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MUTATION UPDATE

Mutations in Severe Combined Immune Deficiency (SCID) Due to JAK3 Deficiency

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During the last 10 years, an increasing number of genes have been identified whose abnormalities account for primary immunodeficiencies, with defects in development and/or function of the immune system. Among them is the *JAK3*-gene, encoding for a tyrosine kinase that is functionally coupled to cytokine receptors which share the common gamma chain. Defects of this gene cause an autosomal recessive form of severe combined immunodeficiency with almost absent T-cells and functionally defective B-cells (T^-B^+ SCID). Herewith, we present molecular information on the first 27 unique mutations identified in the *JAK3* gene, including clinical data on all of the 23 affected patients reported so far. A variety of mutations scattered throughout all seven functional domains of the protein, and with different functional effects, have been identified. Availability of a molecular screening test, based on amplification of genomic DNA, facilitates the diagnostic approach, and has permitted recognition that JAK3 deficiency may also be associated with atypical clinical and immunological features. Development of a structural model of the JAK3 kinase domain has allowed characterization of the functional effects of the various mutations. Most importantly, molecular analysis at the JAK3 locus results in improved genetic counseling, allows early prenatal diagnosis, and prompts appropriate treatment (currently based on hematopoietic stem cell transplantation) in affected families. Hum Mutat 18:255–263, 2001.

KEY WORDS: JAK3; severe combined immune deficiency; immune deficiency; SCID; autosomal recessive; mutation database; tyrosine kinase, intracellular; cytokine receptor

DATABASES:
JAK3 – OMIM: 600173; GDB: 376460; GenBank: U09607; HGMD: JAK3 http://www.uta.fi/imt/bioinfo/JAK3base/ (JAK3 Mutation Database)

BACKGROUND

Severe combined immune deficiency with lack of circulating T cells but a normal number of B lymphocytes (T^-B^+ SCID) accounts for 30–50% of all cases of SCID in humans, and affects approximately 1/50,000 newborns. In its most common form, T^-B^+ SCID is inherited as an X-linked trait (SCIDX1) and is due to mutations of the *IL2RG* gene, encoding for the common γ chain (γ_c), shared by receptors for interleukin-(IL-) 2, IL-4, IL-7, IL-9, and IL-15.

However, the second most common variant of T^-B^+ SCID is inherited as an autosomal recessive trait and is due to mutations of the *JAK3* gene (MIM# 600173).

JAK3 is a member of the Janus Kinase (JAK)

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family, that also includes JAK1 (MIM# 147795), JAK2 (MIM# 147796), and TYK2 (MIM# 176941). All JAK family members are characterized by the presence of seven distinct JAK Homology domains (JH) that have been numbered beginning from the C-terminal end. JH1 is a kinase domain that lacks src-homology 2 (SH2) or SH3 motifs and its kinase activity seems correlated with the ATP-binding residue Lys 855. This activity is regulated by phosphorylation of tyrosine residues in the putative activation loop. Next follows a similar, but non-catalytic pseudokinase domain (JH2) of supposed regulatory function. These tandem domains have given the name to the whole family in that they are reminiscent of the two faced Roman god, Janus. The JH2 domain may interact with the kinase domain JH1 itself [Chen et al., 2000] and with particular signaling proteins, named signal transducers and activators of transcription (STATs) [Fujitani et al., 1997]. The N-terminal JH6-JH7 domains contain a 58-residue string that is necessary and sufficient for binding to the γ_c. The rest of these two domains is required for efficient signal transduction [Cacalano et al., 1999]. The additional domains (JH3–JH5) are thought to contribute to the in vivo assembly of the JAKs, but their functional role is only partly defined. The JH4 domain contains an SH2 motif with unknown significance.

The *JAK3* gene (GenBank# U09607) maps on chromosome 19p12-13.1. Its ORF of 3,372 bp translates into a 1,124 amino acid protein of approximately 125 kDa. The JAK3 protein, an intracellular kinase, is predominantly expressed in cells of the hematopoietic system, where it binds to the γ_c.

