DIAGNÓSTICO MOLECULAR DE HIES

Silvia Danielian
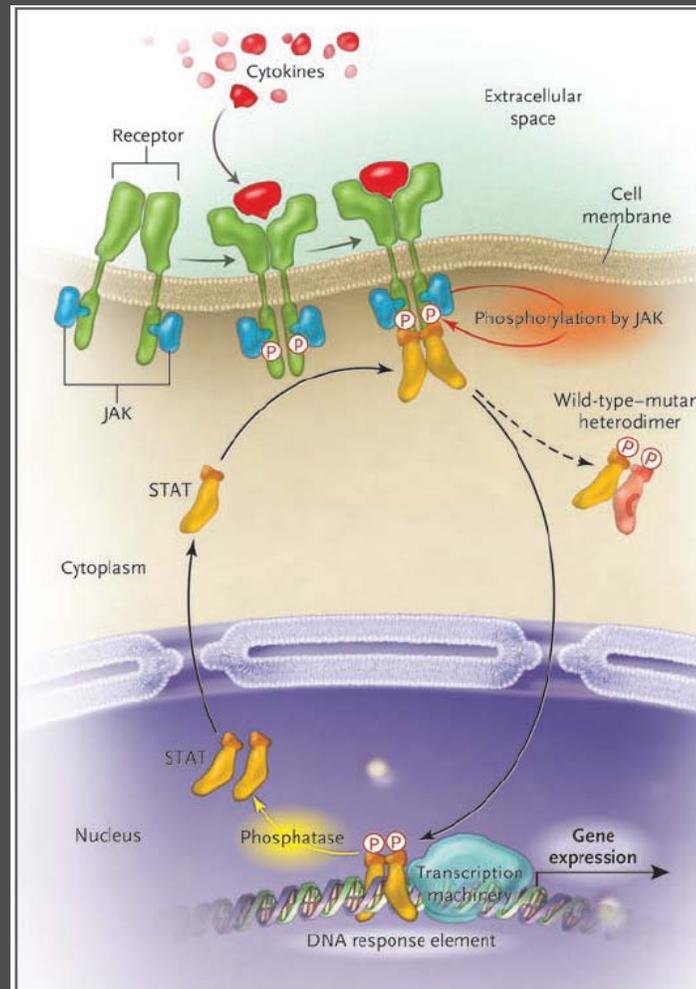
ÁREA BIOLOGÍA MOLECULAR

