COGNITIVE DOMAINS FUNCTION COMPLEMENTATION

BY *NTNG* GENE PARALOGS

Pavel Prosselkov[1,2]*, Denis Polygalov[3], Qi Zhang[1], Thomas J. McHugh[3], and Shigeyoshi

Itohara[1]*

[1]Laboratory of Behavioral Genetics, RIKEN Brain Science Institute, Wakoshi, 351-0198

Saitama, Japan

[2]Graduate School, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo

University, 113-8657 Tokyo, Japan

[3]Laboratory of Circuit and Behavior Physiology, RIKEN Brain Science Institute, 351-0198

Saitama, Japan

*corresponding authors:

prosselkov@brain.riken.jp

sitohara@brain.riken.jp

Keywords: gene duplication, evolution, subfunctionalisation, cognitive complement

ABSTRACT

Gene duplication was proposed by S.Ohno (Ohno, 1970) as a key mechanism of a gene

function evolution. A pair of gene paralogs, *NTNG1* and *NTNG2*, sharing identical gene

and protein structures and encoding similar proteins, forms a functional complement

subfunctionalising (SF) within cognitive domains and forming cognitive

endophenotypes, as detected by Intellectual Quotient (IQ) tests (Prosselkov et al., 2015).

***NTNG* paralogs are associated with autism spectrum disorder (ASD), bipolar disorder**

(BD) and schizophrenia (SCZ), with unique non-overlapping segregation among the

other 15 cognitive disorders (CD), emphasizing an evolutionary gain-dependent link

between advanced cognitive functions and concomitant neurocognitive pathologies.

Complementary expression and human brain transcriptome composition of the

paralogs explains the observed phenomena of their functional complementarity. The

lowest identity among *NTNGs* is found in a middle of encoded by them proteins

designated as uknown (Ukd) domain. *NTNG1* contains anthropoid-specific constrained

regions and both genes contain non-coding conserved sequences underwent accelerated

evolution in human. *NTNG* paralogs SF perturbates "structure drives function" concept

at protein and gene levels. Their functions diversification result in a so-called

"Cognitive Complement (CC)" formation, a product of gene duplication and

subsequent cognitive subfunction bifurcation among *NTNG* gene duplicates.

INTRODUCTION

Gene duplication was proposed by S.Ohno (**Ohno, 1970**) as a key mechanism of a gene function evolution. Complex behaviors arise from a combination of simpler genetic modules that either have evolved separately or co-evolved. Many genes and the proteins they encode have been found to be involved in the cognitive information processing with a single variant or a single gene generally accounting for only a partial phenotypic variation in a complex trait. Cognitive processing as a quintessence of brain functioning can be viewed as a product of intricately interlinked networks generated by deeply embedded into it players with specific or partially overlapping functions. The robustness of the cognitive processing towards its single elements genetic eliminations (to study their function) and its simultaneous fragility expressed in the multiple forms of neurological disorders manifest the existence of cognitive domains interlocked but SF within a unit of cognition formed upon these domains interaction. Previously, we have described a function of a pair of gene paralogs (*NTNG1* and *NTNG2*) involved in human IQ tests performance which underwent hominin-specific evolutionary changes (**Prosselkov et al., 2015**). Hereby, we continue looking at these genes paralogs features focusing on underlying mechanisms of their function segregation and complementation within the cognitive domains.

RESULTS

The previously observed phenomena of functional complementation among the *NTNG* paralogs within cognitive domains (**Prosselkov et al., 2015**) is also manifested in *NTNG*-associated human pathologies diagnosed in most cases (if only not in all) by a cognitive decline (**Figure 1A-1** and **A-2**). Both genes are associated with BD and SCZ – devastating disorders sharing similar etiology (**Lee et al., 2013**) with genetic correlation by multivariate analysis of 0.590 (**Maier et al., 2015**), linked to human creativity (**Power et al., 2015**), and characterized by impulsiveness as a common diagnostic feature (**Reddy et al., 2014**). Recently found associations of both paralogs with ASD (**Sanders et al., 2015**) supports the reported genetic correlation of 0.194 ASD/SCZ pair (**Maier et al., 2015**) and shared module eigengenes detected by PC1 among these two disorders (**Parikshak et al., 2015**). 12 *NTNG1*-linked CDs, ranging from AD to TS, span a broad spectrum of clinical features frequently involving reduced processing speed (PS) and verbal comprehension (VC, **Figure 1A-1**). As for *NTNG2*, working memory (WM) deficit and inability "to bind" events (perceptual organization, PO) are the most prominent diagnostic traits for the SLE and TLE patients (**Figure 1A-2**), with PN characterised by indolent behavior in 90% of the cases (**Cavard et al., 2009**). Thus, both *NTNG* paralogs are associated with a variety of CDs and mostly in a non-overlapping manner, except for ASD, BD and SCZ characterized by shared and wide spectrum of cognitive abnormalities. The clinical etiology of the aforementioned diseases supports the IQ-deduced functional complementation among the *NTNG* paralogs (**Prosselkov et al., 2015**) with (VC/PS) and (WM/PO) deficits being also uniquely segregated among the associated cognitive pathologies.

Since both genes are expected to have identical gene exon/intron compositions but different in their intron lengths (**Yin et al., 2002**) we have reconstructed the paralogs transcriptomes by re-processing the publicly available RNA-seq dataset (**Wu et al., 2012**)

from healthy and SCZ human subjects superior temporal gyrus (STG) post-mortem brain

tissue (Supplementary Table 1a=**ST1a**). A difference is noted instantly at the total expression

levels (genes, exons, individual RNA transcripts) when two gene paralogs are compared

(**Figure 1B-1** and **B-2**). *NTNG2* amount (as a whole gene) is 5 times larger comparing to

NTNG1; exons (2-5) are 3 times, exons (8-9) are 18 times and exon 10 is 4 times higher

expressed for *NTNG2* than for *NTNG1*. The only two exons outlaying the prevailing amount

rule for the *NTNG2* mRNAs are exons 6 and 7, expressed nearly at the same absolute level as

for the *NTNG1* exon paralogs, making them highly underrepresented within the whole

NTNG2 transcriptome. Next, distinct non-alternating splicing modules are formed by exons

(2-5) for *NTNG1* (**Figure 1B-1**), while exons (4-5) and exons (8-9) for *NTNG2* (**Figure 1B-**

2). Two structurally identical RNA transcript paralogs (*NTNG1a* = G1a and *NTNG2a* = G2a)

have been found to exist in both *NTNG* transcriptomes with G2a being expressed at 8-9 times

higher level than G1a. *NTNG1* is uniformly presented across the all analysed 16 human

samples by 2 more protein coding RNAs (G1c and G1d, detected previously in mice brain,

Nakashiba et al., 2000) and by 2 non-coding intron (9-10) derived transcripts (**Figure 1B-1**).