In general, rapid regulation of JAK activity [Imada and Leonard, 2000] is achieved directly by differential autophosphorylation of tyrosine residues [Zhou et al., 1997] in the putative activation loop of the kinase domain and by inhibitory proteins [Yoshimura, 1998], such as JAK binding protein (JAB) [Endo et al., 1997]. Indirect regulation via other molecules, including cytokine inducible SH2 containing protein (CIS) [Matsumoto et al., 1997], suppressor of cytokine signaling (SOCS), STAT-induced STAT inhibitor (SSI) [Narazaki et al., 1998], signal transducing adaptor molecule (STAM) [Takeshita et al., 1997], and protein inhibitor of activated STATs (PIAS) [Chen et al., 1998] has also been described [Liu et al., 1998]. JAK3 expression can be inhibited in vitro by glucocorticoids and prostaglandin E_2 [Bianchi et al., 2000; Kolenko et al., 1999].

Type I and II cytokine receptors lack intrinsic kinase activity. To signal, those cytokine receptors that include γ_c as a component recruit the intracytoplasmatic kinases JAK1 and JAK3. The latter physically (by its JH6/7 domains) and functionally associates with the γ_c subunit close to the membrane proximal intracellular proline-rich Box1/Box2 homology region. JAK1, on the other hand, associates with the major specific transducing subunit of the cytokine receptor. Following interaction between a specific cytokine and its receptor, the receptor subunits get so close to each other that the JAK kinases can cross-phosphorylate and thus activate each other on tyrosine residues within minutes. They then phosphorylate multiple tyrosine residues in the more distal portion of the cytosolic tails of the receptor subunits and create docking sites for particular SH2-domain containing STATs. Once recruited into the cytokine receptor complex, STATs are also phosphorylated on a single tyrosine located around residue 700, which allows them to dimerize via reciprocal phosphotyrosine-SH2 domain interactions, they can then translocate to the nucleus using the importin α/β localization pathway and bind to cognate response elements shared by cytokine responsive genes, and hence drive their transcription [Liu et al., 1998].

Being involved in the same signal transduction pathway, defects in the *IL2RG* or in the *JAK3* genes result in an identical immunological phenotype.

Due to the multiple cytokines using this signaling pathway, an early and severe block in T- and natural killer (NK) cell development combined with impaired B-cell function is typically observed. In particular, in humans IL-7-mediated signaling is necessary for T-cell development, whereas IL-2 promotes peripheral T-cell homeostasis and antigen-driven specific T-cell expansion. Moreover, IL-15 is required for differentiation of NK cells, and IL-4 is important for terminal B-cell differentiation and isotype switching. Therefore, patients with JAK3 deficiency typically lack both T- and NK-lymphocytes, but have an almost normal number of B-lymphocytes, that however are functionally deficient.

Consequently, patients with JAK3-deficient SCID are highly susceptible from the first days of life to severe infections, often sustained by opportunistic pathogens, and die within a few years unless treated by hematopoietic stem cell transplantation. Suspicion of JAK3 deficiency is based upon analysis of the immunological phenotype and complete family history (parental consanguinity, occurrence of typical clinical signs also in females) and confirmed by biochemical and molecular assays. In most cases, levels of the JAK3 protein in lymphocytes and lymphoblastoid B-cell lines derived from JAK3-deficient patients are absent or markedly reduced.

In some cases, with residual expression of mutant JAK3 protein, in vitro stimulation with appropriate cytokines fails to induce tyrosine-phosphorylation of JAK3 and STAT proteins.

