"Herramientas para el diagnóstico genético en el TEA."

Dra. Sara Alvarez
Director Médico, NIMGenetics

Madrid, 7 de Noviembre 2018

NIMGenetics
New Integrated Medical Genetics

Protocolos y algoritmos del uso de la citogenómica en neuropediatría

ACMG PRACTICE GUIDELINES | Genetics inMedicine

Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions

G. Bradley Schaefer, MD[1] and Nancy J. Mendelsohn, MD[2]; for the Professional Practice and Guidelines Committee

Table 4 Template for the clinical genetic diagnostic evaluation of autism spectrum disorder

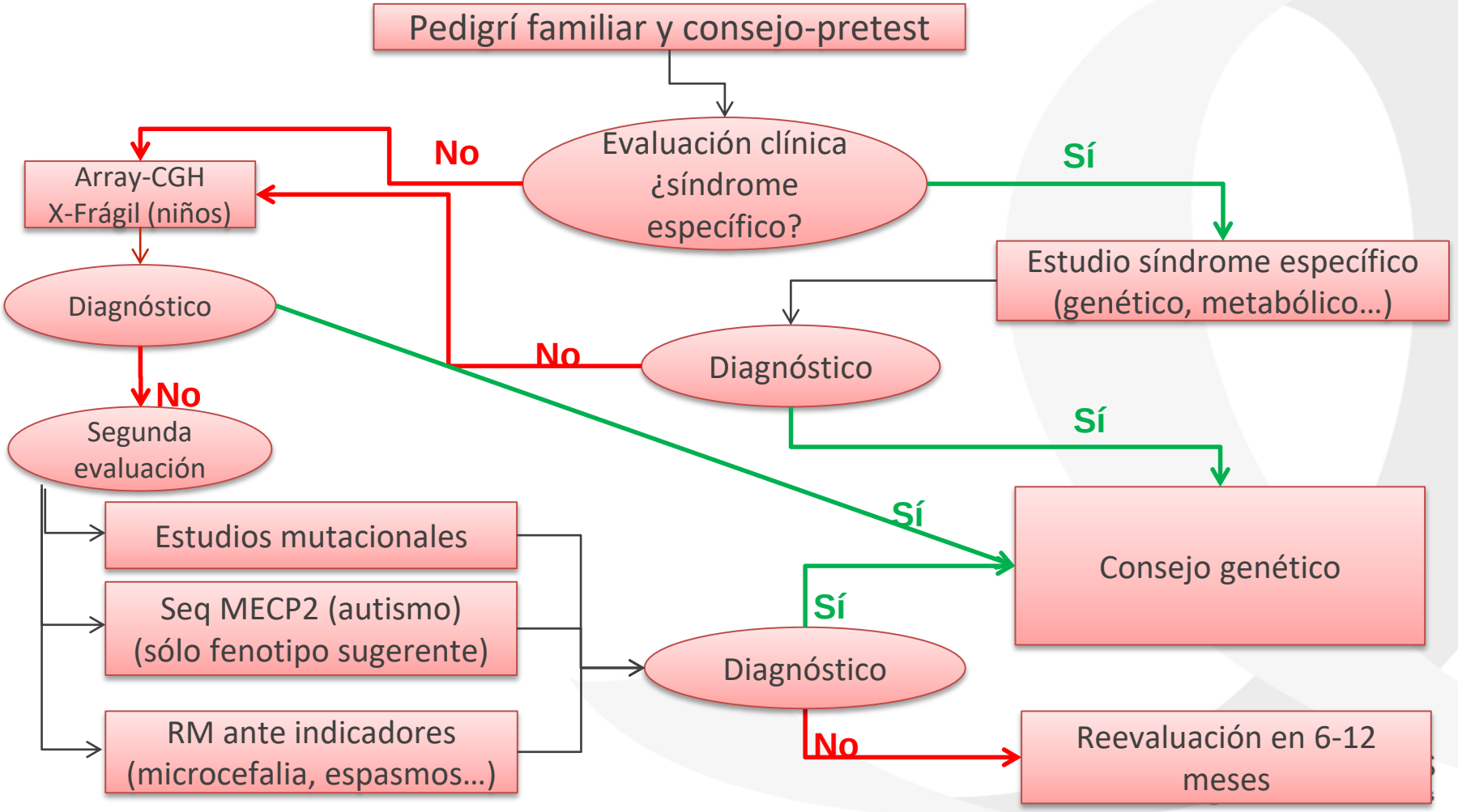
First tier	Second tier
Three-generation family history with pedigree analysis	*MECP2* sequencing to be performed for all females with ASDs
Initial evaluation to identify known syndromes or associated conditions	*MECP2* duplication testing in males, if phenotype is suggestive
Examination with special attention to dysmorphic features	*PTEN* testing only if the head circumference is >2.5 SD above the mean
If specific syndromic diagnosis is suspected, proceed with targeted testing	Brain magnetic resonance imaging only in the presence of specific indicators (e.g., microcephaly, regression, seizures, and history of stupor/coma)
If appropriate clinical indicators present, perform metabolic and/or mitochondrial testing (alternatively, consider a referral to a metabolic specialist)	
Chromosomal microarray: oligonucleotide array-comparative genomic hybridization or single-nucleotide polymorphism array	
DNA testing for fragile X (to be performed routinely for male patients only)[a]	

ASD, autism spectrum disorder; *MECP2*, methyl-CPG-binding protein 2; *PTEN*, phosphatase and tensin homolog.

[a]DNA testing for fragile X in females if indicators present (e.g., family history and phenotype).

NIMGenetics
New Integrated Medical Genetics

Protocolos y algoritmos del uso de la citogenómica en neuropediatría



Arquitectura Genética en el TEA

Síndromes genéticos relacionados con el TEA (10%)

Alt. Cromosómicas (5%):

- Trisomía 21
- Sdr de Turner
- dup (15)(q11q13)
- Etc..

Enf. Mendelianas (3%):

- X-fragil (1-2%)
- Esclerosis tuberosa (1%)
- S. Rett (0.5%)
- Etc..

Sdr. de deleción de genes contiguos (2%):

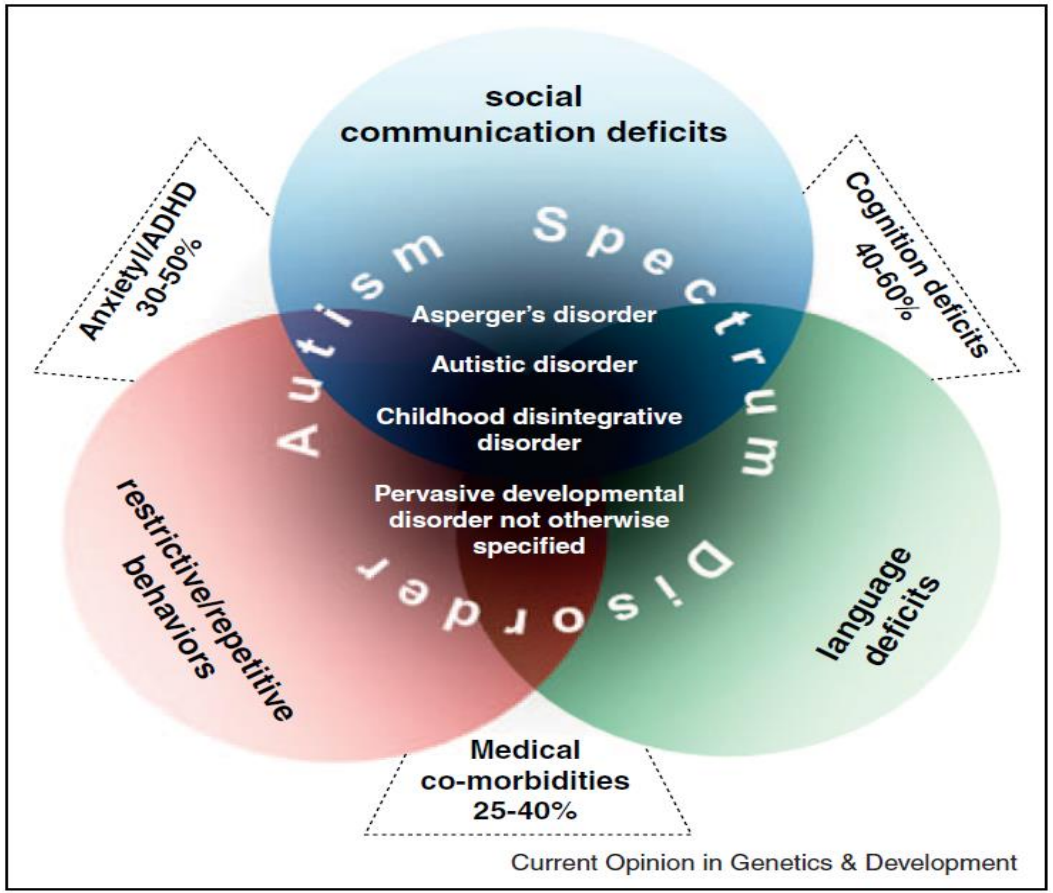
- S. Williams-Beuren
- S. Sotos
- S. Phelan-McDermid
- Etc.

Caracterizados por presentar Fenotipos con:

- Otras alteraciones neurológicas:
  - discapacidad intelectual
  - Epilepsia
  - etc..
- Malformaciones asociadas
- Facies característica

NIMGenetics
New Integrated Medical Genetics

Trastorno del espectro autista (TEA): Entidad compleja clinica y genéticamente



- Trastorno altamente Heterogéneo
- **Alta Prevalencia (>1%) de la población**
- Relación Hombre/Mujer 4:1
- **Alta heredabilidad**

- **Presentación clínica caracterizada por**
  - Dificultad en la Interacción social
  - Deficit comunicación verbal y no verbal
  - Comportamientos o intereses repetitivos

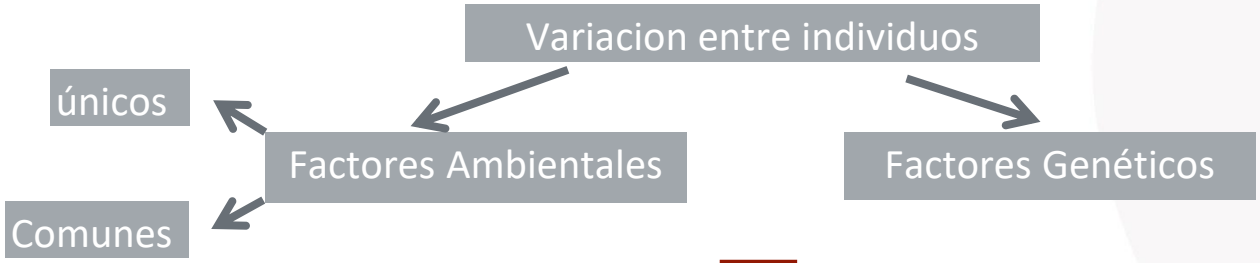
Adicionalmente, esos trastornos pueden asociarse a:

- alteraciones neurológicas (epilepsia, esquizofrenia ó deficit intelectual
- manifestaciones clínicas (pej: dismorfias ó problemas gastrointestinales
- Trastornos del comportamentales (ansiedad, TDAH y otras complicaciones médicas complejas asociadas)

NIMGenetics
New Integrated Medical Genetics

Definición

Proporción de la variación de un rasgo fenotípico entre los individuos de una población que en un momento determinado puede ser atribuible a diferencias genéticas.



Estudios de gemelos/Hermanos

Estudios Clásicos	Estudios recientes (2014)
Heredabilidad del 90%	**Heredabililad de~50%.**

NIMGenetics
New Integrated Medical Genetics

Estudio sueco (1982-2007)

Estudios de gemelos/Hermanos más grande realizado

Registro de todos los niños suecos nacidos entre 1982 y 2007 asociado al registro de todos los niños diagnosticados de TEA antes de los 10 años

1.6 millones de familias con al menos dos hijos:
- 5,799,875 parejas de primos
- 2,642,064 Hermanos de padre y madre
- 432,281 hermanos de madre
- 445,531 hermanos de padre
- 37,570 Mellizos y Gemelos

14,516 casos de TEA
5,689 (39%) de Trastorno autista

Carácterísticas del Estudio:

- Estudio Homogeneo que permite estimar el riesgo de recurrencia
- Controla factores como:
  - Edad Materna
  - Antecedentes Psiquiatricos en la familia
  - Ratio Niños/Niñas
  - Exposición ambiental a factores únicos (NO a factores comunes)

Sandin, SA. Et al JAMA. 2014 May 7;311(17):1770-7

NIMGenetics
New Integrated Medical Genetics

Una heredabilidad estimada del 50% sugiere que los factores genéticos explican el 50% del riesgo

Table 2. Autism Spectrum Disorder and Autistic Disorder Heritability

Models, Terms Included[b]	Model Comparison Measures				Estimated Variance (95% CI)[a]				
	No. of Parameters in the Model	-2 LL	Diff -2 LL	*P* Value[c]	Additive Genetic[d]	Dominant Genetic	Environment		Total Genetic[e]
							Shared	Nonshared	
Autism spectrum disorder									
Full model[f]	14	143 910	NA	NA	0.33 (0.00-0.55)	0.16 (0.00-0.59)	0.05 (0.00-0.17)	0.46 (0.24-0.65)	0.49 (0.21-0.75)
Excluding the dominant genetic term	13	143 910	0.7	.41	0.42 (0.19-0.55)	NA	0.04 (0.00-0.15)	0.54 (0.45-0.66)	0.42 (0.19-0.55)
Excluding the shared environment term	13	143 911	0.8	.38	0.44 (0.24-0.55)	0.13 (0.00-0.51)	NA	0.43 (0.23-0.55)	0.57 (0.45-0.77)
Excluding the additive genetic term	13	143 913	3.0	.08	NA	0.45 (0.18-0.71)	0.14 (0.07-0.20)	0.41 (0.21-0.62)	0.45 (0.18-0.71)
Additive genetic + nonshared environment	12	143 911	1.2	.55	0.50 (0.45-0.56)	NA	NA	0.50 (0.44-0.55)	0.50 (0.45-0.56)
Dominant genetic + nonshared environment	12	143 934	23.8	<.001	NA	1.00 (1.00-1.00)	NA	0.00 (0.00-0.00)	1.00 (1.00-1.00)
Shared + nonshared environment term	12	143 923	13.3	.001	NA	NA	0.24 (0.21-0.26)	0.76 (0.73-0.79)	NA
Nonshared environment term only	11	144 178	268.8	<.001	NA	NA	NA	1.00 (1.00-1.00)	NA

Abbreviations: Diff −2 LL, 2 * difference in log-likelihood between the model and the full model; NA, not applicable; −2 LL, −2 * log-likelihood.