At the same time, *NTNG2* transcriptome is comprised of one extra potentially coding RNA

(G2a-like with exon 2 spliced out but in-frame coding preserved) and 2 assumed to be non-

coding RNAs with exons 6 and 7 retained along with preceding and following them introns.

Quite interesting that these two latter transcripts are the only RNA species with *NTNG2* exon

6 and 7 retained (**Figure 1B-2**). Two more coding (G1f and G1n) and 4 more non-coding for

NTNG1 and 9 extra non-coding for *NTNG2* RNA species have been also assembled from the

available reads but due to inconsistency in their appearance across all 16 STG samples they

are not presented on the figure but summarized in the table (**Figure 1C**, for details refer to

ST1d). Summarising this, quantitative and qualitative complementary differences is a

prominent feature characterising the brain RNA transcriptome of human *NTNG* paralogs.

However, no significant changes at the transcription level of neither whole genes, nor

individual exons, nor reconstructed RNA transcripts have been found for SCZ and healthy

subjects.

Upon calling the presence of IQ-affecting SNPs (**Prosselkov et al., 2015**) across all

STG samples (**ST1c**) it has been revealed that 15 out of 16 subjects were positive for the T-

allele of rs2149171 (exon 4-nested), shown above to attenuate the WM score in SCZ patients,

making a comparison among the allele carrier *vs* non-carrier impossible. Four healthy and

three SCZ samples carry a T-allele of rs3824574 (exon 3-nested, non-affecting IQ), and 1

healthy and 1 SCZ sample each contains a C-allele of rs4915045 (exon10, non-coding part-

nested, and non-affecting IQ). Thus, among the eleven cognitive endophenotype-associated

SNPs only 3 were possible to call out of the available *NTNG* transcriptome.

Distinctly complementary nature of the *NTNG* paralogs segregation within

neurological disorders and RNA transcriptome usage in STG (**Figure 1**) has prompted us to

analyse both genes expression across the entire human brain. We have reconstructed both

genes expression profiles in the human brain areas over the life span from conception (pcw =

post-conception week) to mature age (30-40 yrs old) using the RNA-seq data from BrainSpan

(www.brainspan.org). Similarities and differences are easily noted when the age-dependent

phases of *NTNG1* and *NTNG2* expression profiles are matched (**Figure 2**). Based on the

visual inputs three distinct classifiers have been elaborated: 1. predominantly synchronous

(**Figure 2A(1-4))**, characteristic mostly for the cortical areas; 2. predominantly mixed and

asynchronous (**Figure 2B**), characteristic for the cerebellar cortex and subcortical formations;

and 3. anti-phasic (complementary, **Figure 2C**), characteristic for the MD of thalamus and

hippocampus. All analysed brain areas demonstrated an elevated level of *NTNG2* expression

in comparison to *NTNG1* except for thalamus (**Figure 2C**) with the largest difference

observed is at the time of birth (35-37 pcw) or soon after (4 mo) for the synchronous

classifiers (**Figure 2A**), oscillating increment values across the life span for the mixed (**Figure 2B**) and anti-phasic (**Figure 2C**) classifiers. It is quite intriguing to note that essentially all brain areas show a trend towards the expression difference being negated between the paralogs by reaching the mature age of 30-40 yrs old (nearly or above the mean age used for the IQ testing), except MD where the expression discrepancy is increased. Thus, the observed functional complementation among the *NTNG* paralogs is supported by the anatomical distribution of the genes in human brain and their expression pattern modality over the human subjects lifetime.

A direct comparison of the *NTNG* paralogs shows not only identical intron-exon gene structure (**Figure 1B-1, 2B-2**) but also closely matched exon sizes (**Figure 3A**). There are three exons of identical sizes (exons 4, 8 and 9), another three exons differed by one encoded aa (exons 3, 5 and 6) and there are exons of different sizes (exons 2, 7 and 10). In terms of size the largest difference among the genes is visually presented by the introns: intron (9-10) of *NTNG1* is 52.7 times larger its *NTNG2* paralogous intron with intron (6-7) of *NTNG1* being only 1.43-times larger pointing towards non-equilibria process of non-coding elements elaborations as the process of gene paralogs SF proceeded. Nevertheless, it can be generalised that in average all *NTNG1* introns are several times larger their *NTNG2* analogs (**Figure 3A**). We have shown previously that exons 6 and 7 are differentially used within the brain *NTNG* transcriptome (**Figure 1B-1** and **B-2**) and to explore their potential contribution into the paralogs SF we have built identity matrices with these exons excluded and included (but still producing in-frame existing transcripts, **Figure 3B-1** left and right panels, respectively). Exclusion of both exons from the full-lengths transcripts (thus converting *NTNG1m* to *NTNG1a* and *NTNG2b* to *NTNG2a*, respectively) increases the identity of DNA on 2% (a relatively large effect since both exons together represent only 7.22 and 9.69% of the total coding part of the full-length RNA transcripts, *NTNG1m* and *NTNG2b*, respectively).

This effect becomes even stronger when the encoded by these transcripts proteins are also

compared (**Figure 3B-2**). The spliced out Ukd protein domains (encoded by the exons 6 and

7) increases the proteins identity on 3.8% thus making the middle of both genes (and encoded

proteins) substantially more different among the both gene paralogs. To corroborate this

observation and to explore the importance of other protein parts we have directly compared

the sequences encoded by the full-length transcripts and producing Netrin-G1m and Netrin-

G2b (**Figure 3C**). Similarly to what has been shown on **Figure 3B-1** and **3B-2**, the lowest

identity (17.5%) is represented by the Ukd domain (encoded by the exons 6 and 7) and by the

preceding it exon 5 (a 3'-part of the LE1 domain). Two other areas also show a substantially

low identity, namely the N-terminus (it includes the protein secretory signal indicated by an

arrow) and the outmost C-terminus responsible for the unique feature of Netrin-Gs – the GPI

attachment. Thus, based on the percent identity comparisons among the Netrin-G paralogs it

can be predicted that there are several potential protein parts contributing to the paralogs SF.

