Genetic Findings in Autism:

Toward a Biological Understanding

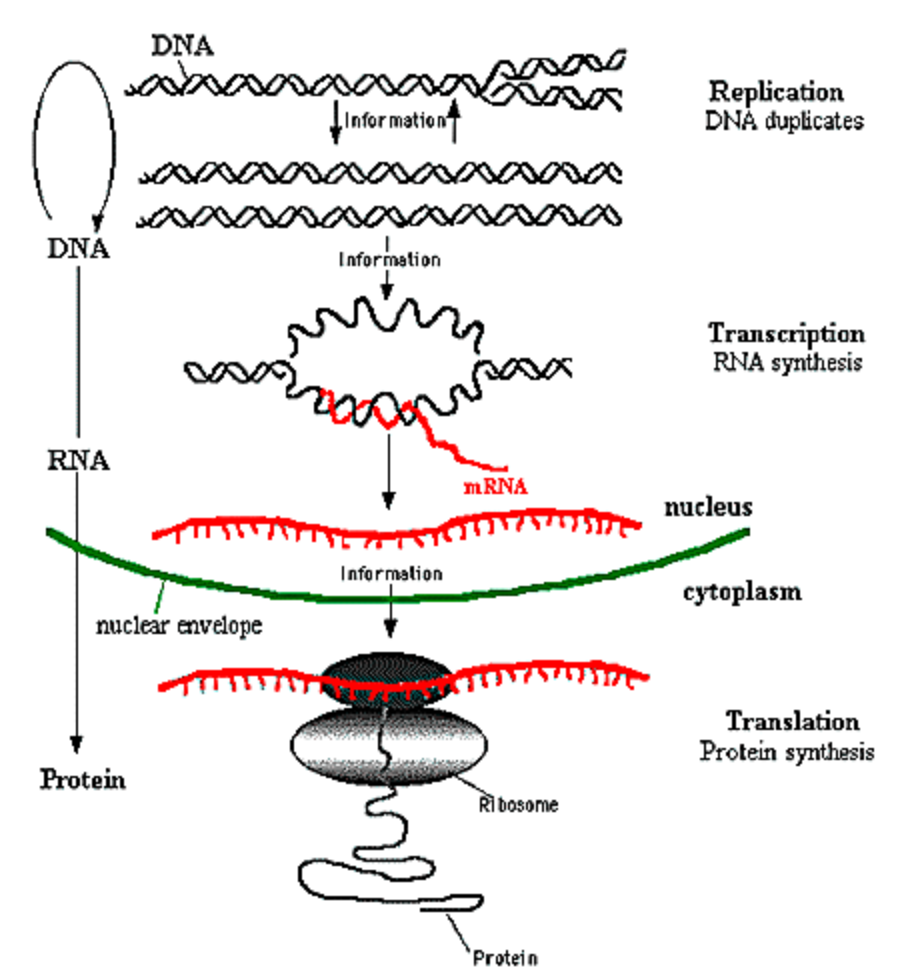
Daniel B. Campbell, Ph.D.
Assistant Professor
Department of Psychiatry and the Behavioral Sciences
Zilkha Neurogenetic Institute
Center for Genomic Psychiatry
Keck School of Medicine
University of Southern California
Los Angeles, CA USA
Email: dbcampbe@usc.edu

The Help Group Summit
October 26, 2013

Outline

- Introduction to Non-Coding RNAs
- Overview of Autism Genetics
- Genome-Wide Association Study results
  - Point to non-coding RNAs
- Exome Sequencing results
  - Point to a transcription factor that regulates … non-coding RNAs
- A New Type of Pharmacology Targets Non-Coding RNAs

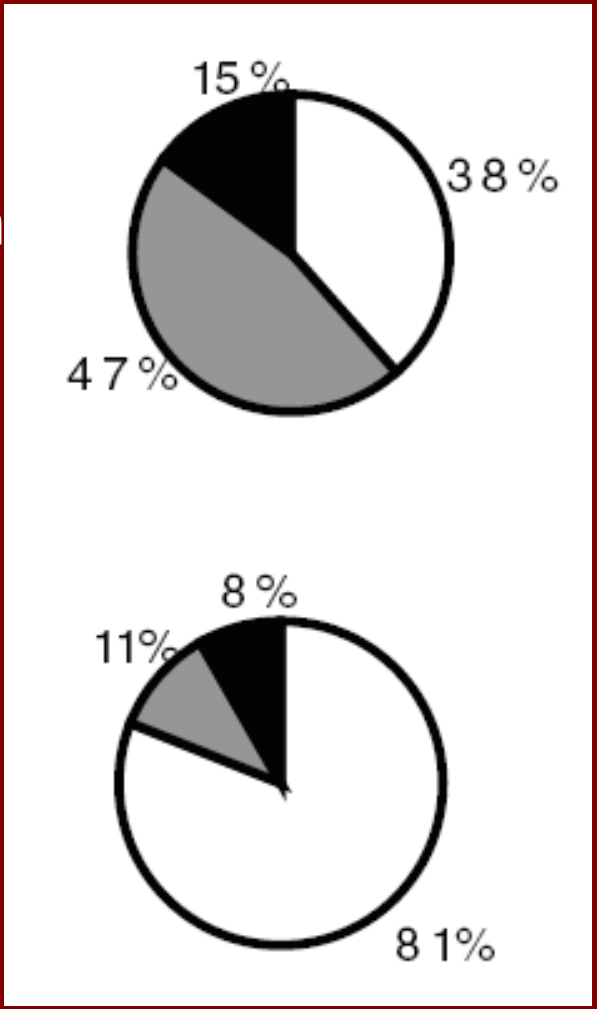
The Central Dogma



In the Human Brain, 62% of All Long RNAs are Non-Coding

Human Brain

Fruit Fly



Kapranov et al. 2010.
BMC Biol.

Non-Coding RNAs and Complexity

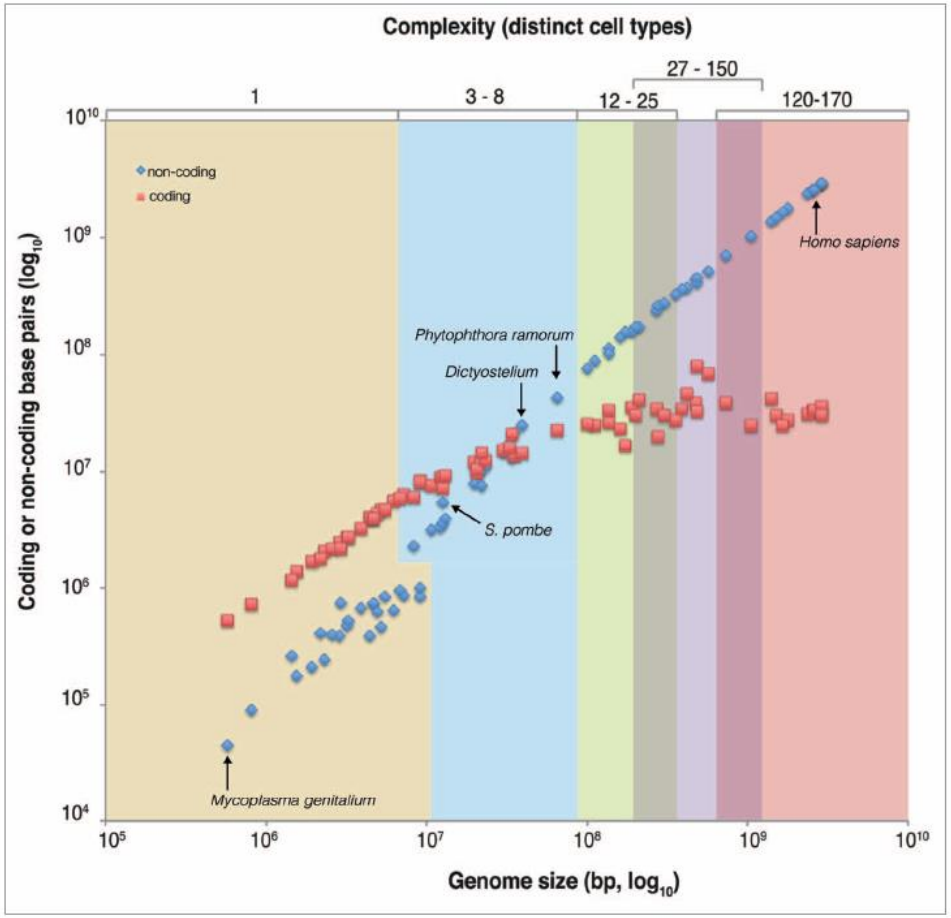


Figure 3. The relationship between biological complexity and genome composition. In this plot, the 73 organisms with a previously defined number of distinct cell types (e.g., relative biological complexity, see **Table S1**; ref. 35) are shown as pairs of data points, with one depicting total protein-coding sequence bases (red) and one total non-protein-coding bases (blue) which cumulatively give the total genome size (x-axis). Non-protein-coding sequence increases exponentially with the number of distinct cell types, while protein-coding sequence is asymptotic. Note that the intersection of the protein-coding and non-protein-coding data sets occurs among simple multicellular organisms.

Liu, Mattick, & Taft. 2013. *Cell Cycle*.

Translating dosage compensation to trisomy 21

Jun Jiang[1], Yuanchun Jing[1], Gregory J. Cost[2], Jen-Chieh Chiang[1], Heather J. Kolpa[1], Allison M. Cotton[3], Dawn M. Carone[1], Benjamin R. Carone[1], David A. Shivak[2], Dmitry Y. Guschin[2], Jocelynn R. Pearl[2], Edward J. Rebar[2], Meg Byron[1], Philip D. Gregory[2], Carolyn J. Brown[3], Fyodor D. Urnov[2], Lisa L. Hall[1] & Jeanne B. Lawrence[1]

Down's syndrome is a common disorder with enormous medical and social costs, caused by trisomy for chromosome 21. We tested the concept that gene imbalance across an extra chromosome can be ***de facto*** corrected by manipulating a single gene, *XIST* (the X-inactivation gene). Using genome editing with zinc finger nucleases, we inserted a large, inducible *XIST* transgene into the *DYRK1A* locus on chromosome 21, in Down's syndrome pluripotent stem cells. The *XIST* non-coding RNA coats chromosome 21 and triggers stable heterochromatin modifications, chromosome-wide transcriptional silencing and DNA methylation to form a 'chromosome 21 Barr body'. This provides a model to study human chromosome inactivation and creates a system to investigate genomic expression changes and cellular pathologies of trisomy 21, free from genetic and epigenetic noise. Notably, deficits in proliferation and neural rosette formation are rapidly reversed upon silencing one chromosome 21. Successful trisomy silencing ***in vitro*** also surmounts the major first step towards potential development of 'chromosome therapy'.

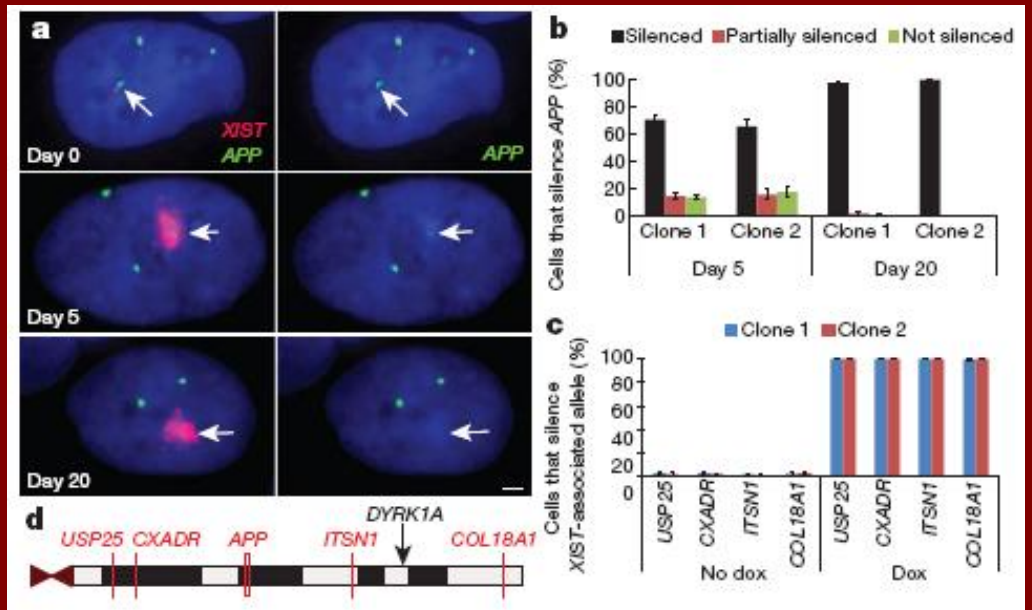


Figure 3 | *XIST* induces long-range silencing in targeted iPS cells. a, RNA

Nature. 2013.

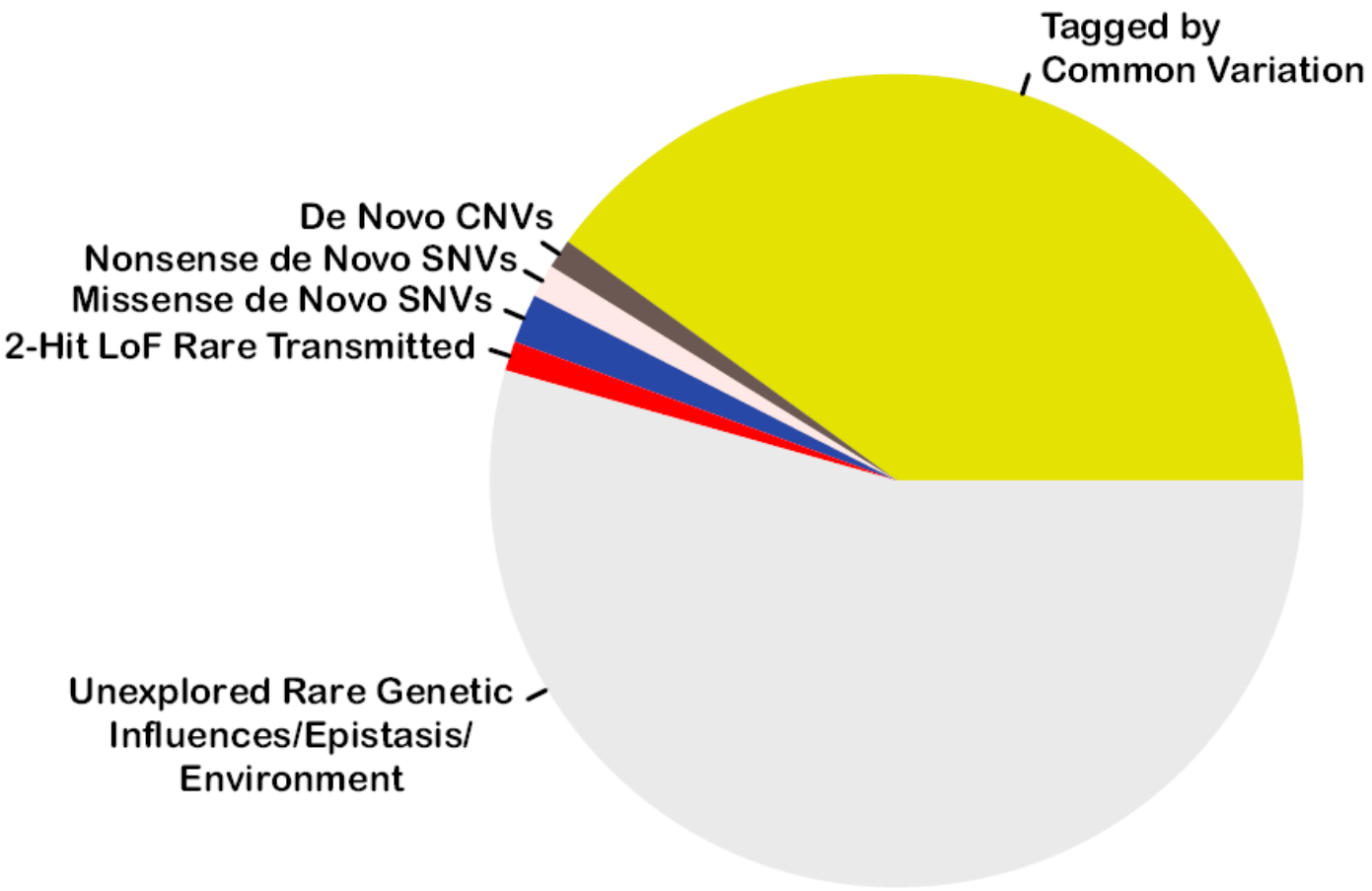
Outline

- Introduction to Non-Coding RNAs
- Overview of Autism Genetics
- Genome-Wide Association Study results
  - Point to non-coding RNAs
- Exome Sequencing results
  - Point to a transcription factor that regulates … non-coding RNAs
- A New Type of Pharmacology Targets Non-Coding RNAs

The Contributions to Autism?