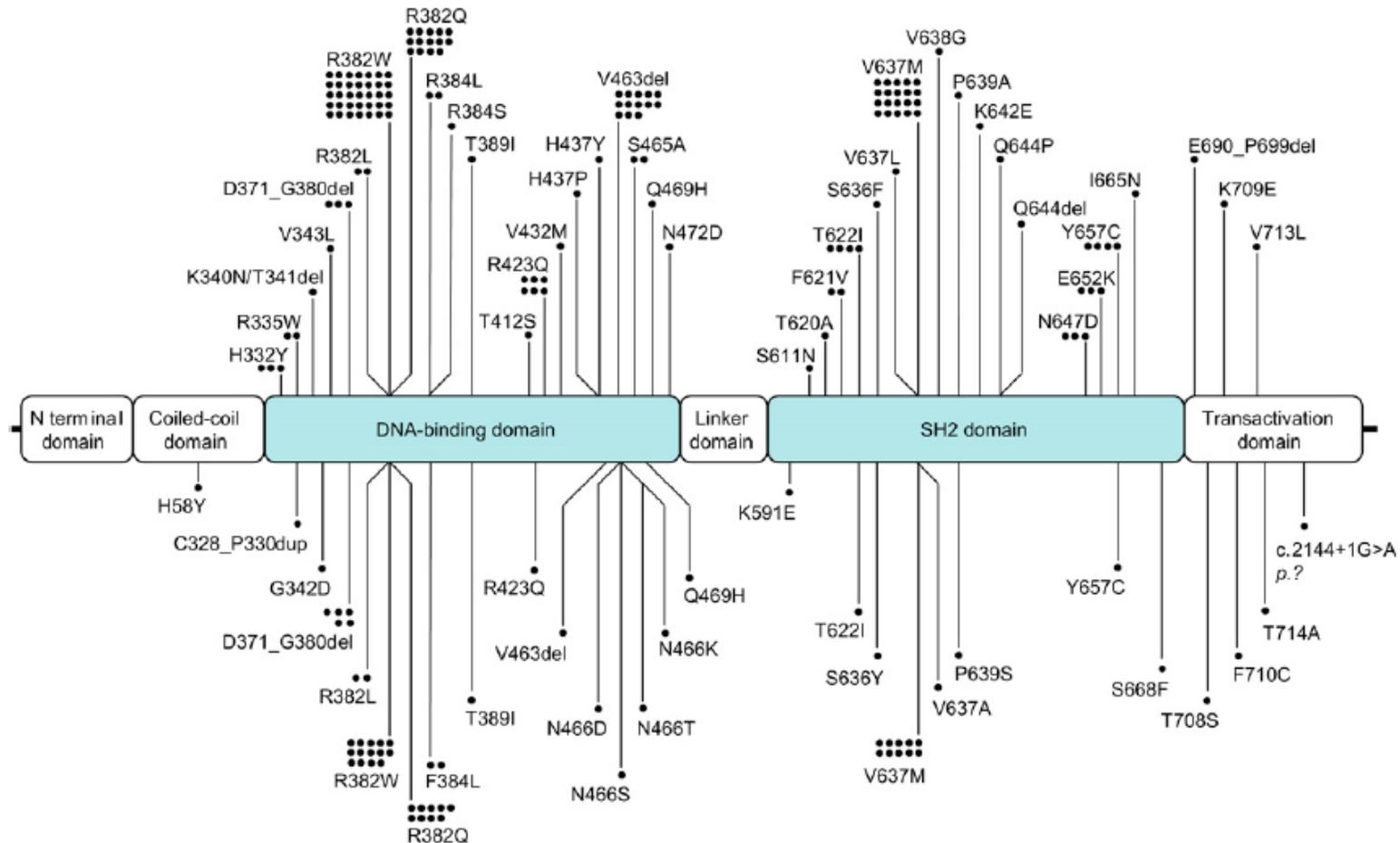**Servicio de Inmunología y Reumatología**

Diagnóstico definitivo de AD-HIES

Presentar, además de las características clínicas y de laboratorio sugestivas, una mutación heterocigota dominante negativa en STAT3.

STAT3: mutaciones en toda la molécula

STAT3: selección de pacientes a estudiar

H58Y	Coiled-coil	42
G342D	DNA-binding	79
C328_P330dup	DNA-binding	41
D371_G380del	DNA-binding	53
D371_G380del	DNA-binding	70
D371_G380del	DNA-binding	83
D371_G380del	DNA-binding	65
D371_G380del	DNA-binding	54
R382W	DNA-binding	76
R382W	DNA-binding	63
R382W	DNA-binding	64
R382W	DNA-binding	57
R382W	DNA-binding	62
R382W	DNA-binding	59
R382W	DNA-binding	61
R382W	DNA-binding	73
R382W	DNA-binding	58
R382W	DNA-binding	29
R382W	DNA-binding	64
R382W	DNA-binding	74
R382W	DNA-binding	48
R382W	DNA-binding	60
R382L	DNA-binding	56
R382L	DNA-binding	45
R382Q	DNA-binding	72