As it has been reported by **Seiradake et al. (2011)**, identical gene and protein domain

compositions result in the identical structural motif with differences only in the spatial

arrangement of the loops facing the post-synaptic Netrin-G's interacting partners, NGL-1 and

NGL-2, respectively (**Figure 3D**). Loop I binding surfaces alignment (**Figure 3C**, blue color)

shows a high level of conservation (with at least 5 amino acids 100% conserved) among the

Netrin-G paralogs, indicating that it is unlikely to be responsible for the cognate ligand

binding specificity. Neither Loop II (**Figure 5C**, yellow color) nor Loop III (**Figure 5C**,

orange color) display a single conserved amino acid shared among the paralogous binding

interfaces (as it originally has been described in **Seiradake et al., 2011**). Thus the

complementary pattern of the pre-postsynaptic interactions mediated via specific Netrin-

G/NGL pairs is reflected in the reciprocally different sizes of the loops binding interfaces

representing another element of the *NTNG*-encoded protein paralogs SF.

DISCUSSION

Complementary contribution of *NTNG* paralogs into human cognitive pathologies.

Involvement of the pre-synaptically expressed axon-localised *NTNGs* in SCZ diagnosis

supports the established view of SCZ as a result of distorted trans-synaptic signaling (**Lips et**

al., 2012), with a recent study proving that axonal connectivity-associated genes form a

functional network visualisable by fMRI (**Richiardi et al., 2015**), and that brain connectivity

predicts the level of fluid intelligence (**Finn et al., 2015; Pamplona et al., 2015**). Both

NTNGs have been found to participate in the brain functional connectivity by the parcellated

connectome reconstruction (**Hawrylycs et al., 2015**). Most of the reported disease

associations link *NTNG1* to SCZ with a variety of other neurologic pathologies (15 in total,

Figure 1A-1), while *NTNG2* pathologic associations (6 in total, **Figure 1A-2**) are quite

limited to those affecting WM or PO. Among them is SLE frequently characterized by WM

deficit (**Shucard et al., 2011**) and also known to represent schizoid-type abnormalities

characteristic for autoimmune pathologies (**Guilloux et al., 2010**; **Eaton et al., 2006**).

Immune activation is known to lead to altered pre-pulse inhibition (a key diagnostic trait for

SCZ) reversed by antipsychotics (**Romero et al., 2007**). The three diseases associated with

both paralogs (ASD, BD and SCZ) are also a primary focus of the recently initiated

PsychENCODE project (**PsychENCODE et al., 2015**). It is also worth to mention the

resemblance of the reported disease associations with the behavioral phenotypes of *Ntng1*

and *Ntng2* gene knockout mice (**Zhang et al., 2016**).

A gene content associated with IQ score often relates to numerous diseases, such as

SCZ, ASD, depression, and others (see **Zhao et al., 2014** for ref.; **Johnson et al., 2015**).

Several genes associated with SCZ have undergone positive selection following the human

brain evolution (**Xu et al., 2015**). Despite the global network properties of the brain

transcriptome are highly conserved among the species there are robust human-specific

disease-associated modules (**Miller et al., 2010**) and human accelerated regions (HARs) - highly conserved parts of genome that underwent accelerated evolution in humans (**Pollard et al., 2006**). HARs can serve as genomic markers for human-specific traits underlying a recent acquisition of modern human cognitive abilities by brain (**Boyd et al., 2015**) but that also "might have led to an increase in structural instability… resulted in a higher risk for neurodegeneration in the aging brain" (**Zhou et al., 2015**), rendering our intellectual abilities genetically fragile (**Crabtree, 2013**) and resulting in a variety of CDs. The role genomic context, epistasis (**Hemani et al., 2014**), plays in the evolution and pathology is manifested by frequently found disease-causing alleles present in animals without obvious pathological symptoms for the host (**Jordan et al., 2015**). Any CD is characterized by general intellectual disability (GID) plus psychiatric symptoms. A genetic perturbation-exerted behavioral cognitive deficit (BCD) in an animal model organism is a poor match to a human CD *per se* due to very poor contextual resemblance between the human GID and animal BCD together with the absence of interpretable psychiatric symptoms. Usefulness of animals as psychiatric models is also compromised by the fact that transcriptome differences within species tissues is smaller than among the homologous tissues of different species (**Barbosa-Morais et al., 2012; Lin et al., 2014**). No wonder that the compounds that "cure" mice models consistently fail in human trials (discussed in **Hyman, 2014**).

***NTNG* paralogs brain transcriptome intrinsic complementarity and possible mechanism for the IQ-affecting mutation alleles effect.** There is no global change at the mRNA level between healthy subjects and SCZ patients (**Figure 1B**). This conclusion is supported by previously published works stating that globally altered mRNA expression of *NTNG1* or *NTNG2* is unlikely to confer disease susceptibility, at least in the temporal lobe (**Eastwood and Harrison, 2008**), and Brodmann's area (**Aoki-Suzuki et al., 2005**). However, the original paper-source of the STG samples RNA-seq along with many other genes (>1,000)

found that *NTNG1* (but not *NTNG2*) falls under the group of genes with significant

alternative promoter usage (**Wu et al., 2012**: **ST6**, *p*<9.05E-10 at FDR <0.5) and *NTNG2*

(but not *NTNG1*) clusters with genes (>700) with significant alternative splicing change (**Wu**

et al., 2012: **ST7**, *p*<6.15E-12 at FDR<0.5) when SCZ and controls are compared. Such

GWAS observation adds an extra layer of complementary regulation to both *NTNG* paralogs

on a top of the described in the results section complementary usage rule for the exons,

formed unspliced splicing modules, resulting transcripts and their comprising exons (**Figure**

1B). Based on the available RNA-seq dataset it was almost impossible to detect RNA with

the matched position of *NTNG* SNPs used for the IQ testing (**ST2c**) except for two coding

exons located (rs2149171 and rs3824574) and exon 10 non-coding area located but

transcribed rs4915045 (in 2 out of 16 samples). This fact points towards indirect effect of the

