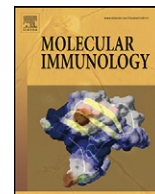




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Short communication

Novel and recurrent *STAT3* mutations in hyper-IgE syndrome patients from different ethnic groups

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ABSTRACT

We performed clinical, immunological and genetic studies of 12 hyper-IgE syndrome (HIES) patients from 4 Hungarian, 2 Lebanese, one Russian, one Polish, and one Swedish families with autosomal dominant (AD) or sporadic forms of the disease to reveal cross-ethnicity of recurrent and novel mutations in the signal transducer and activator of transcription-3 gene (*STAT3*). Four patients from 3 Hungarian families, and one Russian, and one Swedish patient carried the heterozygous R382W germline mutation at the DNA-binding site of *STAT3*. The recurrent V637M mutation affecting the SRC homology 2 (SH2) domain was detected in one Lebanese and one Polish family, and the V463del deletion located in the DNA-binding domain was unveiled in another Lebanese family. A novel H332Y mutation affecting the DNA-binding site of *STAT3* in three Hungarian patients from a Gypsy family was also found. The segregation of this mutation with HIES, restriction fragment length polymorphism analysis of *STAT3* from patients and controls and the negligible production upon IL-6 stimulation of monocyte chemotactic protein-1 by the patient's blood mononuclear cells suggested that the H332Y mutation was disease-causing. These data suggest, that dominant negative mutations of the DNA-binding and SH2 domains of *STAT3* cause AD and sporadic cases of HIES in different ethnic groups with R382W as the predominant mutation found in 5 of the 9 families. Functional and genetic data support that the novel H332Y mutation may result in the loss of function of *STAT3* and leads to the HIES phenotype.

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1. Introduction

The hyper-IgE syndrome (HIES; Job syndrome; OMIM 147060) is a multisystem primary immunodeficiency disorder characterized by pyogenic skin and lung infections, pneumatocele formation,

severe eczema, and extreme elevation of serum IgE (Davis et al., 1966; Grimbacher et al., 2005; Buckley, 2001). Additional manifestations include distinctive facial features, hyperextensibility of the joints, cranial synostosis, scoliosis, abnormal dentition, and pathological fractures (Grimbacher et al., 2005; Buckley, 2001). Central nervous system lesions, coronary artery aneurysms, and Chiari's malformation may also be part of the complex clinical pathology (Ling et al., 2007; Freeman et al., 2007). The most common pathogens of infections in HIES patients include *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Candida*. Staphylococcal skin abscesses typically fail to demonstrate

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erythema and warmth, and hence are called “cold abscesses” (Davis et al., 1966).

Both autosomal recessive (AR) and autosomal dominant (AD) inheritance have been described, but most HIES cases are sporadic (Grimbacher et al., 2005; Renner et al., 2004). Minegishi et al. have recently identified a homozygous mutation of the tyrosine kinase-2 gene (*TYK2*) in a patient with AR-HIES (Minegishi et al., 2006). Recent studies from this group and from another group have showed mutations in the signal transducer and activator of transcription-3 gene (*STAT3*) on chromosome 17q21 as major causes of AD and sporadic HIES (Minegishi et al., 2007; Holland et al., 2007). Mutations were localized in either the DNA-binding domain

or the SH2 domain of *STAT3*. In order to evaluate the universality of the causative mutations and find potential new mutations in different ethnic groups we carried out mutation screen for the entire *STAT3* gene in 12 patients from Hungary, Lebanon, Russia, Poland, and Sweden.

2. Materials and methods

2.1. Patients

In total 12 individuals from 4 Hungarian, 2 Lebanese, one Russian, one Polish, and one Swedish families with AD or sporadic