MUTATIONS AND POLYMORPHISMS

Identification of JAK3 mutants among patients with T^-B^+ SCID was originally based on the candidate gene approach by providing evidence for absent or markedly reduced JAK3 protein expression in lymphoblastoid B cell lines derived from two patients and eventually confirmed by mutation analysis [Macchi et al., 1995; Russell et al., 1995]. Subsequently, several other JAK3 deficient patients have been identified, and in most cases the defect has been characterized at the molecular level (Fig. 1). The recent identification of the genomic organization of the human *JAK3* gene in 23 exons [Schumacher et al., 2000] has made rapid mutation detection feasible, and has also opened the possibility for early prenatal diagnosis [Schumacher et al., 1999]. Moreover, the three-dimensional structure of the JAK3 kinase and pseudokinase domains has recently been modeled [Vihinen et al., 2000], and functional consequences of identified mutations occurring in those domains have been predicted. Figure 2 shows the model of the three-dimensional structure of the JH1 and JH2 domains with indications on where the respective mutations map.

From the more than 2,000 patients with immunodeficiencies in which a genetic defect has been described, only 1% affects the JAK3 protein [Vihinen et al., 2001].

Altogether 23 patients from 21 unrelated families with molecularly defined JAK3 deficiency have been identified by the authors and one more has been described in detail from the U.S. [Russell et al., 1995] and has been included; at least eight others were mentioned in a recent review [Buckley, 2000]. The mutations are collected in the JAK3 database, which can be found at the website: http://www.uta.fi/imt/bioinfo/JAK3base/.

Parental consanguinity was documented in nine families (for a total of 11 patients). In all but two families, affected patients were homozygous for specific *JAK3* mutations. Furthermore, homozygosity for a *JAK3* defect was also identified in two unrelated infants, in whom there was no obvious parental consanguinity. Eleven patients were found to be compound heterozygotes.

A total of 27 unique mutations have been

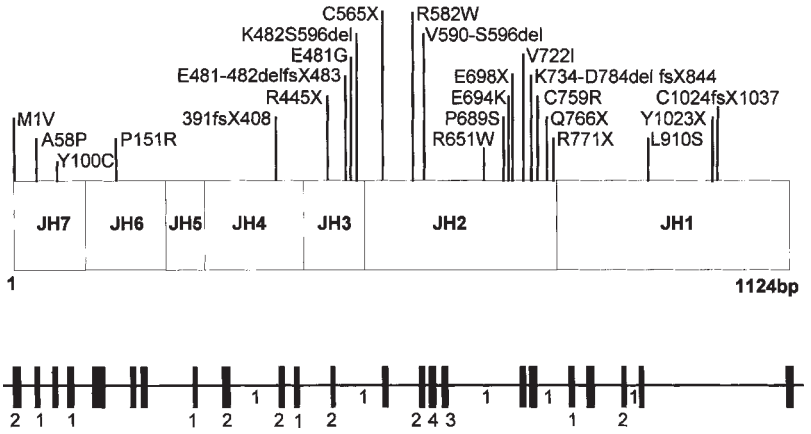

FIGURE 1. **Schematic representation of the organization of the human *JAK3*-gene and protein. In the upper panel the boxes show the JAK homology functional domains (JH1-JH7), with the individual mutations on top. In the lower panel the genomic organization of the gene is shown (in scale) with the number of mutations found indicated below each exon (black boxes) and in the different introns (lines).**