IQ-affecting mutation alleles potentially associated with shorter (secretable) isoforms

generation (**Prosselkov et al., unpublished**) lacking two of the most prominent *NTNG*

features: GPI-link and the Ukd domain through an aberrant splicing factor binding. The GPI-

link is a hallmark of Netrin-G family members (**Nakashiba et al., 2000, 2002**) and without it

the aberrant Netrin-G isoforms are likely to mimic the action of their releasable ancestry

molecules - netrins, still being able to bind to their cognate postsynaptic ligand – NGL but

without forming an axonal-postsynaptic contact. The Ukd domain of Netrin-G1, despite its

so-far unknown function, is involved in lateral binding to the pre-synaptically localised LAR

modulating the binding strength between NGL-1 and Netrin-G1 (**Song et al., 2013**). Work is

currently underway in search for a similar lateral interaction partner for the Netrin-G2 Ukd

domain (**Kim E, personal communications**). The inclusion of Ukd encoding exons 6 and 7

is regulated by the Nova splicing factor (**Ule et al., 2005**) affecting the cortex Netrin-G1 exon

7 but not exon 6, and, simultaneously, Netrin-G2 paralog exons exhibiting an opposite

pattern. In general, it is tempting to speculate that deregulation of *NTNG* transcripts

processing may have a role in the brain-controlled cognitive abilities and associated CDs. Supporting such notion, a decreased level of Netrin-G1c mRNA (exons 6-9 excluded, **Figure 1B-1**) has been reported for BD and SCZ (**Eastwood and Harrison, 2008**) with Netrin-G1d (exons 6 and 7 included but 8-9 excluded, **Figure 1B-1**) and Netrin-G1f (a secretable short isoform consisted of domain VI only and lacking the Ukd and GPI-link) being increased in BD, but not in SCZ, in anterior cingulated cortex (**Eastwood and Harrison, 2010**). Higher Netrin-G1d mRNA expression in fetal brain but low for the Netrin-G1c isoform in the human adult (**Eastwood and Harrison, 2008**) indicates different functionality of these two splice variants joggling with the Ukd domain inclusion. And, according to our other data, if Netrin-G1 Ukd-containing isoforms are the dominant isoforms in adult mouse brain, Netrin-G2 Ukd-containing isoforms are present only at the trace level (**Prosselkov et al., forthcoming**), resembling a similar transcriptome pattern for the human STG samples (**Figure 1B-1** and **B-2**). A similar "dynamic microexon regulation" associated with the protein interactome misregulation has been reported to be linked to ASD (**Irimia et al., 2014**).

Synchronous and complementary expression of *NTNG* paralogs in the human brain supports the IQ-associated cognitive endophenotypes. Influential parieto-frontal integration theory (P-FIT, **Jung and Haier, 2007**) states that general intelligence ("g") is dependent on multiple brain cortical areas such as dlPFC, Broca's and Wernicke's areas, somatosensory and visual cortices (**Colom et al., 2009**). Despite "g" is widely accepted as the only correlate of the intelligence, its unitary nature was challenged by (**Hampshire et al., 2012)** claiming had indentified two independent brain networks (for memory and for reasoning) responsible for the task performance, the idea later criticised for the employed data processing approach (**Haier et al., 2014**). Higher IQ scores (a composite surrogate of "g") have been reportedly associated with the fronto-parietal network (FPN) connectivity (**Song et al., 2008**; **Glascher et al., 2009**). High level of *NTNG* paralogs expression within

the cognition intensively loaded areas of the brain and the distinct patterns of expression

profiles (synchronous, asynchronous/mixed, and complementary, **Figure 2A**) support

associations of *NTNG1* and *NTNG2* with the recorded cognitive endophenotypes (**Prosselkov**

et al., 2015). Based on the expression patterning over the human life-span, among the total 16

analysed brain areas we found two falling under the same "anti-phasic (complementary)"

classifier (**Figure 2C**): HIP and MD. Adding more to that, MD is the only brain area (out of

the 16 presented) where *NTNG1* expression level exceeds that of *NTNG2* making it a

promising candidate for the phenomena of *NTNGs* SF explanation. Two other brain areas

classified by a synchronous paralogs expression deserve a special attention, dlPFC and mPFC

(**Figure 2A-4**). PFC circuitry has been known as a "hub of the brain's WM system" (**Kim et**

al., 2013; Markowitz et al., 2015), which acts through direct HIP afferents (**Spellman et al.,**

2015) and has many connections with other cortical and subcortical areas (**Riga et al., 2014**).

mPFC may function as an intelligence-control switchboard and lPFC, part of the FPN global

connectivity, predicts the WM performance and fluid intelligence (**Cole et al., 2012**).

Interactions of the auditory recognition information fed by the vPFC stream with the

sequence processing by the dorsal stream are crucial for the human language articulation

(**Skeide and Friederici, 2015; Thothathiri and Rattinger, 2015**). The fact that both *NTNG*

paralogs are extensively expressed across PFC (**Figure 2A-2 and A-4**) pinpoints this area as

a key for future molecular studies of the human-unique symbolic communications. And PFC

is not only implicated in many psychiatric disorders, including SCZ (**Gulsuner and**

McClellan, 2014; see also **Riga et al., 2014** for ref.), but is also the only brain structure

unique to primates without known homologs in the animal kingdom (**Wise, 2008**).

Evolution of the protein paralogs encoded by the *NTNGs*. Forkhead box P2 (FOXP2) – a

ubiquitously expressed transcription factor that has been reported to be linked to the

evolution of human language through T303N, N325S substitutions when compared to a

primate ortholog (**Enard et al., 2002**) and is 100% identical to Nea protein (**Krause et al.,**

2007). FOXP2 regulates expression of multiple genes in human and chimpanzee (**Konopka**

et al., 2009), and among them is an M3 gene brain module representative responsible for

general fluid cognitive abilities (**Johnson et al., 2015**), *LRRC4C*, a gene encoding NGL-1 − a

post-synaptic target of Netrin-G1. Similarly to FOXP2, Netrin-G1 is a 100% conserved

protein among the hominins with only 1 mutation found in chimpanzee which is absent in

marmoset (and other primates) and mice proteins (**Prosselkov et al., 2015**). On the other

hand, extinct hominins' Netrin-G2 relatively to modern human contains T346A point

mutation (as per current version of hg19), also found in primates and mouse and known as

rs4962173 (dbSNP missense mutation) representing an ancient substitution from Neandethal

genomes found in modern humans and reflecting a recent acquisition of the novel allele

around 5,300 yrs BC. Nothing is known regarding the functional significance of this mutation

but biochemically a substitution of alanine (A) on a polar threonine (T) could bring an extra

point of regulation, e.g. a phosphorylation or glycosylation (NetPhos2.0 (**Blom et al., 1999**)

assigns a low score for the T346 to be phosphorylated but NetOGlyc4.0 (**Steentoft et al.,**

