Hyper-IgE syndrome with a novel STAT3 mutation-a single center study from India

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Summary

Background: **Hyper IgE syndrome (HIES) is a rare primary immunodeficiency disorder characterized by the triad of elevated IgE and eosinophilia, eczema and recurrent skin and pulmonary infections. Mutation in the STAT3 gene accounts for majority of the autosomal dominant and sporadic forms of HIES.**

Objective**: To report clinical and molecular analyses of patients with Hyper IgE syndrome from a single tertiary care center in India.**

Methods**: Four patients with suspected HIES were studied. Flowcytometry for T_H17 cell numbers and phosphoSTAT3, and STAT3 gene sequencing were performed.**

Results**: T_H17 cells were significantly reduced. Mutations were found in the DNA-binding domain in three and a mutation in the transactivation domain in one patient. One of the mutations detected was a novel mutation (g54792 c.1018A>C p.K340Q) in the DNA binding domain. *Mycobacterial* infection, which is usually not commonly associated with HIES was found in two of our cases, one with a cutaneous abscess in the shoulder, and the other with BCG site reactivation.**

Conclusions**: A novel mutation in the STAT3 is reported. *Mycobacterial* infections can be seen in the spectrum of HIES related infections. *(Asian Pac J Allergy Immunol 2014;32:321-7)***

Keywords: *Hyper-IgE Syndrome, Mycobacterial infection, novel STAT3 mutation, phosphoSTAT3, T_H17 cells*

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Introduction

Davis, Schaller, and Wedgwood first coined the term Job's syndrome[1] for this entity in 1966. They reported two red-haired, fair-skinned girls with frequent sinopulmonary infections, severe dermatitis, and recurrent staphylococcal cold abscesses in the skin. The syndrome was further defined and clarified by Buckley et al. in 1972, who noted similar manifestations in two boys with severe dermatitis, characteristic facies, and elevated IgE levels, leading to the term Buckley's syndrome.[2]

The pathogenesis of Hyper IgE Syndrome (HIES) remained unknown till 2007 when mutations in STAT3 (Signal Transducer and Activator of Transcription 3) were documented in patients with autosomal dominant (AD) and sporadic forms of HIES by two investigating groups simultaneously.[3,4] HIES is clinically a triad of high serum levels of IgE (>2000 IU/ml), recurring staphylococcal skin abscesses, and pneumonia with *pneumatocele* formation. Chronic eczematoid dermatitis, coarse facies, mild eosinophilia, and mucocutaneous candidiasis, are variable features. Dental abnormalities (retained primary teeth, non-eruption of permanent teeth, double rows of teeth), anomalies in midline facial development, and skeletal abnormalities (bone fractures, hyperextensible joints, scoliosis) reflect the multisystem nature with the involvement of seemingly unrelated systems and dissociated manifestations. Both autosomal dominant (AD-HIES) and recessive (AR-HIES) modes of inheritance are seen, with skeletal manifestations being a feature of the former, whereas viral infections and neurologic manifestations are a feature of the AR-HIES.

STAT 3 mutations are the predominant cause of sporadic and familial HIES,[3,4] although other genomic loci may also be involved. STAT3 is involved in the signal transduction of many cytokines, including but not limited to IL-6, IL-10, IL-21, IL-22, and IL-23, and also plays an integral role in wound healing, angiogenesis, cancer, and immunity.[5-10] STAT3 is implicated in the differentiation of IL-17 producing $CD4^+$ T lymphocytes, important for immunity to extracellular bacterial infections and fungi.[11-14] STAT3-related HIES have been consistently shown to have impaired development of T_H17 cells and IL-17 production[15,16] and this defect in the STAT3-IL-17 axis have been proposed as a major pathogenic mechanism of the defective immunity against extracellular bacterial pathogens.[15-17] The other genes that have been implicated in HIES include TYK2[18,19] and DOCK8.[20-23]

Methods

Four cases of STAT-3 related Hyper-IgE Syndrome, from three kindreds, diagnosed at the Postgraduate Institute of Medical Education and Research, Chandigarh, India, were included. Written informed consents were obtained from the patients or their families. The study was approved by the Institute Ethics Committee. The clinical presentation and laboratory findings are summarised in table 1.

