can be used to facilitate the identification of new Jak3-deficient SCID patients, including prenatal diagnosis.

Keywords: *JAK3*; gene structure; gene organisation; SCID; immunodeficiency

Introduction

The Janus protein tyrosine kinase (Jak) family plays a fundamental role in cytokine receptor signal transduction pathways of several haematopoietic cytokines.^{1,2} To date, this family consists of four members, Jak1, Jak2, Jak3 and Tyk2. In contrast to the other members, which have ubiquitous tissue expression, Jak3 is preferentially expressed in lymphoid and myeloid cells.³ Jak3 is physically associated with the cytokine receptor common γ_c chain (γ_c), a receptor subunit shared between the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15.^{4,5} Recently, mutations affecting the expression of

Jak3 were found to be responsible for autosomal recessive severe combined immunodeficiency (SCID) in humans.^{6,7} Patients with SCID due to Jak3 deficiency (Jak3-SCID) have a clinical phenotype that is similar to the X-linked variant of SCID that is caused by mutations of γ_c . Both forms of SCID patients are characterised by persistent severe infections in early life, lack of T cells and NK cells and normal to elevated numbers of nonfunctional B cells. The *JAK3* gene has been localised to human chromosome 19p13.1 and its genomic organisation was characterised by two research groups.^{8,9} Comparison of both reports revealed several discrepancies between the two published structures, including differences in the number of exons that encodes *JAK3*. In this paper, we report on a revised genomic structure of the human *JAK3* gene, including detailed information of the exon–intron boundary sequences that allows development of PCR-based mutation analysis for identification of new patients.

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Materials and Methods

Amplification and Nucleotide Sequence Determination

DNA fragments up to 6.0 kb were amplified from human high molecular weight DNA, using the long and accurate PCR technology (Takara Biomedicals, Gennevilliers, France) according to the manufacturer's instructions with primers based on either the coding region of *JAK3*³ or identified intron sequences. The PCR products were sequenced using the AmplitaqCycle Sequencing kit (Perkin Elmer Applied Biosystems, Langen, Germany) following manufacturer's instructions. The flanking regions of the exons found were used to develop primers in order to amplify the exons separately.

Results

To define the whole exon-intron structure we amplified the entire *JAK3* gene using a long template PCR method. Several pairs of overlapping primers were designed in the coding sequence that encompasses the whole human *JAK3* gene. The PCR fragments obtained were compared in length with the published *JAK3* sequences. The most striking difference observed concerned the fragment length of the amplified C terminal end of *JAK3* between exon 18 and the 3' untranslated region. According to the sequence by Riedy⁸ a fragment of 1024 bp was estimated, but a specific fragment of around 5000 bp was amplified. Sequence analysis of this fragment revealed the presence of three additional exon and intron structures. Another difference in exon organisation was found at exon 7. An additional exon was identified, already described by Villa *et al.*⁹ A schematic representation of the *JAK3* gene in relation to the encoded Janus homology (JH) domains of the

protein has been outlined in Figure 1. JH1, the catalytic domain of *JAK3* is encoded by exons 17–23. To allow the amplification of single exons with intronic primers for future mutation analysis, we sequenced 40–50 bp of intronic sequences flanking the exon-intron boundaries, as shown in Table 1.

Discussion

The genomic structure of human *JAK3* we identified consists of 23 exons and 22 introns (Figure 1), and shows strong homology with the organisation of the murine *Jak3* locus,¹⁰ suggesting a conserved maintenance through evolution. All the splice junctions show the invariant GT/AG dinucleotides (Table 1). It has been suggested that the pseudokinase (JH2) and kinase (JH1) domains of *JAK3* might have evolved from a common ancestor. Comparison of the genomic structure of the two domains demonstrates that each has a distinct organisation (Figure 1). This lack of similarity suggests that the JH1 and JH2 domains arose from different ancestral genes. In a previous study, three different splice variants (S, B, and M forms) of human *JAK3* were found to be expressed in cells of haematopoietic and epithelial origins.¹¹ These isoforms contain identical 5' sequence but diverge at the 3' end. The S form corresponds to the published *JAK3* cDNA sequence,³ whereas the B form lacks the kinase domain. Alignment of the B form sequence with the intron sequences we identified, revealed homology between the 5' site of intron 22 and the 3' end of the B isoform. Thus, the B isoform does not use the splice donor site of intron 22 and generates a read-through transcript. The