- Rare Genetic Variants
  - Whole Exome Sequencing
  - Copy Number Variations
- Common Genetic Variants
  - Genome-Wide Association
  - Candidate Gene Association
- Environmental Factors
- The debate continues …

Global Contribution of Types of Genetic Variation to ASD



Stein et al. 2013. *Neuron*.

Table 1

Loci/gene often affected by CNV in ASD

Locus[a]	Cytoband	Pinto *et al.* [20••] # of events in cases/controls	Sanders *et al.* [38•] # of events in cases/controls	Combined # of events in cases/controls	*P*-value (cases vs. controls)[b]	Frequency in 2120 ASD cases (males)
CNV-16p11.2	16p11.2	4/996; 3/1287	14/1124; 0/872	18/2120; 3/2159	0.001	0.8%
PTCHD1/PTCHD1AS	Xp22.11	7/839; 0/383 males	3/968; 0/403 males	10/1807; 0/786 males	0.038	0.5% (0.6%)
NRXN1	2p16.3	6/996; 0/1287	3/1124; 1/872	9/2120; 1/2159	0.011	0.4%
CNV-7q11.23	7q11.23	0/996; 0/1287	4/1124; 0/872	4/2120; 0/2159	0.06	0.2%
CNV-22q11.2	22q11.2	3/996; 1/1287	1/1124; 0/872	4/2120; 1/2159	0.214	0.2%
CNV-1q21.1	1q21.1	1/996; 3/1287	3/1124; 0/872	4/2120; 3/2159	0.723	0.2%
CNV-15q13.3	15q13.3	2/996; 0/1287	3/1124;0/872	5/2120; 0/2159	0.030	0.2%
CNV-15q11–q13	15q11–q13	1/996; 0/1287	1/1124; 0/872	2/2120; 0/2159	0.245	0.1%
SHANK2	11q13.3	2/996; 0/1287	0/1124; 0/872	2/2120; 0/2159	0.245	0.1%
SHANK3	22q13.33	1/996; 0/1287	0/1124; 0/872	1/2120; 0/2159	0.495	0.05%
NLGN3	Xq13.1	0/839; 0/383 males	1/968; 0/403 males	1/1807; 0/786 males	1	0.05% (0.06%)
NLGN4X	Xp22.3	0/839; 0/383 males	1/968; 0/403 males	1/1807; 0786 males	1	0.05% (0.06%)

[a] *CNV-16p11.2*: encompasses 700 kb and 30 genes, 10 deletions and 8 duplications observed in ASD cases, one deletion and two duplications observed in controls; *PTCHD1/PTCHD1AS*: CNV region involves ~1 Mb region at Xp22.11, 1 deletion of *PTCHD1* and 9 deletions affecting upstream *PTCHD1AS* non-coding RNA gene; *NRXN1*: 8 deletions and 1 duplication observed in cases, one deletion observed in controls; *CNV-7q11.23*: encompasses ~1.4 Mb and 22 genes, all four cases represent apparently reciprocal duplications of 7q11.23 region typically deleted in Williams–Beuren syndrome; *CNV-22q11.2*: encompasses ~2.5 Mb and 56 genes, two deletions and two duplications were observed in ASD cases, one duplication observed in controls; *CNV-1q21.1*: encompasses ~1.5 Mb and 14 genes, four duplications were observed in ASD cases; *CNV-15q13.3*: encompasses ~1.5 Mb and 6 genes, four deletions and one duplication were observed in ASD cases; *CNV-15q11–q13*: encompasses ~5 Mb and 12 genes, two duplications were observed in ASD cases; *SHANK2*: two deletions observed in cases; *SHANK3:* one duplication observed in cases; *NLGN3:* one deletion observed in cases; *NLGN4X*: one duplication observed in cases; for *NRXN1*, *SHANK2*, *SHANK3*, *NLGN3* and *NLGN4X* CNVs are only counted in cases and controls if they affect one or more exons.

[b] Fisher's exact 2-sided *P*-value.

Devlin & Scherer. 2012.

3 Autism Exome Sequencing Papers: April 2012

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]

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], Emre Karakoc[1], Niklas Krumm[1], Bradley P. Coe[1], Roie Levy[1], Arthur Ko[1], Choli Lee[1], Joshua D. Smith[1], Emily H. Turner[1], Ian B. Stanaway[1], Benjamin Vernot[1], Maika Malig[1], Carl Baker[1], Beau Reilly[2], Joshua M. Akey[1], Elhanan Borenstein[1,3,4], Mark J. Rieder[1], Deborah A. Nickerson[1], Raphael Bernier[2], Jay Shendure[1] & Evan E. Eichler[1,5]

LETTER

doi:10.1038/nature11011

Patterns and rates of exonic *de novo* mutations in autism spectrum disorders

Benjamin M. Neale[1,2], Yan Kou[3,4], Li Liu[5], Avi Ma'ayan[3], Kaitlin E. Samocha[1,2], Aniko Sabo[6], Chiao-Feng Lin[7], Christine Stevens[2], Li-San Wang[7], Vladimir Makarov[4,8], Paz Polak[2,9], Seungtai Yoon[4,8], Jared Maguire[2], Emily L. Crawford[10], Nicholas G. Campbell[10], Evan T. Geller[7], Otto Valladares[7], Chad Schafer[5], Han Liu[11], Tuo Zhao[11], Guiqing Cai[4,8], Jayon Lihm[4,8], Ruth Dannenfelser[3], Omar Jabado[12], Zuleyma Peralta[12], Uma Nagaswamy[6], Donna Muzny[6], Jeffrey G. Reid[6], Irene Newsham[6], Yuanqing Wu[6], Lora Lewis[6], Yi Han[6], Benjamin F. Voight[2,13], Elaine Lim[1,2], Elizabeth Rossin[1,2], Andrew Kirby[1,2], Jason Flannick[2], Menachem Fromer[1,2], Khalid Shakir[2], Tim Fennell[2], Kiran Garimella[2], Eric Banks[2], Ryan Poplin[2], Stacey Gabriel[2], Mark DePristo[2], Jack R. Wimbish[14], Braden E. Boone[14], Shawn E. Levy[14], Catalina Betancur[15], Shamil Sunyaev[2,9], Eric Boerwinkle[6,16], Joseph D. Buxbaum[4,8,12,17], Edwin H. Cook Jr[18], Bernie Devlin[19], Richard A. Gibbs[6], Kathryn Roeder[5], Gerard D. Schellenberg[7], James S. Sutcliffe[10] & Mark J. Daly[1,2]

April Exome: Major Findings

Table 1 | Distribution of SNVs between probands and siblings

Category	Total number of SNVs*		SNVs per subject		Per base SNV rate ($\times 10^{-8}$)		*P*†	Odds ratio (95% CI)‡
	Pro $N = 200$	Sib $N = 200$	Pro $N = 200$	Sib $N = 200$	Pro $N = 200$	Sib $N = 200$		
De novo								
				All genes				
All	154	125 §	0.77	0.63	1.58	1.31	0.09	NA
Silent	29	39	0.15	0.20	0.29	0.40	0.28	NA
All non-synonymous	125	87	0.63	0.44	1.29	0.92	**0.01**	1.93 (1.11–3.36)
Missense	110	82	0.55	0.41	1.13	0.86	0.05	1.80 (1.03–3.16)
Nonsense/splice site	15	5	0.08	0.03	0.16	0.05	**0.04**	4.03 (1.32-12.4)
				Brain-expressed genes				
All	137	96	0.69	0.48	1.41	1.01	**0.01**	NA
Silent	23	30	0.12	0.15	0.24	0.31	0.41	NA
All non-synonymous	114	67	0.57	0.34	1.18	0.71	**0.001**	2.22 (1.19–4.13)
Missense	101	64	0.51	0.32	1.04	0.68	**0.005**	2.06 (1.10–3.85)
Nonsense/splice site	13	3	0.07	0.02	0.14	0.03	**0.02**	5.65 (1.44–22.2)

Sanders et al. 2012. *Nature*.

- Each paper finds a few genes with mutations in 2 affected individuals and 0 controls
- In a total of 584 families, the same gene was found to have a *de novo* mutation in no more than 2 (<0.4%) families
  - 4 genes: *SCN2A*, *CHD8*, *NTNG1*, *KATNAL2*
- Each paper concludes that there is no gene that is causal for autism, and that several hundred genes will contribute to risk

The 4th Autism Exome Sequencing Paper

Neuron
Article

De Novo Gene Disruptions in Children on the Autistic Spectrum

Ivan Iossifov,[1,6] Michael Ronemus,[1,6] Dan Levy,[1] Zihua Wang,[1] Inessa Hakker,[1] Julie Rosenbaum,[1] Boris Yamrom,[1] Yoon-ha Lee,[1] Giuseppe Narzisi,[1] Anthony Leotta,[1] Jude Kendall,[1] Ewa Grabowska,[1] Beicong Ma,[1] Steven Marks,[1] Linda Rodgers,[1] Asya Stepansky,[1] Jennifer Troge,[1] Peter Andrews,[1] Mitchell Bekritsky,[1] Kith Pradhan,[1] Elena Ghiban,[1] Melissa Kramer,[1] Jennifer Parla,[1] Ryan Demeter,[2] Lucinda L. Fulton,[2] Robert S. Fulton,[2] Vincent J. Magrini,[2] Kenny Ye,[3] Jennifer C. Darnell,[4] Robert B. Darnell,[4,5] Elaine R. Mardis,[2] Richard K. Wilson,[2] Michael C. Schatz,[1] W. Richard McCombie,[1] and Michael Wigler[1,*]

Table 2. Summary of De Novo Single Nucleotide Variants (SNVs) in 343 SSC Families

	40× (High) Coverage		All Loci							
SNV Effect	Proband	Sibling	Proband	Sibling	Proband F (29)	Proband M (314)	Sibling F (182)	Sibling M (161)	Both	Total
Splice site	4	1	6	3	1	5	1	2	0	9
Nonsense	15	7	19	9	3	16	6	3	2	30
Missense	125	121	207	207	19	188	116	91	3	417
Synonymous	53	42	79	69	8	71	43	26	4	152
Promoter	0	1	1	1	0	1	0	1	0	2
UTR	5	7	8	9	0	8	3	6	0	17
Intron	34	35	59	64	5	54	38	26	1	124
Intergenic	0	2	1	2	0	1	2	0	0	3
Total	236	216	380	364	36	344	209	155	10	754

De novo SNVs were tabulated according to affected status, gender, and type of mutation. Data under "40× coverage" indicate variants in the subset of the exome target region in which all members of a given family were covered by at least 40 sequence reads. The power to detect de novo variants in children from this well-covered portion of the target is very high, and we found no bias in coverage between affected and unaffected children. No significant difference was seen for missense mutations (125 in probands to 121 in unaffected siblings), but larger ratios of nonsense (15:7) and splice site (4:1) mutations were observed in probands relative to unaffected siblings. When we expanded our set of calls to include every variant that passed our thresholds (under "all loci"; see Experimental Procedures), similar ratios were observed. Probands and unaffected siblings are further subdivided based on gender: "proband F" indicates an affected female; "proband M" an affected male; "sibling F" an unaffected female; and "sibling M" an unaffected male. In parentheses, we indicate the number of children with the corresponding affected status and gender. The "both" column shows de novo SNVs that were shared by both siblings from the same family.

Autism Exome Sequencing: June 2012

- 967 families (quads) exome sequenced
- Still no gene with *de novo* LGD mutations in more than 2 (0.2%) families

"Mutations" that Cause Loss of Protein Function are Shockingly Common

A Systematic Survey of Loss-of-Function Variants in Human Protein-Coding Genes

Daniel G. MacArthur,[1,2*] Suganthi Balasubramanian,[3,4] Adam Frankish,[1] Ni Huang,[1] James Morris,[1] Klaudia Walter,[1] Luke Jostins,[1] Lukas Habegger,[3,4] Joseph K. Pickrell,[5] Stephen B. Montgomery,[6,7] Cornelis A. Albers,[1,8] Zhengdong D. Zhang,[9] Donald F. Conrad,[10] Gerton Lunter,[11] Hancheng Zheng,[12] Qasim Ayub,[1] Mark A. DePristo,[13] Eric Banks,[13] Min Hu,[1] Robert E. Handsaker,[13,14] Jeffrey A. Rosenfeld,[15] Menachem Fromer,[13] Mike Jin,[3] Xinmeng Jasmine Mu,[3,4] Ekta Khurana,[3,4] Kai Ye,[16] Mike Kay,[1] Gary Ian Saunders,[1] Marie-Marthe Suner,[1] Toby Hunt,[1] If H. A. Barnes,[1] Clara Amid,[1,17] Denise R. Carvalho-Silva,[1] Alexandra H. Bignell,[1] Catherine Snow,[1] Bryndis Yngvadottir,[1] Suzannah Bumpstead,[1] David N. Cooper,[18] Yali Xue,[1] Irene Gallego Romero,[1,5] 1000 Genomes Project Consortium, Jun Wang,[12] Yingrui Li,[12] Richard A. Gibbs,[19] Steven A. McCarroll,[13,14] Emmanouil T. Dermitzakis,[7] Jonathan K. Pritchard,[5,20] Jeffrey C. Barrett,[1] Jennifer Harrow,[1] Matthew E. Hurles,[1] Mark B. Gerstein,[3,4,21]† Chris Tyler-Smith[1]†

Genome-sequencing studies indicate that all humans carry many genetic variants predicted to cause loss of function (LoF) of protein-coding genes, suggesting unexpected redundancy in the human genome. Here we apply stringent filters to 2951 putative LoF variants obtained from 185 human genomes to determine their true prevalence and properties. We estimate that human genomes typically contain ~100 genuine LoF variants with ~20 genes completely inactivated. We identify rare and likely deleterious LoF alleles, including 26 known and 21 predicted severe disease–causing variants, as well as common LoF variants in nonessential genes. We describe functional and evolutionary differences between LoF-tolerant and recessive disease genes and a method for using these differences to prioritize candidate genes found in clinical sequencing studies.

Science. 2012.

All "Mutations" are Shockingly Common

Table 1 | **Mean number of coding variants in two populations**

Variant type	Mean number of variants (± sd) in African Americans	Mean number of variants (± sd) in European Americans
Novel variants		
Missense	303 (± 32)	192 (± 21)
Nonsense	5 (± 2)	5 (± 2)
Synonymous	209 (± 26)	109 (± 16)
Splice	2 (± 1)	2 (± 1)
Total	520 (± 53)	307 (± 33)
Non-novel variants		
Missense	10,828 (± 342)	9,319 (± 233)
Nonsense	98 (± 8)	89 (± 6)
Synonymous	12,567 (± 416)	10,536 (± 280)
Splice	36 (± 4)	32 (± 3)
Total	23,529 (± 751)	19,976 (± 505)
Total variants		
Missense	11,131 (± 364)	9,511 (± 244)
Nonsense	103(± 8)	93 (± 6)
Synonymous	12,776 (± 434)	10,645 (± 286)
Splice	38 (± 5)	34 (± 4)
Total	24,049 (± 791)	20,283 (± 523)

The table lists the mean number (± standard deviation (sd)) of novel and non-novel coding single nucleotide variants from 100 sampled African Americans and 100 European Americans. Non-novel variants refer to those found in dbSNP131 or in 200 other control

Bamshad et al. 2011. *Nature Reviews Genetics*.