2013) robustly predicts it to be glycosylated, **SM**). Another mutation S371A/V reflects a

selective sweep in Netrin-G2 protein from primates to hominins within a similar to T346A

functional context when a hydrophobic alanine (in chimpanzee, A)/valine (in marmoset, V) is

replaced by a polar serine (S) and a strong positive predictions for glycosylation but not

phosphorylation (**SM**). This poses a question whether these two human-specific protein

substitutions associate with advanced cognitive traits as they may represent a hidden layer of

poorly studied so far protein glycosylation-associated regulatome known to affect the brain

function and diseases (**Baenziger, 2012**; **Baenziger, 2013**). Adding more to this, T346 is

nested on exon 5 just 20 nu away from the affecting WM score rs2274855 (**Prosselkov et al.,**

2015), and, together with S371A/V, they are both located within the lowest percent identity

area (exons (5-7)) of Netrin-Gs (**Figure 3C**) and, proposedly, contributing to the *NTNG*

duplicates SF. There are at least three more protein parts potentially contributing to the gene

paralogs specialised function subdivision (based on the low identity scores, **Figure 3C**): the

secretory peptide, the GPI-link, and the outmost structurally elaborated unstructured loops (I-

III) responsible for the reciprocal binding of Netrin-Gs to their post-synaptic cognate partners,

NGL-1 or NGL-2, both containing a C-terminal PDZ-binding domain (**Kim et al., 2006**). An

interesting finding was reported in (**Arbuckle et al., 2010**) found a presence of SH3(PSD95)

domain binding site (required for the phosphatidylinositol-3-kinase recruitment) in mice

Netrin-G2 (100% identical to human) but not in Netrin-G1. The detected SH3 binding site

overlaps with the Netrin-G2-loop III responsible for the binding specificity to NGL-2

(**Seiradake et al., 2011; Soto et al., 2013; DeNardo et al., 2012**). A plausible working

hypothesis would be that while internalised (and being GPI-link naïve/immature) the pre-

synaptic Netrin-G2 is bound to SH3-PSD95 via loop III but as soon as being secreted

extracellularly (and being attached to the membrane) it is bound to post-synaptic NGL-2.

Corroborating this, in the absence of Netrin-G2 in the KO mice NGL-2 is unstable on the

post-synaptic surface and gets quickly internalised (**Zhang et al., 2016**). We can only

speculate regarding the potential importance of PSD-95(SH3)-Netrin-G2-NGL-2 scaffolding

loop interaction/competition but the ability for Netrin-G1 to bind to SH3 has not been

reported. Following this logic, Netrin-G1 should have a similar binding partner via loop II.

The overall identical structural scaffold among the Netrin-G paralogs (**Figure 3D**) is

likely to represent an anciently preserved one of the primordial protein (encoded by a single

gene in the primitive urochordate *C.intestinalis*) and its contribution to the process of SF

among the *NTNG* paralogs goes against the "structure drives function" concept. It looks like

that it is not the "structure" but rather the "evolution" itself that drives a selection for the best

structural (or unstructural in our case) fit out of the available frameworks provided by the

gene duplicates to fulfill the emerged functional demand in a new ecological niche. The

intricate variability of phenotype is grounded by the conserved nature of genotype and

constrained by the "structure-function" limitations of the coding DNA and is only possible

due to permissive evolutionary continuing elaborations of non-coding areas able to absorb the

most recently acquired elements (having a potential to become regulatory at some point, e.g.

like HAR5 (**Boyd et al., 2015**)) and carried over by neutral drift as proposed by Kimura but

for proteins (**Kimura, 1983**). At the same time, the multiple protein substitutions coinciding

with the SF labor segregation phenomena among the Netrin-G paralogs question their neutral

nature. Both of them undergo a purifying selection from mice to human through the reduction

in size of non-coding DNA (introns) and encoded proteins (the mice Netrin-G2 is 2 aa longer

its human ortholog) further contributing to the host-specific SF. Thus while the non-coding

sequences are used to explore the evolutionary space in time, the restrictive boundaries of the

paralogs SF are determined by the protein (unstructured) elements.

Molecular evolution of the Cognitive Complement (CC). Appearance of the neural crest

(**Abitua et al., 2012**), an event that "affected the chordate evolution in the unprecedented

manner" (**Green et al., 2015**), multipotent progenitor cells (**Stolfi et al., 2015**), and

neurogenic placodes (suggesting a chemosensory and neurosecretory activities, **Abitua et al.,**

2015) in first primitive urochordates/tunicates coincides with the presence of *Ntng* precursor

gene (ENSCING00000024925) later undergoing two rounds of duplication events in lamprey

and found to affect human cognitive abilities (**Prosselkov et al., 2015**). *NTNG* paralogs are

expressed in the human neural crest-forming cells with *NTNG2* 10 times stronger than

NTNG1 (**Rada-Iglesias et al., 2012**), both are differentially expressed in human comparing to

chimpanzee and rhesus monkey with *NTNG2* expression model showing stronger probability

than *NTNG1* (**Iskow et al., 2012**), and both are stronger expressed in human telencephalon

comparing to chimpanzee and macaque (**Konopka et al., 2012**). *NTNG1* has been classified

as a brain module hub gene "whose pattern fundamentally shifted between species" (**Hawrylycs et al., 2015**). Belonging to distinct modules of brain expression regulation (**Liu et al., 2012**, **Konopka et al., 2012**), *NTNGs* are classified as "genes with human-specific expression profiles" (**Liu et al., 2012**). The nearby gene ~260 kbp upstream of *NTNG2* is *MED27* (mediator of RNA polymerase II) has been proposed to be associated with the evolution of human-specific traits (**McLean et al., 2011**). *NTNG1* has also been reported among the "adaptive plasticity genes" (**Ghalambor et al., 2015**) potentiating rapid adaptive evolution in guppies (*NTNG2* was not found among the input RNA for analysis).

