

Autosomal dominant hyper IgE syndrome from a single centre in Chongqing, China (2009-2018)

Qingqing Xiang¹  | Luying Zhang² | Xia Liu³ | Shiyu Wang¹ | Tao Wang¹ | Min Xiao¹ | Xiaodong Zhao⁴  | Liping Jiang¹

¹Clinical Immunology Laboratory, Pediatric Research Institute, Chongqing Key Laboratory of Child Infection and Immunity, Ministry of Education Key Laboratory of Child Development and Disorders, National Clinical Research Center for Child Health and Disorders, China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Children's Hospital of Chongqing Medical University, Chongqing, P.R China

²Department of Hematology and Oncology, Children's Hospital of Chongqing Medical University, Chongqing, P.R China

³Department of Neonatology, Children's Hospital of Chongqing Medical University, Chongqing, P.R China

⁴Laboratory Biosafety-2, Pediatric Research Institute, Chongqing Key Laboratory of Child Infection and Immunity, Ministry of Education Key Laboratory of Child Development and Disorders, National Clinical Research Center for Child Health and Disorders, China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Children's Hospital of Chongqing Medical University, Chongqing, P.R China

Correspondence

Liping Jiang, Clinical Immunology Laboratory, No. 136 2nd Zhongshan Road, Chongqing 400014, China.
Email: jiangliping@hospital.cqmu.edu.cn

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Abstract

Autosomal dominant hyper IgE syndrome (AD-HIES) caused by STAT3 gene mutation is a rare primary immunodeficiency disease. To better understand the disease, we described the clinical characteristics of 20 AD-HIES patients in Chongqing, China and explored the effect of mutations in different domains of STAT3 gene on the function of STAT3 protein by Western blot and confocal microscopy. The mean age at onset was 0.12 years. The mean age at diagnosis was 5.31 years. The most common presentation was eczema, pneumonia, skin abscesses and chronic mucocutaneous candidiasis. Seven patients suffered from BCG complications. R382W/Q were identified in 12 patients, V637M mutation in three patients. Three patients have died. The phosphorylated STAT3 was expressed more in wild-type(WT) and R382W mutant STAT3 in the cytoplasm of COS7 cells with epidermal growth factor(EGF) stimulation, less in the V637M mutation and T620S mutation. Dynamic observation showed that STAT3 cytoplasmic accumulation and nuclear translocation occurred rapidly after EGF stimulation in WT-STAT3-GFP, the time of accumulation and nuclear translocation was later and the expression was less in R382W-STAT3-GFP compared with WT-STAT3-GFP, followed by V637M and T620S mutation. These results suggested that our patients had earlier onset, diagnostic age and higher rate of BCG complications. However, our patients had higher incidence of mortality though the earlier diagnostic age. We did not find a significant genotype/phenotype correlation, but Src homology 2 domain mutations (V637M and T620S) had a greater effect on STAT3 phosphorylation and nuclear translocation than DNA-binding domain mutation (R382W) in vitro.

1 | INTRODUCTION

Hyper-IgE syndrome (HIES) is a rare multisystem primary immunodeficiency disease first described and named as Job syndrome by Davis et al in 1966.¹ In 1972, Buckley et al² reported 2 Job syndrome patients with very high plasma immunoglobulin E levels, which they thus named HIES. According to the scoring system developed by the National Institutes of Health (NIH) in 1999, HIES can be clinically suggested with a total score >40.³ Both autosomal dominant and recessive inheritance have been described in HIES patients.⁴⁻⁷ Autosomal dominant hyper-IgE syndrome (AD-HIES) was characterized by persistent eczema, repeated *Staphylococcus aureus* (*S. aureus*) skin abscesses, pneumonia with abscess and pneumatocele formation, candida infections, and skeletal and connective tissue abnormalities.^{8,9} In 2007, signal transducer and activator of transcription 3 (STAT3) gene mutations were found to be the cause of the AD-HIES.^{7,10} For AD-HIES, the major goal of treatment focuses on control of skin and lung infections using antibiotics, the role of haematopoietic stem cell transplantation in AD-HIES is uncertain.⁸

As a member of the activators of transcription family proteins, STAT3 mediates responses to multiple cytokines and growth factors and plays an important role in regulating proliferation, differentiation, migration, apoptosis and cell survival.^{11,12} STAT3 has six functional domains, including the N-terminal domain, coiled-coil domain, DNA-binding domain (DBD), linker domain, Src homology 2 domain (SH2D) and transactivation domain (TAD).¹³ The most common hotspot mutations in the STAT3 gene are R382W/Q in the DBD and V637M in the SH2D.¹³ There is limited description of the differences between these mutations on the phosphorylation, accumulation and nuclear translocation of the STAT3 protein, which further affect the signalling transduction.

Children's Hospital of Chongqing Medical University is located in Southwest of China, it is one of the major PID centres in mainland China. To better understand the clinical characteristics, gene features, treatment, prognosis of AD-HIES patients in China and possible mechanism of AD-HIES, our study collected 20 AD-HIES patients from 2009

to 2018 in our centre and explored the changes of the phosphorylation and nuclear translocation of the STAT3 protein in AD-HIES related SH2D mutations (V637M and T620S) and DBD mutation (R382W).

2 | MATERIAL AND METHODS

2.1 | Patients

We enrolled 20 patients (≤18 years) diagnosed with AD-HIES based on clinical manifestations and genetic analysis in Children's Hospital of Chongqing Medical University from 2009 to 2018. The clinical suspicion of AD-HIES was strengthened by the NIH scores: NIH scores points >40 are suggestive of AD-HIES; 20-40 is considered indeterminate; <20 is considered unlikely to indicate AD-HIES. We retrospectively analysed the clinical data and gene mutation of the 20 patients. The study was approved by the ethics committee of the Children's Hospital of Chongqing Medical University, and written informed consent was obtained from the parents of the patients.