Table 1
Demographics, and clinical and laboratory findings

Families	Age (y)	Sex	Eczema	Other skin/mucous membrane	Lung involvement manifestations	Skeletal manifestations	Highest IgE levels (kU/L)	Other manifestations
Hungarian								
C1	24	F	Severe	Newborn rash Cold abscess Nail candidiasis	Pneumonia Pneumatocele Bronchiectasis	Pathologic fracture Face of Job Scoliosis (14°) Hyperextensibility	30,000	Fasciitis necroticans
C2	5	M	Severe	Cold abscess	Pneumonia	Face of Job	50,000	–
C3	3	M	Severe	Pyoderma	Pneumonia	Face of Job	2000	–
N1	40	F	Severe	Newborn rash Pneumatocele Nail candidiasis Cold abscess	Pneumonia	Pathologic fracture Face of job Scoliosis (>20°) Hyperextensibility Retained primary teeth	5000	B cell lymphoma Nephrectomy (at age 7)
N2	8	F	Mild	Newborn rash Cold abscess	Pneumonia	Face of Job Hyperextensibility	1400	–
K	5	M	Moderate	Newborn rash Cold abscess Oral candidiasis	Pneumonia	Face of Job Hyperextensibility	20,000	–
S1	9	M	Severe	Skin abscess Oral candidiasis Sinusitis, otitis	Pneumonia	Face of Job Retained primary teeth Hyperextensibility	70,000	Arthropathy
Lebanese								
F1	23	F	Severe	Skin abscesses Newborn rash, Nail candidiasis Adenitis Otitis Dental infections Newborn rash	Pneumonia Pneumatocele	Scoliosis (=30°) Characteristic facies Hyperextensibility Retained primary teeth	30,000	–
CA1	22	M	Severe	Skin abscesses Nail candidiasis Otitis Dental infections	Pneumonia Pneumatocele Pectus excavatum	Characteristic facies	20,000	–
Polish								
B	3	F	Moderate	Pyoderma Purulent Conjunctivitis Purulent otitis Skin candidiasis Nail candidiasis	Pneumonia	Face of Job	30,000	–
Russian								
Z	10	F	NA	NA	NA	NA	NA	
Swedish								
SW	25	M	Moderate	Otitis Skin abscesses Dental infections	Pneumonia Empyema Bronchiectasis	Characteristic facies Osteitis	16,000	Allergy Asthma Food allergy

M, male; F, female; NA, not available; Hungarian patients N1 and K are included in another submitted manuscript as UPN133 and UPN134, respectively.

HIES patients were studied. All studies were performed with the informed consent of the patients or of their parents. Demographics and clinical characteristics of 11 patients are shown in Table 1. Clinical diagnosis of HIES was made by analysis of clinical, immunological, and radiological findings, and segregation of the disease in the families.

2.2. In vitro cytokine assays

Peripheral blood mononuclear cells (PBMC) were isolated from K-EDTA-anticoagulated. Cells were cultured at 5×10^6 /ml density in AIMV medium in 96-well round-bottom plates and treated with 200 ng/ml lipopolysaccharide (LPS) from *Escherichia coli* (Sigma–Aldrich, St. Louis, MO), 2.5 μ g/ml of the TLR7/8 agonist CL075 (Invivogen, San Diego, CA), or 40 ng/ml interleukin (IL)-6 (PeproTech, Rocky Hill, NJ). After 48 h of incubation, supernatants were harvested and cytokine levels were determined with a sandwich enzyme-linked immunosorbent assay (ELISA).

2.3. Mutational analysis

EDTA blood was obtained and genomic DNA was isolated by using standard methods. All 23 exons and exon/intron boundaries were separately amplified by PCR. Sequences were analyzed using the Sequence Analyzer v3.0 software (Amersham Biosciences).

2.4. Restriction fragment length polymorphism (RFLP) assay

The presence of the c.994C>T mutations in family members and in healthy individuals were analyzed by using the Mph11031 restriction enzyme. Exon 9 of the *STAT3* gene was amplified in 230 bp products. In homozygous wild type samples, digestion with Mph11031 yielded 130 and 100 bp restriction fragments. However, due to the c.994C>T heterozygous mutation, genomic DNA was not digested by Mph1103.

3. Results

We found four heterozygous mutations, three in the DNA-binding domain (H332Y, R382W, and V463del), and one in the SH2 domain (V637M) (Table 2; Fig. 1). The V637M mutation was found in two sporadic patients from Lebanon and Poland, and both of them had extremely high IgE levels (Table 1). The V637M sequence variant in Family 7 seemed to be *de novo* since none of the parents and the sibling of the patients carried this mutation (Table 2).

