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Paucity of Genotype-phenotype correlations in *STAT3* mutation positive Hyper IgE Syndrome (HIES)

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Abstract

Autosomal dominant HIES (AD-HIES) is a primary immunodeficiency caused by dominant negative mutations in *STAT3* clustered in the DNA binding and SH2 domains. Although in vitro differences in mutational constructs are observed, clinical phenotypic correlates of these genetic changes have not been described. We reviewed the charts of 65 AD-HIES patients (DNA binding n=35; SH2 n=30), recorded the components of the NIH HIES clinical scoring system as well as brain and coronary artery abnormalities and analyzed data by mutation region in adults and children. Patients with SH2 domain mutations had increased frequency of high palate, broad inter-alar distance, upper respiratory tract infections and, in the pediatric sub-group, significant scoliosis. There was suggestion of increased mortality for patients with DNA binding mutations. Although subtle differences in phenotype were observed to depend on the *STAT3* genotype, overall the clinical phenotypes were similar between individuals with DNA binding and SH2 domain mutations.

Keywords

Autosomal Dominant Hyper-IgE Syndrome(AD-HIES); Job's Syndrome; STAT-3; Genotype-Phenotype

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Introduction

Autosomal dominant Hyper IgE Syndrome (HIES) is a rare primary immunodeficiency first described in 1966 as Job's syndrome and characterized by the triad of eosinophilia, eczematoid dermatitis, and recurrent skin and pulmonary infections (1). In 1972, Buckley et al identified elevations in IgE(2). Autosomal dominant HIES is now recognized as a multi-system disorder including abnormal healing of pneumonias leading to pneumatoceles and bronchiectasis, scoliosis, retained primary teeth, minimal trauma fractures, hyperextensibility, coronary artery aneurysms and tortuosity, focal hyperintensities on brain MRI, Arnold Chiari 1 malformations and characteristic facies with high palate and broad nose (3–10). Dominant-negative mutations in the signal transducer and activator of transcription 3 (*STAT3*) gene account for the vast majority of HIES cases (11–13) and have been found in diverse ethnic groups. However, the pathogenesis of many of the varied clinical features of HIES remains poorly understood.

The majority of mutations in *STAT3* have been identified in the DNA binding and SH2 domains of *STAT3*. Within these regions, there are several mutation hotspots, with several unrelated patients exhibiting the same mutations. These mutated regions show modest differences *in vitro* in *STAT3* phosphorylation (14) and nuclear localization (personal communication M Paulson), but so far there are no known clinical differences between patients with DNA binding and SH2 domain mutations. Therefore, we reviewed a large number of cases of *STAT3* deficient HIES to determine whether phenotypic differences were caused by mutations in these discrete *STAT3* domains.

Methods

Charts of patients with proven *STAT3* mutations were reviewed for features included in the previously published HIES clinical score as well as newly defined clinical features. (11) All patients were enrolled in an NIH IRB approved protocol to study patients with Hyper-IgE Syndrome. Mutations were assigned to either the DNA binding domain (amino acids 320–465) or the SH2 domain (amino acids 585–688). Patients in the two mutation groups (SH2 and DNA binding) were specifically compared for gender, age at diagnosis, known family history before diagnosis, survival time, HIES clinical score, number of boils, candidiasis (vaginal/oral or fingernail), sinusitis/otitis, pneumonia, parenchymal lung abnormalities including cysts, newborn rash, eczema, fatal infection, coronary artery anomalies, characteristic facies, Arnold Chiari I malformation, fractures, hyperextensibility, interalar distance, high palate, retained primary teeth, scoliosis, lymphoma, hyperintensities on brain MRI, highest absolute eosinophil count, absolute neutrophil count and lymphocyte subpopulations (adults), highest IgE and median IgG, IgA and IgM.

Clinical features were documented from the most recent visit in the database. All clinical scoring was performed by a single experienced clinical investigator (JD) who did not know the mutation at the time of scoring. Subset analyses were performed for subjects greater than or equal to 18 years (n=46), subjects under 18 years (n=19) and probands (n=48). We focused on overall comparison of the SH2 and DNA binding groups, but paid special attention to recurrent mutations.