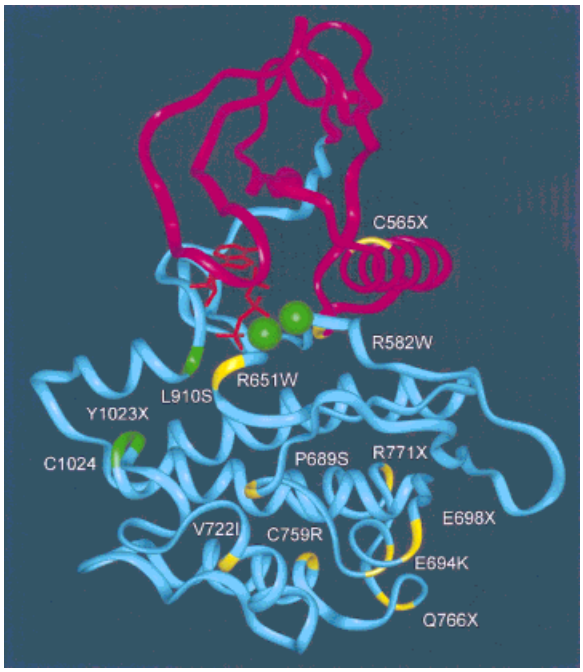

FIGURE 2. **Model of the three-dimensional structure of the JAK3 protein kinase and pseudokinase domains [Vihinen et al., 2000], with location of the disease causing mutations. Kinase domain mutations are in green, pseudokinase domain mutations in their corresponding positions of the kinase domain in yellow. The large deletion in the N-terminus of the pseudokinase domain is in magenta. The ATP analog is in red and Mg2+ ions in green. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]**

identified that affect all seven structural JH domains, although many of them appear to be clustered in the JH2 and JH3 domains. Homozygosity for the R445X mutation was identified in two first degree cousins (patients # 3 and 4); in addition, heterozygosity for the same mutation was found in one unrelated patient (#5). Similarly, the R651W mutation was found homozygous in one (#19) and heterozygous in another unrelated patient (#16). Until now, however, there is no evidence for preferential hot spots or for founder effects.

Among the 29 mutations identified in unrelated families and shown in Table 1, there were 13 missense and seven nonsense mutations, three splice site mutations, two deletions, and one insertion. In addition, two novel polymorphisms were identified.

The C759R substitution results in constitutive phosphorylation of JAK3. That, however, can not be upregulated by cytokine stimulation and does not allow for signal transduction [Candotti et al., 1997].

The R582W substitution leads to two different products, one of which has normal length but is not phosphorylated and the other one, with a 71 aa deletion in the downstream JH2 domain, is also expressed and can be phosphorylated, but is insufficient for signal transduction [Bozzi et al., 1998].

In addition to mutations in the JH3 and JH2 domain, single point mutations in the JH1 kinase domain (at least those affecting the last tyrosine residue at codon 1023) have also been shown to abolish phosphorylation [Mella et al., 2001].

BIOLOGICAL RELEVANCE

The functional importance of JAK3 mutations is illustrated by the multiple cytokines that require this specific kinase to signal.

T^-B^+ SCID is a disease that allows survival into adulthood only upon development of successful bone marrow transplant (BMT) strategies [Fischer et al., 1990]. However more recently, a few individuals have been identified who seem to be affected by a somewhat milder phenotype of the disease (Table 2). One example is a girl who thrived well and was identified as being a JAK3-deficient SCID only at age two years while having persistent candidiasis and recurrent respiratory infections (#14). Postnatal development of autologous, oligoclonal anergic CD3+/CD4+ T-cells with an activated phenotype in JAK3-SCID was described by our group in a patient with a compound heterozygosity in *JAK3* that led to expression of minimal amounts of two mutant forms of the protein, one of which (#7) showed residual function (as assessed by STAT5 phosphorylation in response to IL-2) [Brugnoni et al., 1998]. These data suggest that there might exist another, yet unknown, development pathway for CD4+ T-lymphocytes that could be driven by antigen exposure independently from the γ_c-JAK3 pathway.

The ability to test function of specific and expressed mutants in a phosphorylation assay using physiological substrates (JAK3 itself and STAT5) has enabled us to verify the consequences of the observed mutations [Candotti et al., 1997; Bozzi et al., 1998; Cacalano et al., 1999; Chen et al., 2000]. In fact, demonstration of JAK3 protein in Western assays does not rule out functional JAK3 deficiency until cytokine induced phosphorylation of JAK3 itself and/or STAT5 is excluded.