T_H17 cell assessment

PBMCs were isolated with Ficoll-Hypaque density centrifugation (Sigma Aldrich, St Louis, Mo). T_H17 cells were identified by means of intracellular staining of $CD4^+$ T cells for the production of IL-17. Briefly, 1×10^6 cells from patients and control subjects were stimulated for 6 hrs with 10 ng/ml phorbol 12-myristate 13-acetate and 1 ug/ml ionomycin (Sigma-Aldrich, St Louis, Mo) in the presence of GolgiPlug (BD Biosciences, San Jose, CA). After cell-surface staining with PerCP-conjugated anti-CD4 (BD Biosciences, San Jose, CA), cells were fixed, permeabilized (Cytofix/Cytoperm, BD Biosciences, San Jose, CA), and stained with Alexa Fluor 647-conjugated anti-IL-17A (BD Biosciences, San Jose, CA). An immunoglobulin isotype control was used as a background control. Since a subset of T_H17 cells also produce IFN-γ, $CD4^+$, T cells were also evaluated for IFN-γ production (Fluorescein isothiocyanate-conjugated anti-IFN-γ; BD Biosciences, San Jose, CA). Flow cytometric studies were performed on a FACS ARIA III instrument (BD Biosciences) and analyzed with Cell Quest Pro software (BD Biosciences).

Evaluation of STAT3 phosphorylation

Tyrosine phosphorylation of STAT3 was assessed by flow cytometry using the BD Phosflow reagents as per the manufacturer's instructions (BD Biosciences, San Jose, CA). Briefly, 100 µl of whole blood was stimulated with 100 ng of IL-6 (Peprotech, USA) for 15 minutes at 37°C. Cells were simultaneously fixed and lysed in lyse/fix buffer (BD Biosciences, San Jose, CA). After washing with staining buffer, cells were permeabilized with Perm Buffer III (BD Biosciences, San Jose, Calif) and stained with Alexa Fluor 647 conjugated mouse anti-tyrosine 705 phosphorylated STAT3 (pY705-STAT3) mAb (BD Biosciences, San Jose, CA). Samples were acquired on FACS ARIA III and results were analyzed using Cell Quest Pro software (BD Biosciences).

Mutation analysis

The STAT3 gene was amplified by PCR from genomic DNA (gDNA) using specific oligonucleotide primers. Briefly, gDNA was prepared from venous blood by using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen BioSciences, Union City, USA). The amplified gene fragments were sequenced from Euroffin Genomics India Pvt Ltd, Bangalore,using the ABI Big Dye Terminator mix (Applied Biosystem, Carlsbad CA) and analyzed with a 3730xl DNA Analyzer, BDT version 3.1 (Applied Biosystems, Carlsbad CA). The sequencing data were analysed using Codon Code Aligner software. A polymorphism Phenotyping program (PolyPhen, http://genetics.bwh.harvard.edu/pph) and a SIFT (Sorting Intolerant from Tolerant) program were used to predict the effect of the identified STAT3 mutations. This program predicts whether an amino acid substitution affects protein function, based on sequence homology and the physical properties of amino acids.

Results

Serum IgE levels and absolute eosinophil counts (AEC):

Three of the four patients showed IgE levels above 2000 IU/ml at presentation (45,935 IU/ml, 22,400 IU/ml, and 4300 IU/ml). One patient (case 3) had a lower initial serum IgE of 822 IU/ml but this subsequently increased to 54,0000 IU/ml. The AECs were 295, 856, 603, and 948/mm^3 respectively (normal range 50-350/mm^3).

Table 1. Table showing clinical profiles and laboratory findings of the four cases.