Statistical Methods

The Wilcoxon rank sum test was used to evaluate differences between mutation groups in age at diagnosis, highest absolute eosinophil count, median ANC (absolute neutrophil count), median total IgG, IgA, IgM, highest serum IgE and lymphocyte subsets.

Lymphocyte subsets including CD4, CD8, CD4/CD45RO, CD8/CD45RO, CD20 and CD20/CD27 were assessed in adult patients only due to variation in values throughout childhood and the small number of patients.

The Fisher's exact test was used to test for the association with dichotomous (present/absent) phenotypic characteristics, including mucocutaneous candidiasis, characteristic facies, Arnold-Chiari malformation, coronary artery abnormalities, hyperextensibility, pneumatoceles, newborn rash, high palate, gender, death from all causes, and fatal infection. Most clinical traits were categorized by severity including number of boils, eczema, interalar distance, number of non-traumatic fractures, degree of scoliosis, number of pneumonias, number of retained primary teeth, annual episodes of sinusitis/otitis and number of hyperintensities on brain MRI. The Cochran-Armitage trend test was used to compare the severity of phenotype expression between the SH2 and DNA binding groups. The statistical analysis was done in SAS (version 9.1; SAS Institute Inc, Cary, NC) and R (version 2.8.1; R Foundation for Statistical Computing, Vienna, Austria <http://www.r-project.org>) software. Because these findings are from an exploratory data analysis they were not adjusted for multiple comparisons.

Features that were at least borderline significant ($p < 0.10$) associations by mutation group, as well as features whose intensity could be affected by age (hyperintensities on brain MRI, lifetime number of fractures > 2 , lifetime number of boils, number of episodes of sinusitis in worst year, presence of high palate scoliosis ≥ 10 , and cardiac anomalies), were examined separately by age.

Separate analyses were performed on the 48 individuals who were the probands in families with multiple affecteds. Because earlier diagnosis in siblings and offspring of known HIES patients might affect timing of diagnosis and thereby influence outcome, we examined variables potentially affected by early intervention including candidiasis, lung parenchymal abnormalities, boils, pneumonias, eczema, sinusitis or otitis, and fatal infection to determine if mutations in either region were inherently more severe despite potentially early intervention.

Results

Among the cohort of 65 subjects, 35 (54%) had a mutation in the DNA binding region and 30 (46%) in the SH2 region (Table 1). Individuals with SH2 mutations were diagnosed at a median of 7 years as were individuals with DNA binding domain mutations (Table 1). The 65 subjects had 23 distinct mutations: 11 in the DNA binding region and 12 in the SH2 region. The patient ages ranged from 2–57 years. Mutational hotspots were identified as those at which multiple patients were affected; 77% of DNA binding and 76% of SH2 binding region mutations occurred in hotspots (Supplemental figure 1). In the DNA binding region, these hotspots included 10 patients with substitution of arginine with tryptophan at position 382, 7 patients with substitution of arginine with glutamine at position 382, 6 patients with substitution of arginine with glutamine at position 423 and 4 patients with deletion of valine at position 463. In the SH2 region, these hotspots included 13 patients with substitution of valine with methionine at position 637, 3 patients with substitution of asparagine with aspartic acid at position 647, 3 patients with substitution of glutamic acid with lysine at position 652, 2 patients with substitution of threonine with isoleucine at 622 and 2 patients with substitution of tyrosine with cysteine at 657.

Full Group Analyses

The majority of patients in both SH2 and DNA binding domain mutation groups had similar distributions and extents of the features commonly associated with HIES (Table 2, Figure 1).

The only features with borderline or significant associations with mutation group were high palate ($p=0.03$), increased interalar distance ($p=0.07$) and increased sinusitis and otitis ($p=0.04$), all in the SH2 mutation group. There were no gender based differences. There was no significant difference between the median of peak eosinophil count in patients with SH2 versus DNA binding domain mutations ($p=0.15$). ANC was available for assessment in 28/35 in the DNA binding domain group and 28/30 in the SH2 group. The median ANC of the 2 groups were not significantly different ($p=0.16$). There were 58 patients for whom we were certain of history with regard to intravenous immunoglobulin (IVIG) therapy. Of these, 6/30 (20%) in the DNA group received IVIG and 5/28 (18%) in the SH2 group received IVIG. Serum IgG was evaluated for patients not receiving replacement immunoglobulin therapy. The levels of IgG, IgA and IgM were similar between the two mutation groups with no significant differences found in the median level (p value >0.5 , data not shown).