2.2 | Mutation analysis

Genomic DNA (gDNA) from the patients and family members were extracted from the blood samples using blood genomic DNA extraction kit (Tiangen Biotech). Total mRNA was isolated using the RNeasy Pure Blood Kit (BioTeke) and reverse transcribed into cDNA using the PrimeScriptTM RT Reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa). The STAT3 gene was amplified from cDNA and gDNA by RT-PCR or polymerase chain reaction (PCR) with specific primers (Tables 1 and 2) designed according to human STAT3 mRNA (NM_139276.2) and gDNA sequences (NC_000017.10). PCR products were confirmed by gel electrophoresis and then sent to Sangon Bio. for Sanger sequencing. The sequence near the hotspot mutation site was first amplified. If no mutation is found

TABLE 1 Primer sequences for STAT3 gene in the mRNA level

Exon	Primer sequence	
9-15 ^a	F-AGGAGAGAATCGTGGAGCTGTTTAG	R- CATTCTGGGATTGTTGGTCAGC
18-20 ^b	F-CTGGTGTGAATTATTCAGGGTGTC	R- TGGTGTACACAGATAAACTTGGTC
1-6	F- ATGCCCAATGGAATCAGCT	R-CACGTA CTCCATCGCTGACAAA
7-11	F-TCACTGCGCTGGACCAGAT	R-GTGT TTTGTGCC CAGAATGTTA
11-17	F-GCTGGTCAAATTCCTGAGTTGA	R-AGCCAGACCCAGAAGGAGAAGC
18-23	F-TCAGATCACATGGGCTAAATT	R-TCACATGGGGGAGGTAGC

^aThe primer used to amplify the sequence near the hotspot mutation site(c382).

^bThe primer used to amplify the sequence near the hotspot mutation site(c637).

TABLE 2 Primer sequences for STAT3 gene in the genomic DNA level

Exon	Primer sequence	
1	F-GAGTCCAGGAGTTCAAGGCC	R-CCAAGTAAATGAGGTCCAAATC
2	F-GATGGTGATTCTGTGTTTGTGTG	R-CAGTGGACCGCGTTAACATG
3	F-GCCGACAGAGAGCCTTCACC	R-CCATTGGGTCTGTTGGATTCT
4	F-GCACCCTGCCTCTGTTTCC	R-GTAGGAGGATCGCTTGGGCC
5-7	F-GTATCTTTTTTGGAGTTTTGGCTTC	R-AGTCCCCACGTTGGAGATATAGTAC
8-9	F-GAGCCGAGATTGCACCACTG	R-GAGAGCAAGCAGATAGTATGGC
10	F-GATCCTCTTGCTCAGCCTC	R-GCAGAACGTATCCCAGTCG
11-13	F-GGTGTTATGTTGCGCTGATC	R-CTGTTTCATGTCACTTTGGCCTG
14	F-GGAGTTTTGCTGTGCTGCTTAGAC	R-AGCAAACAGTAATCATTCCACCTTC
15-16	F-TCACTGAGCTGACTCACTCACACTC	R-GCCTAATGCTCAGTAGACATGGC
17-18	F-GTGAGTGCTGCTGTTGGTTC	R-GCCAAGGAGGAGGAACAATG
19	F-GGCTTTCCATTACTCTTTTCTC	R-GTTGAGACCCTGAACACCCTG
20	F-CAGGGTGTTCAAGGTCTCAAC	R-CGAGGTTTCACCATATTGCC
21	F-GCTGCTAGTGCTGGGAGATC	R-GCCATCAAACCTCTGGTCTCC
22	F-GGAGACCAGAGTTTGATGGC	R-GCCACTCACCCAGCCTCAAC
23	F-GGGAGGAGGGTAAATCTGGC	R-GCAGATCACCCACATTCCTC

in the hotspot mutation region, then the whole coding region of cDNA was amplified. cDNA was sequenced first, followed by confirmatory sequencing of gDNA if a STAT3 mutation was identified. Next-generation sequencing (NGS) was done in parts of patients and followed by confirmation by Sanger sequencing. Sequence analyses were performed using Bioedit software and the basic local alignment search tool (BLAST) from the National Center for Biotechnology Information (NCBI; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>). New missense mutation was confirmed by screening in 100 normal alleles from 50 unrelated, healthy Chinese individuals to rule out the possibility of polymorphism in the Chinese population.

2.3 | Detection of STAT3 phosphorylation, accumulation and nuclear translocation

Tyrosine phosphorylation, accumulation and nuclear translocation of STAT3 were assessed in COS7 cells (African green monkey kidney cells) transfected with wild-type (WT) or mutant STAT3. The mutant plasmid T620S-STAT3-GFP was newly constructed with pAcGFP1-N1 vector (Invitrogen, supplemental data). The human STAT3 WT plasmid WT-STAT3-GFP and three mutant plasmids R382W-STAT3-GFP, V637M-STAT3-GFP (the adviser participated in the construction of these plasmids while she was a visiting scholar at the University of Washington; the plasmids were kindly offered by Prof. Hans Ochs) and T620S-STAT3-GFP were transfected into COS7 cells by Lipofectamine 2000 (Invitrogen).¹³