[a] The 95% CIs are 2-sided.

[b] All models adjusted for sex and birth cohort.

[c] *P* value for the testing the hypothesis: the parameters not in the model but in the full model are all equal to zero.

[d] Additive genetic indicates narrow-sense heritability, which only includes the additive genetic component.

[e] Total genetic indicates broad-sense heritability, which includes both the additive and the dominant genetic.

[f] Full model includes terms for additive genetic, dominant genetic, shared environment, and nonshared environment (usually referred to as an ACDE model).

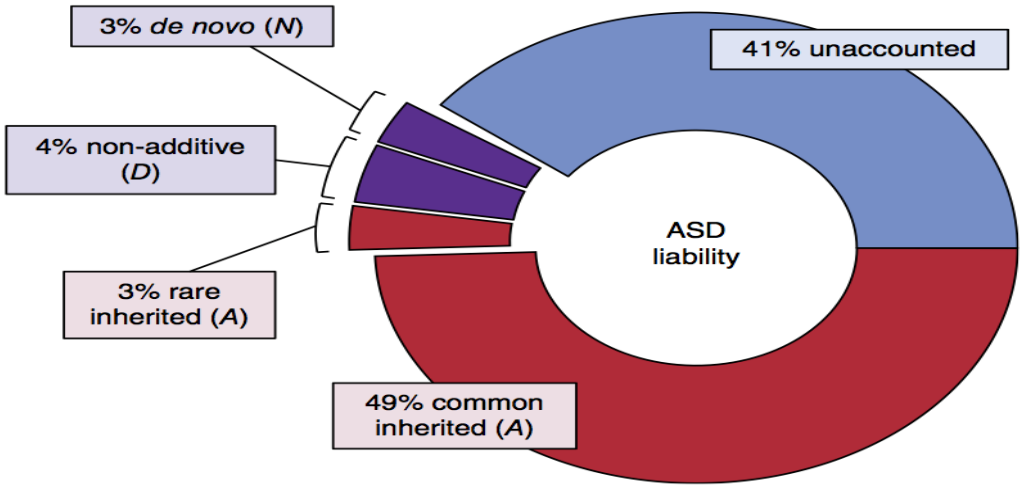
Most genetic risk for autism resides with common variation

Trent Gaugler[1], Lambertus Klei[2], Stephan J Sanders[3,4], Corneliu A Bodea[1], Arthur P Goldberg[5–7], Ann B Lee[1], Milind Mahajan[8], Dina Manaa[8], Yudi Pawitan[9], Jennifer Reichert[5,6], Stephan Ripke[10], Sven Sandin[9], Pamela Sklar[6–8,11,12], Oscar Svantesson[9], Abraham Reichenberg[5,6,13], Christina M Hultman[9], Bernie Devlin[2], Kathryn Roeder[1,14] & Joseph D Buxbaum[5,6,8,11,15,16]

Estudio de 531.906 SNPs

N=3046 individuos

- 466 con TEA
- 2580 controles



NIMGenetics
New Integrated Medical Genetics

Gaugler T et al Nat Genet. 2014 Aug;46(8):881-5.

Most genetic risk for autism resides with common variation

Trent Gaugler[1], Lambertus Klei[2], Stephan J Sanders[3,4], Corneliu A Bodea[1], Arthur P Goldberg[5–7], Ann B Lee[1], Milind Mahajan[8], Dina Manaa[8], Yudi Pawitan[9], Jennifer Reichert[5,6], Stephan Ripke[10], Sven Sandin[9], Pamela Sklar[6–8,11,12], Oscar Svantesson[9], Abraham Reichenberg[5,6,13], Christina M Hultman[9], Bernie Devlin[2], Kathryn Roeder[1,14] & Joseph D Buxbaum[5,6,8,11,15,16]

La mayor parte del riesgo genético para el autismo proviene de versiones de genes que son comunes en la población (SNPs) y no de variantes raras o erróneas (mutaciones)

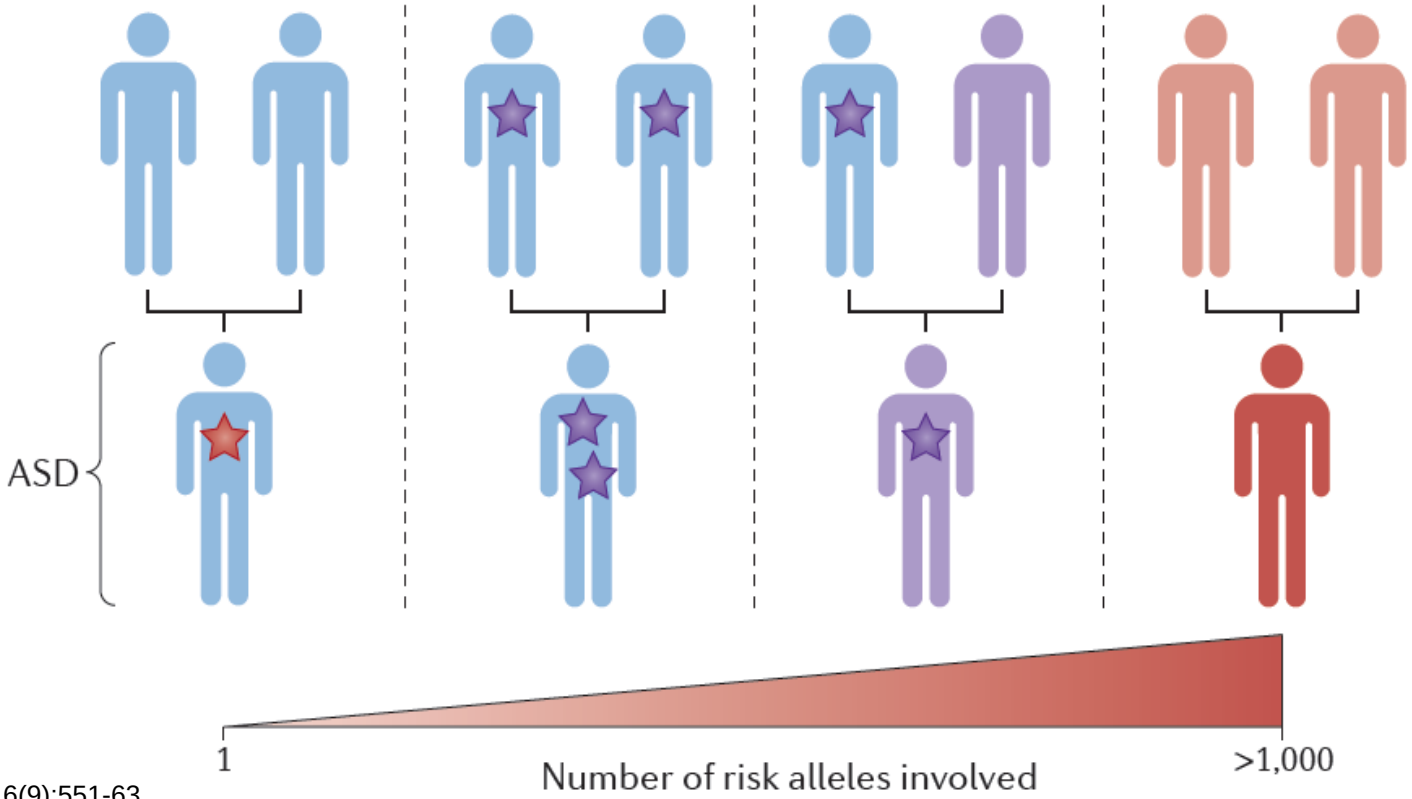
La variación genética probablemente representa aproximadamente el 60 por ciento de la responsabilidad del autismo, con variantes comunes que comprenden la mayor parte de su arquitectura genética

«A pesar de que cada una ejerce sólo un efecto pequeño individualmente, estas variaciones comunes en el código genético suman un impacto sustancial en su conjunto»

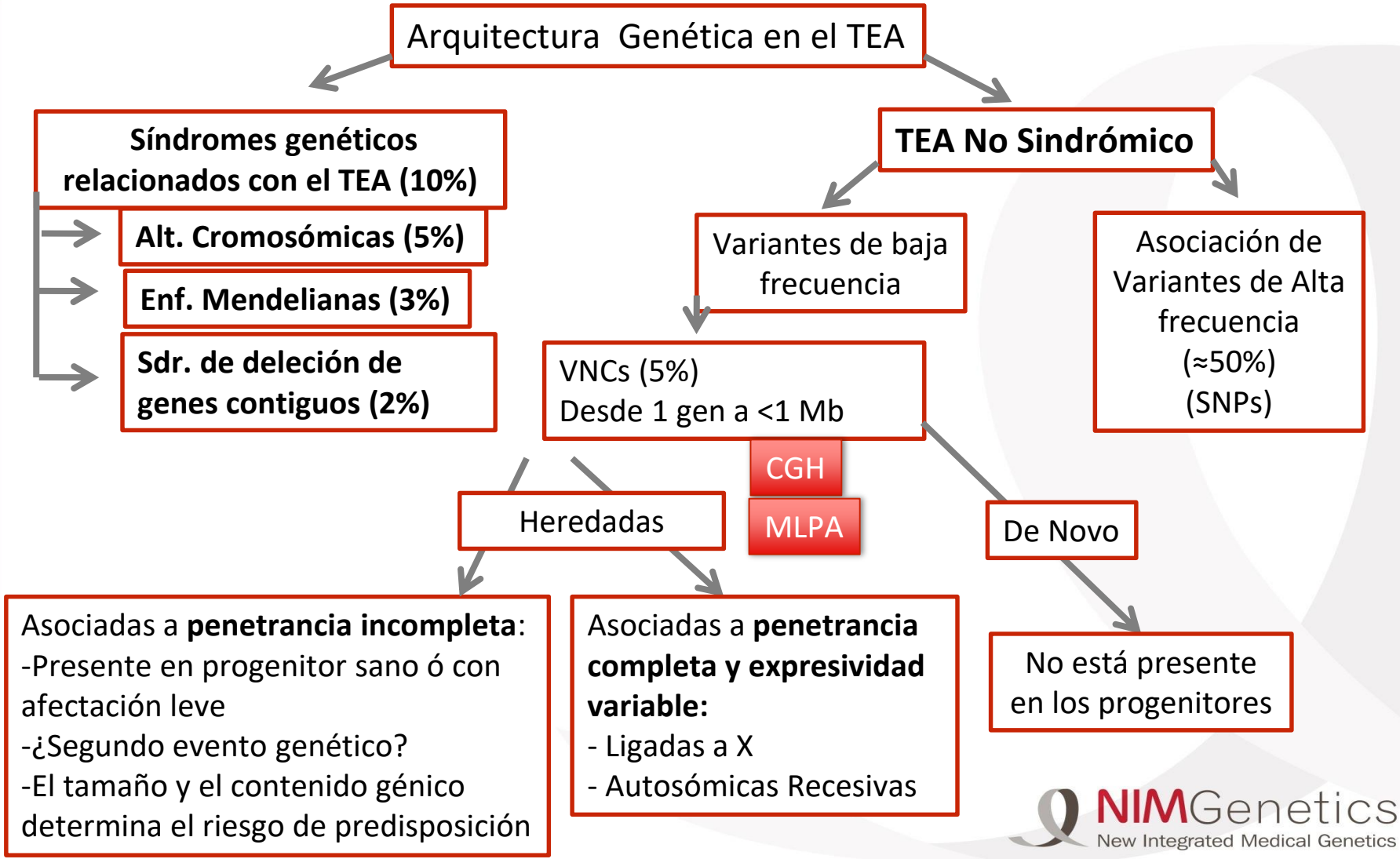
Las mutaciones “de novo” contribuyen a un modesto 2,6% del riesgo total.

NIMGenetics
New Integrated Medical Genetics

Patrones de Herencia en el TEA



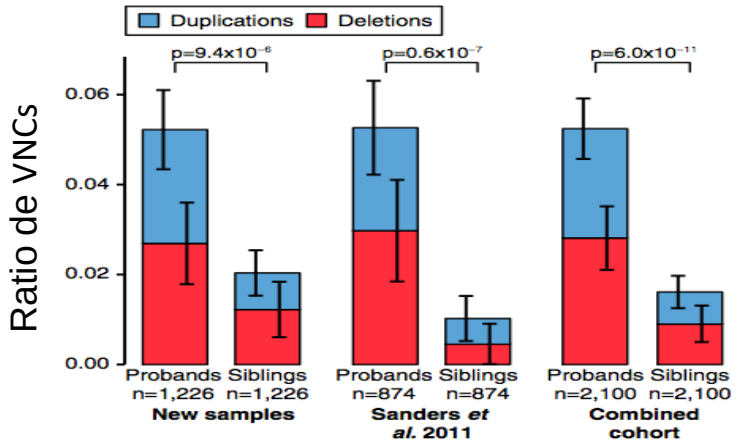
Nat Rev Neurosci. 2015;16(9):551-63.