Case No.	Age at presentation	Sex	Clinical presentation	NIH Score	AEC (cells/µl)	Serum IgE (IU/ml)
1	35 years	Male	Repeated episodes of pneumonia, skin rashes, and skin abscesses since age 15 days. At 13 years, had widespread skin infection. At 18 had cough & hemoptysis treated as TB. At 30 had *Staph* lung abscess. Facial features present (Fig. 1a, 1b). Family history: A son had recurrent pneumonia, pneumothorax and pyoderma at the age of 14 months. Facial features present (Fig 1c). Serum IgE was 2449 IU/ml, NIH Score 42. Expired, genetic studies not done.	52	295	45,935
2	5 years	Male	5 years old son of case No. 1. History of recurrent upper respiratory tract infections and itching. No history of pulmonary infections and no characteristic facial features.	25	856	22,400
3	1 year 10 months	Male	Recurrent skin infections, ear discharge, empyema, recurrent pneumonia, with pneumatocele formation. First episode of pneumonia at 11 months of age. Facial features present (Fig 1d). Swelling over right elbow and shoulder (antibioma). Showed granulomatous inflammation with numerous AFB.	45	603	54,000
4	4 months	Male	Rash since day 2 of life, with anal and oral ulcerations. Recurrent oral and tongue lesions, resulted in destruction of the right lateral border of the tongue (Fig 1d, 1f), scrapings showed fungus. Nodular swelling at BCG site, FNAC showed presence of AFB.	42	948	4300

AFB: Acid fast bacilli. ATT: Anti-tubercular therapy. BCG: Bacillus Calmette-Guerin. FNAC: Fine needle aspiration cytology.

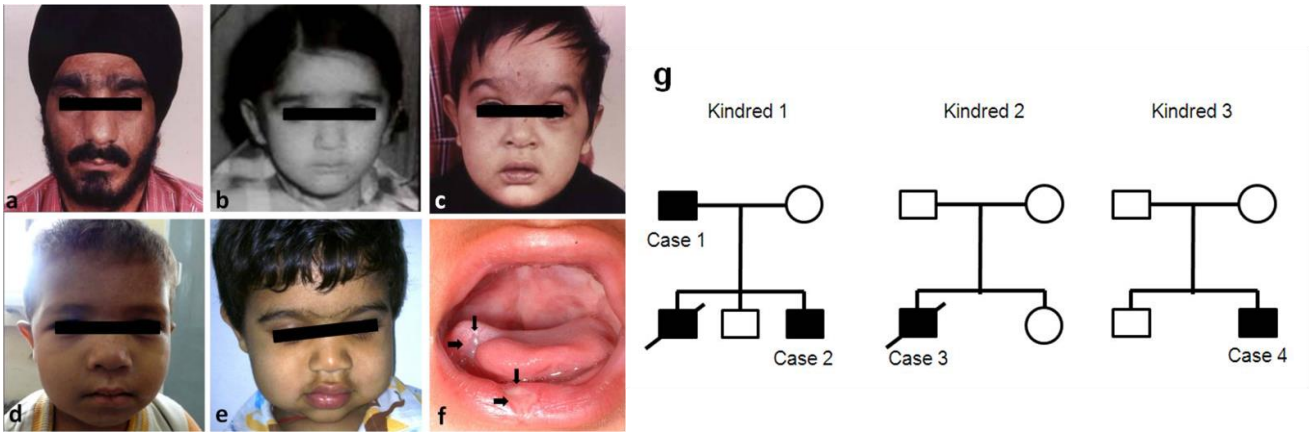

Figure 1. (a) Case 1 showing coarse facial features with broad forehead, bushy eyebrows and bulbous nose. (b) The patient, in his childhood photograph showed the presence of the facial features. (c) Facial appearance of the 14 months old child, elder sibling of case 2 with widely spaced eyebrows, wide forehead, and a broad nasal bridge. This child expired before he could be investigated. Mutation studies could not be done, and hence are not included in this case series (d) Case 3, showing relatively milder facial features. (e) Case 4, despite a cushingoid appearance because of therapy with steroids, a gradual coarsening of facial features could be appreciated. (f) Ulcers in the tongue and lower lip (arrows) with punched out distortion of the right lateral border of the tongue in case 4, photographed at a stage when the acute lesions were healing after steroids. (g) Family trees representing the three kindred.

Percentage of T_H17 cells

All the four cases showed a reduction in the T_H17 cell numbers, which ranged from 0.09 to 0.2% (0.2, 0.09, 0.1 and 0.2% respectively). The data are represented in Table 1 and Figure 3.

Evaluation of Phospho-STAT3 status

Flowcytometry for pSTAT3 could be performed in two patients (case 1 and case 4). A normal pSTAT3 was observed in case 1 with 46.9% of the cells showing pSTAT3 against a control of 52.4 % respectively, while in case 4, only 2.5% of the cells showed phosphorylation. The test could not be performed in cases 2 and 3. Figure 3 shows pSTAT3 activity in terms of change in mean fluorescence intensity (MFI) between the stimulated and un-stimulated cells of cases 1 and 4.