Sciencexpress

Multiplex Targeted Sequencing Identifies Recurrently Mutated Genes in Autism Spectrum Disorders

Brian J. O'Roak,[1] Laura Vives,[1] Wenqing Fu,[1] Jarrett D. Egertson,[1] Ian B. Stanaway,[1] Ian G. Phelps,[2,3] Gemma Carvill,[2,3] Akash Kumar,[1] Choli Lee,[1] Katy Ankenman,[4] Jeff Munson,[4] Joseph B. Hiatt,[1] Emily H. Turner,[1] Roie Levy,[1] Diana R. O'Day,[2] Niklas Krumm,[1] Bradley P. Coe,[1] Beth K. Martin,[1] Elhanan Borenstein,[1,5,6] Deborah A. Nickerson,[1] Heather C. Mefford,[2,3] Dan Doherty,[2,3] Joshua M. Akey,[1] Raphael Bernier,[4] Evan E. Eichler,[1,7]* Jay Shendure[1]*

Exome sequencing studies of autism spectrum disorders (ASD) have identified many de novo mutations, but few recurrently disrupted genes. We therefore developed a modified molecular inversion probe method enabling ultra-low-cost candidate gene resequencing in very large cohorts. To demonstrate the power of this approach, we captured and sequenced 44 candidate genes in 2,446 ASD probands. We discovered 27 de novo events in 16 genes, 59% of which are predicted to truncate proteins or disrupt splicing. We estimate that recurrent disruptive mutations in six genes—*CHD8*, *DYRK1A*, *GRIN2B*, *TBR1*, *PTEN*, and *TBL1XR1*—may contribute to 1% of sporadic ASD. Our data support associations between specific genes and reciprocal subphenotypes (*CHD8*-macrocephaly, *DYRK1A*-microcephaly) and replicate the importance of a β-catenin/chromatin remodeling network to ASD etiology.

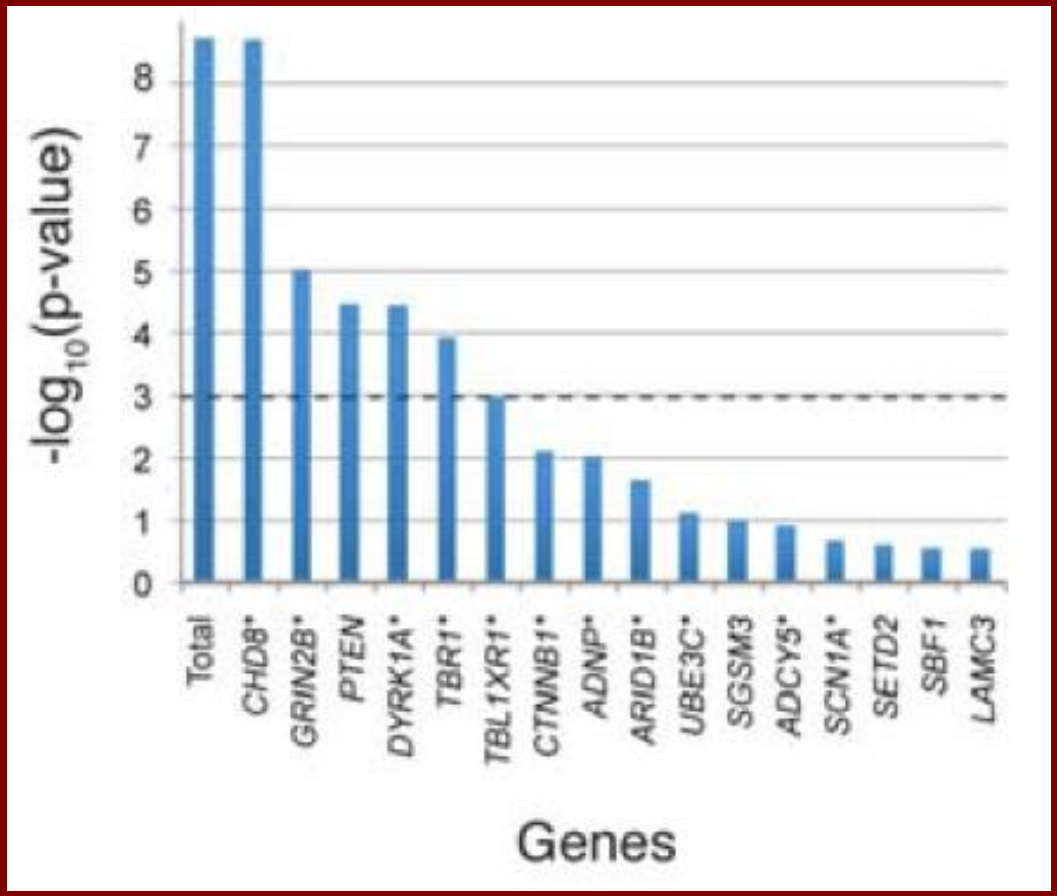
O'Roak et al. 2012 (Nov 15). *Science*.

CHD8: 11 *de novo* LoF mutations

Table 1. Six genes with recurrent de novo mutations. Abbreviations: M-male, F-female, Mut-mutation type, fs-frameshifting indel, ns-nonsense, sp-splice-site, aa-single amino acid deletion, ms-missense, HGVS-Human Genome Variation Society nomenclature; NVIQ-nonverbal intellectual quotient.

Proband	Sex	Gene	Mut	Assay‡	HGVS	NVIQ
12714.p1	M	CHD8*	ns	MIP	p.Ser62X	78
13986.p1	M	CHD8*	fs	MIP	p.Tyr747X	38
11654.p1	F	CHD8*	sp	MIP‖ (*4*)	c.3519-2A>G	41
13844.p1	M	CHD8*	ns	EX	p.Gln1238X	34
14016.p1	M	CHD8*	ns	MIP	p.Arg1337X	92
12991.p1	M	CHD8*	fs	MIP	p.Glu2103ArgfsX3	67
12752.p1	F	CHD8*	fs	EX	p.Leu2120ProfsX13	93
14233.p1	M	CHD8*	fs	MIP	p.Asn2371LysfsX2	19
14406.p1	M	CHD8*	aa	MIP	p.His2498del	98
12099.p1	M	DYRK1A*	fs	MIP‖ (*4*)	p.Ile48LysfsX2	55
13890.p1	F	DYRK1A*	sp	EX	c.1098+1G>A	42
13552.p1	M	DYRK1A*	fs	MIP¶ (*6*)	p.Ala498ProfsX94	66
11691.p1	M	GRIN2B†	fs	MIP§,‖ (*3*)	p.Ser34GlnfsX25	62
13932.p1	M	GRIN2B†	ms	MIP	p.Cys456Tyr	55
12547.p1	M	GRIN2B†	ns	MIP§	p.Trp559X	65
12681.p1	F	GRIN2B†	sp	EX	c.2172-2A>G	65
14433.p1	M	PTEN	ms	MIP	p.Thr131Ile	50
14611.p1	M	PTEN	fs	MIP	p.Cys136MetfsX44	33
11390.p1	F	PTEN	ms	EX	p.Thr167Asn	77
12335.p1	F	TBL1XR1*	ms	EX	p.Leu282Pro	47
14612.p1	M	TBL1XR1*	fs	MIP	p.Ile397SerfsX19	41
11480.p1	M	TBR1†	fs	EX	p.Ala136ProfsX80	41
13814.p1	M	TBR1†	ms	MIP	p.Lys228Glu	78
13796.p1	F	TBR1†	fs	MIP‖ (*4*)	p.Ser351X	63

*Part of 49-member connected component reported in (*3*). †Part of expanded 74-member connected component. ‡Primary assay that identified the variant. ‖¶Proband was exome sequenced by cited study and variant was ‖not reported or ¶reported. §Variant reported in MIP screen from (*3*).



… and association with autism $P<10^{-8}$

… note: next best genes have 3 *de novo* LGD

O'Roak et al. 2012. *Science*.

Table 2

GWA studies in ASDf

Study	Sample	*N*	SNP	Chr band	Position	Gene	MAF	OR	P discovery	P meta or mega analyses
Wang *et al.* [83]	AGRE[a]	3101 subjects; 1299 cases; 780 families	rs4307059	5p14.1	26,003,460	None	0.38	1.19	3.4×10^{-8}	2.1×10^{-10}
	ACC[a]	1204 cases; 6491 controls								
	CAP[b,c]	1390 subjects; 504 cases; 447 families								
	CART[b]	108 cases, 540 controls								
Weiss *et al.* [84]	AGRE[a]	3000 subjects, 780 families	rs10513025	5p15.2	9,676,622	SEMA5A TAS2R1	0.041	0.55	1.7×10^{-6}	2.1×10^{-7}
	NIMH[a]	1233 subjects, 341 families	rs10513026		9,677,106		0.04	0.53	4.5×10^{-6}	na[d]
	Montreal[b]	318 trios	rs16883317		9,701,592		0.038	0.53	7.2×10^{-5}	na[d]
	AGP[b]	1755 trios								
Anney *et al.* [85]	AGP	1369 families, 1385 probands	rs4141463	20p12.1	14,695,221	MACROD2	0.43	0.56	2.1×10^{-8}	4.7×10^{-8}
	AGRE	810 families								

[a] Discovery.
[b] Replication.
[c] Included in Ref. [83] and reported in Ref. [100].
[d] Imputed SNPs. Table is based on Ref. [88].

Devlin & Scherer. 2012.

Outline

- Introduction to Non-Coding RNAs
- Overview of Autism Genetics
- Genome-Wide Association Study results
  - Point to non-coding RNAs
- Exome Sequencing results
  - Point to a transcription factor that regulates … non-coding RNAs
- A New Type of Pharmacology Targets Non-Coding RNAs

nature Vol 459|28 May 2009|doi:10.1038/nature07999

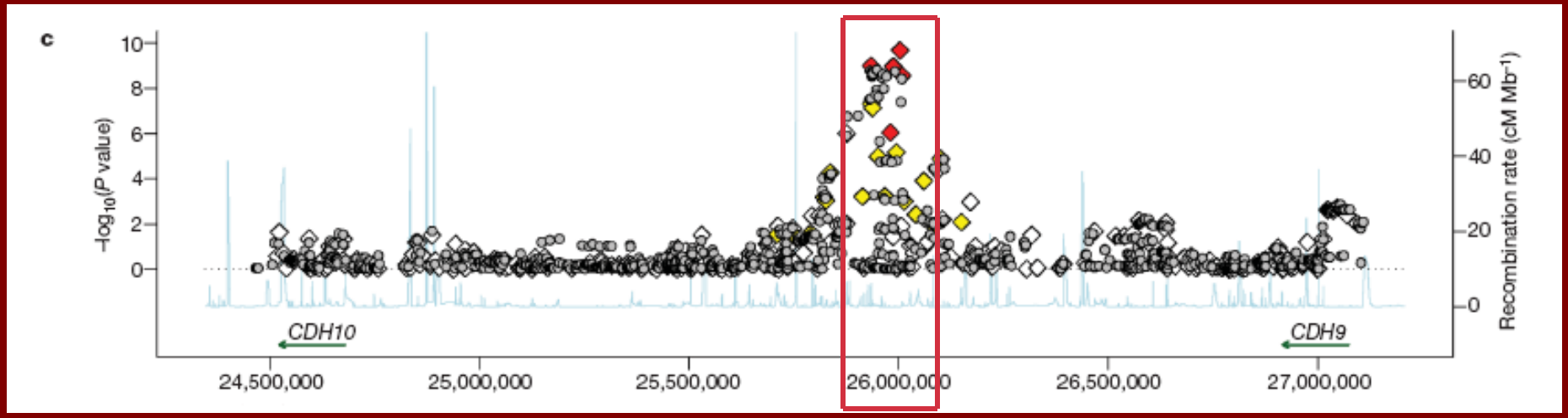
ARTICLES

Common genetic variants on 5p14.1 associate with autism spectrum disorders

Kai Wang[1]*, Haitao Zhang[1]*, Deqiong Ma[2]*, Maja Bucan[3], Joseph T. Glessner[1], Brett S. Abrahams[4], Daria Salyakina[2], Marcin Imielinski[1], Jonathan P. Bradfield[1], Patrick M. A. Sleiman[1], Cecilia E. Kim[1], Cuiping Hou[1], Edward Frackelton[1], Rosetta Chiavacci[1], Nagahide Takahashi[5], Takeshi Sakurai[5], Eric Rappaport[6], Clara M. Lajonchere[7], Jeffrey Munson[8], Annette Estes[8], Olena Korvatska[8], Joseph Piven[9], Lisa I. Sonnenblick[4], Ana I. Alvarez Retuerto[4], Edward I. Herman[4], Hongmei Dong[4], Ted Hutman[4], Marian Sigman[4], Sally Ozonoff[10], Ami Klin[11], Thomas Owley[12], John A. Sweeney[12], Camille W. Brune[12], Rita M. Cantor[13], Raphael Bernier[8], John R. Gilbert[2], Michael L. Cuccaro[2], William M. McMahon[14], Judith Miller[14], Matthew W. State[11], Thomas H. Wassink[15], Hilary Coon[14], Susan E. Levy[6], Robert T. Schultz[6], John I. Nurnberger Jr[16], Jonathan L. Haines[17], James S. Sutcliffe[18], Edwin H. Cook[12], Nancy J. Minshew[19], Joseph D. Buxbaum[5,20], Geraldine Dawson[8], Struan F. A. Grant[1,6], Daniel H. Geschwind[4], Margaret A. Pericak-Vance[2], Gerard D. Schellenberg[21] & Hakon Hakonarson[1,6]

Autism spectrum disorders (ASDs) represent a group of childhood neurodevelopmental and neuropsychiatric disorders characterized by deficits in verbal communication, impairment of social interaction, and restricted and repetitive patterns of interests and behaviour. To identify common genetic risk factors underlying ASDs, here we present the results of genome-wide association studies on a cohort of 780 families (3,101 subjects) with affected children, and a second cohort of 1,204 affected subjects and 6,491 control subjects, all of whom were of European ancestry. Six single nucleotide polymorphisms between cadherin 10 (*CDH10*) and cadherin 9 (*CDH9*)—two genes encoding neuronal cell-adhesion molecules—revealed strong association signals, with the most significant SNP being rs4307059 ($P = 3.4 \times 10^{-8}$, odds ratio = 1.19). These signals were replicated in two independent cohorts, with combined *P* values ranging from 7.4×10^{-8} to 2.1×10^{-10}. Our results implicate neuronal cell-adhesion molecules in the pathogenesis of ASDs, and represent, to our knowledge, the first demonstration of genome-wide significant association of common variants with susceptibility to ASDs.

Genome Wide Association Study (GWAS) Revealed Association of Common Genetic Variants on Chromosome 5



Wang et al. 2009. *Nature*.

Possible Interpretations:

1. The GWAS peak implicates the neighboring *CDH10* and *CDH9* genes in ASD.

rs4307059 Genotype Did Not Correlate with Expression of *CDH9* or *CDH10*

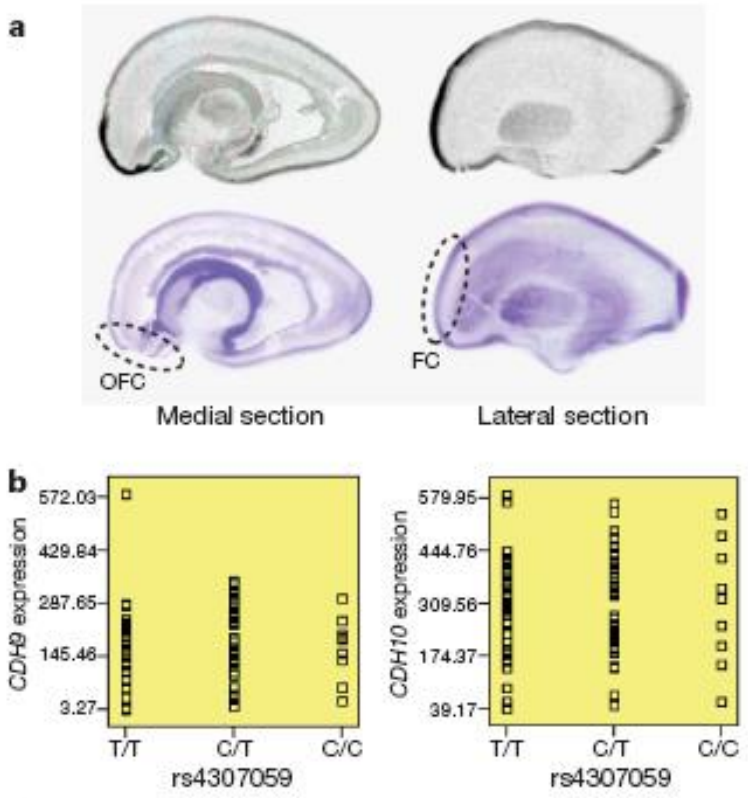
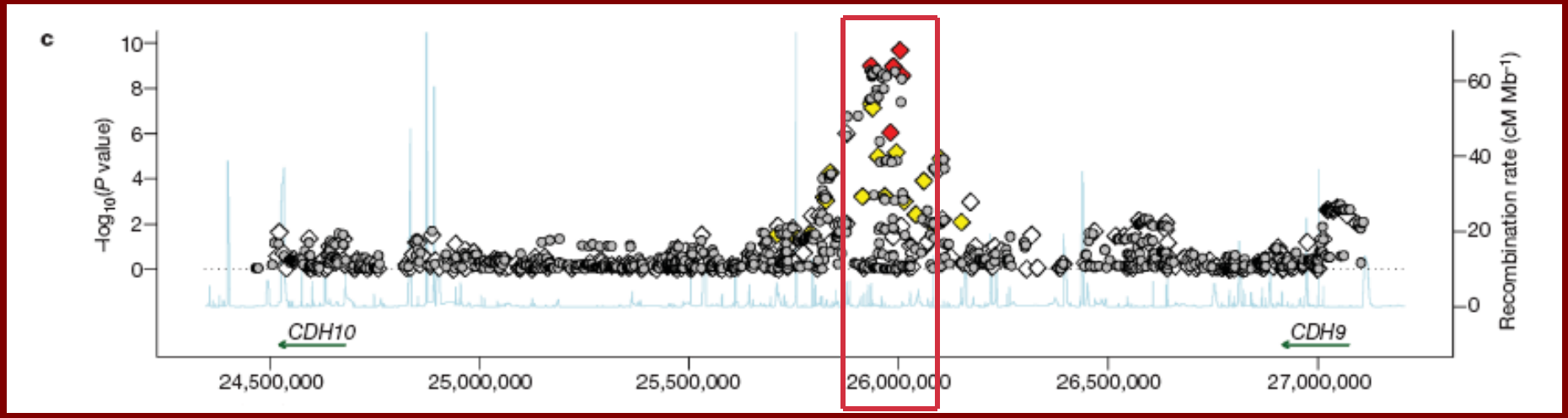


Figure 2 | Examination of brain expression for *CDH10* and *CDH9*. **a**, The *in situ* hybridization of *CDH10* in human fetal brain sectioned in the sagittal plane. Medial and lateral sections from a representative sample are shown above corresponding cresyl-violet-stained marker slides. Orbitofrontal cortex (OFC) and frontal cortex (FC) are highlighted, with marked expression enrichment. **b**, The SNP genotypes of rs4307059 are not associated with *CDH9* or *CDH10* transcript levels in 93 cortical brain tissues.