R382Q	DNA-binding	46
R382Q	DNA-binding	62
R382Q	DNA-binding	62
R382Q	DNA-binding	57
R382Q	DNA-binding	60
R382Q	DNA-binding	61
R382Q	DNA-binding	50
R382Q	DNA-binding	48
F384L	DNA-binding	54
F384L	DNA-binding	71
T389I	DNA-binding	52
R423Q	DNA-binding	59
V463del	DNA-binding	56
N466D	DNA-binding	55
N466S	DNA-binding	41
N466T	DNA-binding	66
N466K	DNA-binding	45
Q469H	DNA-binding	57
K591E	SH2	75
T622I	SH2	47
S636Y	SH2	71
V637M	SH2	68
V637M	SH2	37
V637M	SH2	45
V637M	SH2	58

V637M	SH2	44
V637M	SH2	56
V637M	SH2	59
V637M	SH2	30
V637M	SH2	56
V637M	SH2	58
V637A	SH2	72
P639S	SH2	55
Y657C	SH2	33
S668F	SH2	56
T708S	Transactivation	53
F710C	Transactivation	58
T714A	Transactivation	63
p.?	Transactivation	32

De 100 HIES posibles, 64 presentaron mutaciones en STAT3

Woellner et al J Allergy Clin Immunol February 2010

STAT3: selección de pacientes a estudiar

• **Altos scores NIH fuertemente asociados con mutaciones en STAT3 (P 3.9e-07)**

• **Pero 56% de pacientes HIES sin mutaciones en STAT3 tienen score NIH ≥ 40**

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No mutation	72
No mutation	60
No mutation	49
No mutation	29
No mutation	55
No mutation	38
No mutation	47
No mutation	32
No mutation	49
No mutation	43
No mutation	44

No mutation	48
No mutation	39
No mutation	57
No mutation	35
No mutation	29
No mutation	26
No mutation	38
No mutation	45
No mutation	61
No mutation	42
No mutation	46
No mutation	48
No mutation	44
No mutation	63
No mutation	40
No mutation	53
No mutation	29
No mutation	39
No mutation	19
No mutation	21
No mutation	39
No mutation	29
No mutation	30
No mutation	22
No mutation	49

STAT3: selección de pacientes a estudiar

Mutations in STAT3 and diagnostic guidelines for hyper-IgE syndrome

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STAT3: estrategia para su análisis

H58Y	Coiled-coil	42
G342D	DNA-binding	79
C328_P330dup	DNA-binding	41
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V637M	SH2	44
V637M	SH2	56
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V637M	SH2	56
V637M	SH2	58
V637A	SH2	72
P639S	SH2	55
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T708S	Transactivation	53
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T714A	Transactivation	63
p.?	Transactivation	32

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STAT3: primeros análisis en pacientes argentinos

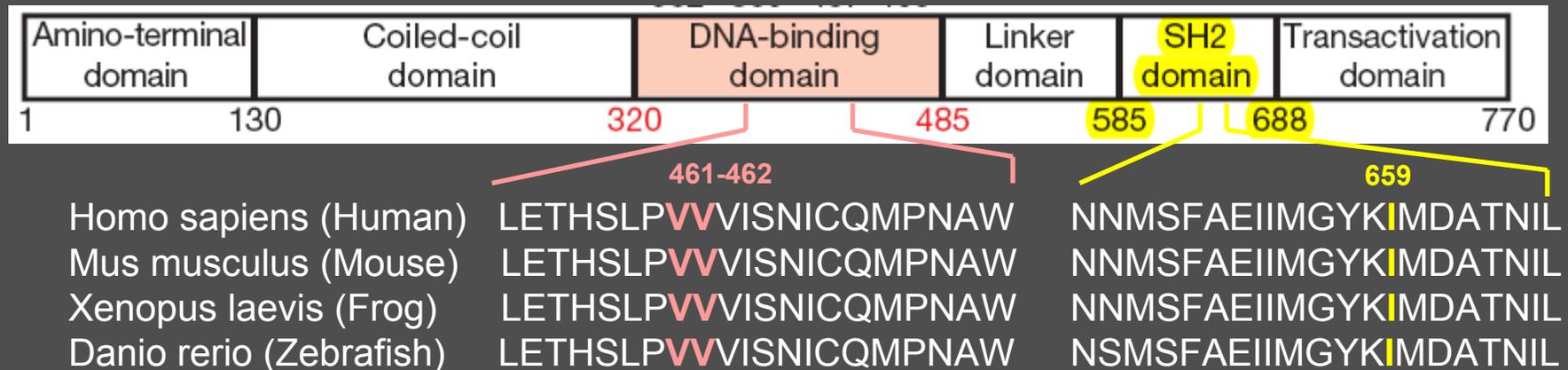| Dominio STAT3	Sitio de mutación	Cambio cDNA	Cambio AA?
DNA-binding	Exon 13	c.1144C>T	**R382W**
DNA-binding	Exon 16	c.1381G>C	**V461L**
DNA-binding	Exon 16	c.1384G>A	**V462M**
DNA-binding	Exon 16	c.1387_1389delGTG	**V463del**
Linker	Exon 19	c.1699A>G	**N567D**
SH2	Exon 21	c.1909G>A	**V637M**
SH2	Exon 21	c.1909G>A	**V637M**
SH2	Exon 21	c.1976T>A	**I659N**