Mutation analysis:

The various mutations observed are shown in Table 2. Case 1 showed an A to C transversion in exon 10 at the nucleotide g54792 position (c.1018 A>C), leading to substitution of lysine at amino acid position 340 for glutamine (p.K340Q) in the DNA binding domain (Figure 4a). This mutation was not found in the single nucleotide polymorphism database (dbSNP; www.ncbi.nlm.nih.gov/projects/SNP) and hence is considered to be a novel mutation. The mutation had a SIFT score of 0.01 (damaging) and a polyphen score of 0.99 (damaging). The same mutation was demonstrated in Case 2. One hundred and two alleles from 51 healthy volunteers were sequenced for exon 10, and no mutations were found.

Case 3 showed a G to A transition in exon 13 at nucleotide position g.58854 (c.1145 G>A), leading to substitution of arginine at amino acid position 382 for glutamine (p.R382Q) in the DNA binding domain (Figure 4b). The mutation had a SIFT score of 0.12 (tolerated) and a polyphen score of 1.00 (probably damaging).

Case 4 showed a C to T transition in exon 22 at nucleotide position g.71311 (c.2141 C>T), leading to substitution of threonine at amino acid position 714 for isoleucine (p.T714I) in the transactivation domain (Figure 4c). The mutation had a SIFT score of 0.43 (tolerated) and a polyphen score of 0.99 (probably damaging).

Discussion

HIES is a relatively rare disorder and diagnosis requires a high index of clinical suspicion. Though definitive clinical scoring systems have been devised, the diagnosis can be challenging, especially in young children. This is exemplified in case number 4, where the clinician suspected the diagnosis, in spite of a very atypical presentation, based solely on the observation of a gradually coarsening facies over a very short follow-up.

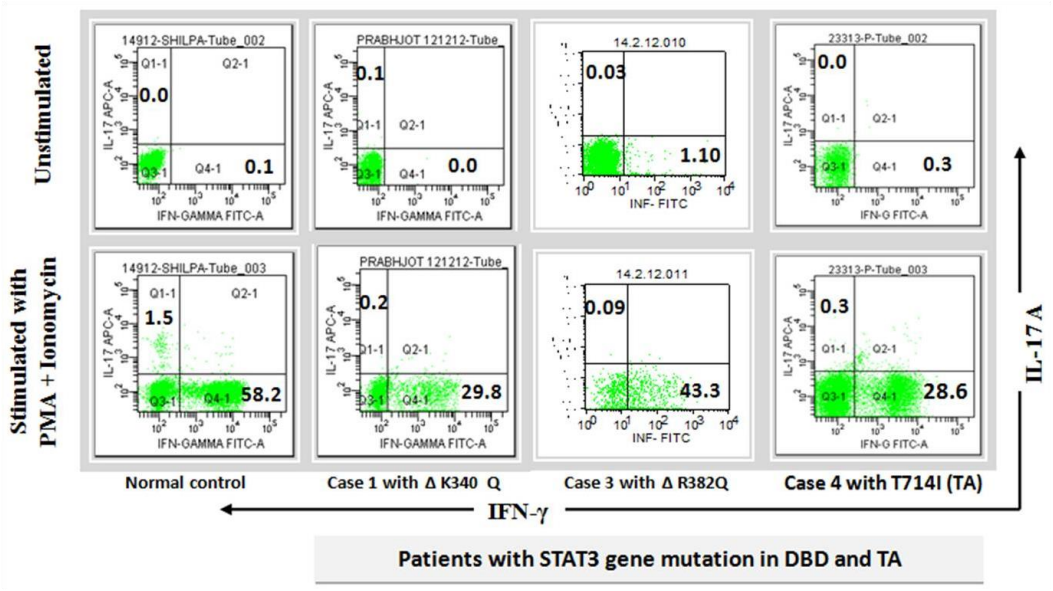

Figure 2. Flowcytometric analysis of $CD4^+$ $IL\text{-}17A^+$ T cells (T_H17 cells) from healthy control and patients with HIES.