The expression of Tyr705 phosphorylated STAT3 (p-STAT3) in the cytoplasm of each group was detected by Western blot. The cytoplasmic proteins were extracted with nuclear and cytoplasmic protein extraction kits (KeyGEN) after stimulation with epidermal growth factor (EGF, 100 ng/mL) (PeproTech) for 20 minutes at 36 hours post-transfection. Briefly, 5×10^6 /mL of cells was centrifuged at 4°C, 500 g, 10 minutes, washed twice with cold PBS. 100 µL of precooled Buffer A (add 1 µL of DTT, 5 µL of 100 mmol/L PMSF, 5 µL of protease inhibitor per ml of Buffer A before use) was added for every 20 µL of cell, vortexed for 15 seconds at maximum speed, and placed on ice for 15 minutes. Then, added 5.5 µL of precooled Buffer B, vortexed for 5 seconds and placed on ice for 1 minute. After max vortexed for 5 seconds and centrifuged at 4°C, 16 000 g, 5 minutes, supernatant was transferred to another precooled clean microcentrifuge tube and place on ice to obtain cytosolic protein immediately. BCA method was used to determine the protein concentration. After standard SDS-PAGE and protein transfer, blots were probed with antibodies to total STAT3 (1:1000; Epitomics), Tyr705 p-STAT3 (1:18000; Epitomics) and actin (Beijing 4A Biotech Corporation). Blots were developed by using secondary horseradish peroxidase-conjugated polyclonal antibodies (1:5000; Hua'an Biotech) and ECL Plus Western blot detection reagents (Beyotime Biotech). β-actin was used as an internal control for normalizing protein loading. The images were analysed with Image J software. Ratio of p-STAT3/ β-actin was used for statistical analysis. Experiments were repeated three times.

The accumulation and nuclear translocation of STAT3-GFP were continuously observed by confocal microscopy. Briefly, 24

TABLE 3 Clinical manifestation of 20 AD-HIES patients

Patient/ Sex	Province	Age of onset	Age of diagnosis	Status	NIH score	STAT3 Mutation	Domain	Pneumonia	Parenchymal lung anomalies
P1/M	Guizhou	3 mo	4.17 y	Dead	62	c.1859 C>G T620S	SH2D	+	Pneumatocoles
P2/F	Shandong	6 mo	3 y	12+ y	50	c.1909 G>A V637M	SH2D	+	—
P3/M	Guangdong	3 d	8 y	Dead	72	c.1145 G>A R382Q	DBD	+	Pneumatocoles
P4/F	Hubei	3 d	3.27 y	10+ y	62	c.1909 G>A V637M	SH2D	+	Pneumatocoles
P5/F	Guangdong	3 d	2 y	9+ y	56	c.1825 A>G R609G	SH2D	+	—
P6/F	Chongqing	1 d	7 y	14+ y	77	c.1909 G>A V637M	SH2D	+	Pneumatocoles Bronchiectasis
P7/M	Sichuan	7 d	1.58 y	7+ y	71	c.1144 C>T R382W	DBD	+	Pneumatocoles
P8/M	Chongqing	2 d	8 y	13+ y	58	c.1907 C>T S636F	SH2D	+	Pneumatocoles
P9/M	Sichuan	40 d	8.58 y	14+ y	56	c.1144 C>T R382W	DBD	+	Pneumatocoles Bronchiectasis
P10/M	Yunnan	20 d	4.92 y	Lost	43	c.1407 G>C Q469H	DBD	—	—
P11/M	Shanxi	2.87 mo	3.33 y	7 y	41	c.1145 G>A R382Q	DBD	+	—
P12/M	Gansu	10 d	2 y	7+ y	43	c.1144 C>T R382W	DBD	+	—
P13/M	Sichuan	5 mo	3 y	Dead	56	g.76304 T>G C712G	TAD	+	Pneumatocoles
P14/M	Sichuan	2 d	12 y	15+ y	55	c.1144 C>T R382W	DBD	+	Pneumatocoles
P15/M	Sichuan	20 d	6.67 y	9 y	48	c.1144 C>T R382W	DBD	+	—
P16/F	Henan	1 d	12 y	14+ y	55	c.1294 G>A V432M	DBD	+	—
P17/F	Henan	2 mo	6.58 y	9 y	48	c.1144 C>T R382W	DBD	+	—
P18/M	Fujian	1 mo	2.75 y	4+ y	61	c.1139+5 G>A	DBD	+	Pneumatocoles Bronchiectasis
P19/M	Chongqing	2 mo	1.25 y	3+ y	53	c.1144 C>T R382W	DBD	+	—
P20/M	Yunnan	3 d	6 y	7+ y	43	c.2113 T>C Y705H	TAD	+	—

F, female, M, male; —, negative; +, positive; No, without BCG vaccination.

hours after transfection, cells were stimulated with EGF (100 ng/mL) for 20 minutes, and STAT3-GFP were observed every 4 minutes within 1 hour. The nucleus was dyed by DAPI (1:1000; Beyotime Biotech) one hour after stimulation with EGF to observe the accumulation of STAT3-GFP in the nucleus.

2.4 | Statistics

Statistical analysis was accomplished using SPSS 20.0. All the data were statistically analysed by unpaired *t* test. *P* < 0.05 was considered significant.