TABLE 1. Mutations of the JAK3 Gene in SCID

Genomic alteration	Mutated exon	Protein alteration	Patient	Homozygous/ heterozygous	Protein expression	Reference
g.96A>G	1	M1V	22a & b	Hetero	Undetectable	Mella et al. [2001]
g.268G>C	1	A58P	14	Hetero		Mella et al. [2001]
g.394A>G	2	Y100C	1	Homo	+	Macchi et al. [1995]
g.547C>G	4	P151R	2	Hetero	n.d.	Schumacher et al. [2000]
1267insG	8	391fsX408	21	Hetero	Undetectable	Russell et al. [1995]
g.1428C>T	9	R445X	3,4	Homo	Undetectable	Schumacher et al. [2000]
g.1428C>T	9	R445X	5	Hetero	Undetectable	Candotti et al. [1997]
IVS9-2A>G	9	E481-482del *fs*X483	6	Homo	Undetectable	Candotti et al. [1997]
g.1537A>G	10	E481G	7	Hetero	++	Candotti et al. [1997]
g.1536-1882del	10	K482-S596del	7	Hetero	+	Candotti et al. [1997]
g.1790C>A	11	C565X	21	Hetero	Undetectable	Russell et al. [1995]
g.1839C>T	12	R582W	8	Homo	++	Bozzi et al. [1998]
g.1862C>T	12	V590-S596del	9	Homo	+	Candotti et al. [1997]
IVS12-1G>A	13	Splice site	18	Hetero	n.d.	Mella et al. [2001]
g.2046C>T	14	R651W	19	Homo	n.d.	Mella et al. [2001]
g.2046C>T	14	R651W	16	Hetero	n.d.	Mella et al. [2001]
g.2160C>T	15	P689S	15	Homo	n.d.	Mella et al. [2001]
g.2175G>A	15	E694K	16	Hetero	n.d.	Mella et al. [2001]
g.2187G>T	15	E698X	18	Hetero	n.d.	Mella et al. [2001]
g.2259G>A	15	V722I	10[a]	Hetero	n.d.	Schumacher et al. [2000]
g.2370T>C	16	C759R	5	Hetero	++	Candotti et al. [1997]
g.2391C>T	16	Q766X	17	Homo	Undetectable	Mella et al. [2001]
g.2406C>T	16	R771X	20	Homo		Mella et al. [2001]
IVS16+2T>C	16	K734-D784del *fs*X844	11a & b	Homo	Undetectable	Villa et al. [1996]
IVS18+3G>C	18	Splice site	22a & b	Hetero	Undetectable	Mella et al. [2001]
g.2824T>C	19	L910S	12	Hetero	++	Schumacher et al. [2000]
g.3164C>A	21	Y1023X	12	Hetero	Undetectable	Schumacher et al. [2000]
g.3167del	21	C1024fsX1037	14	Hetero		Mella et al. [2001]
IVS21+2T>A	21	Splice site	13	Homo	Undetectable	Schumacher et al. [2000]
c+13a	7	Polimorphism				
c-30t	14	Polimorphism				

[a]Mutation found in a parent of a child deceased with SCID, before the diagnosis could be achieved.
Residue numbering within the JAK3 nucleotide and amino acid sequence corresponds to the published human JAK3 sequence [Kawamura et al., 1994] and subsequent corrections, with the ATG at base 96.

In vitro mutagenesis studies and the identification of naturally occurring mutations with different consequences (lack of protein expression, functionally null mutants, and proteins with reduced or deregulated activity) in different domains has been helpful in characterizing the functional activity of the various domains. As an example, mutations that affect the contiguous tyrosine residues 980 and 981 have different functional effects: Whereas the former is essential for normal catalytic activity, mutations of the latter increase catalytic activity [Liu et al., 1997].

Moreover, deregulation of JAK3 expression and/or function has also been implied to be involved in malignancy [Tortolani et al., 1995; Migone et al., 1995; Takemoto et al., 1997]; however, as yet the potential role of somatic mutations of the *JAK3* gene in this process can only be speculated.