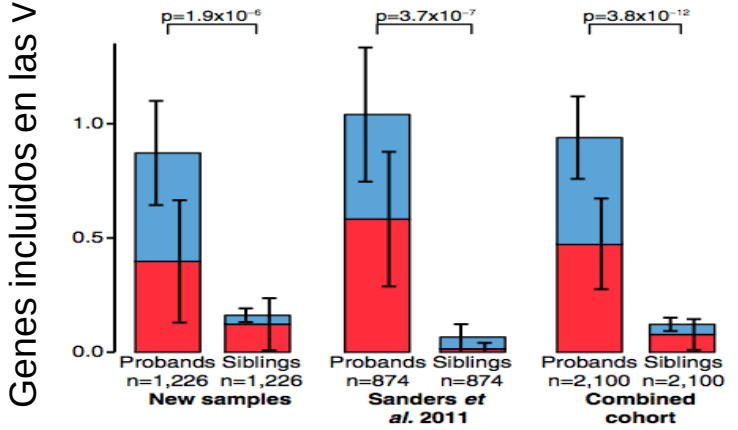

Arquitectura Genética en el TEA
Síndromes genéticos relacionados con el TEA (10%)
Alt. Cromosómicas (5%)
Enf. Mendelianas (3%)
Sdr. de deleción de genes contiguos (2%)
TEA No Sindrómico
Variantes de baja frecuencia
Asociación de Variantes de Alta frecuencia (≈50%) (SNPs)
VNCs (5%)
Desde 1 gen a <1 Mb
CGH
MLPA
Heredadas
De Novo
Asociadas a penetrancia incompleta:
-Presente en progenitor sano ó con afectación leve
-¿Segundo evento genético?
-El tamaño y el contenido génico determina el riesgo de predisposición
Asociadas a penetrancia completa y expresividad variable:
- Ligadas a X
- Autosómicas Recesivas
No está presente en los progenitores
NIMGenetics
New Integrated Medical Genetics

N=10.220 individuos (2591 familias)

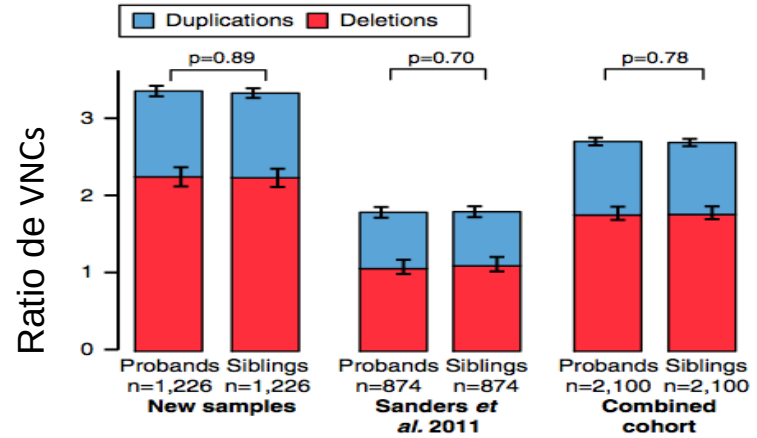
AGP: Autism Genome Project SSC: Simons Simplex Collection

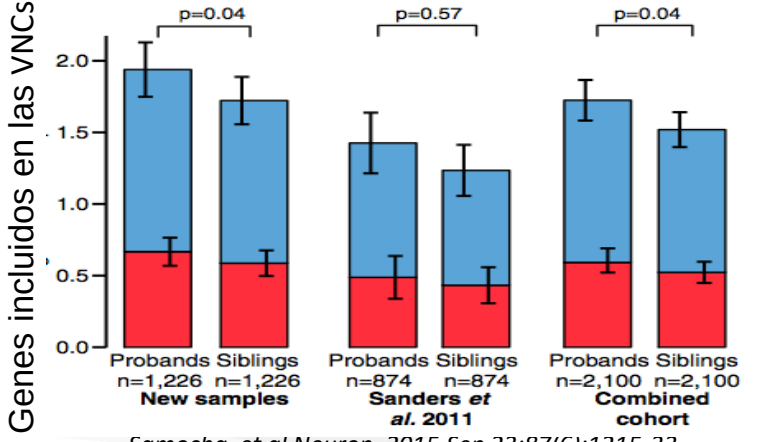
Las VNCs “de novo” se asocian a TEA





Las VNCs de baja frecuencia heredadas muestran una leve asociación con TEA





Samocha, et al Neuron. 2015 Sep 23;87(6):1215-33.

N=10.220 individuos (2591 familias)

AGP: Autism Genome Project
SSC: Simons Simplex Collection

Las VNCs de novo recurrentes permiten identificar 8 regiones genómicas asociadas a predispoición a TEA

Table 2. Regions with Multiple dnCNVs in the SSC and AGP (FDR ≤ 0.1)

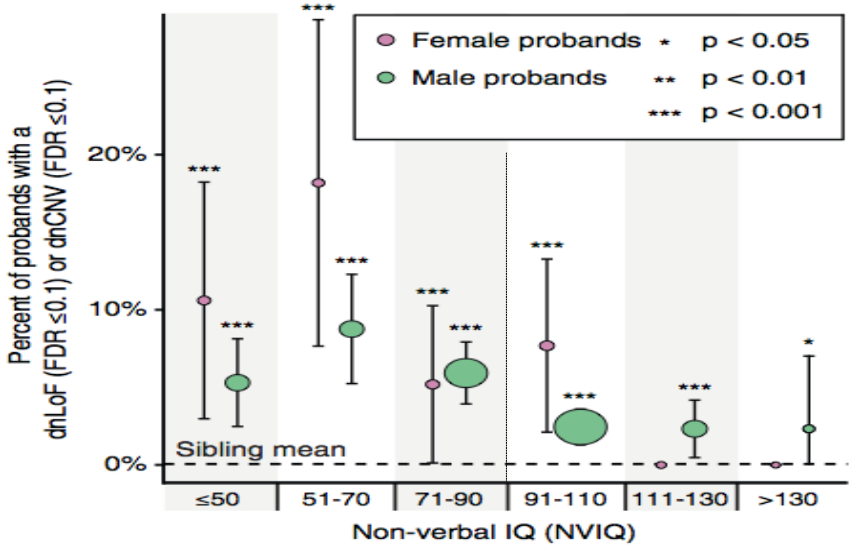
Band	Location (hg19)	dnCNVs (del/dup)	RefSeq Genes	Genes[a]	p Value (Corrected)	q Value (FDR)
1q21.1	chr1:146,467,203-147,801,691	9 (1/8)	13	–	6×10^{-9}	2×10^{-9}
2p16.3	chr2:50,145,643-51,259,674[b]	8 (7/1)	1	*NRXN1*	1×10^{-7}	4×10^{-8}
3q29	chr3:195,747,398-196,191,434	4 (4/0)	7	–	0.07	0.02
7q11.23	chr7:72,773,570-74,144,177	5 (1/4)	22	–	0.005	0.0008
7q11.23	chr7:72,773,570-73,158,061[c]	6 (1/5)	10	–	0.0002	0.00003
7q11.23	chr7:73,978,801-74,144,177[c]	6 (1/5)	2	*GTF2I*, *GTF2IRD1*	0.0002	0.00003
15q11.2-13.1	chr15:23,683,783-28,446,765	10 (0/10)	13	–	$<1 \times 10^{-10}$	$<1 \times 10^{-10}$
15q12	chr15:26,971,834-27,548,820[d]	11 (0/11)	3	*GABRA5*, *GABRB3*, *GABRG3*	$<1 \times 10^{-10}$	$<1 \times 10^{-10}$
15q13.2-13.3	chr15:30,943,512-32,515,849	5 (3/2)	7	–	0.005	0.0008
16p11.2	chr16:29,655,864-30,195,048	19 (12/7)	27	–	$<1 \times 10^{-10}$	$<1 \times 10^{-10}$
22q11.21	chr22:18,889,490-21,463,730	8 (4/4)	45	–	1×10^{-7}	4×10^{-8}
22q13.33	chr22:51,123,505-51,174,548	4 (4/0)	1	*SHANK3*	0.07	0.02

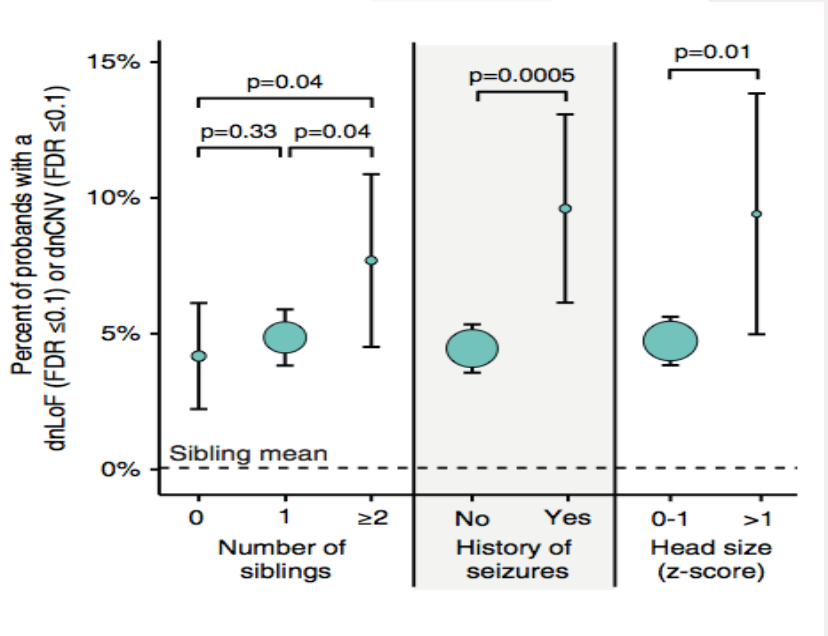
[a]Where ≤3 genes are present they are listed to clarify the genomic location.
[b]Eight dnCNVs overlap at least one exon of this gene.
[c]These are the regions of intersection between two atypical dnCNVs and the Williams-Beuren Syndrome locus (see Figure S5).
[d]This is the region of intersection between an atypical dnCNV and the 15q11.2-13.1 locus (see Figure 6F).

SFARI SIMONS FOUNDATION AUTISM RESEARCH INITIATIVE

NIMGenetics New Integrated Medical Genetics

Fenotipo asociado a las mutaciones de novo





“ La presencia de mutaciones de novo se asocia a un bajo CINV y se observa más frecuent ementes en pacientes con hermanos sanos, historia previa de convulsiones febriles y no fe briles o desviaciones de >1SD del perímetro cefálico”

NIMGenetics
New Integrated Medical Genetics

Fenotipo

Varón de 7 años, autismo no sindrómico.

Cariotipo Normal

X-Frágil Normal

Array 180K Normal

CGH array de diseño específico

NIMGenetics
New Integrated Medical Genetics

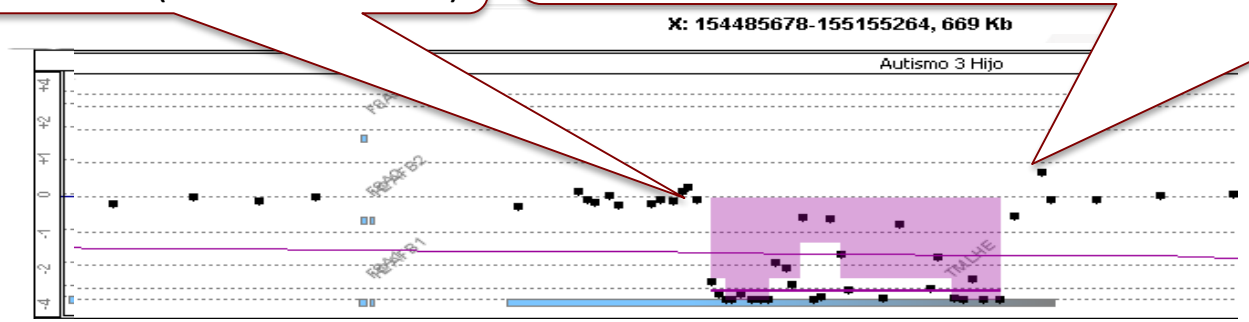
16

Array 180K de diseño específico

Deleción hemicigota Xq28 de 70 Kb
Exon 2 del gen *TMLHE* (OMIM * 300777)

Diagnostico:
Susceptibilidad a autismo ligada al X 6 (#300872)