Skin abscess	Candidiasis	BCG complication	Eczema	Characteristic face	Retained primary teeth	Scoliosis	Fractures	Hyperextensibility
+	Oral	—	+	+	-	-	-	+
+	Oral Fingernails	+	+	+	-	-	-	+
+	Oral	No	+	+	+	10°(L)	-	+
+	Oral	+	+	+	-	-	-	+
+	Oral	+	+	+	-	-	+	+
+	Oral	—	+	+	+	-	-	+
+	—	+	+	+	-	-	-	+
—	Oral	No	+	+	-	-	-	+
+	Fingernails	—	+	+	+	-	-	+
+	—	—	+	+	+	-	-	+
+	Oral	—	+	+	-	-	+	+
+	Oral Fingernails	—	+	+	-	-	-	+
—	Oral	—	+	+	-	-	-	+
—	—	—	+	+	-	-	+	+
+	—	—	+	+	+	-	-	+
+	Fingernails	+	+	+	+	-	-	+
+	—	+	+	+	-	-	-	+
—	—	—	+	+	-	10°(R)	-	+
+	Oral	+	+	+	-	-	+	+
+	Fingernails	—	+	+	+	-	-	+

3 | RESULTS

3.1 | Patients demographics

From 2009 to 2018, we identified 20 patients (14 males, six females) with AD-HIES. A total of 13 patients were diagnosed after 2012, and the other seven patients (P1-P7) were

previously reported.¹⁴ All patients (100%, 20/20) were not from consanguineous families and did not have family histories of the disease. The 20 patients were from 11 provinces in mainland China, and 11 patients (55%) came from southwestern China, including five patients (25%) in Sichuan, three patients (15%) in Chongqing, two patients (10%) in Yunnan and one patient (5%) in Guizhou and so on.

The mean age at onset was 0.12 years (median 15 days, range 1 day to 6 months), 13 patients (65%, 13/20) developed symptoms within 1 month, 18 patients (90%, 18/20) developed symptoms within 3 months, and the remaining two patients (10%, 2/20) developed symptoms within 4–6 months of age. The mean age at diagnosis was 5.31 years (median 4.54 years, range 1.25 years to 12 years). All the patients were diagnosed after 1 year of age, and 11 patients (55%, 11/20) were diagnosed by 5 years of age. By the end of December 2018, one patient was lost to follow-up, and 19 patients (95%, 19/20) were followed up. A total of three patients (15%, 3/20, P1, P3 and P13) have died, and the remaining 16 patients are still alive, with the age range from 3 to 15 years (Table 3).

3.2 | Clinical characteristics

All patients (100%, 20/20) had the characteristic manifestations of HIES, with a range of NIH scores of 41–77 points. Nineteen patients (95%, 19/20) had the history of pneumonia except P10, 15 patients (79%, 15/19) were tested for pulmonary pathogens by sputum culture, and 13 patients had positive cultures. Six patients (40%, 6/15) had repeated *S. aureus* infections, one of whom had *methicillin-resistant Staphylococcus aureus* (MRSA). Other bacteria, such as *Streptococcus pneumoniae* (25%, 3/15), *Haemophilus influenzae* (25%, 3/15) and *Pseudomonas aeruginosa* (25%, 3/15), *Klebsiella pneumoniae* (6.7%, 1/15), *Acinetobacter baumannii* (6.7%, 1/15) were also detected. One patient had fungal pneumonia (*Aspergillus*). Recurrent pneumonia was associated with secondary pneumatoceles in 10 patients (50%, 10/20) and bronchiectasis in three patients (15%, 3/20). Three patients (30%, 3/10) underwent partial lung lobectomy for pneumatoceles. Recurrent skin infections were also observed in this group, 17 patients (85%, 17/20) had a history of recurrent *S. aureus* skin and/or viscera abscesses, and two patients had liver abscesses required surgical excision to control infection. Beside bacterial infections, AD-HIES patients were prone to fungal infections. Fourteen patients (70%, 14/20) had chronic mucocutaneous candidiasis (CMC), 11 patients (55%, 11/20) had recurrent oral thrush in infancy, two of whom complicated with chronic hyperkeratotic fingernails caused by *Candida albicans*, three patients (15%, 3/20) had chronic hyperkeratotic fingernails alone. Other bacterial infections include recurrent and chronic otitis media in eight patients (40%, 8/20), sepsis in three patients (15%, 3/20), tonsillitis in two patients (10%, 2/20), recurrent and chronic sinusitis in one patient (5%, 1/20) and meningitis in one patient (5%, 1/20). Virus infections were found in four patients (20%, 4/20): Epstein-Barr virus infection in two patients, Varicella virus infection in one patient, Measles virus infection in one patient (Table 3).

A total of 18 patients (90%, 18/20) received BCG vaccination according to the National Vaccination Program, while two patients missed the BCG vaccination due to recurrent and severe eczema. Seven (38.8%, 7/18) cases suffered from BCG complications, of whom six patients (33.3%, 6/18) had definitive BCGosis [suppuration at inoculation site, enlargement of ipsilateral axillary lymph nodes, tuberculosis (TB) manifestations in a short time after BCG inoculation, etc.]. One patient (5.6%, 1/18) had TB infection in the lung (Table 3).

Except for the typical manifestations of infection, all patients (100%, 20/20) had recurrent eczema and characteristic facial features of high Ig E syndrome and hyperextensibility. Other skeletal abnormalities were less common in this group, with retained primary teeth in five patients (25%, 5/20), pathologic fractures in four patients (20%, 4/20), and scoliosis in two patients (10%, 2/20). None of our patients had midline anomaly or developed lymphomas (Table 3).