To better understand the genotype–phenotype correlation, development of mutation databases is of foremost importance. Three-dimensional models of protein structure allow prediction of the effect of reported mutations not only at the protein sequence level but also for their steric and electrostatic configuration changes with particular attention to functional binding sites [Vihinen et al., 2000]. The JAK3 database contains information also on the structural and functional consequences of the various mutations.

CLINICAL RELEVANCE

Early recognition of T^-B^+ SCID is of highest priority, and until the diagnosis of a severe im-

TABLE 2. Clinical and Laboratory Data From 24 Patients With JAK3-Deficient SCID

Patient	Sex	Age at diagnosis (mo)	Lymph/ mL	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)	CD16/56 (%)	PHA	lgG	lgM	lgA	lgE	BMT donor[a]	Age at BMT (mo)	Outcome
1	M	5	4332	53	53	1.5	23	1	Absent	39	37	30	3	MUD	12	A/w
2	F	10	2520	11	7	2	70	2	Absent	n.d.	121	11	49	Identical	11	A/w
3	F	4	612	18	1	8	28	34	Absent	66	12	6	n.d.	MUD	9	A/w
4	F	1		<3	n.d.	n.d.	92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	None		Deceased
5	M	2	2304	0.5	0.5	0.5	93	1	Absent	224	36	6	2	Haplo	3	A/w
6	M	3	1364	<1	<1	<1	77	<1	Absent	132	22	6	n.d.	Haplo	5	A/w
7	M	1.5	3000	2	1	2	74	13	Absent	865	117	6	1100	Haplo	15	Deceased
8	F	9	792	0	n.d.	n.d.	>80	n.d.	Absent	Absent	n.d.	n.d.	n.d.	Identical (father)	9	A/w
9	M?		700	1	n.d.	n.d.	96	1	Absent					Haplo		A/w?
10																Deceased
11a	F	7	2074	1	1	0	94	1	Absent	49	57	6	n.d.	Haplo	8	A/w
11b	M	Prenat	916	1	1	1	87	0.5	Absent	160	15	6	3	Haplo	4	A/w
12	M	7	3104	0.5	0.5	0.5	92	0.5	Absent	152	54	23	2	Haplo	7	A, severe neurol. imp
13		3	3210	0	0	0	97	0.5	Minimal	4610	230	70		No		Deceased
14	F	19	1630	8	4	4.6	91.3	0.3	Absent	1670	171	259	24.6	MUD	27	A/w
15		4	3470	2	0	0	22	n.d.	Minimal	9.6 U/ml	14 U/ml	1U/ mll		Haplo		A/w, warts
16		3	5640m	48	18	35	45	1	n.d.		90	20		Haplo		Deceased
17		5	1020	0	0	0	94	1	Absent	1400	570	260		MP		A/w
18		Birth	1020	1	n.d.	n.d.	26	n.d.	Minimal	112 U/ml	34	2		MSD		A/w, warts
19		3	?	0	0	0	#1274	#30	Absent	140	200	20		Haplo		Deceased
20		6	970	31	12	20	66	2	n.d.	Absent						Deceased 8 months
21	F		ca.3000	#204	#204	#58	#2592	#29	Minimal	n.d.	50	n.d.	2U/mL			
22a	F	72	770	60	21	44	24	42	Weak	1149	90	678		No		A/w, severe warts
22b	M	7	820	17	5	9	73	9	Weak	290	147	27		Identical	108	Deceased

[a]BMT, bone marrow transplant; MUD, matched unrelated donor; MSD, mismatched doner; MP, matched parent; Identical, HLA-identical donor; Haplo, Haploidentical donor; A/w, alive and well.

munodeficiency can be ruled out, affected infants should be hospitalized in a protective environment with adequate antimicrobial prophylaxis and administration of live vaccines must be avoided. If blood products are needed, they have to be irradiated to avoid graft versus host disease caused by transfused T-cells, which cannot be rejected by T^-B^+ SCID patients. Lymphopenia is the laboratory hallmark for suspicion, whereas thrush, intractable diarrhea, and severe recurrent airway infections are the most typical clinical signs and symptoms. Once an infant becomes seriously infected (especially in his lungs) [Stephan et al., 1993], the chances of survival, even with BMT, decline rapidly. In addition, young age at BMT is one of the best predictors for survival [Buckley et al., 1999].