Wang et al. *Nature*. 2009.

Genome Wide Association Study (GWAS) Revealed Association of Common Genetic Variants on Chromosome 5

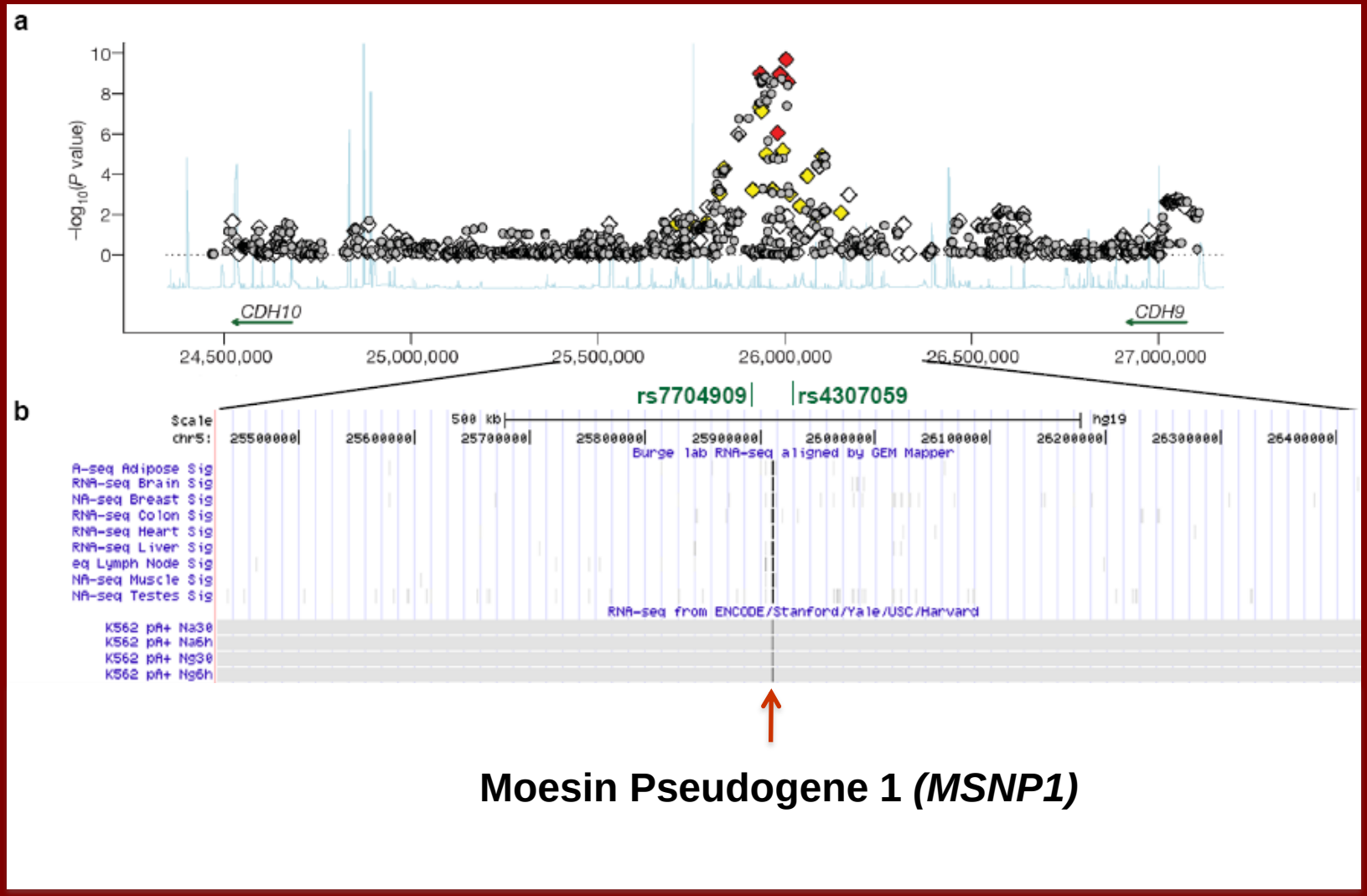


Wang et al. 2009. *Nature*.

Possible Interpretations:

1. The GWAS peak implicates the neighboring *CDH10* and *CDH9* genes in ASD.
2. The GWAS data indicate that no common variants contribute to ASD.
3. The GWAS peak indicates significant contribution of a functional, non-protein-coding genetic element to ASD risk.

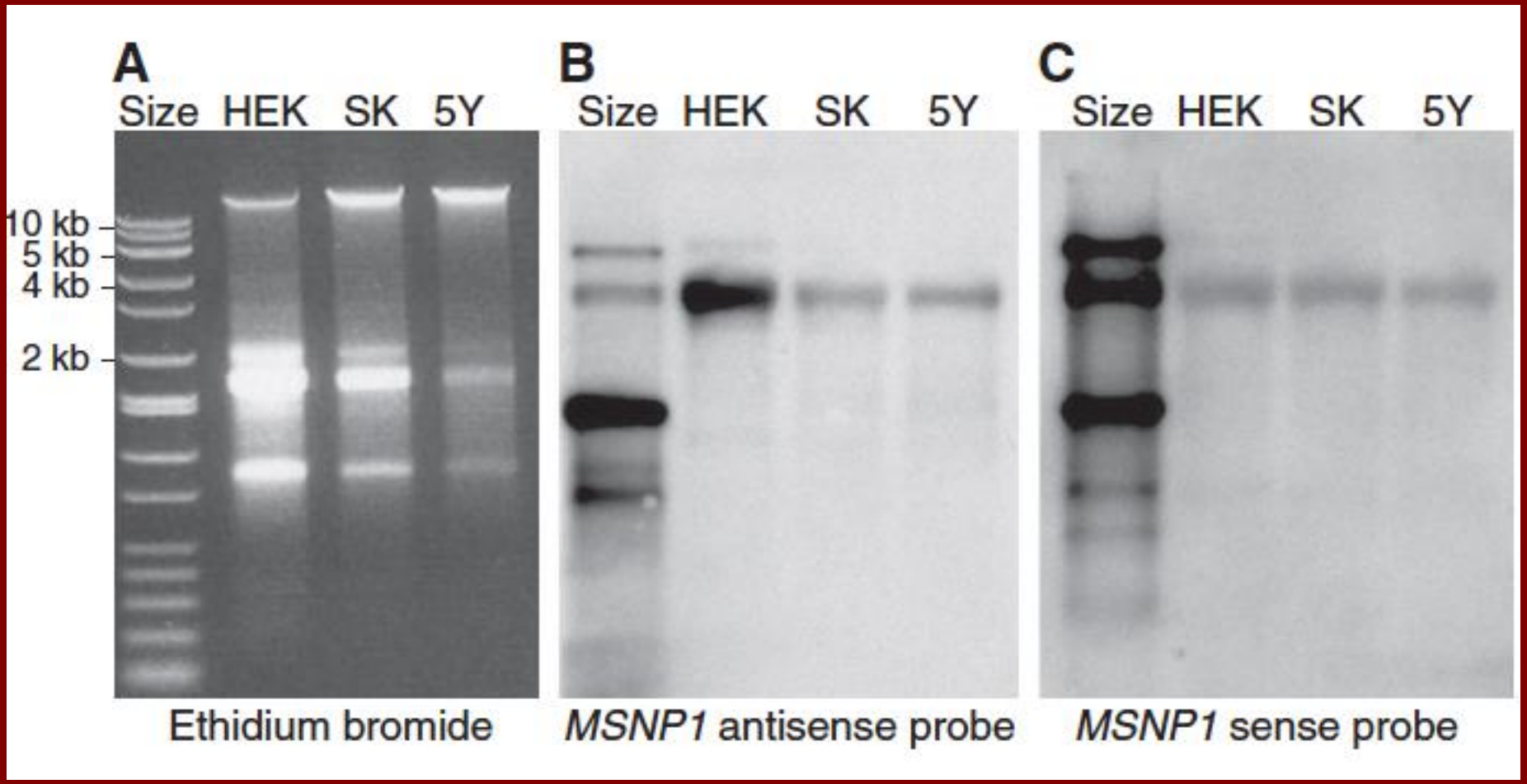
A Long Non-Coding RNA is Expressed Directly Under the ASD GWAS Peak



Moesin Pseudogene 1 *(MSNP1)*

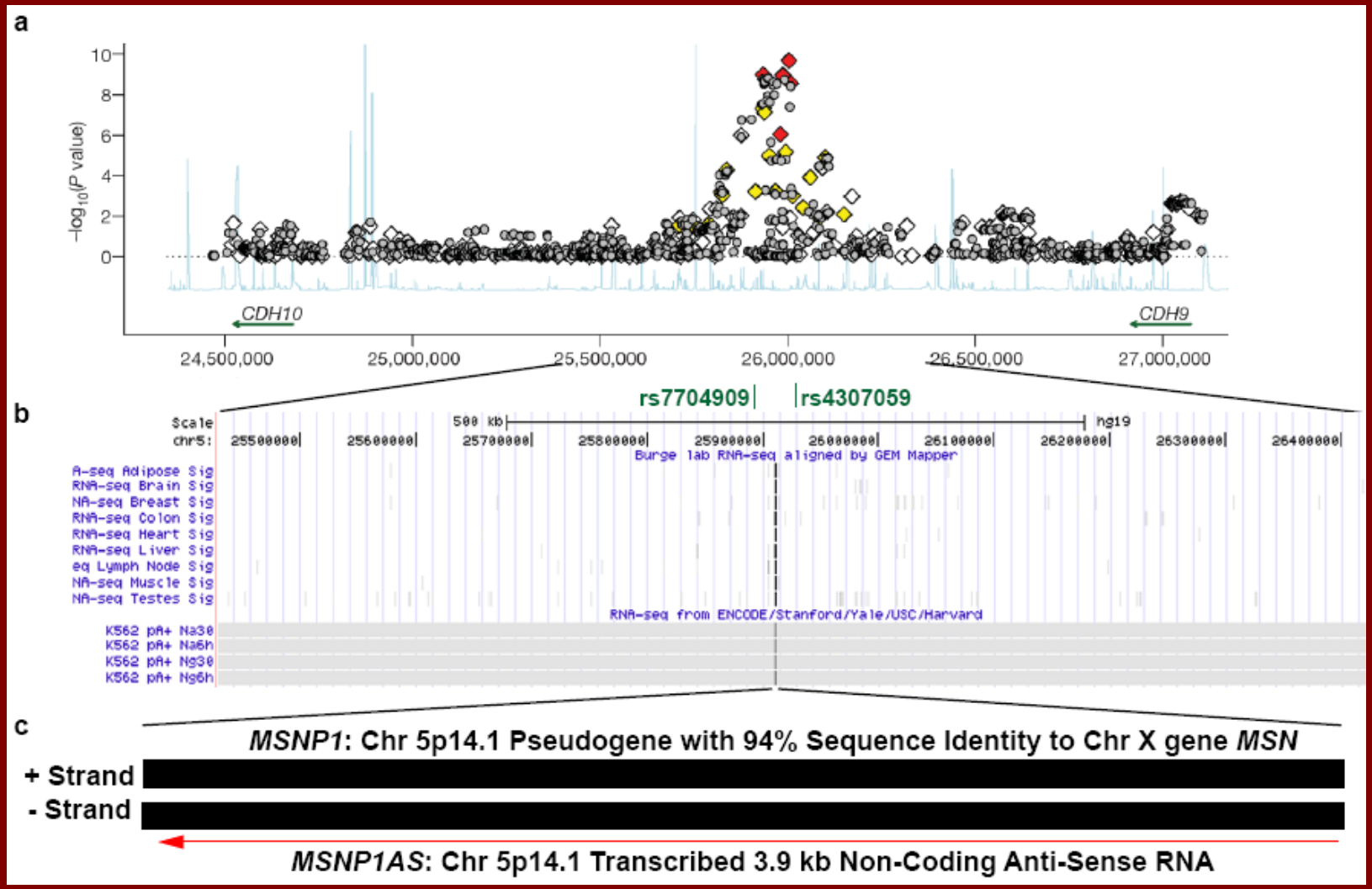
UCSC Genome Browser

Northern Hybridization: The Long Non-Coding RNA is Complementary to *MSNP1*



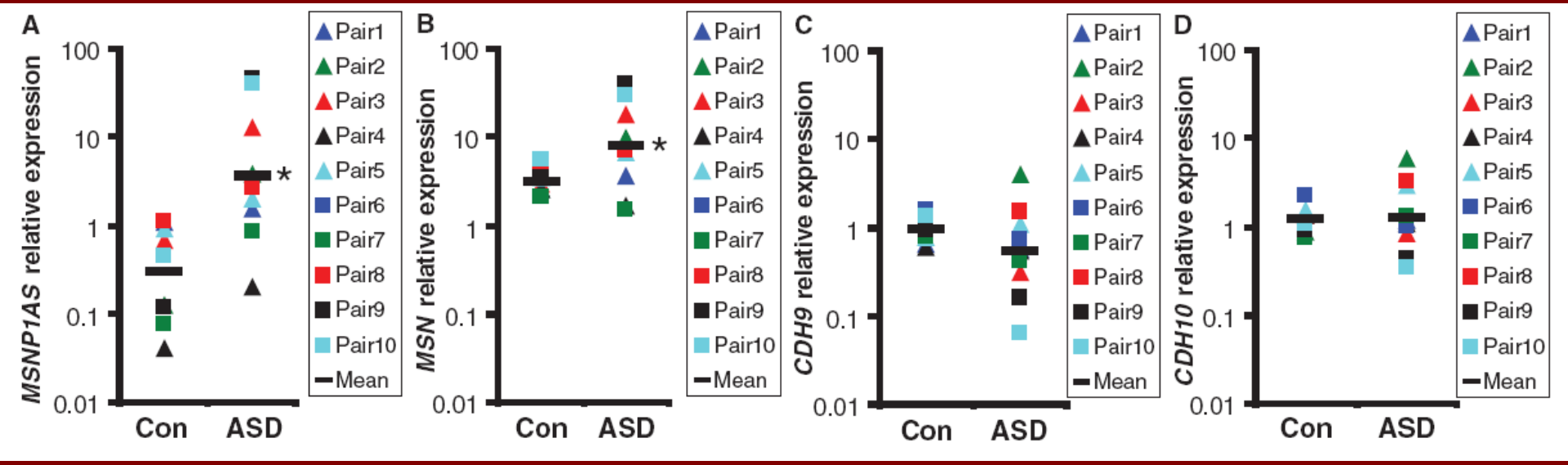
Kerin et al. 2012. *Science Translational Medicine*.