3.3 | STAT3 mutations

We identified 11 STAT3 heterozygous mutations (Table 3), two of which were not reported (P13, C712G; P18, c.1139+5G>T). A total of 19 patients were de novo mutations, the parents of the patients did not have the same mutations as their children. The mother of P18 had the same mutation as P18; however, she did not have any symptom. Twelve patients (60%, 12/20) carried STAT3 mutations affecting the DBD, six (30%, 6/20) affecting the SH2D and two (10%, 2/20) affecting the TAD. Similar to previous reports, the R382W/Q mutation in the DBD was the most frequent STAT3 mutation observed in our cohort, accounting for 45% of mutations (9/20). The second most frequent hotspot, V637M, located in the SH2D, was detected in 15% of the 20 patients (3/20). As our group has reported,¹⁴ our group originally identified two missense mutations (P1, T620S; and P5, R609G). Other mutations (S636F, Q469H, Y705H, V432M) were identified in other four patients.

3.4 | Treatment and prognosis

Attention should be paid to the care of patients in daily life. We suggest that all AD-HIES patients take lifelong prophylactic sulphamethoxazole and itraconazole for prevention of bacterial and fungal infection. By the end of December 2018, one patient (P10) was lost to follow-up, and 3 patients (15%, 3/20, P1, P3 and P13) died. P1 and P3 died of respiratory complications after multiple pulmonary infections and pneumatocele formation, P13 died of intracranial hypertension caused by meningitis. The remaining 16 patients were still alive. Six patients (37.5%, 6/16) persisted in antibiotic/

antifungal prophylaxis only, one patient (6.25%, 1/16) had only IVIG monthly, three patients (18.75%, 3/16) had both antibiotic/antifungal prophylaxis and IVIG, six patients (37.5%, 6/16) had neither oral medicine nor IVIG. Among three patients (P5, P18, P20) insisted on both oral medicine and IVIG, one patient (P18) had pulmonary fungal infection and two patients (P5, P20) had fewer infections. The patients had oral medicine or IVIG only had less infections than before prophylaxis. However, six patients (37.5%, 6/16) had neither oral medicine nor IVIG, one patient had no infection, one patient had tuberculosis treatment, and other four patients still had poorly controlled infections.

3.5 | Detection of phosphorylation and nuclear translocation of STAT3

The phosphorylation of STAT3 in COS7 cells transfected with each mutant was examined by Western blot (Figure 1). The p-STAT3 was detected in WT STAT3 and R382W mutant STAT3 in the cytoplasm of COS7 cells with EGF stimulation ($P > 0.05$). The p-STAT3 was expressed less in V637M mutant cell, and nearly absent in T620S mutant cell compared with WT STAT3 cell and R382W mutant cells ($P < 0.05$). However, no significant differences of p-STAT3 levels between V637M mutant and T620S mutant cell were observed ($P > 0.05$).

We subsequently explored the nuclear translocation of STAT3 mutants by confocal microscopy (Figure 2A, Table 4). WT-STAT3-GFP accumulated in the cytoplasm within 8 minutes of EGF stimulation, began to enter the nucleus after approximately 20 minutes, and prominent accumulation was seen in the nucleus within 1 hour. Nuclear export of WT-STAT3-GFP started approximately 2.5 hours

after stimulation, a small amount of export was observed after 4 hours, and export had almost stopped after 7 hours. A small amount R382W-STAT3-GFP accumulation was seen near the cell membrane at 24 minutes, translocate to the nucleus began at 32 minutes, and obvious accumulation was seen in the nucleus at 50-60 minutes. The export of R382W-STAT3-GFP stopped within 4 hours. A small amount of V637M-STAT3-GFP accumulation was seen in the cytoplasm at 24 minutes, and translocate to the nucleus began at 36 minutes. Little accumulation of V637M-STAT3-GFP was observed around the nuclear membrane at 1 hour, and nuclear export was not obvious. The accumulation and nuclear translocation of T620S-STAT3-GFP were barely observable within 1 hour. The results of DAPI nuclear localization after 1 hour of EGF stimulation further validated this observation. More WT-STAT3-GFP than STAT3-GFP mutants accumulated in the nucleus. R382-STAT3-GFP accumulated at a lower level than WT-STAT3-GFP, V637M-STAT3-GFP accumulated a little in the cytoplasm and rarely entered the nucleus, while T620S-STAT3-GFP exhibited almost no STAT3 accumulation in the cytoplasm and almost no nuclear entry (Figure 2B).

4 | DISCUSSION

We report here the detailed clinical characteristics, molecular features, treatment and prognosis of 20 AD-HIES patients in Chongqing centre, China. Currently, there is no national PID registry in China, meaning no accurate prevalence data on AD-HIES. Up to this date, a total of 40 Chinese AD-HIES patients with STAT3 deficiency have been reported in English and Chinese literature (including seven patients our group previously reported and two patients from Taiwan).^{15–18} Combining these 13 new cases