The mutation V463del occurred only in the patient who had extremely high IgE level in Family 6 that has several members with moderately elevated IgE levels and other HIES related phenotypes.

Table 2
Mutations in the DNA binding and SH2 domains of STAT3

Family	Ethnic group	Mutations		Domain
		Nucleotide change	Amino acid change	
Family 1	Gypsy			
C1	Proband (F)	CAT/TAT	H332Y	DNA binding
C2	Son of C1	CAT/TAT	H332Y	DNA binding
C3	Son of C1	CAT/TAT	H332Y	DNA binding
C4	Father of C1	None	None	-
C5	Mother of C1	None	None	-
Family 2	Hungarian			
N1	Proband (F)	CGG/TGG	R382W	DNA binding
N2	Daughter of N1	CGG/TGG	R382W	DNA binding
N3	Son of N1	None	None	-
N4	Husband of N1	None	None	-
Family 3	Hungarian			
K	Proband (M)	CGG/TGG	R382W	DNA binding
Family 4	Hungarian			
S1	Proband (M)	CGG/TGG	R382W	DNA binding
S2	Sister of S1	None	None	-
S3	Sister of S1	None	None	-
S5	Mother of S1	None	None	-
Family 5	Russian			
Z	Proband (F)	CGG/TGG	R382W	DNA binding
Family 6	Lebanese			
F1	Proband (F)	GTG/-	V463del	DNA binding
F2	Second cousin	Not at the site	Not at the site	
F3	First cousin	Not at the site	Not at the site	
Family 7	Lebanese			
CA1	Proband (M)	GTG/ATG	V637M	SH2
CA2	Father of CA1	Not at the site	Not at the site	
CA3	Mother of CA1	Not at the site	Not at the site	
CA4	Sister of CA1	Not at the site	Not at the site	
Family 8	Polish			
B	Proband (F)	GTG/ATG	V637M	SH2
Family9	Swedish			
SW	Proband (M)	CGG/TGG	R382W	DNA binding

Hungarian patients N1 and K are included in another submitted manuscript as UPN133 and UPN134, respectively.

However, we could not find this mutation in relatives in this family, thus the mutation is deductive *de novo*.

The R382W mutation was the most frequent sequence variant found in five of the 9 families. In Family 2, both the affected mother and her daughter carried the mutation, whereas the unaffected husband and son were wild type (Table 2). Thus the mutated

HIES mutations, this study

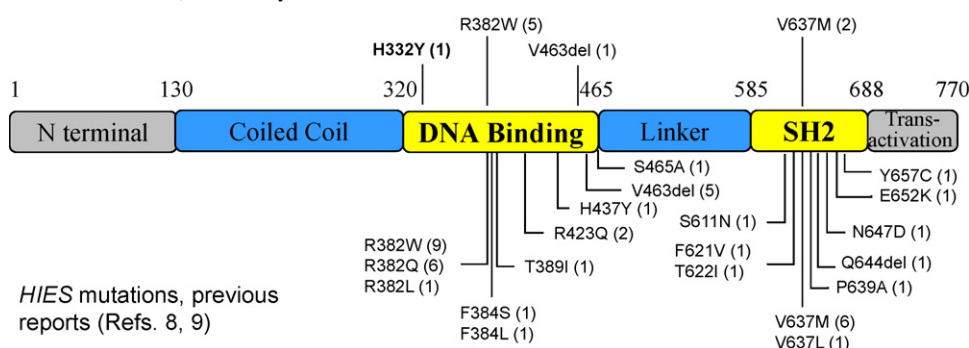


Fig. 1. Domain organization of STAT3 and mutations described recently and reported in this study. The number of families with different mutations are indicated in brackets.

allele was inherited in a dominant manner. In Family 3, an adopted child with no available history of the family also carried the R382W mutation. Three further sporadic cases with R382W mutation were detected in a Hungarian, a Russian and a Swedish family.