Complementarity among the *NTNG* paralogs and encoded by them proteins has been reported previously: brain expression complementary pattern (in almost self-exclusive manner) defined by the 5'-UTR-localised *cis*-regulatory elements (**Yaguchi et al., 2014**); complementary distribution within the hippocampal laminar structures (**Nishimura-Akiyoshi et al., 2007**); axon-dendrite synaptic ending resulting in differential control over the neuronal circuit plasticity (**Matsukawa et al., 2014**); mutually-exclusive binding pattern to post-synaptic partners, NGL-1 and NGL-2, dictated by the protein unstructural elements (**Seiradake et al., 2011**); alternative promoter usage *vs* alternative mRNA splicing (**Wu et al., 2012**) and increased coefficient of variation (CV, **ST1d**) for *NTNG1* expression but not *NTNG2* in SCZ patients (similar to **Zhang et al., 2015**); KO mice behavioral phenotypes and subcellular signaling partners complementarity (**Zhang et al., 2016**); "differential stability" brain modules expression (*NTNG1* is expressed in the dorsal thalamus (M11) as a hub gene (Pearson's 0.92) while *NTNG2* is in neocortex and claustrum module (M6, Pearson's 0.65)) (**Hawrylycs et al., 2015**); hypocretin neurons-specific expression of *NTNG1* (but not *NTNG2*) as a sleep modulator (**Yelin-Bekerman et al., 2015**); top-down *vs* bottom-up information flows gating in mice and differential responsiveness to neuronal stimuli (**Prosselkov et al., forthcoming**); and human IQ-compiling cognitive domains

complementation (**Prosselkov et al., 2015**). The current study reports the *NTNGs*

complementarity association with the CDs (**Figure 1A**); mRNA splicing pattern

complementary at the quantitative and qualitative levels via differential use of the middle-

located exons (**Figure 1B**); brain complementary oscillatory expression over the human life

span observed in the intensive cognitively loaded brain areas (**Figure 2**); AE of the paralogs-

segregated unique non-coding elements (**Figure 3A**); complementary pattern of the protein

orthologs (mice-to-human) protein sequence evolution. Such multi-level complementation is

likely to reflect a shared evolutionary origin from a single gene in a primitive vertebrate

organism 700 mln yrs ago and its subsequent functional segregation among the evolution-

generated gene duplicates in jawless fish, such as lamprey.

Occupying independent but intercalating functional niches, *NTNG1* and *NTNG2* do

not compensate but complement each other's function forming a "functional complement" of

genes. Half a billion yrs ago the doubled gene dosage led to the gradual SF and manifested in

a function complementation within the cognitive domains, at least in human. We would like

to coin such gene pair as a Cognitive Complement (CC).

CONCLUSION

The emerged functional redundancy, as an outcome of gene duplication, leads to function

subdivision and its bifurcation among the gene paralogs resulting in the paralogs SF. A

functional compensation is known to exist among the evolutionary unrelated genes but has

not been reported among the gene paralogs, more frequently characterized by the function

complementation. Gene paralogs structural identity (at both, gene and protein levels) does not

provide a substrate for functional compensation but rather for complementation, perturbating

"structure drives function" rule. A gene duplication event of a tunicate *NTNG* primordial

gene and the subsequent process of its function specialisation (driven by the new ecological

niches appearance and evolution) among the gene duplicates made them to SF into distinct

cognitive domains in a complementary manner forming a CC. In our forthcoming work we

are to describe how *Ntng* mice genes function resembles that of human orthologs (**Prosselkov**

et al., forthcoming).

MATERIALS AND METHODS

Human brain *NTNG* transcriptome reconstruction. Relates to **Figure 1B** and **1-C**. The

original source of the dataset was produced by (**Wu et al., 2012**: E-MATB-1030) and the

downloaded .bam files used for the re-processing are listed in **ST1a**. All reconstructed

transcripts are presented in **ST1d** standalone Excel file. Two samples were excluded from the

analysis due to failed "per base sequence quality" measure, and zero expression level for

NTNG1a and *NTNG1int(9-10)* otherwise consistently expressed throughout other samples

(**ST1b**). SAMtools software was used for the SNPs calling from the available RNA-seq

datasets (**ST1c**). For details refer to **SM**.

Human brain expression profiling for *NTNGs* across the life span. The original source of

data was www.brainspan.org. All available samples were initially included into the analysis

but two of them excluded at a later stage (MD for 12-13 pcw and mPFC for 16-19 pcw) due

to high deviation (6-7 times) from the mean for other replicas. The mean expression values

per each brain area as RPKM were plotted against the sampling age. Profiles classification

was done visually considering the trend over the all plotted points as an average.

***NTNG1* (*NTNG1m*) and *NTNG2* (*NTNG2b*) full-length mRNA transcripts assembly.**

Relates to **Figure 3B.** Human *NTNG1m* brain transcript has been reported previously

(**Meerabux et al., 2005**) and we have also confirmed its ortholog presence in the mice brain

via full-length cloning (**Prosselkov et al., unpublished**). Since NCBI contains only its partial

CDS (AY764265), we used the RNA-seq-generated exons (**Figure 1B**) to reconstruct its full-

length and to generate an ORF of the encoded Netrin-G1m. Similarly, human *NTNG2b* was

reconstructed from the RNA-seq dataset and from Ensemble as follows. Exon 5 sequence

was deduced from ENST00000372179, other exons were from ENST00000467453 (no

longer available on the current version of Ensemble) except for exon 6 deduced by running

three independent alignments against the human genomic DNA with the mice 3'-intron (5-6),

exon 6, and 5'-intron (6-7) concomitantly confirmed by the generated full-length ORF for

Netrin-G2b. The reconstructed protein was predicted to encode 587 amino acids, which is in

a close proximity to the mice netrin-G2b ortholog of 589 residues (**Prosselkov et al., forth.**).

Full-lengths gene structures of *NTNG* paralogs reconstruction. Relates to **Figure 3A.**

Both, the obtained above from the STG brain samples RNA-seq and the reconstructed full-

lengths transcripts carrying all stably expressed exons were used to confirm the intron-exon

junctions positioning for *NTNG1* and *NTNG2*. Due to observed variability in the intron (1-2)

and exon 10 sizes their boundaries were left unmarked.

SUPPLEMENTARY MATERIALS (**SM**)

Contain Supplementary Methods (RNA-seq of STG re-processing and SNPs detection) and

Supplementary Tables (**ST1a-d, ST2**) as a single compiled pdf file. Reconstructed RNA-seq

(.gtf) of the STG is presented as a standalone Excel file (**ST1d**). Also included: Netrin-G2b

predicted phosphorylation and *O*-glycosylation, Netrin-G1 *vs* Netrin-G2 Ukd alignment

(**McWilliam et al., 2013)**, predicted secretory peptide cleavage and GPI attachments.