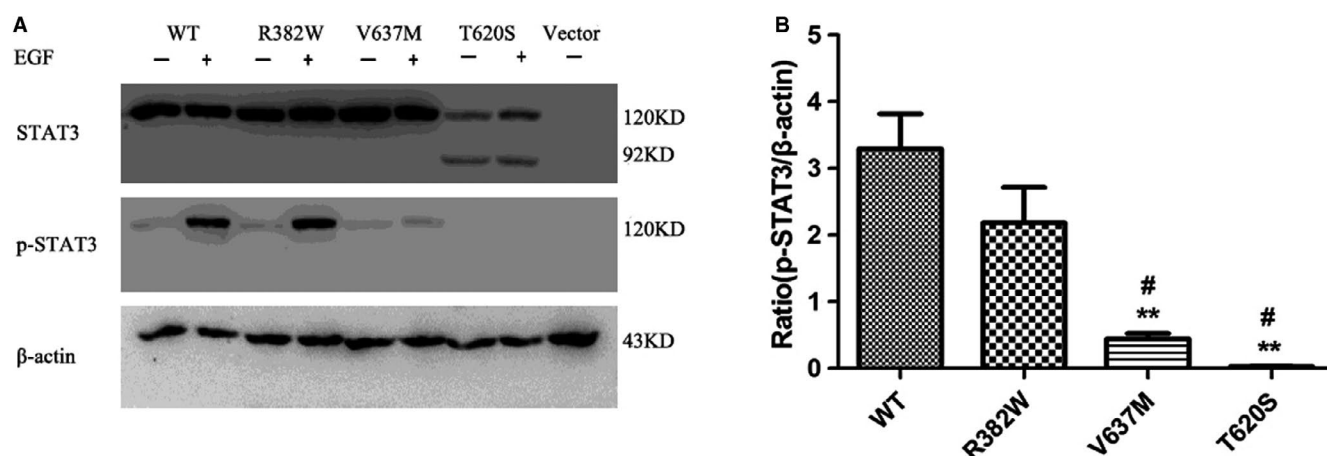


FIGURE 1 Expression of p-STAT3 protein in the cytoplasm of COS7 cells transfected with WT and mutant plasmids. COS7 cells expressing WT or mutant STAT3 were untreated or stimulated with EGF for 20 min, p-STAT3 protein in cytoplasm was analysed by Western blot (A). B, The relative quantity of p-STAT3 protein (ratio of p-STAT3/β-actin) described in A. Data were presented as means ± SD (n = 3). ** $p < 0.01$ compared with WT group. # $P < 0.05$ compared with R382W group

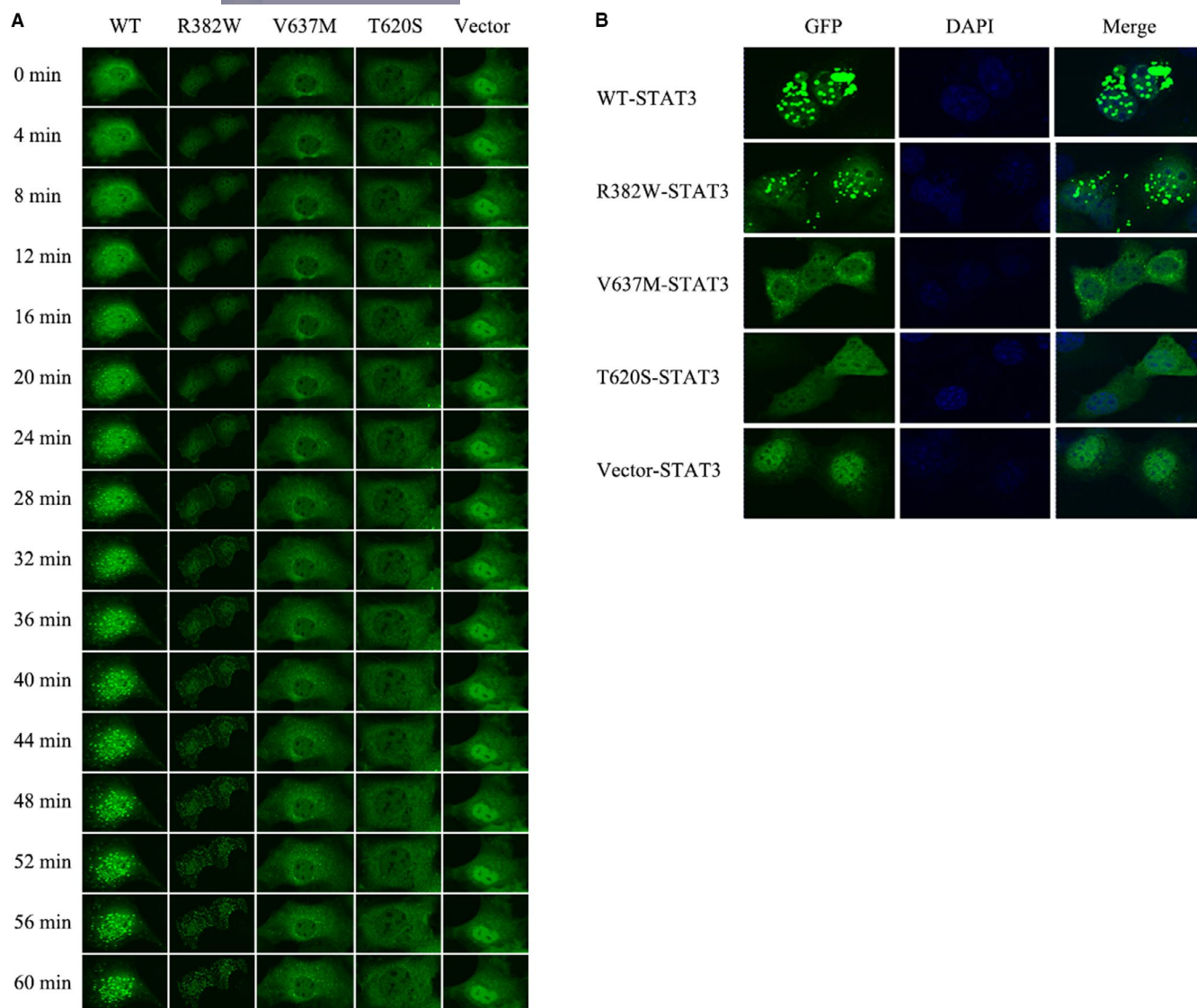


FIGURE 2 Accumulation and nuclear translocation of STAT3. COS7 cells expressing WT or mutant STAT3-GFP were stimulated with EGF for 20 min, STAT3-GFP were observed every 4 min within one hour by confocal microscopy (400 $\times$), following staining with DAPI (blue nuclear stain) one hour after stimulation with EGF. A, Representative images of accumulation and nuclear translocation of STAT3 in cells transfected with WT or mutant STAT3-GFP within 1 h. B, Representative images of DAPI nuclear localization 1 h after EGF stimulation. Three independent experiments were done