The novel heterozygous mutation at amino acid position 332 was found in a Gypsy family (Family 1) from Hungary (Table 2). The mutation was located in exon 9 and within the DNA-binding domain of STAT3. In the family the affected female and both of her sons carried a nucleotide change from CAT to TAT that caused amino acid change from histidine (H) to tyrosine (Y). However none of the parents of the proband had the mutation though the father had an elevated IgE level.

To exclude the possibility of polymorphism, and to reveal the pathological relevance of the c.994C>T mutation, RFLP analysis of the *STAT3* gene was performed (Fig. 1a). The results showed that this mutation was not present in 150 controls (25 Hungarian, 25 Gypsy, 50 Lebanese and 50 Swedish). These data together with the absence of mutation in the parents without disease manifestations suggested that these genetic changes were disease-associated.

Concentrations of TNF- α were 2–3-fold higher in the supernatant of LPS stimulated PBMCs from the two children in Family 1 (Patients C2 and C3) with the novel H332Y mutation, than in supernatants of control cells from a 5-year-old healthy boy (Fig. 2b, left columns). These findings were in concert with previous data showing that leukocytes of patients from HIES produce a higher amount of TNF- α than those from healthy individuals when stimulated with the TLR4 receptor agonist, LPS (Minegishi et al., 2007; Holland et al., 2007). In addition, we found that TNF- α was also elevated in supernatants of HIES children's cells when stimulated with the TLR7/8 receptor agonist CLO75 compared to control cells (Fig. 2b, middle columns). In contrast to results with TLR4 and TLR7/8 stimulation, IL-6 induced a negligible secretion of MCP-1 protein by PBMCs from HIES patients than by cells from controls suggesting an impaired IL-6-mediated signaling in HIES. These data further indicated that the H332Y mutation was disease-causing in affected members of the Gypsy family showing characteristics of multisystemic HIES phenotype.

4. Discussion

We described here three recurrent and one novel mutation in the *STAT3* gene causing AD and sporadic cases of HIES in 9 families from different ethnic groups. The disease-causing role of the novel H332Y mutation was supported by the lack of mutated alleles in healthy controls and family members without HIES phenotype, and the impaired IL-6-induced release of MCP-1 in affected individuals. The H332Y mutation is predicted to damage DNA binding according to bioinformatics analysis with sift and polyphen (Ng and Henikoff, 2002; Ramensky et al., 2002). The essential importance of His332 (as well as Arg382) in the DNA binding of STAT3 (and STAT1), where both residues are in close contact with the phosphates of DNA is clearly demonstrated in previous studies on the three-dimensional structure of STAT3 and STAT1 bound to DNA (Becker et al., 1998; Chen et al., 1998). Such a deleterious structural change in the DNA-binding domain resulting from each of these mutations is also very probably caused by the V463del mutation due to the crucial location of Val463 in the DNA-binding domain of STAT3 (and STAT1). A deletion at 463 is likely to affect the positioning of the DNA interactive residues S465 and N466 (Becker et al., 1998; Chen et al., 1998). So, in the light of these previous structural studies it seems likely that the H332Y mutation has the same deleterious consequence on the DNA binding of STAT3 as the two other (R382W and V463del) and, as a result, on the disease development.

To date all 20 mutations identified in HIES patients explained the majority of sporadic and AD familial HIES cases in Ameri-