MSNP1AS is the Long Non-Coding RNA Directly Under the ASD GWAS Peak



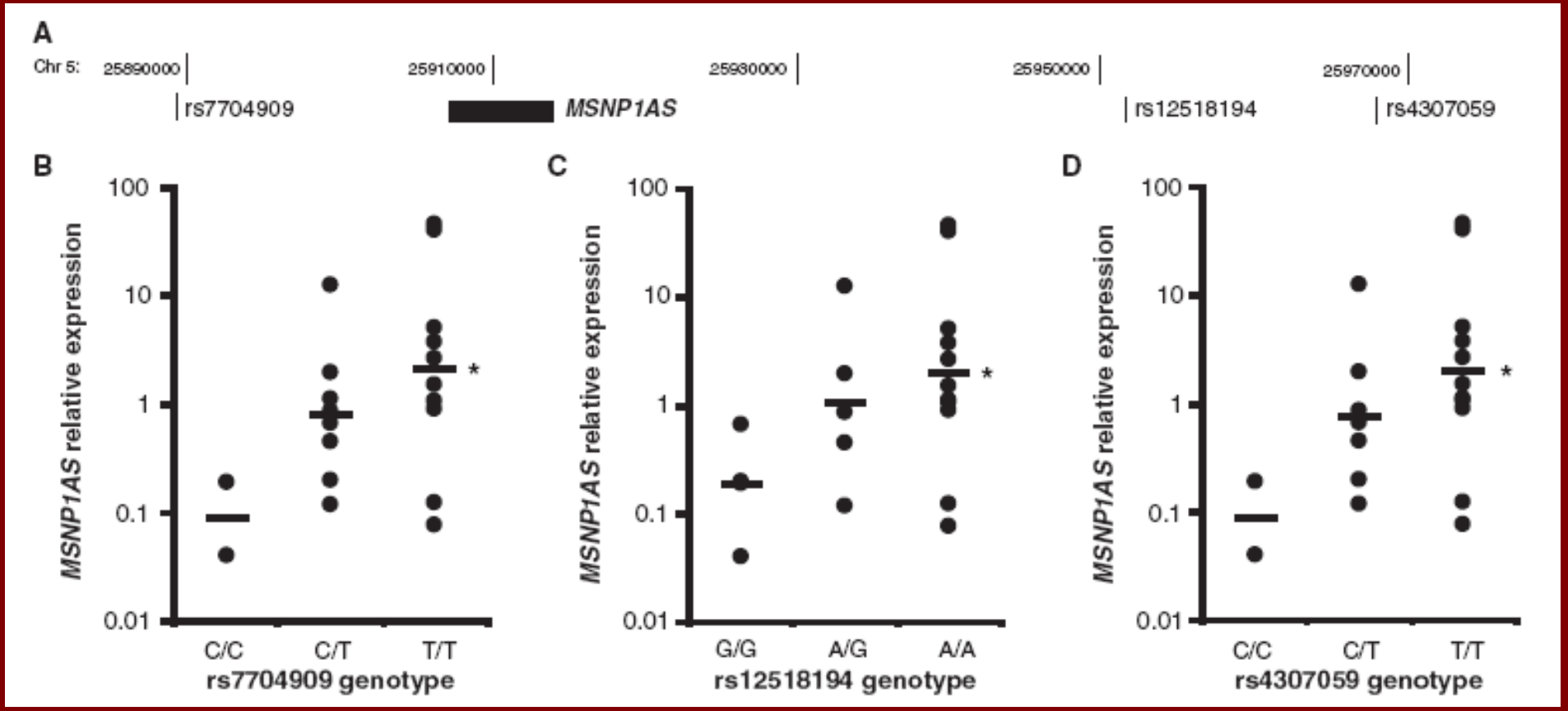
Kerin et al. 2012. *Science Translational Medicine*.

Postmortem Temporal Cortex: *MSNP1AS* Expression is Increased 12.7-Fold in ASD



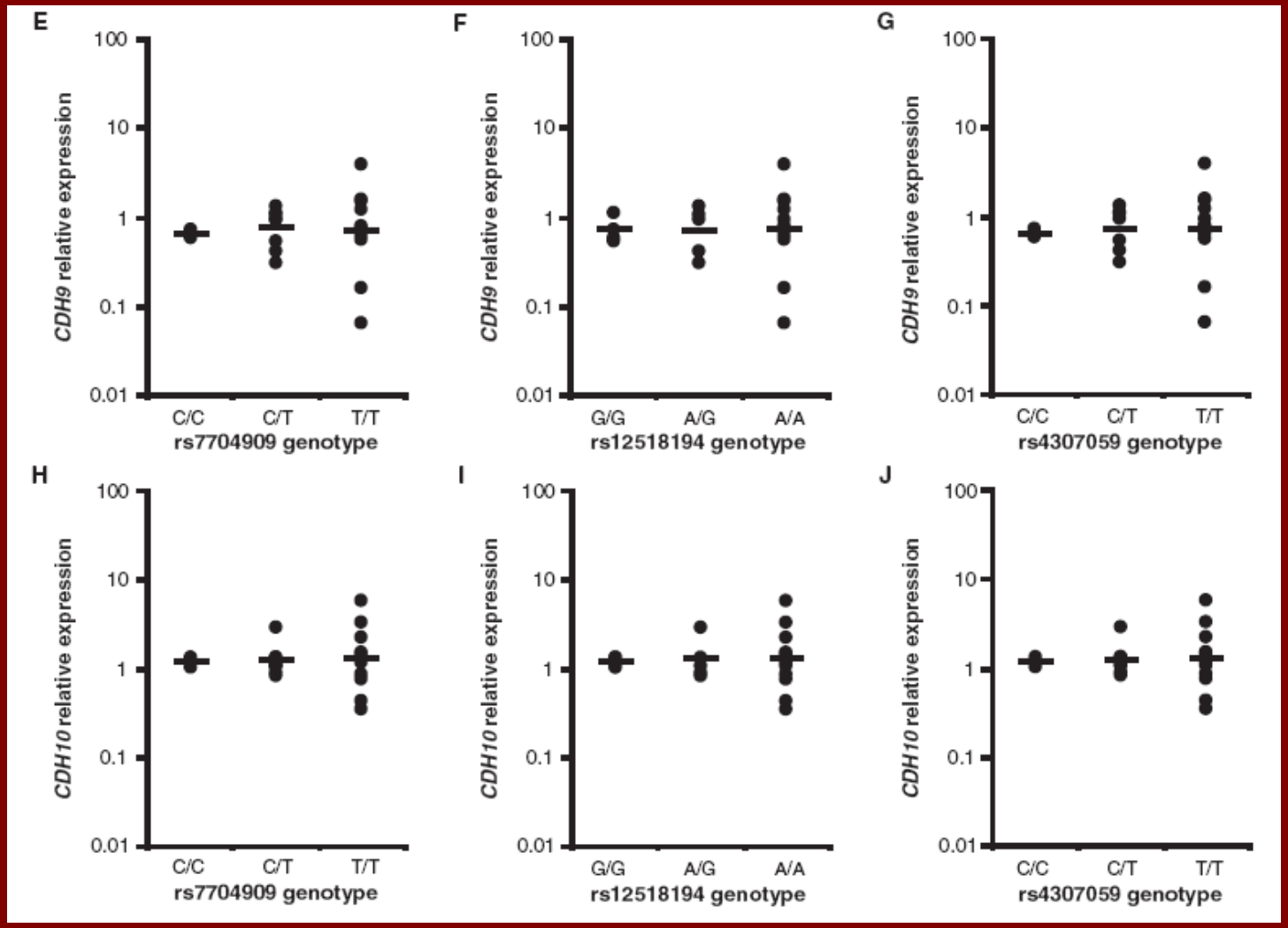
Kerin et al. 2012. *Science Translational Medicine*.

MSNP1AS Expression is Correlated with ASD Risk Allele Genotypes



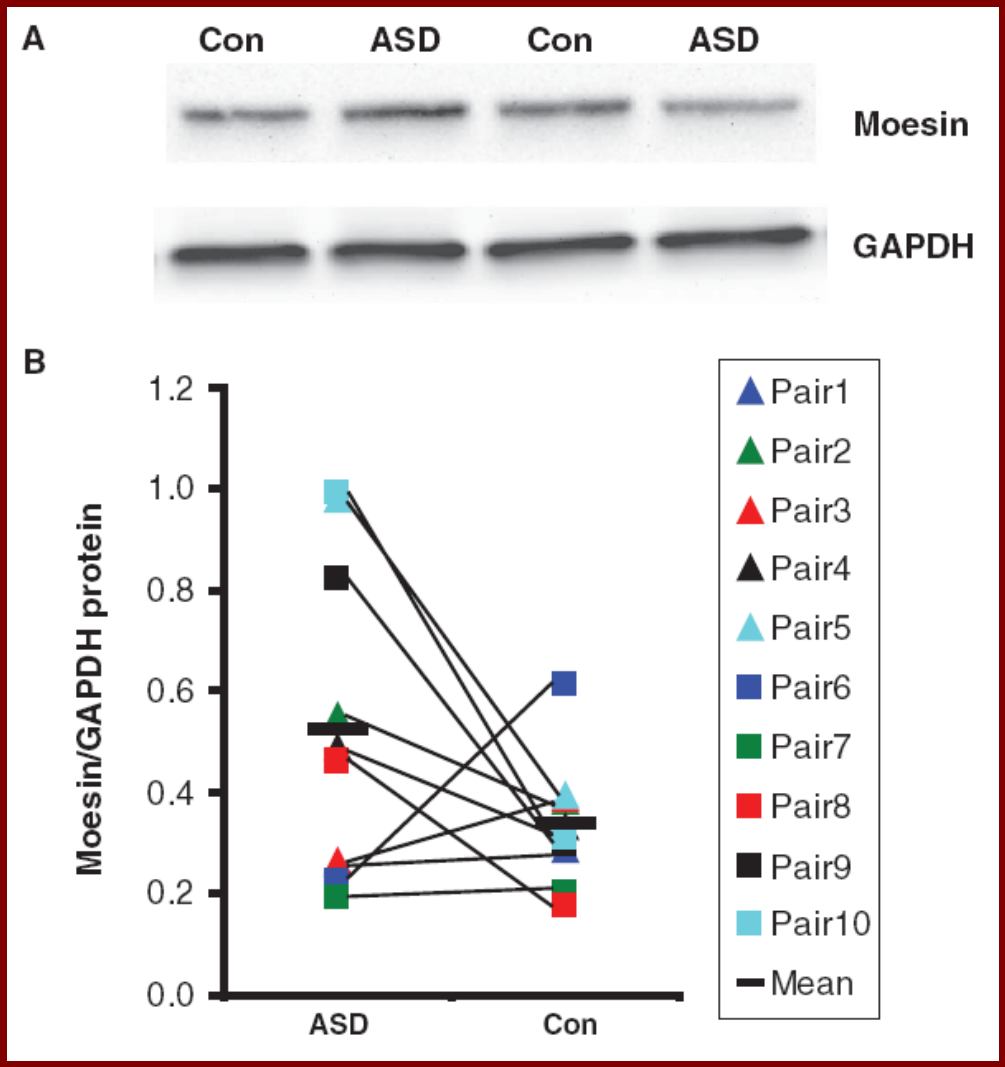
Kerin et al. 2012. *Science Translational Medicine*.

Neither *CDH9* nor *CDH10* Expression is Correlated with ASD Risk Allele Genotype



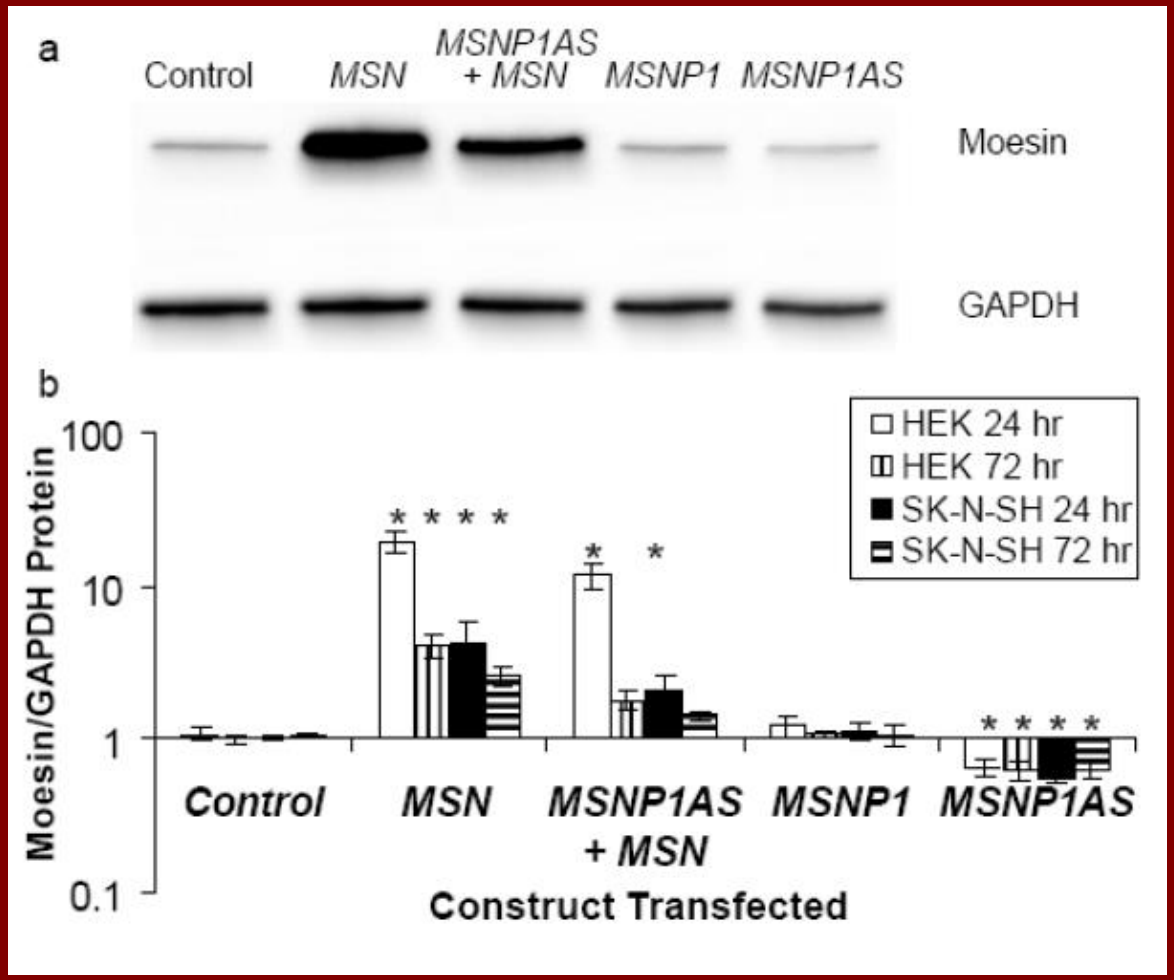
Kerin et al. 2012. *Science Translational Medicine*.

Despite 2.4-fold Increase in *MSN* RNA, Moesin Protein Levels are Unchanged



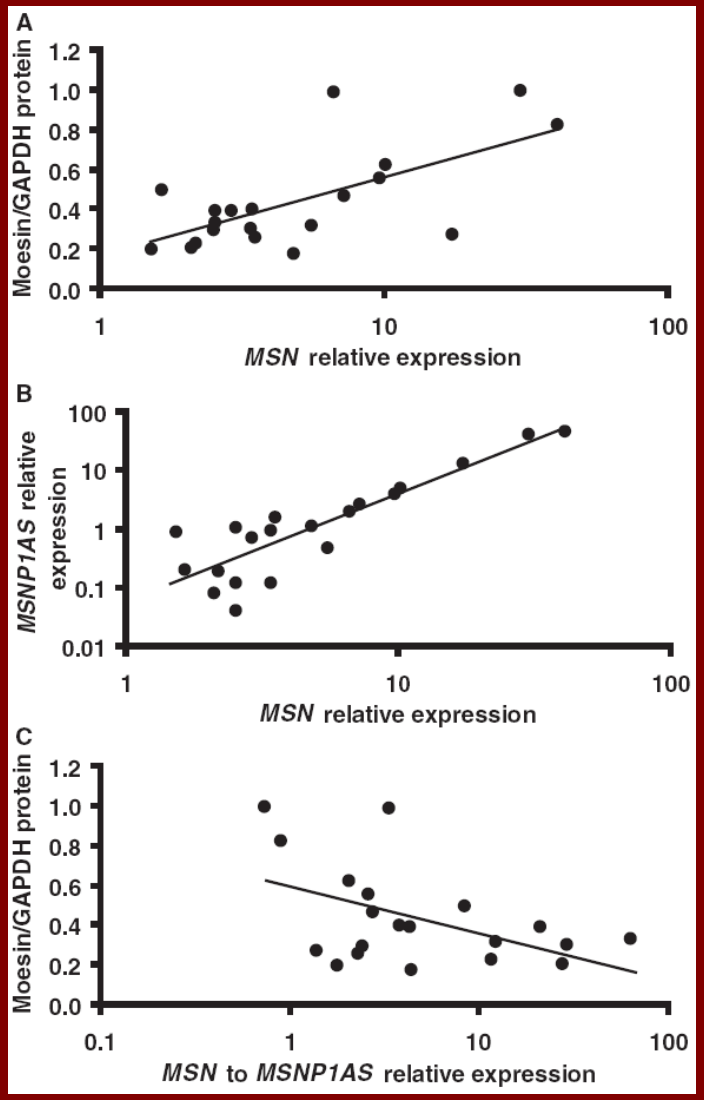
Kerin et al. 2012. *Science Translational Medicine*.