Subgroup Analyses

Among the patients younger than 18 years, 10 (52%) had a mutation in the DNA binding region and 9 (48%) in the SH2 region. The mean age of patients with DNA binding mutations was 12 years and the mean age of patients with SH2 mutations was 10.5 years. There was more scoliosis seen in the SH2 group than the DNA group ($p=0.04$), particularly for curvatures greater than 15 degrees. The association between the SH2 group and increased incidence of scoliosis was not present in those over 18 years, potentially indicating an earlier age of scoliosis onset in the SH2 group. The median IgE was significantly higher in the DNA binding group than the SH2 group, without a significant difference in age ($p=0.01$). However, the DNA binding group did not have increased severity of any other features in the under 18 age group and IgE levels do not appear to correlate with increased risk of atopic symptoms in HIES patients. (Table 3; Figure 2).

In those older than 18 years, 25 (54%) had mutations in the DNA binding region and 21 (46%) in the SH2 region. The mean age at last follow up of patients with DNA binding mutations was 34.5 years and the mean age of patients with SH2 mutations was 35 years. However, in this age subgroup, the SH2 group had higher incidence of high palate ($p=0.03$), increased interalar distance ($p=0.04$) and increased frequency of episodes of sinusitis/otitis ($p=0.02$). (Table 3) Lymphocyte phenotyping was performed for a subset of the adult patients. There were no significant differences in the median lymphocyte subsets between the mutation groups (Supplemental Table 1). There was a wider inner quartile range observed for the CD8 and CD4 cell populations; however this may have been due to the relatively small group sizes.

The probands were examined separately because the natural history of the disease could be affected by earlier diagnosis in family members. Of the probands, 29 (60%) had mutations in the DNA binding region and 19 (40%) in the SH2 region. No significant differences were found between probands in the two mutation groups for features that might have been affected by early recognition or initiation of prophylactic antimicrobials, such as the frequency or severity of candidiasis, bronchiectasis and pneumatocoeles, pneumonia, boils, eczema, sinusitis and otitis or fatal infection. Therefore, there was no apparent difference in the severity or presentation of disease in those who were the first cases diagnosed in their families. (Table 4)

Individual Mutations and Phenotypic Variation within families

Some subjects had overall lower HIES scores, and some were cases or from kindreds with unique mutations. However, with few or only one subject it is impossible to draw conclusions about phenotype. With increased identification of what are now less common mutations, clusters of varying phenotype may be identified. However, the significant

phenotypic variation within some families suggests the presence of modifying genetic or epigenetic factors that influence phenotype. (Table 4 and Supplementary Table 2)

Mortality

In the overall HIES cohort, nine deaths occurred at ages 11 – 50.5 years. Although these patients all had more than 3 pneumonias and pneumatocoles, these features were common (> 60%) in the cohort. Seven of the nine deaths were associated with infection; all six with DNA binding domain mutations, and one of three with SH2 domain mutations. Within the DNA binding domain mutations, five out of these six patients had mutations at the hotspot arginine 382, while the sixth death was at arginine 423. One patient with SH2 domain mutation at valine 637 died of infection related causes.

Discussion

STAT3 is crucial to many cells and many functions, consistent with the diverse consequences of its mutation. *STAT3* mutations leading to HIES are predominantly associated with normal protein expression with dominant negative function due to missense and in-frame deletions in the SH2 and DNA binding domains. Constructs of *STAT3* mutations causing autosomal dominant HIES inhibit the activity of the wildtype allele leading to overall activity that is typically less than 50% of wildtype but not absent (13). *In vitro* differences are seen between constructs with mutations in the DNA binding and SH2 domains. These differences have included decreased *STAT3* phosphorylation following IL-6 stimulation and impaired nuclear translocation in SH2 constructs following IL-6 stimulation (14 and personal communication M. Paulson) and impaired nuclear localization in DNA binding constructs (personal communication, M Paulson). Upon receptor binding by numerous cytokines, especially those using the common cytokine signaling chain gp130, Janus kinases (JAKs) phosphorylate the intracellular portion of the cognate cytokine receptor and the associated *STAT* molecule. After *STATs* are freed from the receptor into the cytosol, they homo- or heterodimerize via their SH2 domains. Dimerized *STATs* translocate to the nucleus where they bind DNA sequences of target genes with their DNA binding domains, leading to transcription. Importantly, *STAT3* can translocate to the nucleus in either phosphorylated or non-phosphorylated states. (15) Since the SH2 and DNA binding domains of *STAT3* perform distinct functions and mutations in these regions lead to *in vitro* differences, it seemed likely that the clinical phenotype might vary based on the specific molecular defect.