STAT3: análisis de mutaciones nuevas

• **Ninguna de las mutaciones fue hallada en 50 cromosomas de individuos control.**

• **Ninguna de las sustituciones fue reportada como SNP en el database NCBI.**

Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos
missense	T	Phe [F]	2	727
contig reference	C	Ser [S]	2	727
frame shift			1	630
frame shift	(15bp)		1	630
contig reference	G	Gly [G]	1	630
missense	C	Ser [S]	3	609
contig reference	A	Arg [R]	3	609
synonymous	C	Arg [R]	1	609
contig reference	A	Arg [R]	1	609
synonymous	G	Leu [L]	3	608
contig reference	A	Leu [L]	3	608
frame shift		Ile [I]	2	597
frame shift	A	Asn [N]	2	597
contig reference	TC	Ile [I]	2	597

Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos
missense	C	His [H]	3	288
contig reference	G	Gln [Q]	3	288
synonymous	T	Asp [D]	3	237
contig reference	C	Asp [D]	3	237
frame shift		Asp [D]	1	150
frame shift	G	Gly [G]	1	150
contig reference	GA	Glu [E]	1	150
missense	A	Ile [I]	3	143
contig reference	G	Met [M]	3	143
synonymous	C	Arg [R]	3	103
contig reference	G	Arg [R]	3	103
frame shift			3	49
contig reference	A	Lys [K]	3	49
synonymous	C	Pro [P]	3	36
contig reference	T	Pro [P]	3	36
missense	A	Lys [K]	1	32
contig reference	C	Gln [Q]	1	32

STAT3: análisis de mutaciones nuevas

Amino-terminal domain	Coiled-coil domain	DNA-binding domain	Linker domain	SH2 domain	Transactivation domain
1	130	320	485	585	688	770

461-462

|		659
Homo sapiens (Human)	LETHSLPVVVISNICQMPNAW	NNMSFAEIIMGYKIMDATNIL
Mus musculus (Mouse)	LETHSLPVVVISNICQMPNAW	NNMSFAEIIMGYKIMDATNIL
Xenopus laevis (Frog)	LETHSLPVVVISNICQMPNAW	NNMSFAEIIMGYKIMDATNIL
Danio rerio (Zebrafish)	LETHSLPVVVISNICQMPNAW	NSMSFAEIIMGYKIMDATNIL

- **Aminoácidos altamente conservados en varias especies**

- Predicción de consecuencia funcional del cambio:

PolyPhen (Polymorphism Phenotyping) program (//genetics.bwh.harvard.edu/pph/)

Sorting Intolerant From Tolerant program (//sift.jcvi.org/)