Over-Expression of *MSNP1AS* Causes a Decrease in Moesin Protein



Kerin et al. 2012. *Science Translational Medicine*.

Correlations Among *MSN*, *MSNP1AS*, and Moesin Protein in Postmortem Cortex



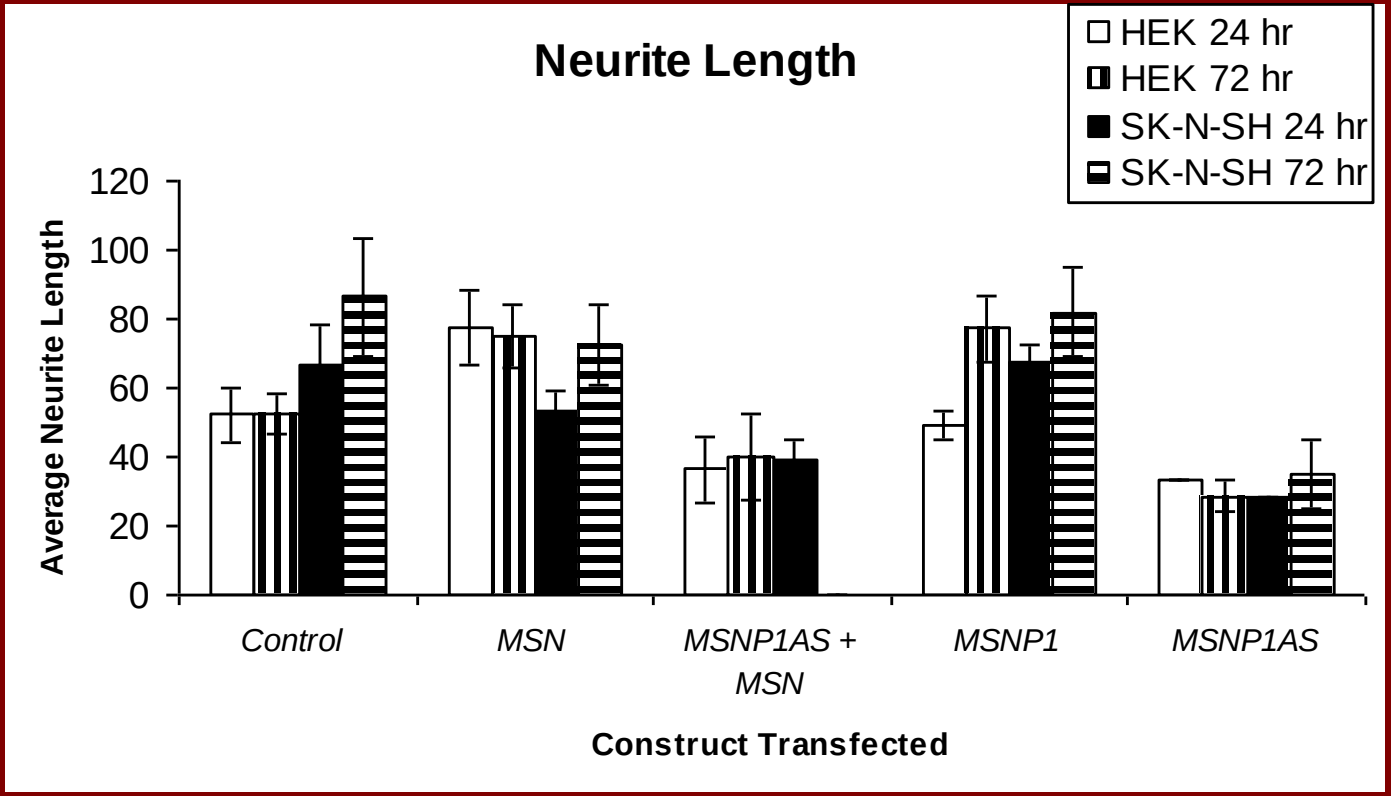
MSN is the major determinant of moesin protein levels

MSN and *MSNP1AS* appear to be co-regulated

MSNP1AS contributes to the regulation of moesin protein

Kerin et al. 2012. *Science Translational Medicine*.

Over-Expression of *MSNP1AS* Causes a Decrease in the Average Neurite Length



Preliminary Data

MSNP1AS Summary

- *MSNP1AS* is the second anti-sense of a pseudogene demonstrated to regulate expression of a gene on a different chromosome
  - First was Oct4-pg5 regulation of Oct4 (Hawkins & Morris, 2010)
- Moesin is an X chromosome-encoded protein that acts:
  - (1) presynaptically to maintain axonal growth cones;
  - (2) postsynaptically to induce dendritic spine formation; and
  - (3) at the immune synapse (APCs-lymphocytes)

Table 2

GWA studies in ASDf

Study	Sample	*N*	SNP	Chr band	Position	Gene	MAF	OR	P discovery	P meta or mega analyses
Wang *et al.* [83]	AGRE[a]	3101 subjects; 1299 cases; 780 families	rs4307059	5p14.1	26,003,460	None	0.38	1.19	3.4×10^{-8}	2.1×10^{-10}
	ACC[a]	1204 cases; 6491 controls								
	CAP[b,c]	1390 subjects; 504 cases; 447 families								
	CART[b]	108 cases, 540 controls								
Weiss *et al.* [84]	AGRE[a]	3000 subjects, 780 families	rs10513025	5p15.2	9,676,622	SEMA5A TAS2R1	0.041	0.55	1.7×10^{-6}	2.1×10^{-7}
	NIMH[a]	1233 subjects, 341 families	rs10513026		9,677,106		0.04	0.53	4.5×10^{-6}	na[d]
	Montreal[b]	318 trios	rs16883317		9,701,592		0.038	0.53	7.2×10^{-5}	na[d]
	AGP[b]	1755 trios								
Anney *et al.* [85]	AGP	1369 families, 1385 probands	rs4141463	20p12.1	14,695,221	MACROD2	0.43	0.56	2.1×10^{-8}	4.7×10^{-8}
	AGRE	810 families								

[a] Discovery.
[b] Replication.
[c] Included in Ref. [83] and reported in Ref. [100].
[d] Imputed SNPs. Table is based on Ref. [88].

Devlin & Scherer. 2012.

Human Molecular Genetics, 2010, Vol. 19, No. 20 4072–4082
doi:10.1093/hmg/ddq307
Advance Access published on July 27, 2010

A genome-wide scan for common alleles affecting risk for autism

Richard Anney[1], Lambertus Klei[2], Dalila Pinto[3], Regina Regan[4], Judith Conroy[4], Tiago R. Magalhaes[5,6], Catarina Correia[5,6], Brett S. Abrahams[7], Nuala Sykes[8], Alistair T. Pagnamenta[8], Joana Almeida[9], Elena Bacchelli[10], Anthony J. Bailey[11,†], Gillian Baird[12], Agatino Battaglia[13,†], Tom Berney[14], Nadia Bolshakova[1], Sven Bölte[15], Patrick F. Bolton[16], Thomas Bourgeron[17], Sean Brennan[1], Jessica Brian[18], Andrew R. Carson[3], Guillermo Casallo[3], Jillian Casey[4], Su H. Chu[20], Lynne Cochrane[1], Christina Corsello[19], Emily L. Crawford[21], Andrew Crossett[20], Geraldine Dawson[22,23,†], Maretha de Jonge[24], Richard Delorme[25], Irene Drmic[18], Eftichia Duketis[15], Frederico Duque[9], Annette Estes[26], Penny Farrar[8], Bridget A. Fernandez[31], Susan E. Folstein[32], Eric Fombonne[33], Christine M. Freitag[15,†], John Gilbert[32], Christopher Gillberg[34], Joseph T. Glessner[35], Jeremy Goldberg[36], Jonathan Green[37], Stephen J. Guter[38], Hakon Hakonarson[35,39,†], Elizabeth A. Heron[1], Matthew Hill[1], Richard Holt[8], Jennifer L. Howe[3], Gillian Hughes[1], Vanessa Hus[19], Roberta Igliozzi[13], Cecilia Kim[35], Sabine M. Klauck[40,†], Alexander Kolevzon[41], Olena Korvatska[27], Vlad Kustanovich[42], Clara M. Lajonchere[42], Janine A. Lamb[43], Magdalena Laskawiec[11], Marion Leboyer[44], Ann Le Couteur[14], Bennett L. Leventhal[45,46], Anath C. Lionel[3], Xiao-Qing Liu[3], Catherine Lord[19], Linda Lotspeich[47], Sabata C. Lund[21], Elena Maestrini[10,†], William Mahoney[48], Carine Mantoulan[59], Christian R. Marshall[3], Helen McConachie[14], Christopher J. McDougle[49], Jane McGrath[1], William M. McMahon[50,†], Nadine M. Melhem[2], Alison Merikangas[1], Ohsuke Migita[3], Nancy J. Minshew[51,52], Ghazala K. Mirza[8], Jeff Munson[28], Stanley F. Nelson[53,†], Carolyn Noakes[18], Abdul Noor[54], Gudrun Nygren[34], Guiomar Oliveira[9,†], Katerina Papanikolaou[55], Jeremy R. Parr[56], Barbara Parrini[13], Tara Paton[3], Andrew Pickles[57], Joseph Piven[58,†], David J Posey[49], Annemarie Poustka[40,‡], Fritz Poustka[15], Aparna Prasad[3], Jiannis Ragoussis[8], Katy Renshaw[11], Jessica Rickaby[3], Wendy Roberts[18], Kathryn Roeder[20], Bernadette Roge[59], Michael L. Rutter[60], Laura J. Bierut[61], John P. Rice[61], Jeff Salt[38], Katherine Sansom[3], Daisuke Sato[3], Ricardo Segurado[1], Lili Senman[18], Naisha Shah[4], Val C. Sheffield[62], Latha Soorya[41], Inês Sousa[8], Vera Stoppioni[63], Christina Strawbridge[36], Raffaella Tancredi[13], Katherine Tansey[1], Bhooma Thiruvahindrapduram[3], Ann P. Thompson[36], Susanne Thomson[21], Ana Tryfon[41], John Tsiantis[55], Herman Van Engeland[24], John B. Vincent[54], Fred Volkmar[64], Simon Wallace[11], Kai Wang[35], Zhouzhi Wang[3], Thomas H. Wassink[65,†], Kirsty Wing[8], Kerstin Wittemeyer[59], Shawn Wood[2], Brian L. Yaspan[21], Danielle Zurawiecki[41], Lonnie Zwaigenbaum[66], Catalina Betancur[67,†], Joseph D. Buxbaum[41,†], Rita M. Cantor[53,†], Edwin H. Cook[38,†],

[†]Lead AGP investigators who contributed equally to this project.
[‡]Deceased.

© The Author 2010. Published by Oxford University Press.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/2.5), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Hilary Coon[50,†], Michael L. Cuccaro[32], Louise Gallagher[1,†], Daniel H. Geschwind[7,†], Michael Gill[1,†], Jonathan L. Haines[68,†], Judith Miller[50], Anthony P. Monaco[8,†], John I. Nurnberger Jr.[49,†], Andrew D. Paterson[3,†], Margaret A. Pericak-Vance[32,†], Gerard D. Schellenberg[69,†], Stephen W. Scherer[3,†], James S. Sutcliffe[21,†], Peter Szatmari[36,†], Astrid M. Vicente[5,6,†], Veronica J. Vieland[70,†], Ellen M. Wijsman[29,30,†], Bernie Devlin[2,*,†], Sean Ennis[4,†] and Joachim Hallmayer[47,†]

Although autism spectrum disorders (ASDs) have a substantial genetic basis, most of the known genetic risk has been traced to rare variants, principally copy number variants (CNVs). To identify common risk variation, the Autism Genome Project (AGP) Consortium genotyped 1558 rigorously defined ASD families for 1 million single-nucleotide polymorphisms (SNPs) and analyzed these SNP genotypes for association with ASD. In one of four primary association analyses, the association signal for marker rs4141463, located within *MACROD2*, crossed the genome-wide association significance threshold of $P < 5 \times 10^{-8}$. When a smaller replication sample was analyzed, the risk allele at rs4141463 was again over-transmitted; yet, consistent with the winner's curse, its effect size in the replication sample was much smaller; and, for the combined samples, the association signal barely fell below the $P < 5 \times 10^{-8}$ threshold. Exploratory analyses of phenotypic subtypes yielded no significant associations after correction for multiple testing. They did, however, yield strong signals within several genes, *KIAA0564*, *PLD5*, *POU6F2*, *ST8SIA2* and *TAF1C*.

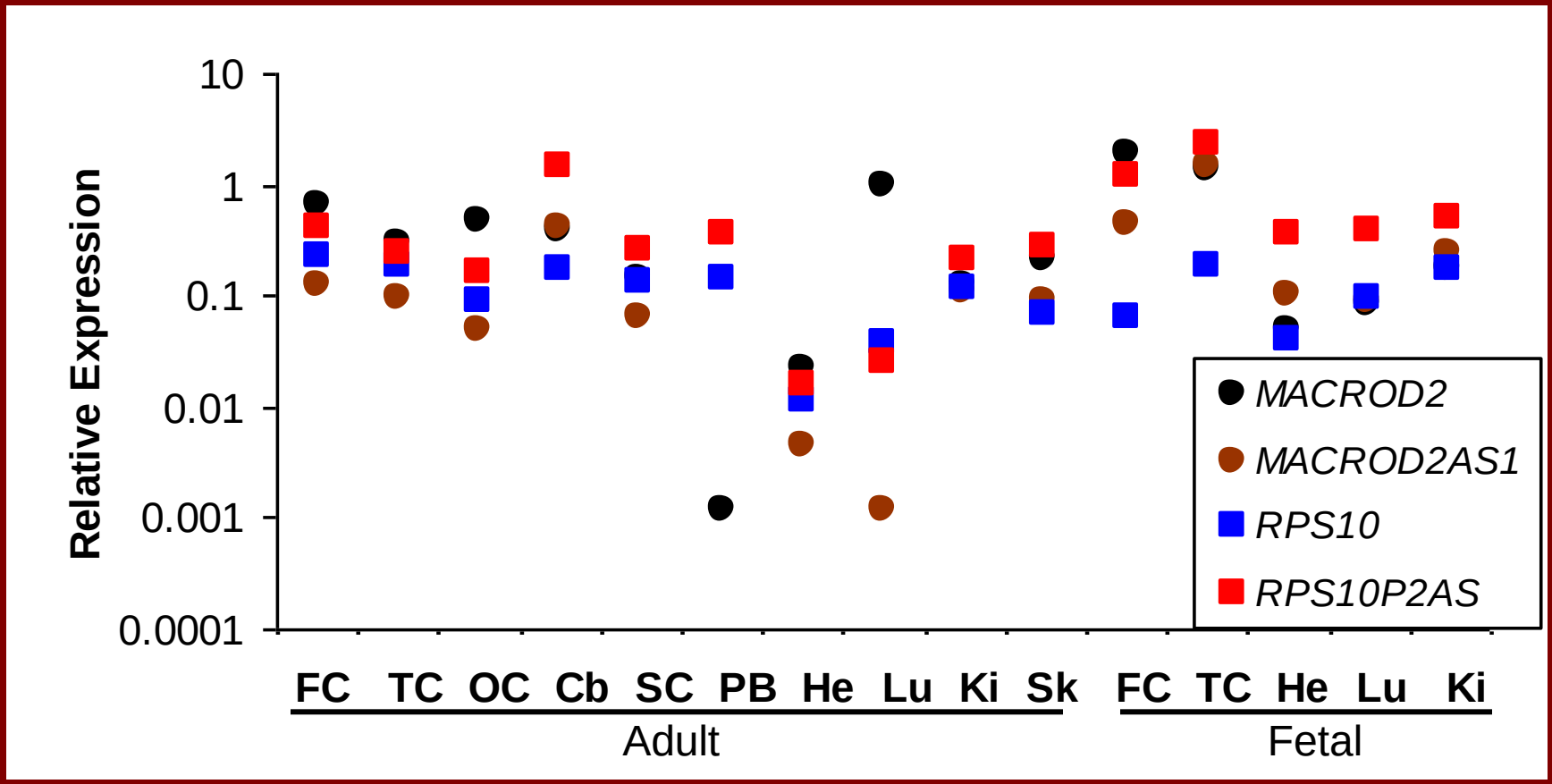
An Uncharacterized Long Non-Coding RNA is Highly Expressed Near rs4141463