The absence of NK cells observed in JAK3-deficient patients is a fortunate coincidence in that it facilitates engraftment (with respect to the T^-B^- forms of SCID) especially after haploidentical BMT (often the only possibility to graft those patients who lack HLA-identical family or unrelated donors). Due to the presence of recipient's B-cells, B-cell engraftment is more difficult to achieve, especially if no chemotherapeutic conditioning is done. However, successful engraftment of T-cells from an HLA-identical donor is sufficient to regain a good humoral response based on the collaboration between donor T cells and autologous B cells [Haddad et al., 1998].

DIAGNOSTIC RELEVANCE

While immunological assays allow rapid diagnosis of T^-B^+ SCID, a precise definition of the gene defect can only be achieved with more extensive biochemical and molecular analysis. In particular, most patients with JAK3 deficiency show absent or markedly reduced levels of JAK3 protein, as detected by Western blotting. Yet, residual expression of the protein can be observed in a substantial proportion of patients. In such cases, functional analysis of JAK3 and STAT5 tyrosine phosphorylation in response to IL-2 (by immunoprecipitation and immunoblotting assays) may facilitate diagnosis of JAK3 defects. However, this is a laborious process that requires availability of fresh peripheral blood mononuclear cells or EBV-transformed B-cell lines derived from patients. Molecular characterization of *JAK3* gene mutation(s) is critical in order to reach definitive diagnosis and provide accurate genetic counseling, including prenatal diagnosis. Mutation analysis can be performed at either the cDNA or at the genomic DNA level. While the former is more rapid, it requires viable cells, and may not provide sufficient information for prenatal diagnosis.

Genomic screening of each of the exons and flanking splice sites with a single strand conformational polymorphism/heteroduplex formation assay [Schumacher et al., 2000], followed by sequencing is the strategy most commonly used to detect DNA abnormalities.

The heterogeneity of defects in different genes along one functional pathway is paralleled by the heterogeneity of immunological phenotypes caused by different mutations in the same gene, thus only detailed molecular and immunological investigation allows dissection of the spectrum of human SCID.

Molecular determination of the cause of SCID in a given patient is pivotal not only for the patient, but also for the family, as it allows appropriate genetic counseling and early prenatal diagnosis. The latter gives new options to the family other than abortion, including 'safe delivery' with pre-emptive stem cell transplantation, as well as intra-uterine transplantation of hematopoietic stem cells, which has so far been attempted in a few cases with promising results [Wengler et al., 1996; Flake and Zanjani, 1997].

FUTURE PROSPECTS

Since the identification of the first patients with JAK3-deficient SCID in 1994, major progress has been achieved, especially at the genetic and physiological level. Genetic testing is now available not only for affected individuals, but also for carrier detection and prenatal diagnosis.

Much more remains to be done in terms of therapy as actually only hematopoietic stem cell transplantation can cure the disease. However, JAK3-deficiency is a candidate for gene therapy. The first attempts in the murine knock out model have shown that transfer of a *JAK3* retroviral vector to repopulating hematopoietic stem cells resulted not only in increased T- and B-lymphocytes but also restored cellular and humoral function of the immune system [Bunting et al., 1998; Bunting et al., 1999]. In vitro biochemical correction of JAK3-deficient human B-cell derived

cell lines has already been successfully accomplished [Candotti et al., 1996].

Given the role of JAK3 in human B- and T-cell malignancies, pharmacological JAK3 inhibitors are currently being studied and may become useful immunomodulating drugs, not only in the treatment of malignancies [Waldmann, 2000; Uckun et al., 1999] but also for other immune-mediated diseases [Wang et al., 2000; Brown et al., 2000].

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