with the 40 cases reported in the literature gives a total number of AD-HIES cases to 53 in China. The NIH clinical AD-HIES scoring system is an important evaluation system for AD-HIES: scores ≥ 40 points suggest AD-HIES. We had 34 patients in our centre with suspected AD-HIES, 20 patients were confirmed to have STAT3 mutation. All 20 patients had the typical clinical manifestations of AD-HIES, with NIH scores above 40. For patients with high NIH score but failed to detect STAT3 gene, we found eight cases of DOCK8 mutation, two cases of Tyk2 mutation and PGM3 mutation was not found by NGS. For those 4 patients who still haven't found gene mutations, we have regular clinical follow-up and expect to find new gene by analysing the NGS sequence data in the future. In addition, seven cases

of SPINK5 mutation were identified by department of dermatology of our hospital with characteristic congenital ichthyosis and hair shaft abnormalities, which were absent in other subtypes of HIES.

The onset age of all 20 patients was less than 6 months old considering the eczema, the mean onset age is 0.12 years old. Compared with other studies, including the shanghai centre in China, the onset age in our centre was earlier, may be due to other studies defining the first infection as the onset manifestation.^{13,15,19–21} In addition, the mean diagnostic age was 5.31 years, the diagnostic age was earlier compared with French study (6.8 years),²⁰ the USA study (13.8 years)²¹ and the study of shanghai centre (10.35 years)¹⁵ in China. However, there was a huge

TABLE 4 Differences in the accumulation and nuclear translocation of STAT3 in COS7 cells transfected with WT-STAT3-GFP and mutant STAT3-GFP

	WT-STAT3	R382W-STAT3	V637M-STAT3	T620S-STAT3	CONTROL
Time of accumulation in cytoplasm	8 min	24 min	24 min	—	—
Time of nuclear translocation	20 min	32 min	36 min	—	—
Accumulation of 1 h after stimulation in nucleus	+++	++	+	—	—
Time of nuclear export	5-7 h	Within 4 h	—	—	—

+, positive; —, negative; CONTROL, vector.

lag time between onset of symptoms and diagnosis age in some patients (P3, P6, P8, P9, P14 and P16). On one hand, we defined eczema without specificity as the onset manifestation, on the other hand, it may implied the lack of recognition of this immunodeficiency disease in primary healthcare workers of China. We hope that Chinese doctors could do better in the identification and diagnosis of PID in the next few years with the understanding of the disease and the improvement of PID registry.

The most common clinical manifestation of our patients is recurrent eczema (100%) and the main infectious manifestations are pneumonia (95%), 'cold' *S. aureus* skin abscesses (85%) and CMC (70%). Respiratory tract infections have a great impact on the quality of life of HIES patients, AD-HIES patients were more likely to develop pneumatoceles after pulmonary *S. aureus* infection than patients with other immunodeficiency disorders.⁸ In our group, 95% of the patients had the history of pneumonia except P10, the incidence of pneumatoceles (50%) in our study was similar with French study (52%)²⁰ and Shanghai centre (41.2%),¹⁵ but lower than those in the USA report (71.1%)¹³ and another international study (74.6%).¹⁹ The lower incidence of pneumatoceles may be related to the early diagnosis, early infection prevention and avoidance of further changes in the pulmonary parenchyma, as well as the younger age in our patients. Further analysis of the pulmonary pathogens yielded similar results to those in other studies, *S. aureus* was the main pathogen, followed by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Pseudomonas aeruginosa*.^{15,20,21} However, we also detected *Klebsiella pneumoniae* and *Acinetobacter baumannii* in our patients, which were not detected in French reports.²⁰ Fungal pneumonia was less observed in this study. About 85% of the patients in our group developed *S. aureus* skin infections. This frequency of *S. aureus* skin infections is higher than in shanghai centre (52.9%)¹⁵ and similar to those reported in France (73.3%)²⁰ and USIDNET Registry (74.4%).²¹ About 70% of the patients in our cohort developed CMC, and the incidence of CMC was lower than in the French study (85%)¹⁶ and higher than in the Shanghai centre (58.8%)¹⁷ and USIDNET Registry (20.7%).²¹

Another major symptom of was infection after BCG vaccination, 38.8% of the patients suffered from BCG complications in our group. The incidence of BCG complications in our study was similar with Shanghai centre (37.5%),¹⁵ but higher than French study (0%).²⁰ China requires that newborns be vaccinated with BCG within 24 hours of birth, which may partly explain the higher BCG vaccination complications. Except for the typical manifestations of infection, all the patients (100%) in our group had typical facial features of high IgE syndrome and hyperextensibility. Other skeletal abnormalities such as retained primary teeth (25%), pathologic fractures (20%) and scoliosis (10%) were less common in our group, which may be related to the younger age of our patients, while other studies included more adult cases.^{15,19–21}