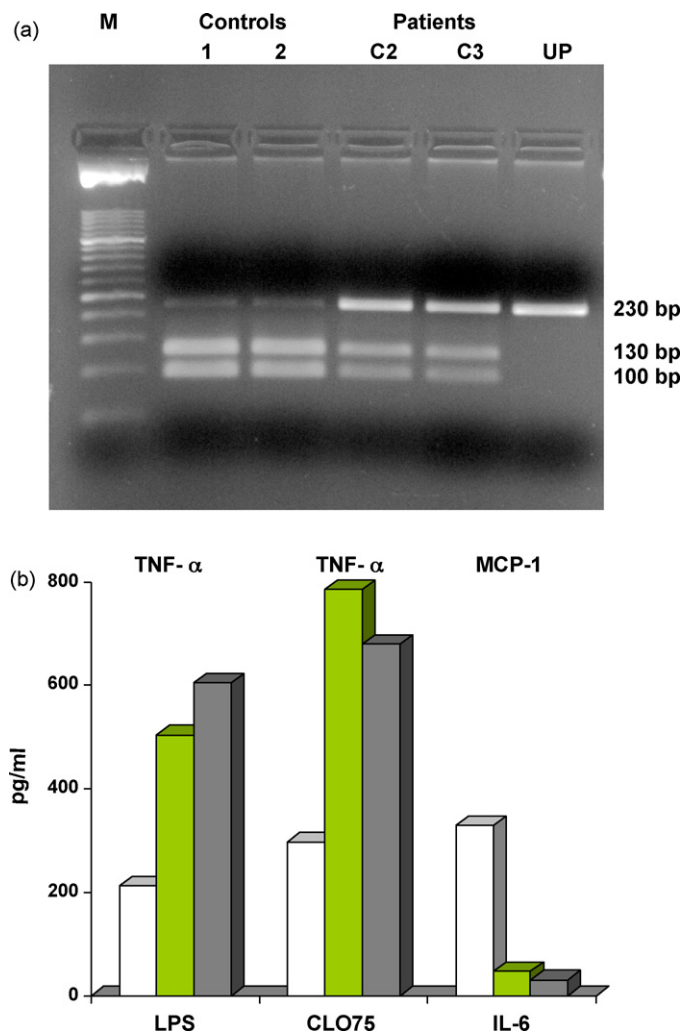


Fig. 2. (a) Restriction digestion analysis of the c.994C>T mutation of the *STAT3* gene. A 2% agarose gel electrophoresis of Mph1103 digests of the amplified exon 9 is shown in controls and two patients from family C (see also Table 1). In homozygous wild type exon 9 (Controls 1 and 2) Mph1103 digestions yielded a 130 bp and a 100 bp restriction fragments. Similar digestion patterns were found in the two Patients who carried one wild type alleles. However, the c.994C>T mutation interferes with the genomic DNA digestion by Mph1103 as indicated in the figure by the presence of a 230 bp band. M, molecular weight marker; UP, untreated product of the PCR amplification. (b) Decreased interleukin (IL)-6-stimulated cytokine response in patients with the p.H332Y mutation of *STAT3*. Production of tumor necrosis factor (TNF)- α and monocyte chemoattractant protein (MCP)-1 in response to lipopolysaccharide (200 ng/ml, left columns), CLO75, a toll-like receptor 7/8 agonist (2.5 μ g/ml, middle columns), and IL-6 (40 ng/ml, right columns) by peripheral blood mononuclear cells. Cytokine concentration was measured in supernatants of cells (5×10^6 /ml) from healthy controls (white columns), and Patients C2 (green), and C3 (grey) of Family 1 (see also Table 2). All experiments were performed in triplicates.

can and Japanese ethnic groups (Minegishi et al., 2007; Holland et al., 2007). None of those mutations is homozygous, which is consistent with results from studies in mice (Takeda et al., 1997). It seems that all *STAT3* mutation carriers showed HIES clinical features often including skeletal/dental abnormalities, which is consistent with the heterozygous manner of mutations. However, one notable phenomenon in our study is that two sporadic mutation carriers from Lebanon are both consanguineous children from whom a homozygous mutation usually will be expected based on an assumed autosomal recessive inheritance pattern of a corresponding phenotype. HIES is a complex multisystem disorder and other genetic or nongenetic factors are likely involved in the disease development. Therefore, a systematic genetic study such as

genome-wide linkage and genome-wide association will help to outline the genetics of HIES. Although other genomic loci may also be involved in the pathology of HIES, the discovery of *STAT3* mutations has improved our understanding of the pleiotropic consequences of these mutations due to the central crossroads of *STAT3* in the signaling pathways of numerous cytokines in different tissues (Chen et al., 2007; Mathur et al., 2007; Yang et al., 2007). The knowledge of the genetic mechanisms leading to development of the disorder established a platform for people to study other related phenotypes that might share the same genetic factors. Furthermore, identified mutations have a potential role in a panel development for clinical use, aiding diagnosis (for example prenatal diagnosis and genetic counseling) and ultimately even for individualizing therapy based on genetic variation profiles.

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