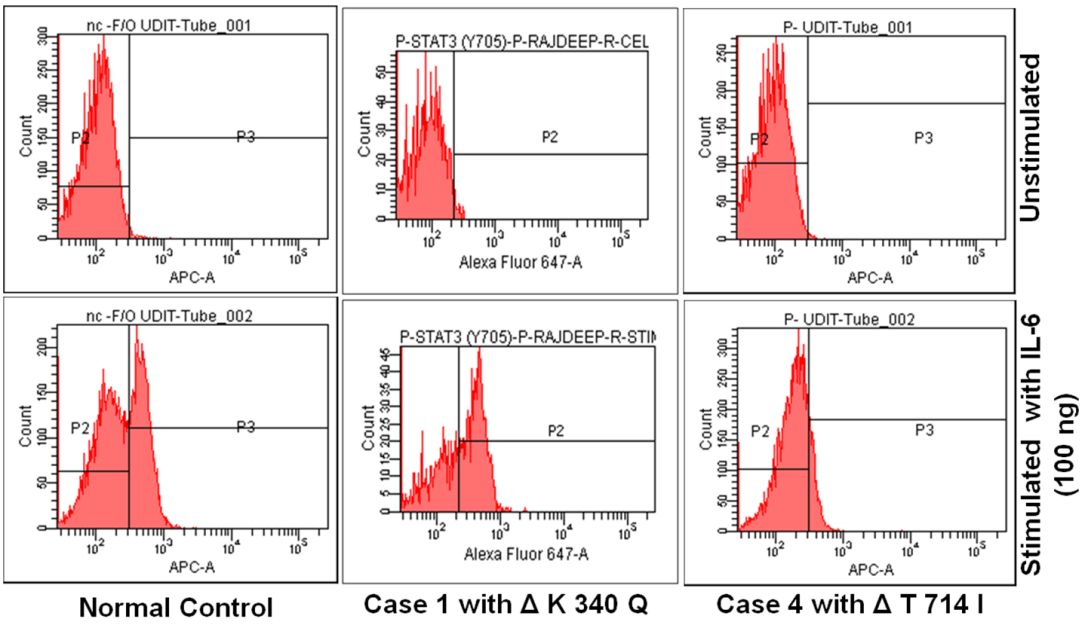

Figure 3. Flowcytometric analysis of STAT3 phosphorylation (Y705) from healthy controls and two patients with mutations in the DBD (ΔK340Q) and TA (ΔT714I) domains.

Pneumonias in HIES are mainly caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, or *Haemophilus influenzae*. The *pneumatocele* that typically follow the resolution of cured bacterial pneumonias are frequently superinfected by *Pseudomonas aeruginosa* and *Aspergillus fumigatus*.[1,2,25,26] Mycobacterial infections, though reported,[27,30] are not a common feature observed in HIES; however, Case 3 had an abscess in the arm which was positive for Mycobacterium tuberculosis. Case 4 showed BCG site infection. It seems likely that the occurrence of Mycobacterial infections in HIES might not be uncommon in countries where mycobacterium tuberculosis is endemic. BCG vaccination in cases where the diagnosis of HIES is made early enough, should be undertaken cautiously and a drug prophylaxis should be considered.

Mucocutaneous fungal disease can be seen in up to 43%-85% of HIES, including 64% of patients with oral candidiasis during the neonatal period.[31-33] Along with a low T_H17 cell count, HIES can thus have features overlapping with chronic mucocutaneous candidiasis. Patients with HIES however also present with other predominant features, such as eczema, skin abscesses and pneumonias, while isolated significant and destructive mucocutaneous candidiasis is not a common presenting feature. Case 4 in our report presented predominantly with oral and anal ulcerations very early in life, i.e. at two months of age, which prompted the initial diagnosis of neonatal Behcet's disease. This is a rare presenting feature in HIES.

Table 2. Table showing the various mutations detected.

Case No.	T_H 17 cells (%)	pSTAT3	Exon	Mutation	Type	Domain
1	0.2%	Normal	10	g.54792, c.1018 A>C, p.K340Q	Heterozygous point mutation, novel	DBD*
2	0.1%	Not Done	10	g.54792, c.1018 A>C, p.K340Q	Heterozygous point mutation, novel	DBD*
3	0.09%	Not Done	13	g.58854, c.1145 G>A, p.R382Q	Heterozygous point mutation	DBD*
4	0.2%.	Reduced	22	g.71311, c.2141 C >T, p.T714I	Heterozygous point mutation	TA†

*DBD- DNA binding domain, †TA- Trans-activation domain.