ACKNOWLEDGEMENTS

This work was in part supported by the "Funding Program for World-Leading Innovative

R&D on Science and Technology (FIRST Program)" initiated by the Council for Science and

Technology Policy (CSTP), and KAKENHI 15H04290 from the Japan Society for the

Promotion of Science (JSPS).

COMPETING INTERESTS

Authors would like to express a lack of any competing interests associated with the work.

REFERENCES

Abitua PB, Wagner E, Navarrete IA, Levine M. 2012. Identification of a rudimentary neural

crest in a non-vertebrate chordate. *Nature* **492:**104-107. doi: 10.1038/nature11589.

Abitua PB, Gainous TB, Kaczmarczyk AN, Winchell CJ, Hudson C, Kamata K, Nakagawa

M, Tsuda M, Kusakabe, TG, Levine M. 2015. The pre-vertebrate origins of neurogenic

placodes. *Nature* **524:**462-465. doi: 10.1038/nature14657.

Akula N, Barb, J, Jiang, X, Wendland, JR, Choi, KH, Sen, SK, Hou, L, Chen, DTW, Laje, G,

Johnson, K, Lipska, BK, Kleinman, JE, Corrada-Bravo, H, Detera-Wadleigh, S, Munson,

PJ, McMahon, FJ. 2014. RNA-sequencing of the brain transcriptome implicates

dysregulation of neuroplasticity, circadian rhythms and GTPase binding in bipolar

disorder. *Molecular Psychiatry* **19:**1179-1185. doi: 10.1038/mp.2013.170.

Aoki-Suzuki M, Yamada K, Meerabux J, Iwayama-Shigeno Y, Ohba H, Iwamoto K, Takao

H, Toyota T, Suto Y, Nakatani N, Dean B, Nishimura S, Seki K, Kato T, Itohara S,

Nishikawa T, Yoshikawa, T. 2005. A Family-Based Association Study and Gene

Expression Analyses of Netrin-G1 and -G2 Genes in Schizophrenia. *Biological Psychiatry*

57:382–393. doi: 10.1016/j.biopsych.2004.11.022.

Arbuckle MI, Komiyama NH, Delaney A, Coba M, Garry EM, Rosie R, Allchorne AJ,

Forsyth LH, Bence M, Carlisle HJ, O'Dell TJ, Mitchell R, Fleetwood-Walker SM, Grant

SGN. 2010. The SH3 domain of postsynaptic density 95 mediates inflammatory pain

through phosphatidylinositol-3-kinase recruitment. *Embo Reports* **11:**473-478. doi:

10.1038/embor.2010.63.

Archer HL, Evans JC, Millar DS, Thompson PW, Kerr AM, Leonard H, Christodoulou J,

Ravine D, Lazarou L, Grove L, Verity C, Whatley SD, Pitz DT, Sampson JR, Clarke AJ.

2006. *NTNG1* mutations are a rare cause of Rett syndrome. *American Journal of Medical*

Genetics Part A **140A:**691-694. doi: 10.1002/ajmg.a.31133.

Ayalew M, Le-Niculescu H, Levey DF, Jain N, Changala B, Patel SD, Winiger E, Breier A,

Shekhar A, Amdur R, Koller D, Nurnberger JI, Corvin A, Geyer M, Tsuang MT, Salomon

D, Schork NJ, Fanous AH, O'Donovan MC, Niculescu AB. 2012. Convergent functional

genomics of schizophrenia: from comprehensive understanding to genetic risk prediction.

Molecular Psychiatry **17:**887-905. doi: 10.1038/mp.2012.37.

Baenziger JU. 2012. Moving the *O*-glycoproteome from form to function. *Proceedings of*

the National Academy of Sciences of the United States of America **109:**9672–9673. doi:

10.1073/pnas.1206735109.

Baenziger JU. 2013. *O*-mannosylation of cadherins. *Proceedings of the National Academy of*

Sciences   of   the   United   States   of   America   **110:**20858-20859.   doi:

10.1073/pnas.1321827111.

Barbosa-Morais NL, Irimia M, Pan Q, Xiong HY, Gueroussov S, Lee LJ, Slobodeniuc V,

Kutter C, Watt S, Çolak R, Kim T, Misquitta-Ali CM, Wilson MD, Kim PM, Odom DT,

Frey BJ, Blencowe BJ. 2012. The Evolutionary Landscape of Alternative Splicing in

Vertebrate Species. *Science* **338:**1587-1593. doi: 10.1126/science.1230612.

Bisgaard A-M, Rasmussen LN, Moller HU, Kirchhoff M, Bryndorf T. 2007. Interstitial

deletion of the short arm of chromosome 1 (1p13.1p21.1) in a girl with mental retardation,

short   stature   and   colobomata.   *Clinical   Dysmorphology*   **16:**109-112.   doi:

10.1097/01.mcd.0000228425.89660.bf.

Blom N, Gammeltoft S, Brunak S. 1999. Sequence and structure-based prediction of

eukaryotic protein phosphorylation sites. *Journal of Molecular Biology* **294:**1351-1362.

doi: 10.1006/jmbi.1999.3310.

Boraska V, Franklin CS, Floyd JAB, Thornton LM, Huckins LM, Southam L, Rayner NW,

Tachmazidou I, Klump KL, Treasure J, Lewis CM, Schmidt U, Tozzi F, Kiezebrink K,

Hebebrand J, Gorwood P, Adan RAH, Kas MJH, Favaro A, Santonastaso P, Fernández-

Aranda F, Gratacos M, Rybakowsk F, Dmitrzak-Weglarz M, Kaprio J, Keski-RahkonenA,

Raevuori A, Van Furth EF, Slof-Op 't Landt MCT, Hudson JI, Reichborn-Kjennerud T,

Knudsen GPS, Monteleone P, Kaplan AS, Karwautz A, Hakonarson H, Berrettini WH,

Guo Y, Li D, Schork NJ, Komaki G, Ando T, Inoko H, Esko T, Fischer K, Männik K,

Metspalu A, Baker JH, Cone RD, Dackor J, DeSocio JE, Hilliard CE, O'Toole JK, Pantel

J, Szatkiewicz JP, Taico C, Zerwas S, Trace SE, Davis OSP, Helder S, Bühren K,

Burghardt R, de Zwaan M, Egberts K, Ehrlich S, Herpertz-Dahlmann B, Herzog W,

Imgart H, Scherag A, Scherag S, Zipfel S, Boni C, Ramoz N, Versini A, Brandys MK,