Probando Array 180K-Autismo



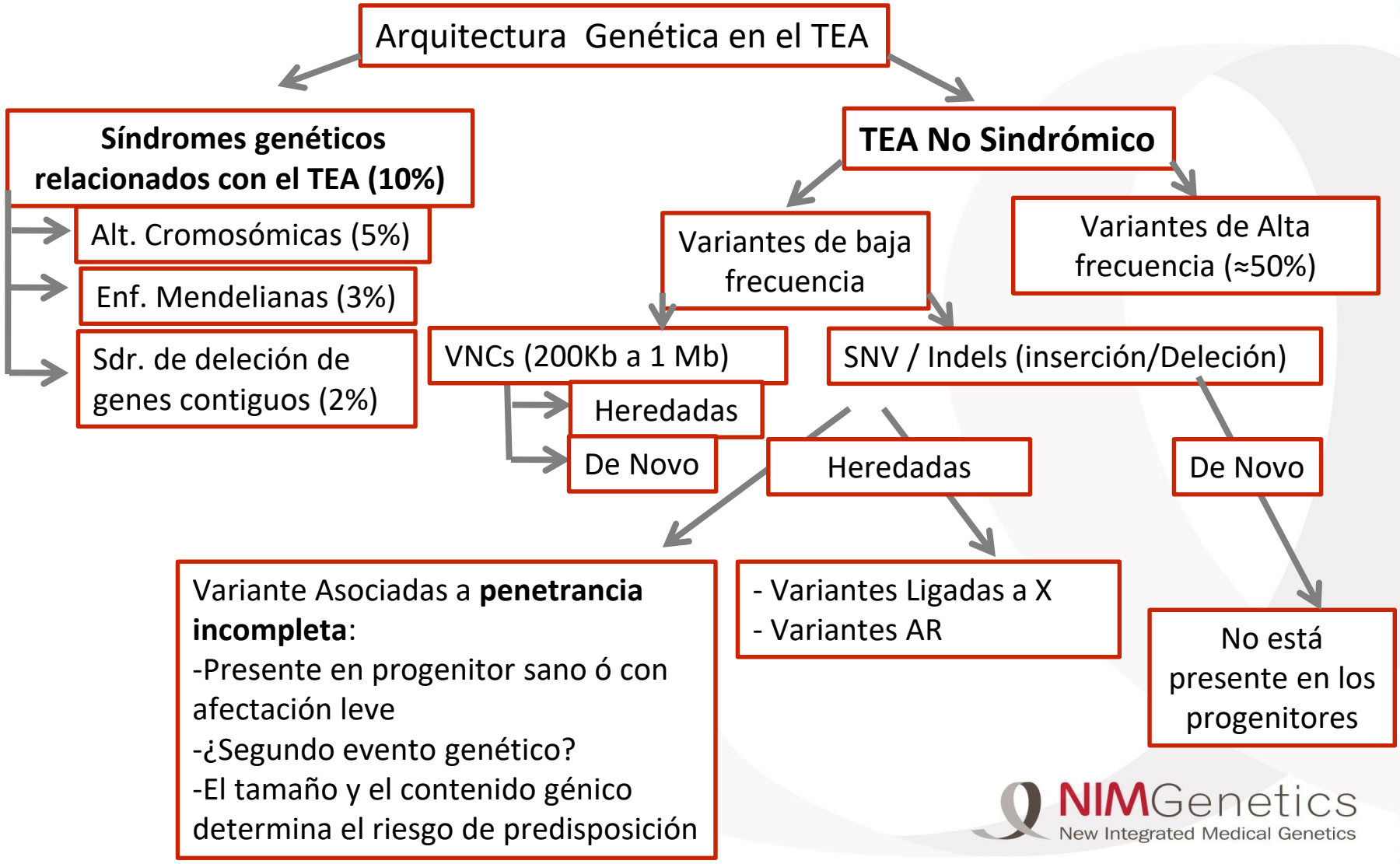
Presentación Clínica:

-Discapacidad intelectual/retraso del desarrollo
-Trastorno espectro autista (incluyendo Asperger)
- Expresividad variable

Human Molecular Genetics, 2011, Vol. 20, No. 22 ***4360–4370***
doi:10.1093/hmg/ddr363
Advance Access published on August 24, 2011

Use of array CGH to detect exonic copy number variants throughout the genome in autism families detects a novel deletion in *TMLHE*

NIMGenetics
New Integrated Medical Genetics


Arquitectura Genética en el TEA
Síndromes genéticos relacionados con el TEA (10%)
Alt. Cromosómicas (5%)
Enf. Mendelianas (3%)
Sdr. de deleción de genes contiguos (2%)
TEA No Sindrómico
Variantes de baja frecuencia
Variantes de Alta frecuencia (≈50%)
VNCs (200Kb a 1 Mb)
SNV / Indels (inserción/Deleción)
Heredadas
De Novo
Heredadas
De Novo
Variante Asociadas a penetrancia incompleta:
-Presente en progenitor sano ó con afectación leve
-¿Segundo evento genético?
-El tamaño y el contenido génico determina el riesgo de predisposición
- Variantes Ligadas a X
- Variantes AR
No está presente en los progenitores
NIMGenetics
New Integrated Medical Genetics

LETTER

doi:10.1038/nature10945

De novo mutations revealed by whole-exome sequencing are strongly associated with autism

Stephan J. Sanders[1], Michael T. Murtha[1], Abha R. Gupta[2]*, John D. Murdoch[1]*, Melanie J. Raubeson[1]*, A. Jeremy Willsey[1]*, . Walker[1], Gordon T. Ober[1], Robert D. Bjornson[5], Lifton[4], Murat Günel[7],

LETTER

doi:10.1038/nature10989

Sporadic autism exomes reveal a highly interconnected protein network of *de novo* mutations

Brian J. O'Roak[1], Laura Vives[1], Santhosh Girirajan[1], En
Joshua D. Smith[1], Emily H. Turner[1], Ian B. Stanaway
Elhanan Borenstein[1,3,4], Mark J. Rieder[1], Deborah A.

nature genetics

Excess of rare, inherited truncating mutations in autism

Niklas Krumm[1,5], Tychele N Turner[1,5], Carl Baker[1], Laura Vives[1], Kiana Mohajeri[1], Kali Witherspoon[1], Archana Raja[1,2], Bradley P Coe[1], Holly A Stessman[1], Zong-Xiao He[3], Suzanne M Leal[3], Raphael Bernier[4] & Evan E Eichler[1,2]

To assess the relative impact of inherited and *de novo* variants on autism risk, we generated a comprehensive set of exonic single-nucleotide variants (SNVs) and copy number variants (CNVs) from 2,377 families with autism. We find that private, inherited truncating SNVs in conserved genes are enriched in probands (odds ratio = 1.14, $P = 0.0002$) in comparison to unaffected siblings, an effect involving significant maternal transmission bias to sons. We also observe a bias for inherited CNVs, specifically for small (<100 kb), maternally inherited events ($P = 0.01$) that are enriched in CHD8 target genes ($P = 7.4 \times 10^{-3}$). Using a logistic regression model, we show that private truncating SNVs and rare, inherited CNVs are statistically independent risk factors for autism, with odds ratios of 1.11 ($P = 0.0002$) and 1.23 ($P = 0.01$), respectively. This analysis identifies a second class of candidate genes (for example, *RIMS1*, *CUL7* and *LZTR1*) where transmitted mutations may create a sensitized background but are unlikely to be completely penetrant.

Las Técnicas de secuenciación masiva aceleran y simplifican el diagnóstico Genético

1.- Permiten analizar simultáneamente **múltiples genes** incluidos en el diagnóstico diferencial a un coste y en un tiempo que se acerca al del estudio de un gen único.

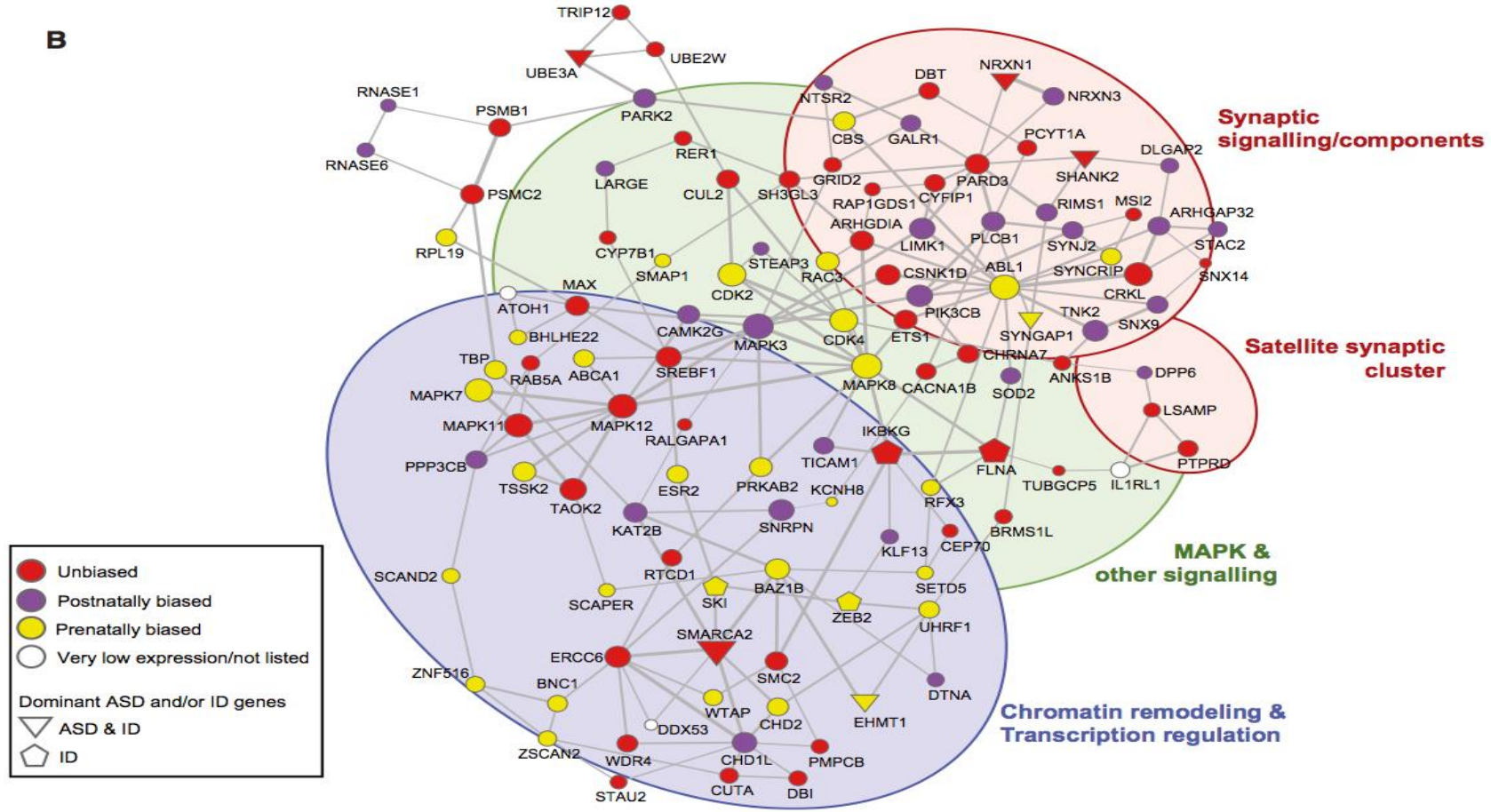
2.- Facilitan **el análisis de genes previamente no analizados** por tecnologías convencionales

3.- Permiten identificar genes no incluidos en el diagnóstico diferencial. La posibilidad de **"echar la red"** es especialmente importante en:

- En enfermedades raras ó con alteraciones en genes de reciente identificación
- En presentaciones clínicas parciales ó atípicas

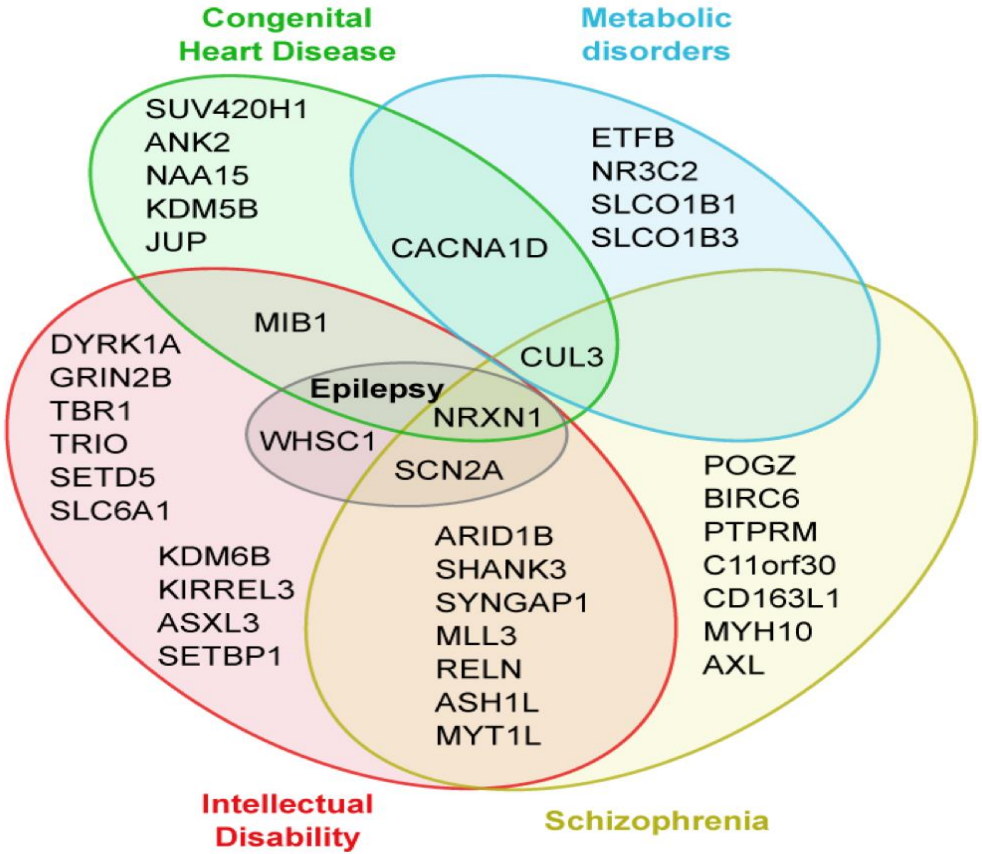
NIMGenetics
New Integrated Medical Genetics

Los genes asociados a TEA están implicados en la sinapsis, la transcripción y la regulación de la cromatina



Pinto D et al Am J Hum Genet. 2014 May 1;94(5):677-94

Los genes asociados a TEA están implicados en diversos trastornos de origen genético