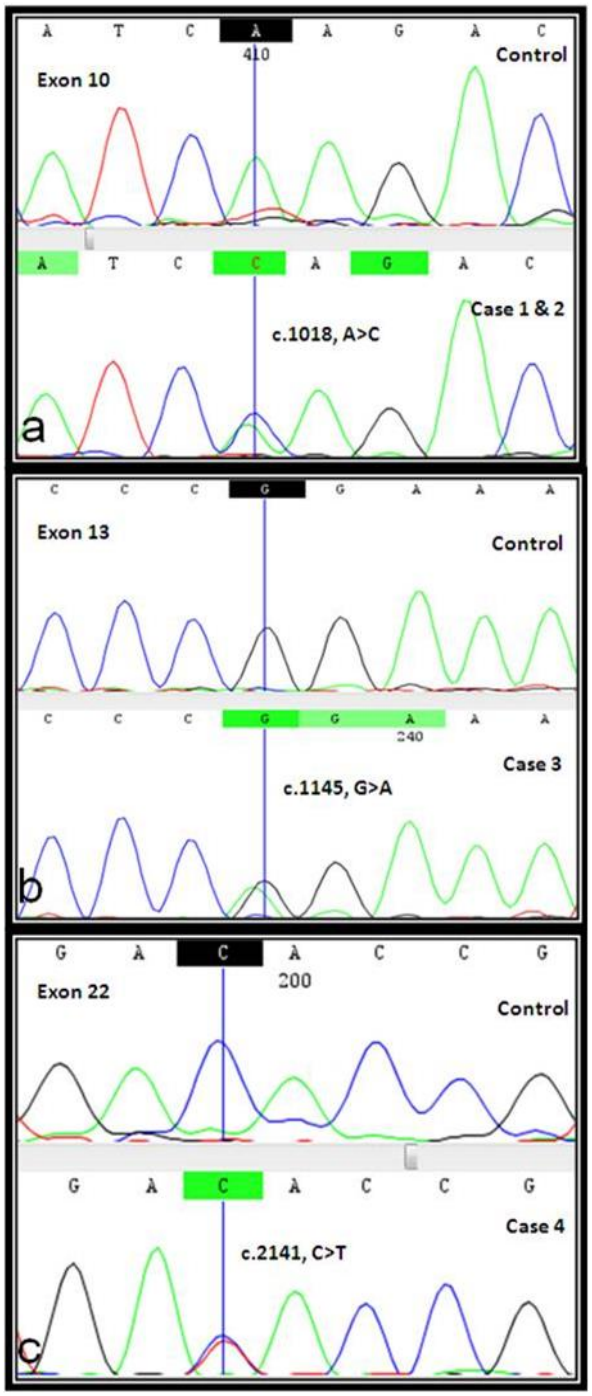

Figure 4. (a) Sequencing results for case 1 and case 2 showing a novel heterozygous mutation (Exon 10, c.1018 A>C, p.K340Q) in DNA binding domain of STAT3 gene. (b) Case 3 showing a heterozygous peak (Exon 13, c.1145 G>A, p.R382Q) in DNA binding domain of STAT3 gene. (c) Case 4 showing a heterozygous peak (Exon 22, c.2141C >T, p.T714I) in transactivation domain of STAT3 gene.

The mutation demonstrated in the first kindred, with a clear cut autosomal dominant phenotype, is a novel mutation and hasn't yet been reported in literature. The mutations in case 3[3,4] and case 4[29] have been previously reported and were sporadic mutations.

Mutations involving the DNA binding domain along with the SH2 domain comprise the mutation hotspots in patients with HIES. STAT3 phosphorylation has been shown to be reduced in SH2 domain mutations, whereas with mutations in the DNA binding domain, STAT3 phosphorylation is often normal. [34,35] Mutations in the transactivation domain, which primarily affect the phosphorylation site, are likely to affect phosphorylation of STAT3 to a greater extent. Hence, pSTAT3 status in our patients correlated with the mutations.

This is the first single centre based report of STAT3 mutation analysis from the Indian subcontinent. More cases need to be screened on a multi-institutional nation-wide collaborative basis.

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