Consistent with previous reports, the majority of patients had very high IgE, peripheral eosinophilia, moderate-severe eczema, newborn rash, greater than two pneumonias, mucocutaneous candidiasis, pneumatocoles or bronchiectasis, minimal trauma fractures, retention of primary teeth, and some degree of characteristic facies. Despite *in vitro* correlates of specific mutations, we found very few significant differences between the two mutation groups, and those that were found were of modest significance. Previous small studies searching for genotype/phenotype correlation in *STAT3* have reached similar conclusions (14,16). The differences we did observe were increased non-immunologic features in the SH2 domain group, including high palate and increased interalar distance, as well as increased scoliosis in younger patients. The increased frequency of sinusitis and otitis observed in SH2 patients may reflect complications of the facial anatomic findings that are more common in SH2 mutations.

The DNA binding region hot spot mutation at arginine 382 may carry be associated with an increased mortality risk as five of the seven infection related deaths carried this mutation. The sixth infection related death in the DNA binding domain group also occurred in a patient with a mutation leading to substitution of the positively charged arginine for a non-

positively charged amino acid. However, the trend of increased infection related deaths in patients with hot spot mutations in the DNA binding domain was not statistically significant. The lack of significance may be related to the low overall numbers of deaths in our cohort and the size of the population overall. In a separate cohort of HIES patients queried regarding their frequency of Varicella-Zoster reactivation, we again observed a non-statistically significant trend toward increased Varicella-Zoster re-activation in patients with the DNA binding domain mutations compared to those with SH2 domain mutations. If this trend of increased infection related deaths and Varicella-Zoster re-activation in patients with DNA binding domain mutations persists as we continue to follow this cohort, it will merit further investigation of whether these sites perform special functions that are unusually linked to infection susceptibility or invasion

Although our sample size was larger than any previously reported cohort, we still lack the statistical power to definitively identify modest associations. Because these findings are from an exploratory data analysis they were not adjusted for multiple comparisons. Therefore, the apparent associations should be treated as preliminary and needing confirmation.

The clinical phenotype of HIES is overall similar between DNA binding and SH2 domain mutations in *STAT3*. How these mutations lead to these phenotypic features is unclear and warrants further study. As more individuals with *STAT3* mutations are identified, including more outside of the hotspot regions, more clear distinctions may become apparent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Clinical score phenotypic findings demonstrated by most HIES patients. Of these, only interalar distance greater than 2 standard deviations, high palate and annual frequency of sinusitis/otitis were significantly more associated with SH2 region mutations in the analysis of all HIES patients.

Figure 2.

In the under 18 years of age subgroup, IgE was higher in patients with the DNA binding domain mutations than those with SH2 region mutations. This difference was not seen in those 18 years of age and older

Table 1

Patient demographics by DNA mutation Region (N=65).

	All N=65	DNA Mutation N=35	SH2 Mutation N=30	P-value*
Median age at diagnosis in years (IQR ⁺)	7 (3, 18)	7 (2, 20)	7 (3, 14)	0.94
Median age at Last contact/death in years (IQR)	28 (15, 39)	28 (15, 40)	27.2 (16, 35)	0.84
Male n (%)	28 (43.1%)	17 (48.6%)	11 (36.7%)	0.45
Race n (%)				
White	49 (75.4%)	25 (71.4%)	24 (80.0%)	0.63
Black	7 (10.8%)	4 (11.4%)	3 (10.0%)	
Hispanic	3 (4.6%)	1 (2.9%)	2 (6.7%)	
Asian	3 (4.6%)	3 (8.6%)	0 (0.0%)	
Other	3 (4.6%)	2 (5.7%)	1 (3.3%)	

* P-values determined by Fisher's exact test for race and gender. Wilcoxon Rank test for both age variables.