Silvia De Rubeis,et al, Nature. 2014 Nov 13; 515(7526): 209–215

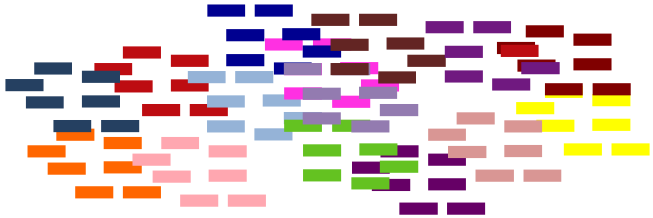
IIMGenetics
New Integrated Medical Genetics

Dos Diferentes Aproximaciones en función del Fenotipo

EXOMA DIRIGIDO

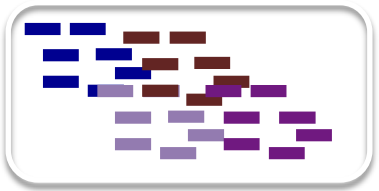
EXOMA TRIO

Secuenciación Exónica



19.000 genes

Selección de Genes



N genes

Análisis de Variantes

Panel Virtual

Genes seleccionados

Indicación:

- Fenotipo bien definido
- Enfermedad No progresiva

Exoma Clínico

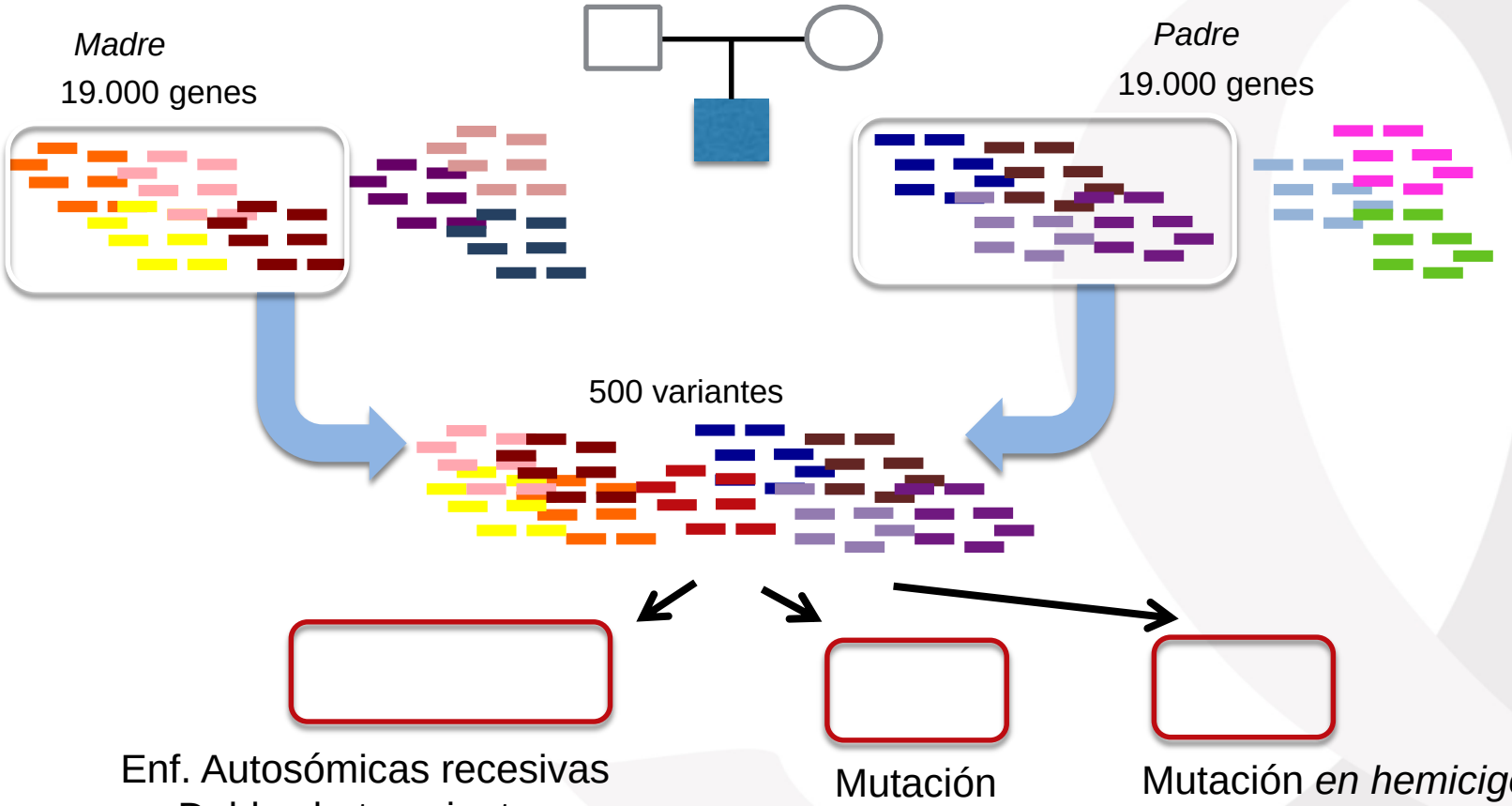
>5700 Genes OMIM

Indicación:

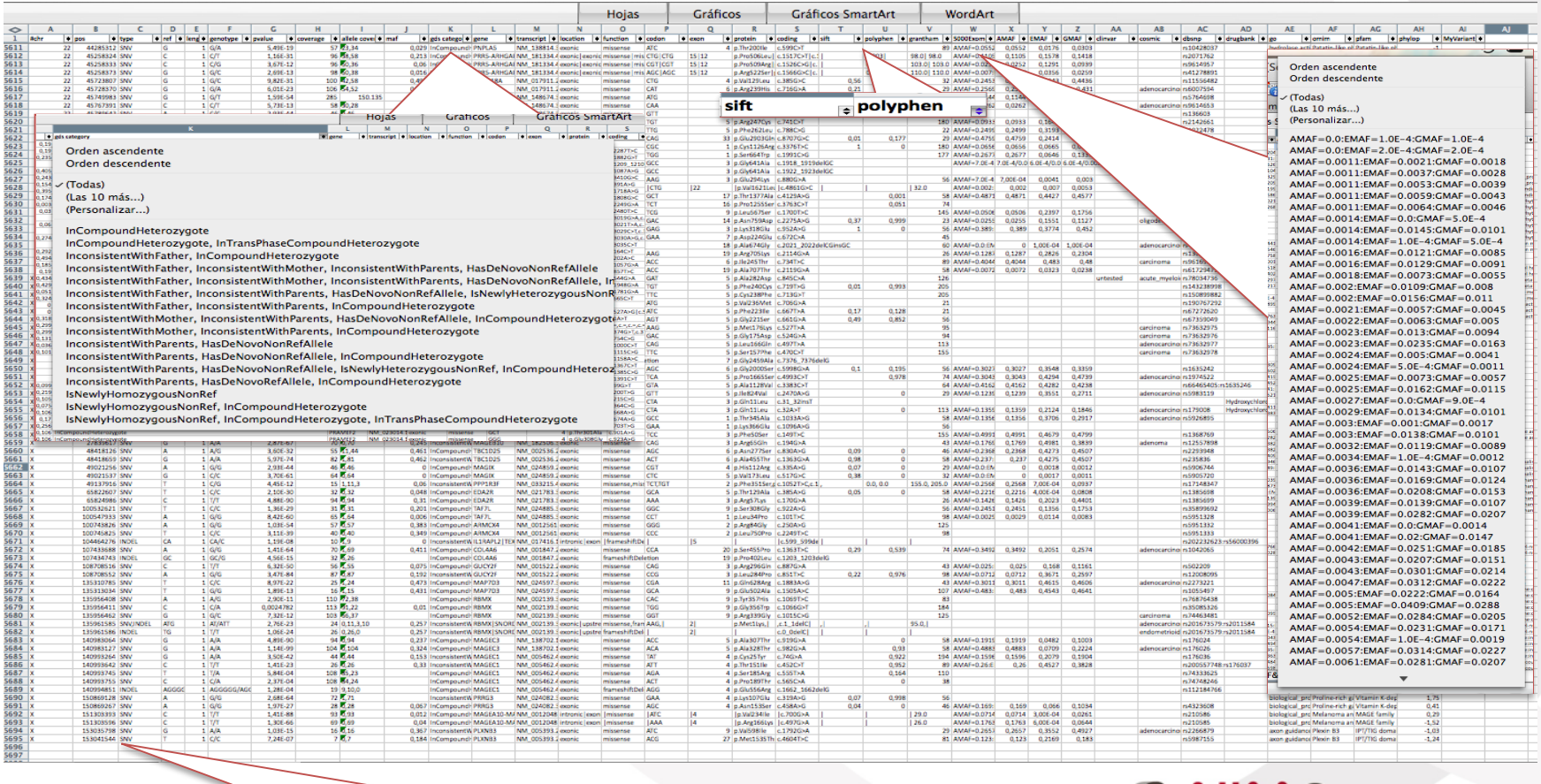
- Fenotipo > complejo y severo
- Enfermedad progresiva

NIMGenetics
New Integrated Medical Genetics

Análisis en Trios

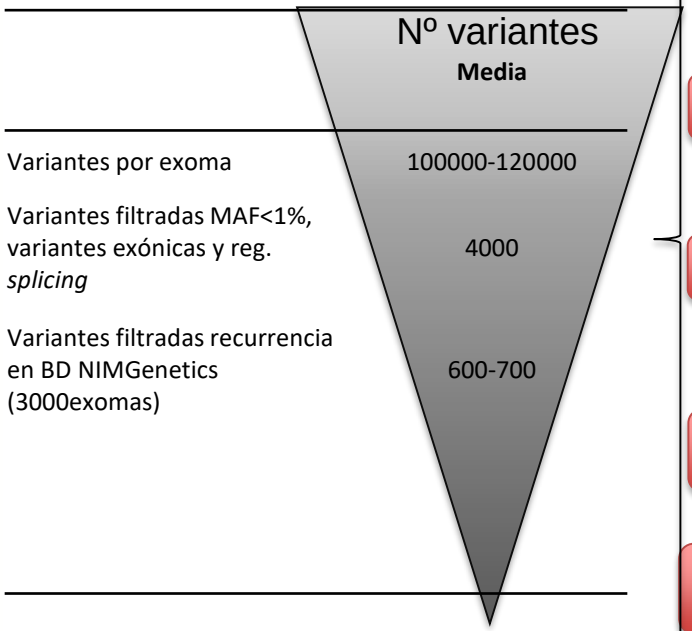


NIMGenetics
New Integrated Medical Genetics



Median of 41037 variants per exoma

Priorización de las variantes



CALIDAD
- **Métricas de calidad:** *p-value, Phred score*, cobertura
- **Base de datos local:** artefactos
- **Visualización IGV:** artefactos de la secuenciación

FUNCIONAL
- **Efecto :** *missense, frameshift, nonsense, stoploss*
- **Localización:** exónica, *splice site*
- **Predicción de patogenicidad**

HERENCIA
- **Patrón de herencia de patología:** AR, XLR, AD, XLD, digénica
- **Segregación familiar de la variante**

ASOCIACIÓN FENOTIPO
- **Base de datos específicas:** OMIM, HGMD-Pro, ClinVar, SFARI
- **Correlación genotipo-fenotipo**

FRECUENCIA POBLACIONA
- **BD frequencias poblaciones:** dbSNP, EVS, ExAC, gnomAD, CIBERER *Spanish Variant Server, Wallderly*

Categorización de las variantes:

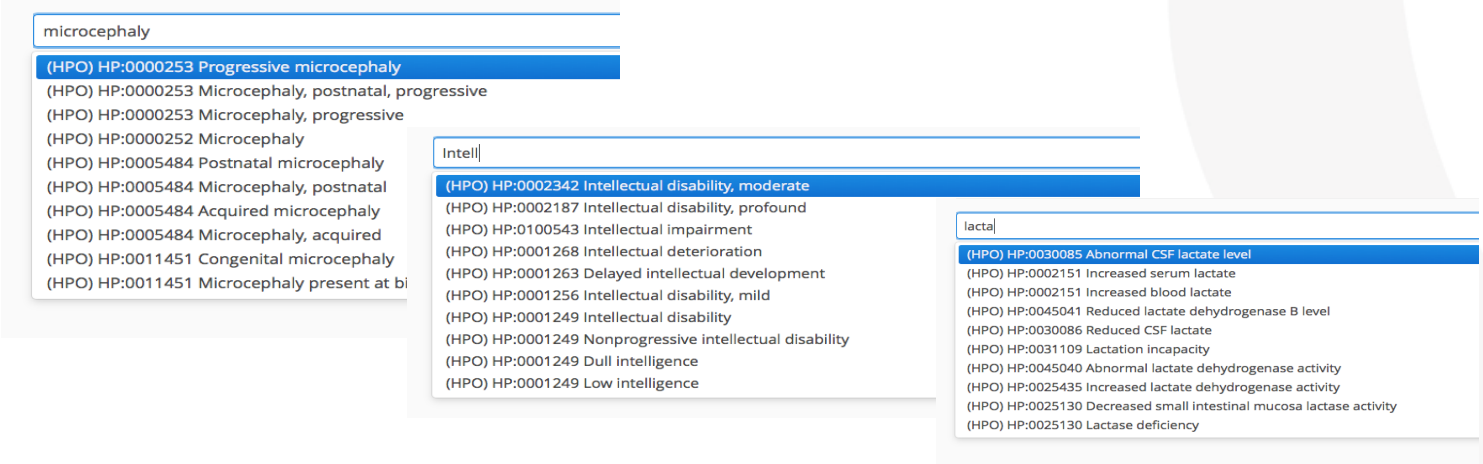
➢ **Recomendaciones del Colegio Americano ACM**

(Richards S et al, Genet Med. 2015 May;17(5):405-24)

- ✓Mutaciones Patogénicas
- ✓Mutaciones de Significado Incierto (VUS)
- ✓Mutaciones probablemente Benignas
- ✓Mutaciones Benignas

The Human Phenotype Ontology

- ✓ Cada término HPO describe una alteración fenotípica
- ✓ Desarrollado a partir de la literatura Médica, Orphanet, DECIPHER, y OMIM
- ✓ Actualmente, 11.000 términos
- ✓ >115,000 anotaciones en enfermedades hereditarias y 4000 en enfermedades comunes



El conjunto de los términos HPO determinará el perfil de la enfermedad

NIMGenetics
New Integrated Medical Genetics

La utilización de términos comunes (HPOs), lógicamente relacionados permite la integración en las bases de datos

phenomizer

The Monarch Initiative

SimulConsult®
A Simultaneous Consult On Your Patient's Diagnosis

PHENOTIPS®

Identificación de Síndromes y Genes Candidatos

OMIM®

DECIPHER
GRCh37

orphanet

NIMGenetics
New Integrated Medical Genetics

Las pequeñas VNCs de novo (<200Kb) y las mutaciones de pérdida de función de novo se concentran en los mismos genes.