Figure 1 Exon-intron organisation of human *JAK3* as related to the functional domains of the protein. The boxes represent the exons, whilst the dashed line represents introns (not in scale). The positions of the JAK homology domains JH1–JH7 are indicated at the bottom

Table 1 Exon-intron boundaries of *JAK3* gene

	Exon	Intron	Exon
1	GGCCAGCG ²⁷⁹	gtgagtgcatccctccctagtgggatcgggcccagagggaaggatg----	GCATCCTG
2	AGGATTGG ⁴⁰³	gtaggaaagtccccccagccccagggattgtacaatttt-----	CTTTTACT
3	TTGCCAG ⁵¹⁵	gtggggttctgcctgggggtttgaccagggggttggggggtc----	CACGGCAG
4	ACTGTCAG ⁶⁶¹	gtgagagccaccagggctgtggggagcggccctctgtctggga----	CTACAAGG
5	AACAGGAG ⁹⁵⁶	gtgagggcgggactctcccgctggcgggggcccaatcgtgg----	GTGCTCCA
6	AGATTTA ¹⁰⁷⁹	gtgggtgcaggattccccctccccctcagccttaaccccg-----	GAGGCGGA
7	CCATCAC ¹²³⁷	gtaaggacctgtacccccattccggctctgtggcaactcagg-----	TCTGGACT
8	GTGTCGAG ¹³⁴⁹	gtcggctactgctagggtaggtggagggtgctgctgg-----	AACCCCTT
9	ACCGAAG ¹⁵³⁶	gtgagcccccttccctccctggaaatgagtggtgctgatatggg----	AAAAGTCC
10	TGGAGTGG ¹⁶⁶⁴	gtaaggagccctggggaatatgaggcgatatacctcagttctggg----	CATGAGAA
11	GCATGGAG ¹⁷⁹⁶	gtgagagcaaatgtggaccagacttttggagtcgggggctg-----	TCATTCTT
12	TGGAGACA ¹⁸⁸¹	gtgagagccccccaccaccaccaccaccctgctcacc-----	GCACCATG
13	ACTATCTG ²⁰⁰⁹	gtgagtgctcctctgctctccaccctccattccccaggg-----	GAGGACAA
14	CCTGGAGA ²¹⁴²	gttaagttcctggaggtagggtagggagggggctgaacagggcaagg--	TGCTCACC
15	CTGCTAAG ²²⁹⁴	gtcagagccccctcaccgggcacgggtctccggaacccccac----	AAACTCCA
16	CTCTTCAG ²⁴⁴⁵	gtgcccgttggggacgggttgggtggggaggggctgtgatgt----	ACTATCAG
17	TGGGCAAG ²⁵⁸⁵	gtaaggtagggcagggccagggtagggtagggaggcaggcagcatcc--	GGCAACTT
18	TGGCCCGG ²⁷⁷⁵	gtgagccagctcccgggatgagtaaccaagacgtatgggtg-----	GCGGCGAG
19	TCTGCAAG ²⁹⁰⁰	gtcgagggggcgccccgggagcttgggggattcagctgg-----	GGCATGGA
20	ATTTTCTG ³⁰⁷³	gtggggaacccgcgcctagggctngccctatttccccagg-----	GTATGCCG
21	CCTCGGCC ³¹⁹¹	gtgagtcggcttccccagagccccccagccttctctccctc-----	GAGTTCTT
22	CTGCTCAG ³³⁰²	gtgagcgccgcagggctagcctcagtttccctcagttctgtag-----	GTTCAAGA

Capitals indicate the 3' nucleotide sequences of the indicated exon, followed by the 5' sequences of the adjacent intron (lower case), the 3' sequences of the intron (lower case), and the 5' sequences of the next exon (capitals). Splice consensus sequences are in bold. Numbering of exon positions corresponds to cDNA sequence by Kawamura³.

3' region of the M form shows no significant homology with the genomic human sequence.

The reported findings of various mutations in the *JAK3* gene resulting in a SCID phenotype in humans demonstrates the importance of *Jak3* in cytokine signal transduction and lymphoid cell development.^{6,7,12} *Jak3* expression is restricted to haematopoietic cell lineage; therefore it is necessary to perform prenatal diagnosis at the genomic DNA level, including carrier testing in the families concerned. The results reported here will facilitate identification of mutations in future *Jak3* deficient SCID patients and may be useful to gain more insight into the molecular basis of the defect.

Reported sequences have been deposited in the EMBL database (accession nos AJ224652, AJ224653, AJ224654, AJ224655, AJ224656, AJ224657).

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