STAT3: análisis de mutaciones nuevas
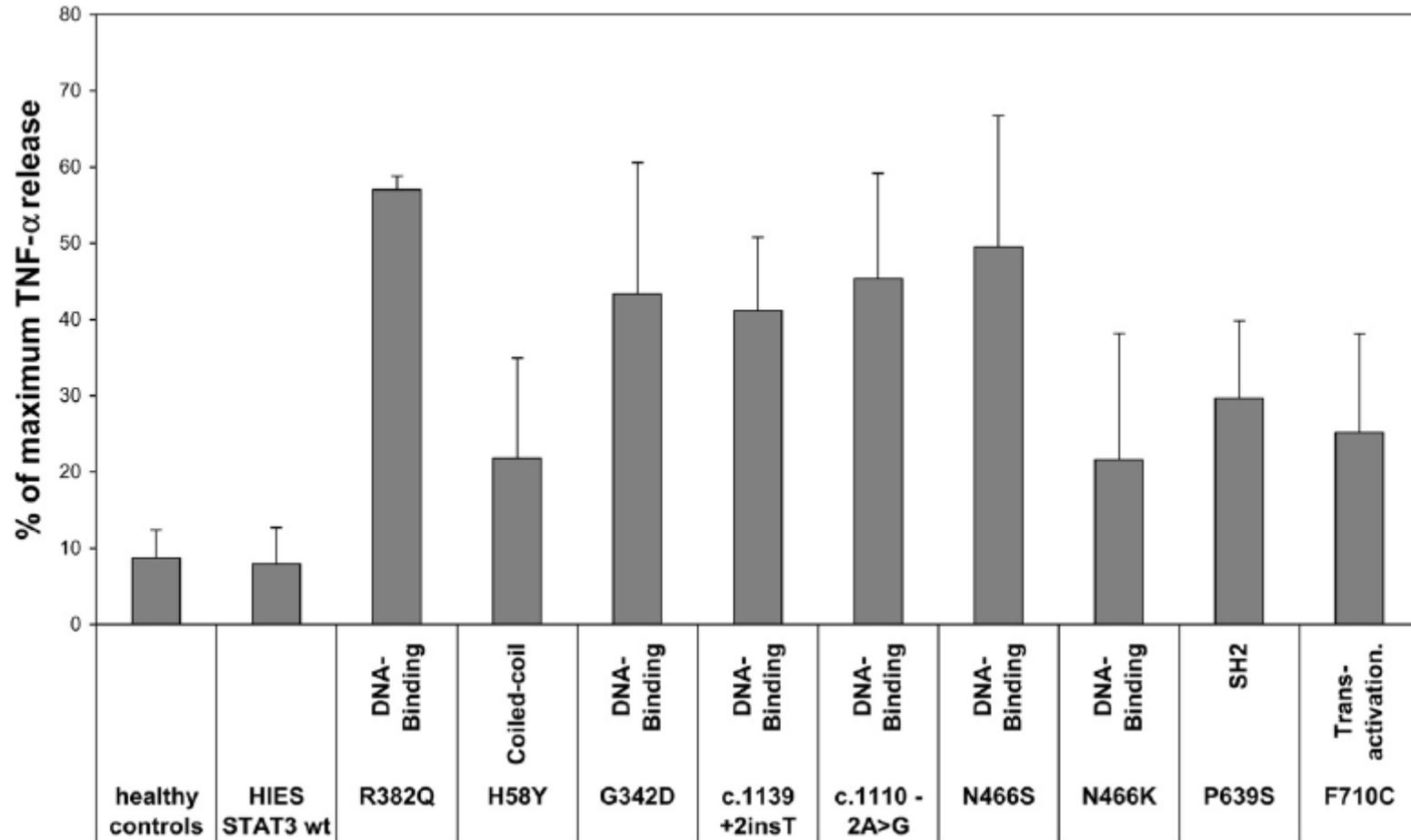
Desarrollar estudios moleculares permite

- Colaborar en el diagnóstico del paciente y en el asesoramiento familiar

- Abrir nuevos campos en investigación

- Contribuir con las bases de datos públicas de mutaciones

Médicos

Estudios moleculares

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Emma Prieto

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