Los genes incluidos en Las grandes VNCs (>200Kb <1MB) y los identidicados con mutaciones de novo son distintos

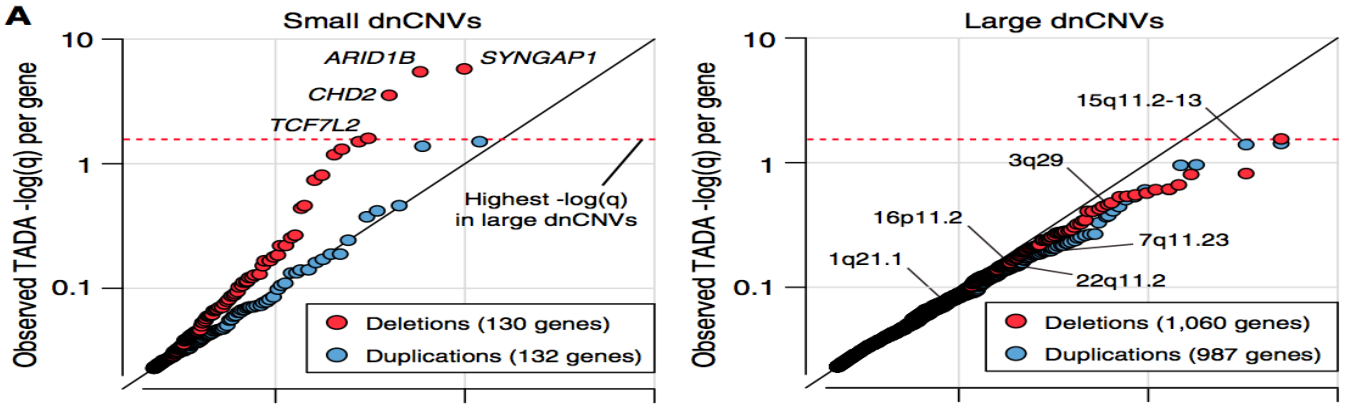


Table 4. Integrating Small De Novo Deletions in TADA Identified 65 ASD Genes

dnLoF Count	FDR ≤ 0.01	0.01 < FDR ≤ 0.05	0.05 < FDR ≤ 0.1
≥2	*ADNP*, *ANK2*, ***ARID1B***, *ASH1L*, ***CHD2***, *CHD8*, *CUL3*, *DSCAM*, *DYRK1A*, *GRIN2B*, *KATNAL2*, *KDM5B*, ***KMT2C***, *NCKAP1*, *POGZ*, *SCN2A*, *SUV420H1*, ***SYNGAP1***, *TBR1*, ***TCF7L2***, ***TNRC6B***, *WAC*	*BCL11A*, *FOXP1*, *GIGYF1*, *ILF2*, *KDM6B*, *PHF2*, *RANBP17*, *SPAST*, *WDFY3*	*DIP2A*, *KMT2E*
1	***NRXN1***, *PTEN*, ***SETD5***, ***SHANK2***, ***SHANK3***, ***TRIP12***	*DNMT3A*, *GABRB3*, ***KAT2B***, *MFRP*, *MYT1L*, *P2RX5*	*AKAP9*, *APH1A*, *CTTNBP2*, *ERBB2IP*, *ETFB*, *INTS6*, *IRF2BPL*, ***MBD5***, *NAA15*, *NINL*, *OR52M1*, *PTK7*, *TRIO*, *USP45*
0	–	*MIB1*, *SLC6A1*, *ZNF559*	*ACHE*, *CAPN12*, ***NLGN3***

Genes with a small de novo deletion are in bold. FDR, false discovery rate.

El 10% de los TEA presenta alteraciones genéticas “de novo” en regiones ó genes previamente implicados en trastornos del neurodesarrollo

Category of de novo mutation	Percent of cohort with a mutation				Percent of cases with a mutation contributing to ASD risk (95% CI)		
	Probands			Siblings	Probands		
	All	Male	Female	All	All	Male	Female
Deletions	3.1%	2.7%	6.0%	1.0%	2.2% (1.1%–3.2%)	1.8% (0.8%–2.5%)	5.0% (2.3%–8.4%)
Duplications	2.7%	2.7%	2.8%	0.8%	1.9% (1.2%–2.6%)	1.9% (1.0%–3.0%)	2.0% (0.2%–4.3%)
All CNVs	5.8%	5.3%	8.7%	1.7%	4.1% (2.6-5.7%)	3.6% (2.3%–4.9%)	7.0% (3.2%–11.4%)
Nonsense	5.9%	6.0%	5.0%	2.8%	3.1% (1.4-4.4%)	3.2% (1.8%–4.9%)	2.2% (0.0%–6.2%)
Splice Site	2.4%	1.9%	6.0%	1.1%	1.3% (0.5-2.3%)	0.7% (0.0%–1.5%)	4.8% (1.5%–8.8%)
Frameshift	7.8%	7.7%	8.7%	4.8%	3.0% (1.2-4.8%)	2.9% (1.2%–4.4%)	3.9% (0.2%–7.5%)
All LoF	15.4%	14.9%	18.8%	8.5%	6.9% (4.9-8.9%)	6.4% (3.9%–8.8%)	10.3% (6.3%–16.2%)
All LoFs and CNVs	20.6%	19.7%	26.6%	10.1%	10.5% (7.8-13.1%)	9.6% (6.8%–12.0%)	16.6% (11.4%–22.6%)

En mujeres con TEA se observa una mayor acumulacion de variates de novo ¿Efecto protector?

NIMGenetics
New Integrated Medical Genetics

Samocha, et al Neuron. 2015 Sep 23;87(6):1215-33.
Iossifov, et al Nature (2014) 515, 216–221.

Original Investigation

Molecular Diagnostic Yield of Chromosomal Microarray Analysis and Whole-Exome Sequencing in Children With Autism Spectrum Disorder

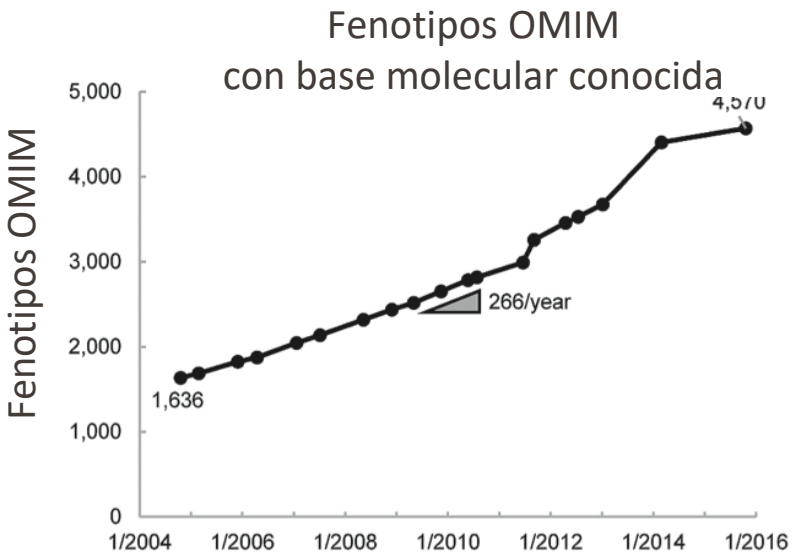
Positive Results	Essential Group	Equivocal Group	Complex Group	*P* Value for 3-Group Comparison
CMA, No./total No.	7/168	4/37	13/53	<.001
% (95% CI)	4.2 (1.7-8.4)	10.8 (3.0-25.4)	24.5 (13.8-38.3)	
WES, No./total No.	2/64	2/7	4/24	.02
% (95% CI)	3.1 (0.0-10.8)	28.6 (3.7-71.0)	16.7 (4.7-37.4)	
CMA and/or WES, No./total No.	4/64	2/7	9/24	.001
% (95% CI)	6.3 (1.7-15.2)	28.6 (3.7-71.0)	37.5 (18.8-59.4)	

Alteraciones Dismorfológicas ó microcefalia

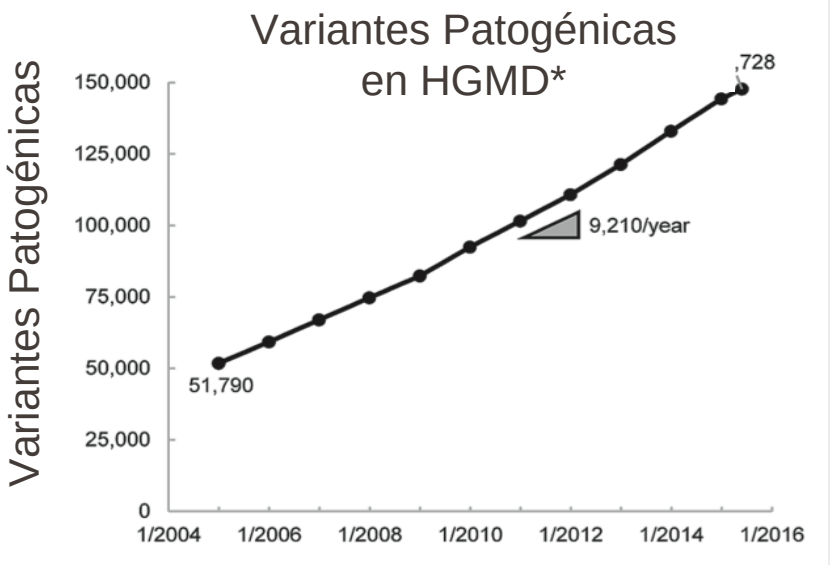
Tammimies K et al. JAMA. 2015 Sep 1;314(9):895-903.

NIMGenetics
New Integrated Medical Genetics

Incremento progresivo de la información genética



> Genes Candidatos confirmados



Mejor interpretación de las variantes

Mayor automatización y Desarrollo de bases de datos actualizadas son las bases para asegurar una máxima rentabilidad de los estudios de NGS

*HGMD: Database of human gene mutation data

Wenger AM, et al Genet Med. 2016 Jul 21.

NIMGenetics
New Integrated Medical Genetics

ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Systematic reanalysis of clinical exome data yields additional diagnoses: implications for providers

Aaron M. Wenger, PhD[1], Harendra Guturu, PhD[1], Jonathan A. Bernstein, MD, PhD[1] and Gill Bejerano, PhD[1,2,3]

N= 40 casos analizados por exoma SIN diagnóstico Genético
Nº de casos diagnosticados tras el reanálisis: 4 (10%)

Case	Causative mutation	Diagnosis from exome data reanalysis	Date of clinical exome report	Publication date of article that ties causative gene to disease
1	*KMT2A* c.3464G>A p.C1155Y	Wiedemann-Steiner Syndrome (OMIM 605130)	July 2012	August 2012
2	*DEAF1* c.737G>C p.R246T	Autosomal-dominant mental retardation 24 (OMIM 615828)	November 2013	May 2014; case reports in December 2010 and November 2012
3	*IFIH1* c.2159G>A p.R720Q	Aicardi-Goutieres syndrome 7 (OMIM 615846)	September 2014	May 2014
4	*PIK3R1* c.1135C>T p.R379W	SHORT syndrome (OMIM 269880)	October 2012	July 2013

Four participants with nondiagnostic clinical exome sequencing received a diagnosis from exome data reanalysis. All four participants were diagnosed with autosomal-dominant disorders due to missense variants. Analysis of parents confirmed that all mutations are de novo in the probands.

Wenger AM, et al Genet Med. 2016 Jul 21.

NIMGenetics
New Integrated Medical Genetics

Fenotipo:
Niña de 3 años y 4 meses de edad que sufre un cuadro de retraso cognitivo y comportamiento autista con esterotipias. fenotipo que recuerda en algunos aspectos al del Síndrome de Williams, aunque no se enmarcarían en ese cuadro respecto al fenotipo conductual.