Because AD-HIES is a multisystem disorder and infections in patients are usually well controlled by conservative therapies, HSCT is not recommended unless haematopoietic malignancy occurs.⁸ For AD-HIES, the major goal of treatment focuses on control of skin and lung infections. Our patients benefit from prophylactic antibiotics including oral administration of sulphamethoxazole and itraconazole as well as IVIG for prevention of bacterial and fungal infection. However, the mortality rate of our patients (15%) was similar with Shanghai centre (17.7%),¹⁵ but higher than reports abroad (3.6% in USIDNET Registry,²¹ 7% in France²⁰) although the diagnostic age was earlier. On one hand, paediatric immunologists need to strengthen the follow-up management of patients, on the other hand, it may be related to patients' adherence to infection prevention and IVIG compliance, as the rate of antibacterial prophylaxis, antifungal prophylaxis and IVIG in our patients were lower than those in French study.²⁰

STAT3 is the only gene known to cause AD-HIES. We identified 11 STAT3 heterozygous mutations, of which two mutations (P1, T620S; P5, R609G) were originally identified by our group and two mutations (P13, C712G; P18, c.1139+5G>T) were not reported. All patients did not have family histories of the disease. P13 mainly presented with severe pneumonia and meningitis, and died of intracranial hypertension caused by meningitis, which was rare in AD-HIES patients. As one of PID, AD-HIES is susceptible to infection.

Meningitis in this patient was difficult to control and lead to death. We speculated that it was related to the STAT3 mutation. The result of whole-exome sequencing (WES) showed that P18 also had SPINK5 heterozygous de novo mutation (c.602+1 G>T, not reported, data were not shown by WuXi NextCODE). The P18(STAT3 c.1139+5G>T) had typical manifestations of STAT3 mutation with recurrent and severe pneumonia and pneumatoceles as well as reduced Th17 cells (data were not shown), but not the characteristic manifestations of SPINK5 mutation, such as congenital ichthyosis or hair shaft abnormalities.²² So we speculated that STAT3 mutation was the pathogenic gene of the patient. However, the mother of P18 had the same STAT3 mutation as P18, she did not have any symptom. It may related to the incomplete penetrance.

In 2007, mutations in STAT3 were identified as the cause of AD-HIES.^{7,10} STAT3 protein has six functional domains, different domains of the protein have distinct functions. As we known, STAT3 was initially recognized as a signal transducer for IL-6 and EGF.²³ After cytokines bind to this receptor, the STAT3 protein is phosphorylated by the receptor-associated JAK enzymes and then form dimers, which is mediated by the SH2D. Subsequently, activated STAT3 protein translocate to the nucleus binding to its regulatory target gene promoter, which is mediated by the DBD. After STAT3 activates specific signalling pathways, it is dephosphorylated by tyrosine phosphatases and is returned to the cytoplasm.^{24,25} In our study, 12 patients carried STAT3 mutations affecting the DBD, six affecting the SH2D, and two affecting the TAD. Like others, on the basis of the clinical features, we did not observe a significant genotype/phenotype correlation.^{13,19} However, this maybe due to the total number of patients described to date is limited. To further verify the effect of different domains of STAT3 gene mutations on STAT3 protein phosphorylation and nuclear translocation, the WT and mutant STAT3-GFP plasmids were transfected into COS7 cells.

The results of Western blot in our study showed that mutations in the SH2D (V637M, T620S) but not those in the DBD (R382W) affect activation-induced Tyr705 phosphorylation of STAT3, it is consistent with the Western blot results of Renner et al¹³ and He et al²⁶ Dynamic observations showed that the STAT3 accumulation in the cytoplasm and nuclear translocation were delayed, the expression was lower, and the nuclear export occurred earlier in cells transfected with the R382W mutant than in cells transfected with WT-STAT3-GFP. It indicated that R382W located in the DBD, may also affect STAT3 accumulation, phosphorylation and nuclear translocation, and lead to a decline in STAT3 protein signal transduction and transcriptional activation, it is consistent with the results of pY705-STAT3 in T cells of HIES patients reported by Khatib et al²⁷ However, we did not see an obvious difference between in cells transfected with WT and

R382W STAT3 by Western blot. In addition, the dynamic observation of STAT3 in cells transfected with WT-STAT3-GFP, V637M-STAT3-GFP and T620S-STAT3-GFP also indicated that the mutation of the SH2D not only affected the phosphorylation but also the accumulation and nuclear translocation of STAT3. However, other studies^{28,29} showed that STAT3 nuclear translocation is independent of tyrosine phosphorylation, which differs from the effect of SH2D mutations on STAT3 phosphorylation and nuclear translocation that were observed in our results. As mentioned above, the SH2D mediated the formation of homodimers or heterodimers, further affecting signal transduction. The reduced dimer formation may account for the effects on the nuclear translocation of STAT3 in the SH2D mutants in our study, but the exact mechanism is unclear.

In conclusion, we reported here the clinical characteristics, molecular features, treatment and prognosis of 20 AD-HIES patients in southwest of China. Our patients had earlier onset and diagnostic age and higher rate of BCG complications. Lifelong prophylactic sulphamethoxazole and itraconazole as well as IVIG reduced infections. However, our patients had higher incidence of mortality though the earlier diagnostic age. We did not find a clear correlation between the genotype and phenotype, but SH2D mutations had a greater effect on STAT3 phosphorylation and nuclear translocation than DBD mutations in vitro.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Qingqing Xiang involved in designing the study, collecting clinical data, performing the experiments, analysis and interpretation of data and writing the manuscript. Luying Zhang, Xia Liu, Shiyu Wang, Tao Wang and Min Xiao involved in collecting clinical data. Xiaodong Zhao involved in diagnosis and referring the patients. Liping Jiang involved in designing and supervising the study and approving the final version of the manuscript.

ORCID

Qingqing Xiang  <https://orcid.org/0000-0003-1575-0843>
Xiaodong Zhao  <https://orcid.org/0000-0003-2054-8711>

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