RPS10P2AS

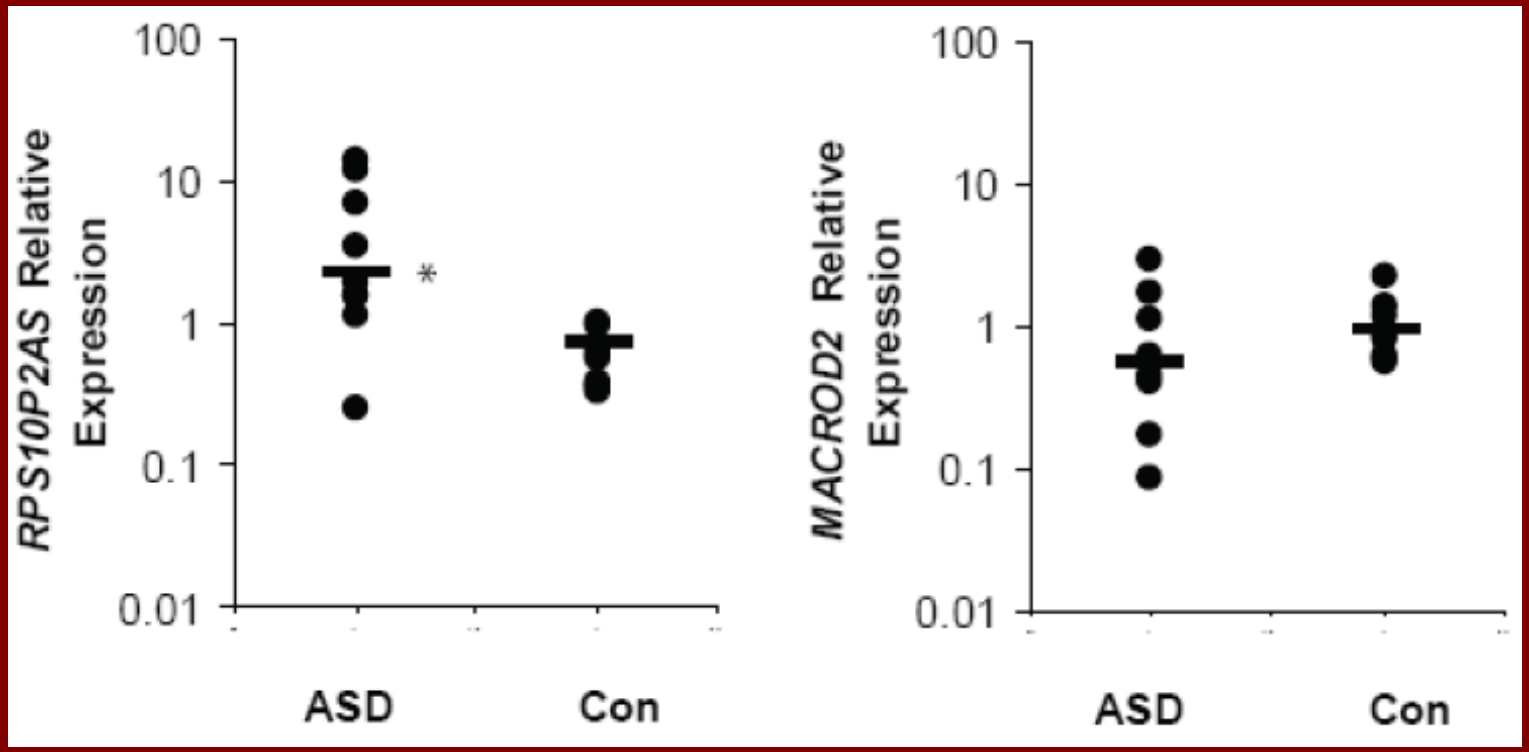
Ribosomal Protein S10 Pseudogene 2, Anti-sense

UCSC Genome Browser

Expression: The Uncharacterized *RPS10P2AS* is Highly Expressed in Multiple Tissues



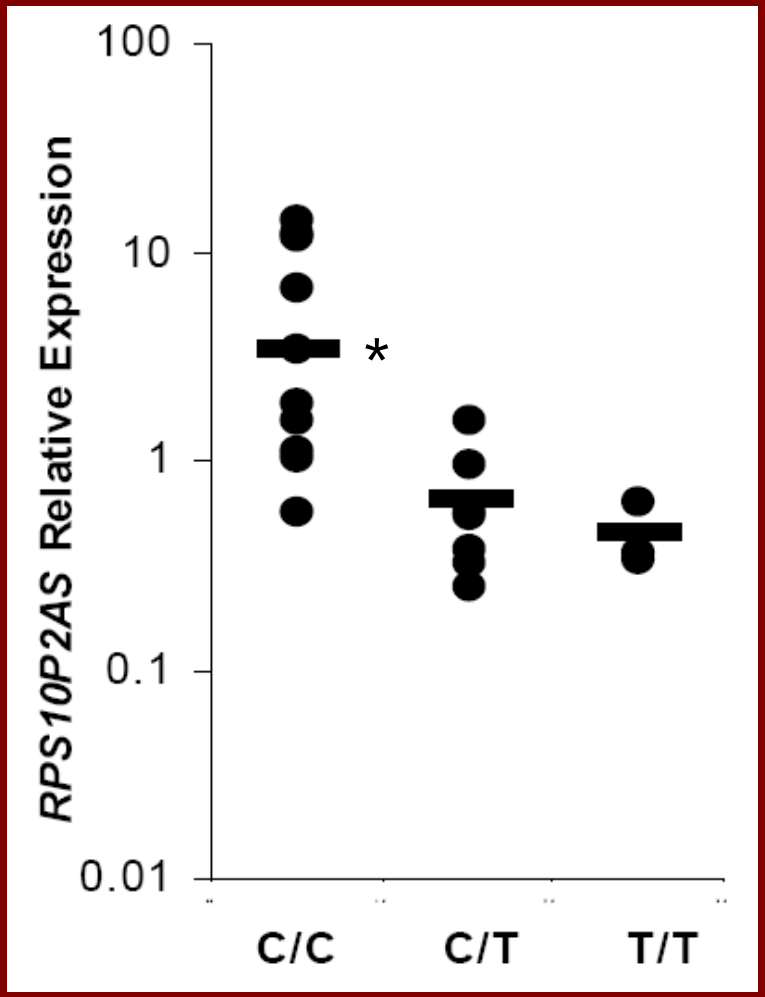
The Non-Coding RNA *RPS10P2AS* is Increased in Expression in Postmortem Autism Brain



RPS10P2AS
5-fold increase
P=0.002

MACROD2
No Sign Diff

Expression of the Non-Coding RNA *RPS10P2AS* is Correlated with the Autism-Associated rs4141463 C/C Genotype



RPS10P2AS Summary

- Under an autism GWAS peak
- Expression is increased in postmortem brains of individuals with autism
- Increased expression is correlated with the autism GWAS allele
- Function? …

Outline

- Introduction to Non-Coding RNAs
- Overview of Autism Genetics
- Genome-Wide Association Study results
  - Point to non-coding RNAs
- Exome Sequencing results
  - Point to a transcription factor that regulates … non-coding RNAs
- A New Type of Pharmacology Targets Non-Coding RNAs

Sciencexpress

Multiplex Targeted Sequencing Identifies Recurrently Mutated Genes in Autism Spectrum Disorders

Brian J. O'Roak,[1] Laura Vives,[1] Wenqing Fu,[1] Jarrett D. Egertson,[1] Ian B. Stanaway,[1] Ian G. Phelps,[2,3] Gemma Carvill,[2,3] Akash Kumar,[1] Choli Lee,[1] Katy Ankenman,[4] Jeff Munson,[4] Joseph B. Hiatt,[1] Emily H. Turner,[1] Roie Levy,[1] Diana R. O'Day,[2] Niklas Krumm,[1] Bradley P. Coe,[1] Beth K. Martin,[1] Elhanan Borenstein,[1,5,6] Deborah A. Nickerson,[1] Heather C. Mefford,[2,3] Dan Doherty,[2,3] Joshua M. Akey,[1] Raphael Bernier,[4] Evan E. Eichler,[1,7]* Jay Shendure[1]*

Exome sequencing studies of autism spectrum disorders (ASD) have identified many de novo mutations, but few recurrently disrupted genes. We therefore developed a modified molecular inversion probe method enabling ultra-low-cost candidate gene resequencing in very large cohorts. To demonstrate the power of this approach, we captured and sequenced 44 candidate genes in 2,446 ASD probands. We discovered 27 de novo events in 16 genes, 59% of which are predicted to truncate proteins or disrupt splicing. We estimate that recurrent disruptive mutations in six genes—*CHD8*, *DYRK1A*, *GRIN2B*, *TBR1*, *PTEN*, and *TBL1XR1*—may contribute to 1% of sporadic ASD. Our data support associations between specific genes and reciprocal subphenotypes (*CHD8*-macrocephaly, *DYRK1A*-microcephaly) and replicate the importance of a β-catenin/chromatin remodeling network to ASD etiology.

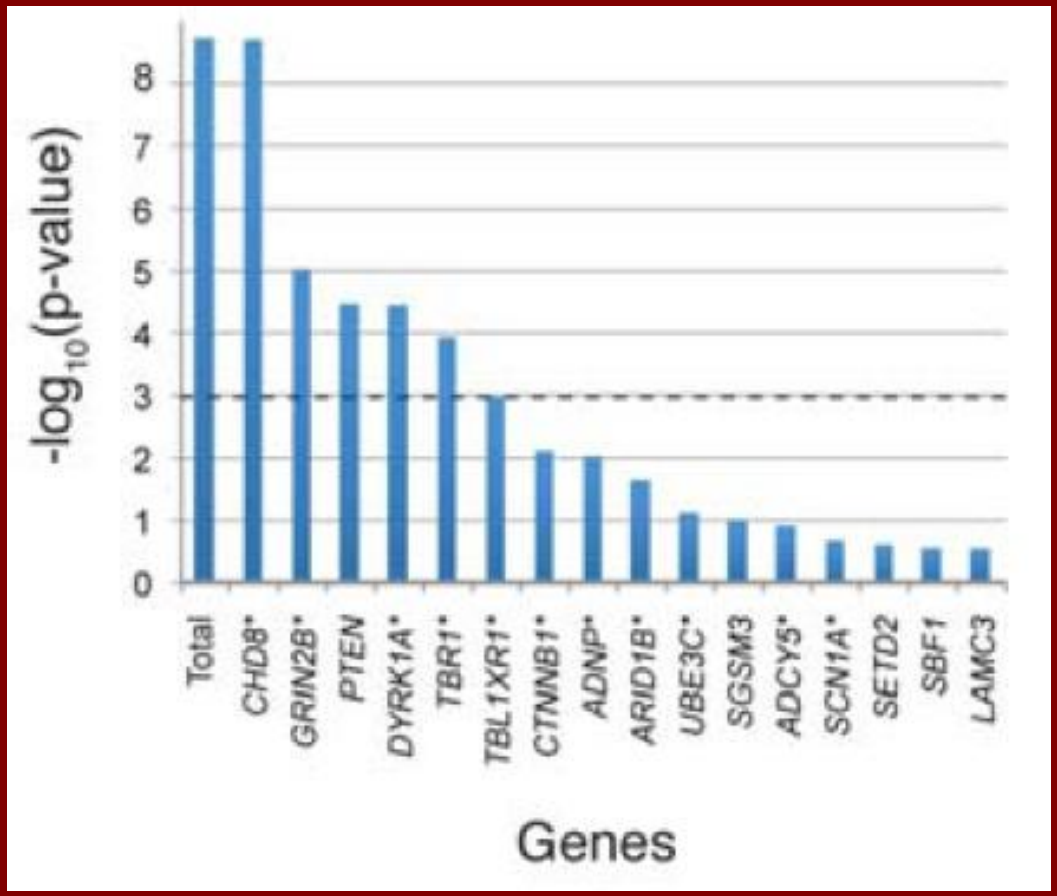
O'Roak et al. 2012 (Nov 15). *Science*.

CHD8: 11 *de novo* LoF mutations

Table 1. Six genes with recurrent de novo mutations. Abbreviations: M-male, F-female, Mut-mutation type, fs-frameshifting indel, ns-nonsense, sp-splice-site, aa-single amino acid deletion, ms-missense, HGVS-Human Genome Variation Society nomenclature; NVIQ-nonverbal intellectual quotient.

Proband	Sex	Gene	Mut	Assay‡	HGVS	NVIQ
12714.p1	M	CHD8*	ns	MIP	p.Ser62X	78
13986.p1	M	CHD8*	fs	MIP	p.Tyr747X	38
11654.p1	F	CHD8*	sp	MIP‖ (*4*)	c.3519-2A>G	41
13844.p1	M	CHD8*	ns	EX	p.Gln1238X	34
14016.p1	M	CHD8*	ns	MIP	p.Arg1337X	92
12991.p1	M	CHD8*	fs	MIP	p.Glu2103ArgfsX3	67
12752.p1	F	CHD8*	fs	EX	p.Leu2120ProfsX13	93
14233.p1	M	CHD8*	fs	MIP	p.Asn2371LysfsX2	19
14406.p1	M	CHD8*	aa	MIP	p.His2498del	98
12099.p1	M	DYRK1A*	fs	MIP‖ (*4*)	p.Ile48LysfsX2	55
13890.p1	F	DYRK1A*	sp	EX	c.1098+1G>A	42
13552.p1	M	DYRK1A*	fs	MIP¶ (*6*)	p.Ala498ProfsX94	66
11691.p1	M	GRIN2B†	fs	MIP§,‖ (*3*)	p.Ser34GlnfsX25	62
13932.p1	M	GRIN2B†	ms	MIP	p.Cys456Tyr	55
12547.p1	M	GRIN2B†	ns	MIP§	p.Trp559X	65
12681.p1	F	GRIN2B†	sp	EX	c.2172-2A>G	65
14433.p1	M	PTEN	ms	MIP	p.Thr131Ile	50
14611.p1	M	PTEN	fs	MIP	p.Cys136MetfsX44	33
11390.p1	F	PTEN	ms	EX	p.Thr167Asn	77
12335.p1	F	TBL1XR1*	ms	EX	p.Leu282Pro	47
14612.p1	M	TBL1XR1*	fs	MIP	p.Ile397SerfsX19	41
11480.p1	M	TBR1†	fs	EX	p.Ala136ProfsX80	41
13814.p1	M	TBR1†	ms	MIP	p.Lys228Glu	78
13796.p1	F	TBR1†	fs	MIP‖ (*4*)	p.Ser351X	63

*Part of 49-member connected component reported in (*3*). †Part of expanded 74-member connected component. ‡Primary assay that identified the variant. ‖¶Proband was exome sequenced by cited study and variant was ‖not reported or ¶reported. §Variant reported in MIP screen from (*3*).



… and association with autism $P<10^{-8}$

… note: next best genes have 3 *de novo* LGD

O'Roak et al. 2012. *Science*.