AF: una hermana gemela que presenta el mismo fenotipo

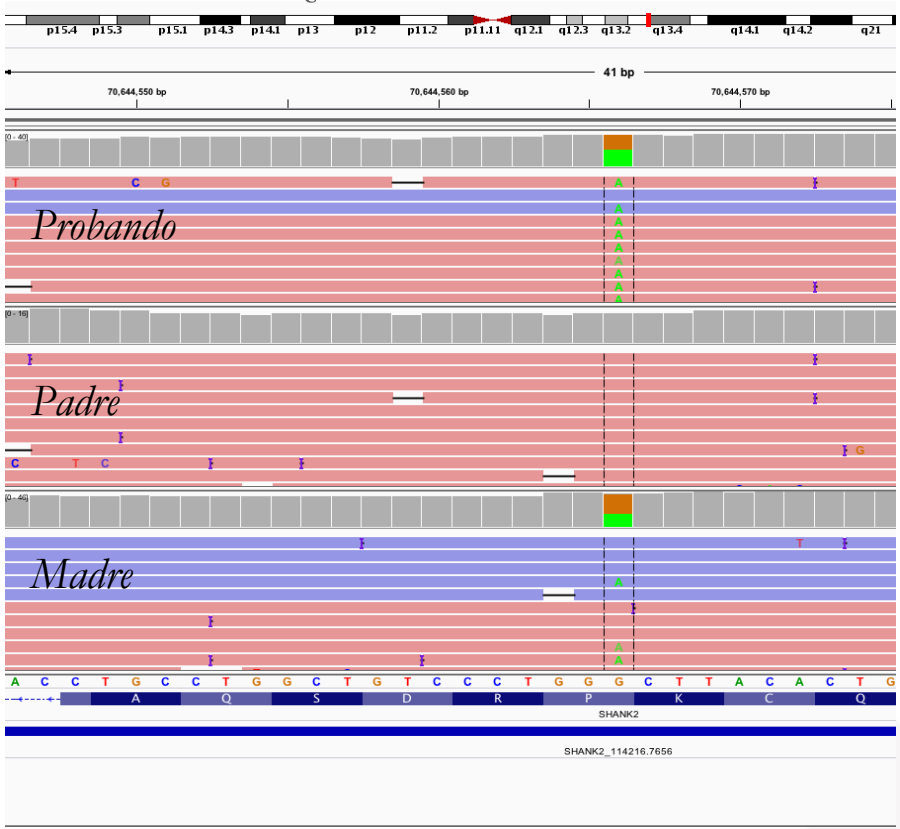
Cariotipo Normal
FISH de la región causal de los Síndromes de Angelman / Prader-Willi NEGATIVO
Estudio mediante MLPA de deleciones subteloméricas NEGATIVO
Síndrome de Rett: mutaciones y deleciones/duplicaciones en el gen MECP2 NEGATIVO
Array 180K Autismo NORMAL

Exoma Trio

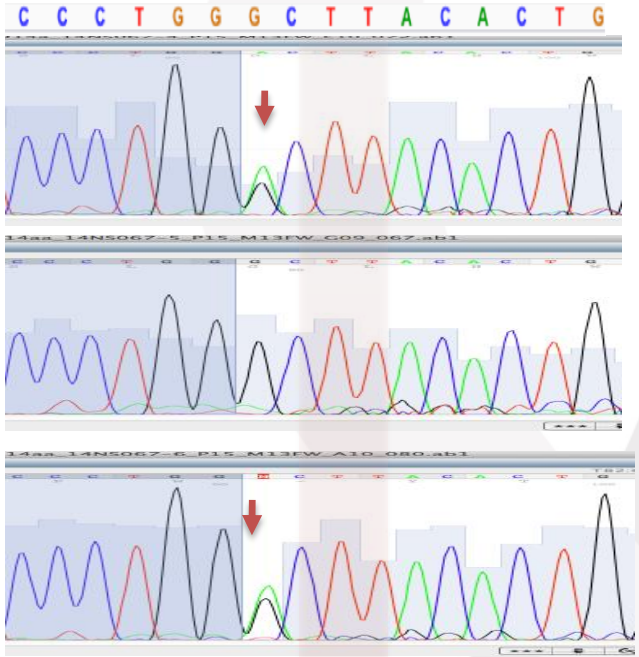
14NS0067 (ASD)

SHANK2 c.622C>T (p.Pro208Ser)

Visualización en IGV



Validación Sanger



NIMGenetics
New Integrated Medical Genetics

SHANK2 c.622C>T (p.Pro208Ser)

Variant Predictors

SIFT	PolyPhen2	LRT	MutationTaster	Mutation Assessor	FATHMM
D	D	N	N	H	D

OPEN ACCESS Freely available online PLoS GENETICS

Genetic and Functional Analyses of *SHANK2* Mutations Suggest a Multiple Hit Model of Autism Spectrum Disorders

PLoS Genetics (2012); 8(2): e1002521

nature genetics

Mutations in the *SHANK2* synaptic scaffolding gene in autism spectrum disorder and mental retardation

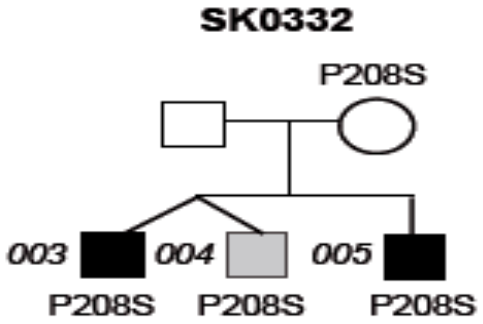
Simone Berkel[1], Christian R Marshall[2], Birgit Weiss[1], Jennifer Howe[2], Ralph Roeth[1], Ute Moog[3], Volker Endris[1], Wendy Roberts[4], Peter Szatmari[5], Dalila Pinto[2], Michael Bonin[6], Angelika Riess[6], Hartmut Engels[7], Rolf Sprengel[8], Stephen W Scherer[2,9] & Gudrun A Rappold[1]

Nat Genet. 2010 Jun;42(6):489-91.

Inherited and *de novo* SHANK2 variants associated with autism spectrum disorder impair neuronal morphogenesis and physiology

Simone Berkel[1], Wannan Tang[3], Mario Treviño[3], Miriam Vogt[4], Horst Andreas Obenhaus[3], Peter Gass[4], Stephen Wayne Scherer[5,6], Rolf Sprengel[3], Gerhard Schratt[2,7] and Gudrun Anna Rappold[1,2,*]

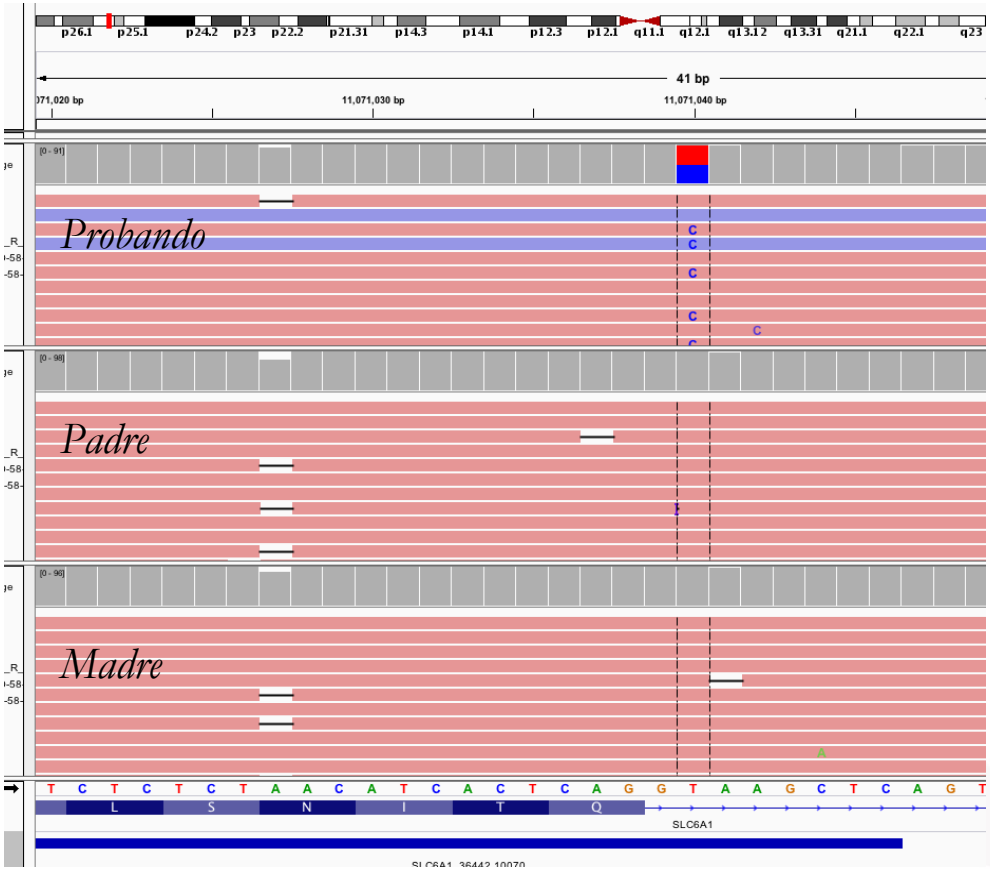
Human Molecular Genetics, 2012, Vol. 21, No. 2 344–357



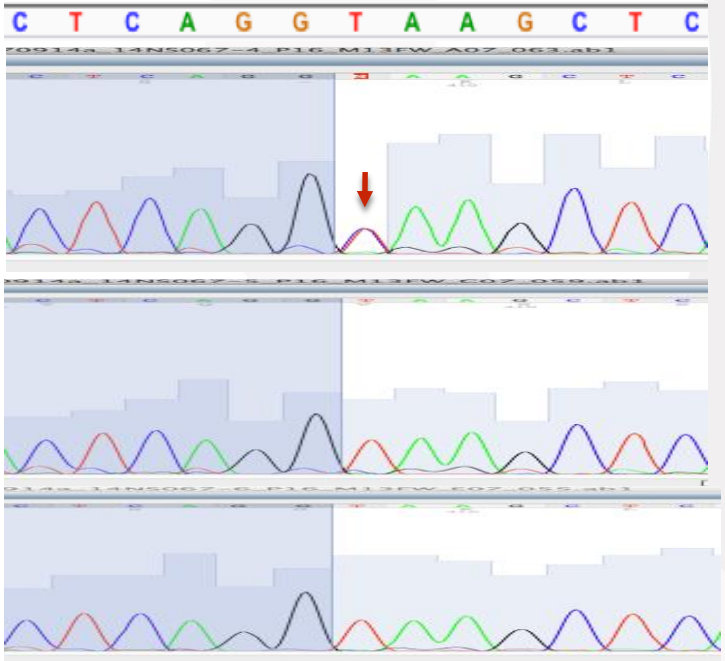
14NS0067 (ASD)

*SLC6A1 (*c.1323+2T>C *) "de novo" mutation*

Visualización en IGV



Validación Sanger



NIMGenetics
New Integrated Medical Genetics

SLC6A1 (c.1323+2T>C) *"de novo" mutation*

Predictores de Splicing

Donor Sites

	SSF [0-100]	MaxEnt [0-12]	NNSPLICE [0-1]	GeneSplicer [0-15]	HSF [0-100]
Threshold	*≥ 70*	*≥ 0*	*≥ 0.4*	*≥ 0*	*≥ 60*
Exon 12 - c.1278					= 66.62
Exon 12 - c.1303					= 67.43
Exon 12 - c.1323 N	**94.22 ⇒ 91.79** *(-2.6%)*	**9.88 ⇒ —**	**1.00 ⇒ —**	**13.12 ⇒ —**	**97.82 ⇒ —**
Intron 12 - c.1323+9		= 5.59	= 0.79	**8.32 ⇒ 9.17** *(+10.2%)*	= 80.52

Natural Splice Site

LETTER

doi:10.1038/nature10945

***De novo* mutations revealed by whole-exome sequencing are strongly associated with autism**

Stephan J. Sanders[1], Michael T. Murtha[1], Abha R. Gupta[2]*, John D. Murdoch[1]*, Melanie J. Raubeson[1]*, A. Jeremy Willsey[1]*, A. Gulhan Ercan-Sencicek[1]*, Nicholas M. DiLullo[1]*, Neelroop N. Parikshak[3], Jason L. Stein[3], Michael F. Walker[1], Gordon T. Ober[1], Nicole A. Teran[1], Youeun Song[1], Paul El-Fishawy[1], Ryan C. Murtha[1], Murim Choi[4], John D. Overton[4], Robert D. Bjornson[5], Nicholas J. Carriero[5], Kyle A. Meyer[6], Kaya Bilguvar[7], Shrikant M. Mane[8], Nenad Sestan[6], Richard P. Lifton[4], Murat Günel[7], Kathryn Roeder[9], Daniel H. Geschwind[3], Bernie Devlin[10] & Matthew W. State[1]

Nature. 2012 Apr 4;485(7397):237-41

Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study

Anita Rauch, Dagmar Wieczorek*, Elisabeth Graf*, Thomas Wieland*, Sabine Endele, Thomas Schwarzmayr, Beate Albrecht, Deborah Bartholdi, Jasmin Beygo, Nataliya Di Donato, Andreas Dufke, Kirsten Cremer, Maja Hempel, Denise Horn, Juliane Hoyer, Pascal Joset, Albrecht Röpke, Ute Moog, Angelika Riess, Christian T Thiel, Andreas Tzschach, Antje Wiesener, Eva Wohlleber, Christiane Zweier, Arif B Ekici, Alexander M Zink, Andreas Rump, Christa Meisinger, Harald Grallert, Heinrich Sticht, Annette Schenck, Hartmut Engels, Gudrun Rappold, Evelin Schröck, Peter Wieacker, Olaf Riess, Thomas Meitinger, André Reis†, Tim M Strom†*

Lancet. 2012 Nov 10;380(9854):1674-82.