⁺ IQR = Interquartile Range

Table 2

Common Features of HIES patients by mutation region.

Feature	DNA region mutations N=35	SH2 region mutations N=30	P value
HIES Score median (IQR ⁺)	73 (60, 82)	79 (65, 86)	0.193 ^{\$}
IgE Median (IQR)	11,500 (7240, 21800)	10,100 (5602, 17640)	0.330 ^{\$}
Highest absolute eosinophil count median (IQR)	936 (810, 1420)	845 (524, 971)	0.154 ^{\$}
>3 pneumonias	23(66%)	18(60%)	0.965 [^]
Pneumatoceles or bronchiectasis	24 (69%)	23 (77%)	0.582 [*]
>4 boils	27 (77%)	20 (67%)	0.479 [^]
Candidiasis			
None	4 (11.8%)	6 (20.7%)	0.57 [^]
Oral/vaginal	13 (38.2%)	9 (31.0%)	
Fingernail	17 (50.0%)	14 (48.3%)	
Newborn Rash	29 (83%)	22(76%)	0.544 [*]
Moderate-Severe Eczema	34 (97%)	27 (90%)	0.302 [^]
>1 retained primary teeth	27(77%)	19 (63%)	0.750 [^]
>10 degree scoliosis	18 (51%)	21(70%)	0.282 [^]
>2 fractures	13 (37%)	15 (54%)	0.112 [^]
Hyperextensibility	23 (66%)	20(77%)	0.404 [*]
Facial Changes	28 (80%)	25 (86%)	0.741 [*]
Hyperintensities on brain MRI	16 (62%)	15 (56%)	0.974 [^]
Arnold Chiari Malformations	13 (42%)	9 (35%)	0.598 [*]
Coronary Artery abnormalities	12(63%)	8(50%)	0.506 [*]

* P-values determined by Fisher's exact test.

^{\$} P-values determined by Wilcoxon test.[^] P-values determined by Cochran-Armitage Trend test⁺ IQR = Interquartile Range

Percentages are based on the number of non-missing values

Table 3

Patient characteristics that were significantly different between the SH2 and DNA binding domain groups by age subgroup. Unless otherwise noted, number (percent) are presented (N= 19).

Phenotype	Severity	DNA Mutation N=10	SH2 Mutation N=9	P value*
Subjects less than 18 years of age				
Subjects under 18 years of age				
Scoliosis (degrees)	<10	9 (90.0%)	5 (55.6%)	0.036
	10–14	1 (10.0%)	0 (0.0%)	
	15–20	0 (0.0%)	4 (44.4%)	
	>20	0 (0.0%)	0 (0.0%)	
Highest IgE	Median, (IQR ⁺)	18850 (13900,22500)	5030 (2240, 10100)	0.013
Subjects 18 years of age or older				
Sinusitis Otitis (number of episodes in worst year)	1–2	5 (20.0)	1 (4.8)	0.021
	3	2 (8.0)	0 (0.0)	
	4–6	6 (24.0)	3 (14.3)	
	>6	12 (48.0)	17 (81.0)	
High Palate	Present	16 (66.7)	19 (85.0)	0.027
	Absent	8 (33.3)	1 (5.0)	
Increase in Interalar distance	<1 SD	6 (24.0)	0 (0.0)	0.035
	1–2 SD	3 (12.0)	3 (15.0)	
	>2SD	16 (64.0)	17 (85.0)	

* P-values determined by Fisher's exact test for binary variables, Cochran-Armitage trend test for ordinal variables, and Wilcoxon Rank test for highest IGE.

⁺IQR = Interquartile Range

Percentages provided are based on the number of non-missing values.

Phenotypic variability within families despite identical STAT3 genotype

	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December
1st	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
2nd	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7
3rd	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
4th	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	
5th	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	
6th	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	
7th	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
8th	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
9th	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	
10th	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	
11th	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
12th	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
13th	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
14th	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	
15th	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	
16th	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
17th	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
18th	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	
19th	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	
20th	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
21st	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
22nd	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	
23rd	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	
24th	7	8	9	10	11	12																		

⁺Duchenne Muscular Dystrophy