CHD8 = Chromodomain Helicase DNA-Binding Protein 8

- Known to … ?
  - *Chd8* knockout mouse is embryonic lethal before a brain appears (Nishiyama et al. 2004. *Mol Cell Biol*.)
  - CHD8 protein is known to interact with a handful of other proteins in cancer cells
    - Histone H1 and β-catenin (Nishiyama et al. 2012. *Mol Cell Biol*.)
    - Androgen Receptor (Menon et al. 2010. *Mol Endocrinology*.)
    - RNA Polymerase III (Yuan et al. 2009. *Mol Cell Biol.*)
- Bottom line: CHD8 interacts with multiple proteins, but its function has not been studied in the brain or neurons
- We found: *CHD8* over-expression in human neuronal cell lines increased expression of the non-coding RNA *MSNP1AS*

Outline

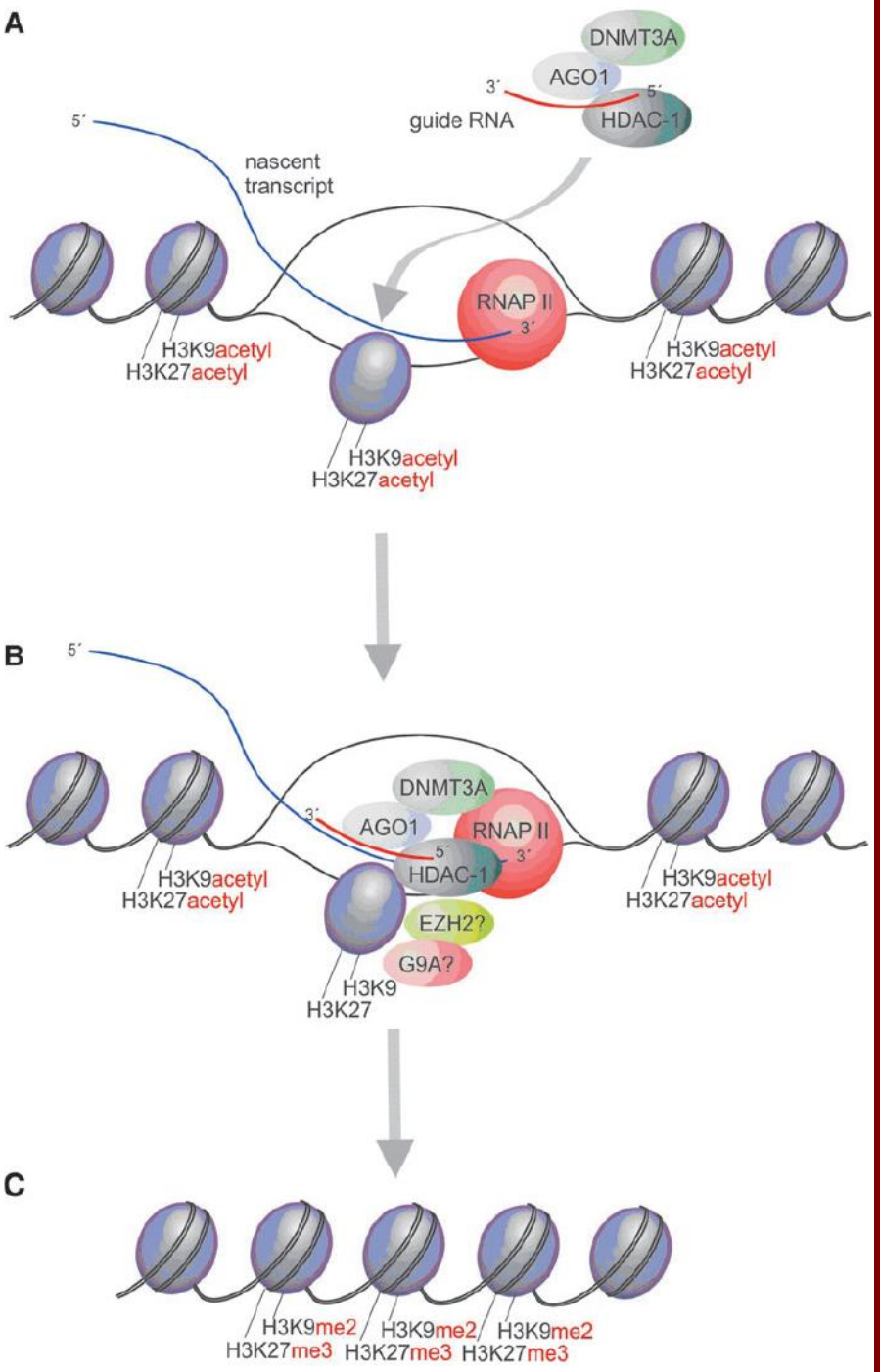
- Introduction to Non-Coding RNAs
- Overview of Autism Genetics
- Genome-Wide Association Study results
  - Point to non-coding RNAs
- Exome Sequencing results
  - Point to a transcription factor that regulates … non-coding RNAs
- A New Type of Pharmacology Targets Non-Coding RNAs

NUCLEIC ACID THERAPEUTICS
Volume 23, Number 1, 2013
© Mary Ann Liebert, Inc.
DOI: 10.1089/nat.2012.0412

Long Non-Coding RNA Targeting and Transcriptional De-Repression

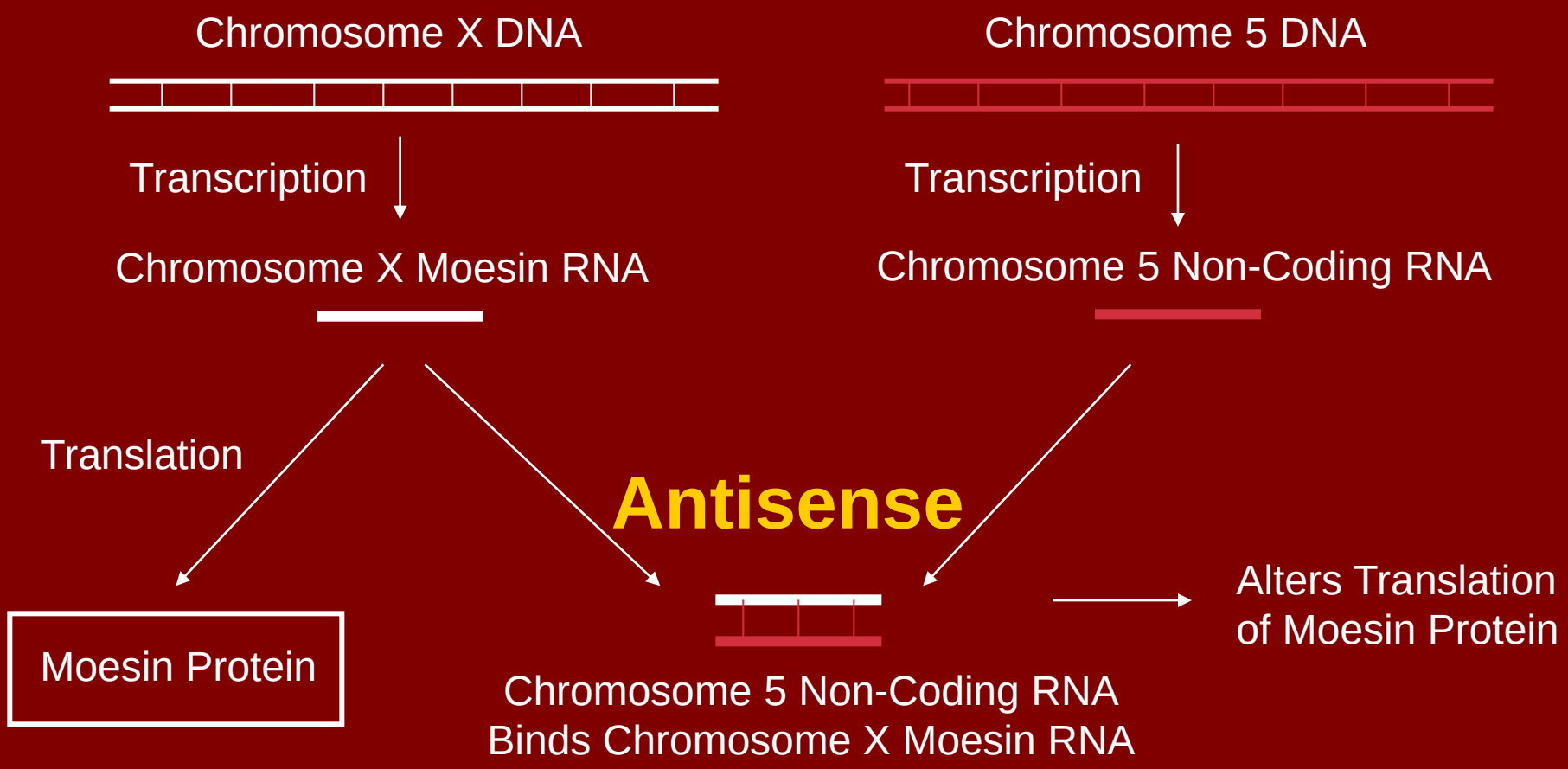
Marc S. Weinberg[1,2] and Kevin V. Morris[1,3]

Our current understanding of the molecular events that functionally characterize cellular biology continues to be revised. Recent observations find that the vast majority of the human genome is transcribed and may be functionally relevant. Many of these previously unrecognized transcripts, both short and long non-coding RNAs, have been found to be active modulators of protein coding gene function. While such observations were in the past relegated to imprinted genes, it is now becoming apparent that several different genes in differentiated cells may be under some form of non-coding RNA based regulatory control. Emerging evidence suggests that some of these long non-coding RNAs are functional in controlling gene transcription by the targeted recruitment of epigenetic silencing complexes to homology-containing loci in the genome. Most notably when these repressor non-coding RNAs are targeted using small RNA-based inhibitors (such as with RNA interference), a de-repression of the targeted gene can occur resulting in activation of gene expression. Knowledge of this emerging RNA based epigenetic regulatory network has implications not only in cellular evolution but also for the development of an entirely new area of pharmacology.

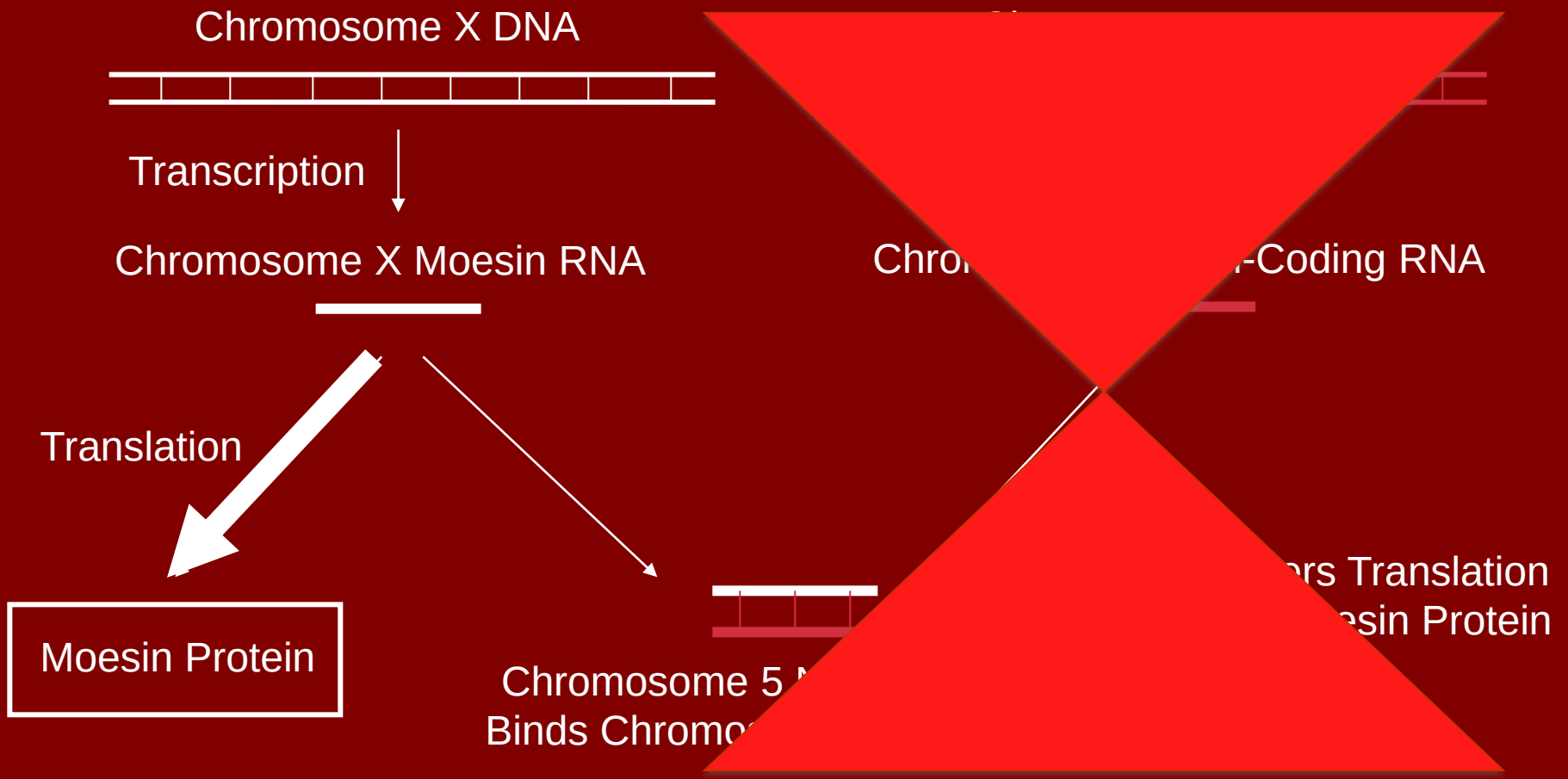


Weinberg & Morris. 2013. *Nucleic Acid Therapies*.

Proposed Mechanism of *MSNP1AS* Long Non-Coding RNA

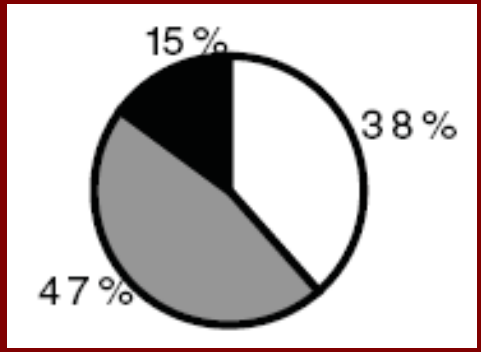


“Antisense to the Antisense”

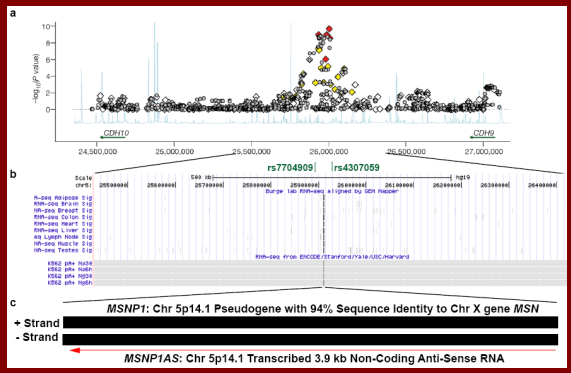


Conclusions

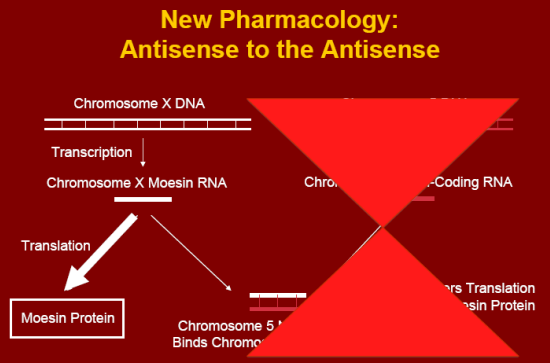
Non-coding RNAs are abundant in human brain



Non-coding RNAs are the functional elements revealed by autism GWAS



Non-coding RNAs are treatment targets in HIV, cancer, diabetes



The Team

Campbell Lab (ZNI):
Nicole Grepo
Brent Wilkinson
Jessica DeWitt
Grace Kim
Christina Zdawczyk
Ani Misirian
Emily Holmes
Sarah Danehower
Kasey Rivas
Tara Kerin
Anita Ramanathan
Elisabeth Rutledge
Ranjita Raghavan
Young Kim
Giovanni Dandekar
Elizabeth Cortez-Toledo
Rachel Marshall

USC Collaborators:
Pat Levitt
Heather Volk
Gerry Coetzee
Jim Knowles
Carlos Pato
Kai Wang
Oleg Evgrafov
Marcelo Coba
Barbara Thompson
Wange Lu
Rob McConnell
Carrie Breton

Outside Collaborators:
Kevin Morris (Scripps)
Judy Van de Water (UC-Davis)
Paul Ashwood (UC-Davis)
Isaac Pessah (UC-Davis)
Irva Hertz-Picciotto (UC-Davis)
Susan Swedo (NIMH)
Audrey Thurm (NIMH)
Lisa Croen (Kaiser Permanente)

Funding:
NIMH
Autism Speaks

Resources:
Autism Tissue Program