3p25.3 Microdeletion of GABA Transporters *SLC6A1* and *SLC6A11* Results in Intellectual Disability, Epilepsy and Stereotypic Behavior

Nicola Dikow,[1]* Bianca Maas,[1] Stephanie Karch,[2] Martin Granzow,[1] Johannes W.G. Janssen,[1] Anna Jauch,[1] Katrin Hinderhofer,[1] Christian Sutter,[1] Susanne Schubert-Bast,[2] Britt Marie Anderlid,[3] Bruno Dallapiccola,[4] Nathalie Van der Aa,[5] and Ute Moog[1]

[1]Institute of Human Genetics, Heidelberg University, Heidelberg, Germany
[2]Center for Child and Adolescent Medicine Pediatric Neurology, Heidelberg University Hospital, Heidelberg, Germany
[3]Institution of Molecular Medicine and Surgery, CMM, Karolinska Institutet and Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden
[4]Ospedale Pediatrico Bambino Gesu—IRCCS Roma, Italy
[5]Department of Medical Genetics, Antwerp University Hospital and University of Antwerp, Antwerp, Belgium

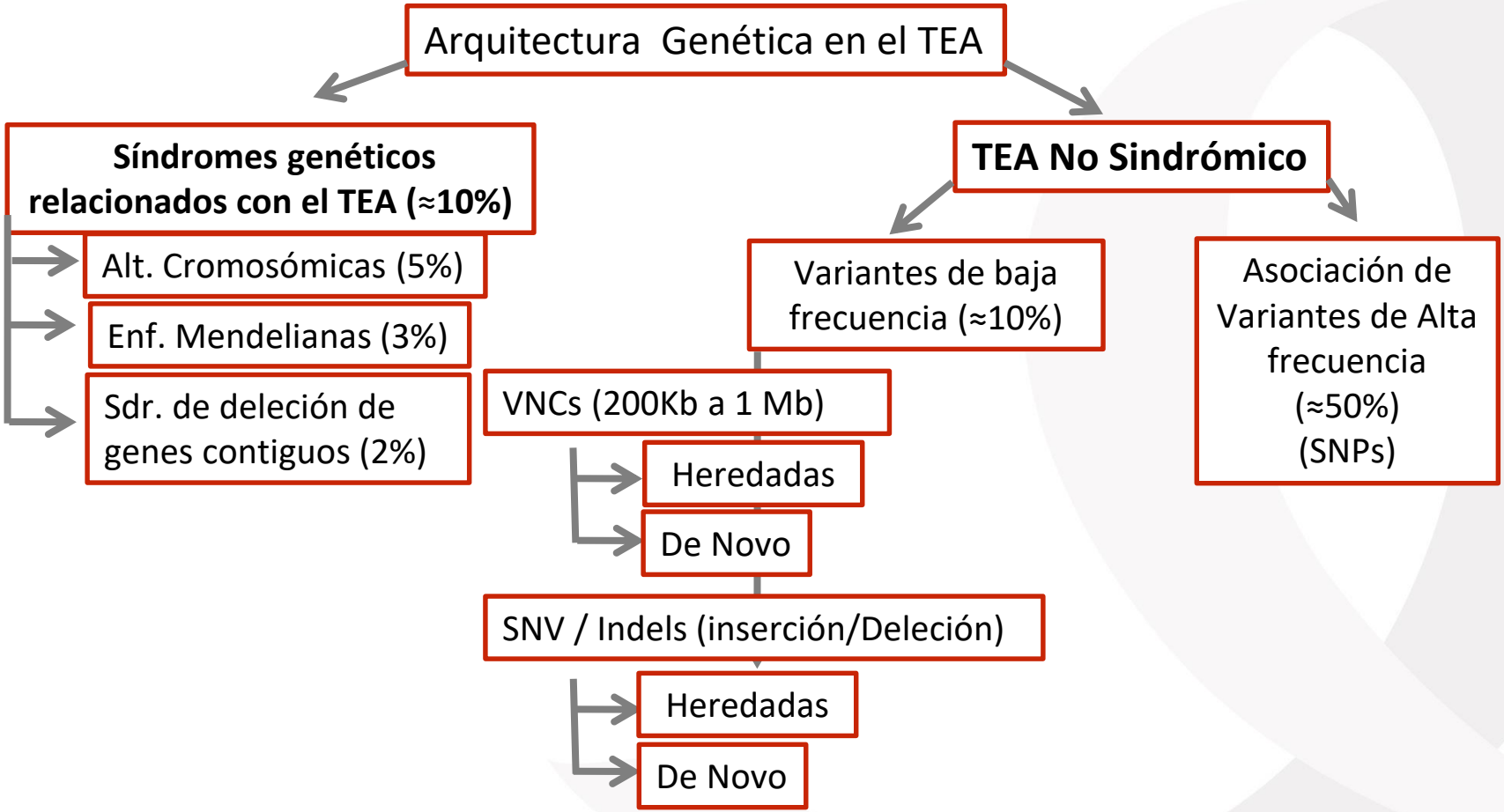
Manuscript Received: 2 May 2014; Manuscript Accepted: 10 August 2014

Am J Med Genet A. 2014 Dec;164A(12):3061-8.

Mutations in the GABA Transporter *SLC6A1* Cause Epilepsy with Myoclonic-Atonic Seizures

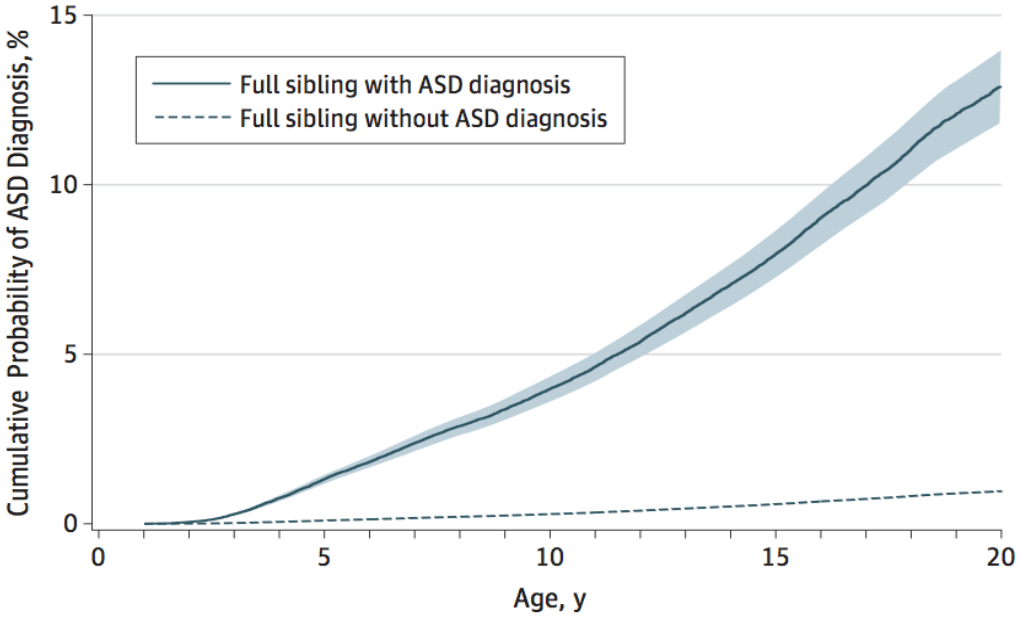
Gemma L. Carvill,[1] Jacinta M. McMahon,[2] Amy Schneider,[2] Matthew Zemel,[1] Candace T. Myers,[1] Julia Saykally,[1] John Nguyen,[1] Angela Robbiano,[3] Federico Zara,[3] Nicola Specchio,[4] Oriano Mecarelli,[5] Robert L. Smith,[6] Richard J. Leventer,[7,8,9] Rikke S. Møller,[10,11] Marina Nikanorova,[10] Petia Dimova,[12] Albena Jordanova,[13,14,15] Steven Petrou,[16] EuroEPINOMICS Rare Epilepsy Syndrome Myoclonic-Astatic Epilepsy & Dravet working group, Ingo Helbig,[17,18] Pasquale Striano,[19] Sarah Weckhuysen,[13,14,20] Samuel F. Berkovic,[2] Ingrid E. Scheffer,[2,7,16,21,*] and Heather C. Mefford[1,21,*]

The American Journal of Human Genetics 96, 808–815, May 7, 2015



NIMGenetics
New Integrated Medical Genetics

Figure 1. Age-Cumulative Probabilities for ASD Diagnosis in Siblings With a Full Sibling With ASD and in Siblings With a Full Sibling Without an ASD Diagnosis



RRR Hermano de padre y madre = 10.3

RRR en Hermano de madre = 3.3

RRR en Hermano de padre = 2.9

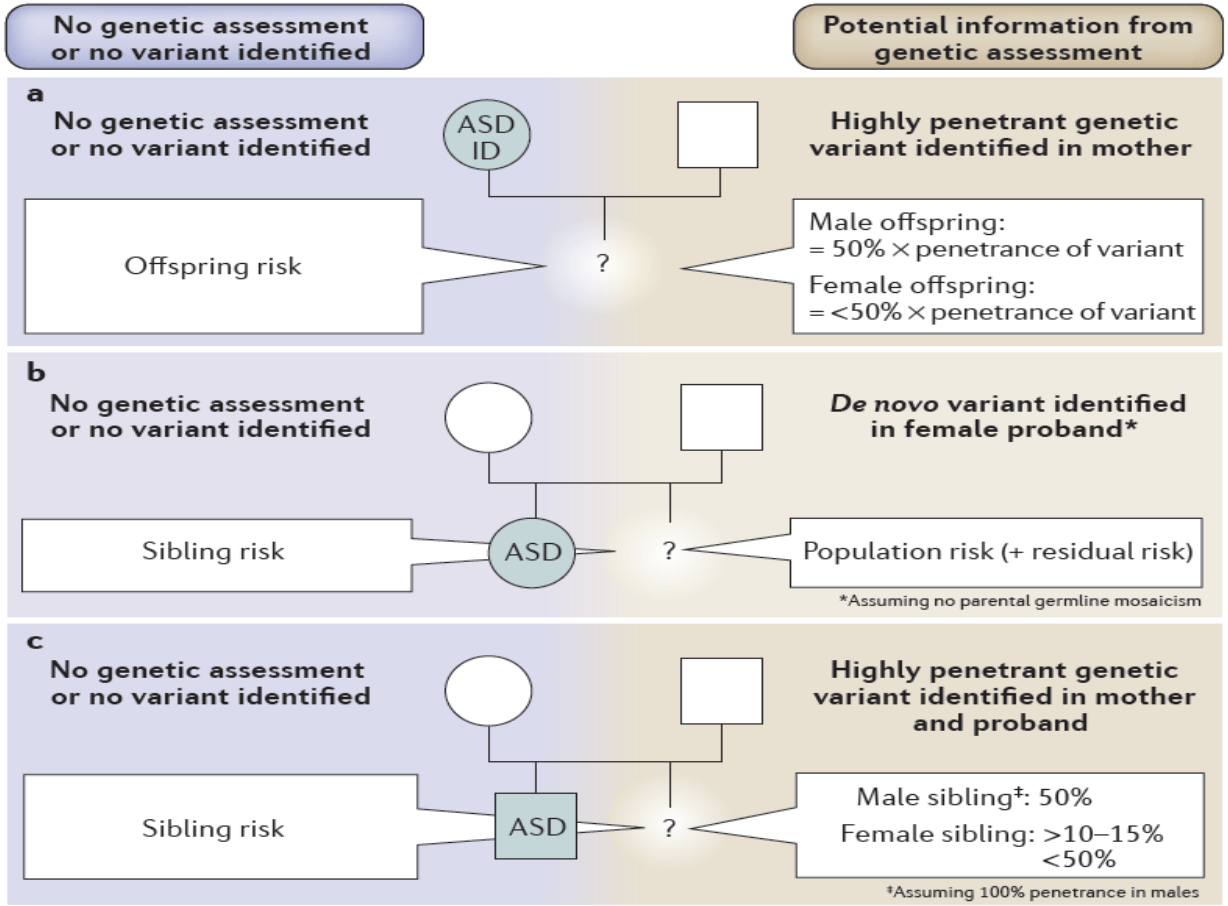
RRR en primos = 2

Riesgo de la población general =1%

NIMGenetics
New Integrated Medical Genetics

Sandin, SA. Et al JAMA. 2014 May 7;311(17):1770-7

Recurrencia estimada en la descendencia en diferentes escenarios en ausencia de una información genética específica



Madre Afecta con TEA y DI

- Si Penetrancia 100% riesgo de recurrencia será del 50%.
- Si penetrancia del 8% el riesgo de recurrencia será del 4%

Padres sanos con HIJA Afecta con TEA

Padres sanos con HIJO Afecta con TEA

NIMGenetics
New Integrated Medical Genetics

Estrategias para trasladar el conocimiento a la práctica clínica

State-of-the-art research knowledge of ASD genetics	Clinical need	Requirements to bridge the gap	Helpful strategies
Numerous rare *de novo* and inherited genetic variants can increase ASD risk in an individual	The ability to inform the affected individual and family about the contribution of the identified genetic variant	• Sufficient confidence in determining causality between the variant and ASD risk	• Reliably and comprehensively collect genotype–phenotype data into accessible databases on a global scale • Strive for uniform implementation of genetic-testing guidelines • Educate health care professionals about clinical genetic reasoning
Genetic variants display variable penetrance	The ability to inform the affected individual and family about recurrence rate	• Identification of the factors (genetic and environmental) driving the variable penetrance	• Evaluate phenotypes as continuous traits in the familial context
Genetic variants are often associated with other phenotypes within or outside the CNS (pleiotropy)	The ability to inform the affected individual and family about other associated phenotypes and to screen or treat if appropriate	• Identification of all other phenotypes associated with the genetic variant • Identification of the factors (genetic and environmental) driving the pleiotropy	• Stimulate broad phenotyping (including assessment of non-CNS-related phenotypes) in genetic studies • View ASDs as medical disorders • Abandon the dichotomy of syndromic versus non-syndromic classification
Genetic risk variants converge on shared biological mechanisms	Effective treatment strategies	• Personalized medicine	• Use genetic information to select individuals for specific treatment trials • Use biological insights to develop new molecular compounds
Different opinions about genetic testing exist in the autism community	A balanced and respectful view of possible ethical concerns related to genetic testing	• Improve insight into the perspectives of the autism community	• Encourage studies investigating different perspectives, using quantitative and qualitative methods • Increase participation of the autism community in the research agenda

ASD, autism spectrum disorder; CNS, central nervous system.

Gracias

salvarez@nimgenetics.com

NIMGenetics
New Integrated Medical Genetics