Danner UN, de Kovel C, Hendriks J, Koeleman BPC, Ophoff RA, Strengman E, van

Elburg AA, Bruson A, Clementi M, Degortes D, Forzan M, Tenconi E, Docampo E,

Escaramís G, Jiménez-Murcia S, Lissowska J, Rajewski A, Szeszenia-Dabrowska N,

Slopien A, Hauser J, Karhunen L, Meulenbelt I, Slagboom PE, Tortorella A, Maj M,

Dedoussis G, Dikeos D, Gonidakis F, Tziouvas K, Tsitsika A, Papezova H, Slachtova L,

Martaskova D, Kennedy JL, Levitan RD, Yilmaz Z, Huemer J, Koubek D, Merl E,

Wagner G, Lichtenstein P, Breen G, Cohen-Woods S, Farmer A, McGuffin P, Cichon S,

Giegling I, Herms S, Rujescu D, Schreiber S, Wichmann H-E, Dina C, Sladek R,

Gambaro G, Soranzo N, Julia A, Marsal S, Rabionet R, Gaborieau V, Dick DM, Palotie A,

Ripatti S, Widén E, Andreassen OA, Espeseth T, Lundervold A, Reinvang I, Steen VM,

Le Hellard S, Mattingsdal M, Ntalla I, Bencko V, Foretova L, Janout V, Navratilova M,

Gallinger S, Pinto D, Scherer SW, Aschauer H, Carlberg L, Schosser A, Alfredsson L,

Ding B, Klareskog L, Padyukov L, Courtet P, Guillaume S, Jaussent I, Finan C, Kalsi G,

Roberts M, Logan DW, Peltonen L, Ritchie GRS, Barrett JC, The Wellcome Trust Case

Control Consortium 3, Estivill X, Hinney A, Sullivan PF, Collier DA, Zeggini E, Bulik

CM. 2014. A genome-wide association study of anorexia nervosa. *Molecular Psychiatry*

19:1084-1094. doi: 1085–1094. 10.1038/mp.2013.187.

Borg I, Freude K, Kubart SK, Hoffmann K, Menzel C, Laccone F, Firth H, Ferguson-Smith

MA, Tommerup N, Ropers HH, Sargan D, Kalscheuer VM. 2005. Disruption of Netrin G1

by a balanced chromosome translocation in a girl with Rett syndrome. *European Journal*

of Human Genetics **13:**921-927. doi: 10.1038/sj.ejhg.5201429.

Boyd JL, Skove SL, Rouanet JP, Pilaz L-J, Bepler T, Gordân R, Wray GA, Silver DL. 2015.

Human-Chimpanzee Differences in a FZD8 Enhancer Alter Cell-Cycle Dynamics in the

Developing Neocortex. *Current Biology* **25:**772-779. doi: 10.1016/j.cub.2015.01.041.

Cavard C, Audebourg A, Letourneur F, Audard V, Beuvon F, Cagnard N, Radenen, B,

Varlet P, Vacher-Lavenu M-C, Perret C, Terris B. 2009. Gene expression profiling

provides insights into the pathways involved in solid pseudopapillary neoplasm of the

pancreas. *Journal of Pathology* **218:**201-209. doi: 10.1002/path.2524.

Cole MW, Yarkoni T, Repovs G, Anticevic A, Braver TS. 2012. Global Connectivity of

Prefrontal Cortex Predicts Cognitive Control and Intelligence. *Journal of Neuroscience*

32:8988-8999. doi: 10.1523/jneurosci.0536-12.2012.

Colom R, Haier RJ, Head K, Álvarez-Linerac J, Ángeles Quiroga M, Shih PC, Jung RE.

2009. Gray matter correlates of fluid, crystallized, and spatial intelligence: Testing the P-

FIT model. *Intelligence* **37:**124-135. doi: 10.1016/j.intell. 2008.07.007.

Crabtree GR. 2013. Our fragile intellect. Part I. *Trends in Genetics* **29:**1-3. doi:

10.1016/j.tig.2012.10.002.

DeNardo LA, de Wit J, Otto-Hitt S, Ghosh A. 2012. NGL-2 Regulates Input-Specific

Synapse Development in CA1 Pyramidal Neurons. *Neuron* **76:**762-775. doi:

10.1016/j.neuron.2012.10.013.

D'Gama AM, Pochareddy S, Li M, Jamuar SS, Reiff RE, Lam A-TN, Sestan N, Walsh CA.

2015. Targeted DNA Sequencing from Autism Spectrum Disorder Brains Implicates

Multiple Genetic Mechanisms. *Neuron* **88:**910-917. doi: 10.1016/j.neuron.2015.11.009.

Eastwood SL, Harrison PJ. 2008. Decreased mRNA expression of netrin-G1 and netrin-G2

in the temporal lobe in schizophrenia and bipolar disorder. *Neuropsychopharmacology*

33:933-945. doi: 10.1038/sj.npp.1301457.

Eastwood SL, Harrison PJ. 2010. Markers of Glutamate Synaptic Transmission and

Plasticity Are Increased in the Anterior Cingulate Cortex in Bipolar Disorder. *Biological*

Psychiatry **67:**1010-1016. doi: 10.1016/j.biopsych.2009.12.004.

Eaton WW, Byrne M, Ewald H, Mors O, Chen CY, Agerbo E, Mortensen PB. 2006.

Association of schizophrenia and autoimmune diseases: Linkage of Danish National

Registers. *American Journal of Psychiatry* **163:**521-528. doi: 10.1176/appi.ajp.163.3.521.

Eisenhaber B, Bork P, Yuan YP, Loffler G, Eisenhaber F. 2000. Automated annotation of

GPI anchor sites: case study *C. elegans. Trends in Biochemical Sciences* **25:**340-341. doi:

10.1016/s0968-0004(00)01601-7.

Enard W, Przeworski M, Fisher SE, Lai CSL, Wiebe V, Kitano T, Monaco AP, Pääbo S.

2002. Molecular evolution of *FOXP2*, a gene involved in speech and language. *Nature*

418:869-872. doi: 10.1038/nature01025.

Finn ES, Shen X, Scheinost D, Rosenberg MD, Huang J, Chun MM, Papademetris X,

Constable RT. 2015. Functional connectome fingerprinting: identifying individuals using

patterns of brain connectivity. *Nature Neuroscience* **online.** doi:10.1038/nn.4135.

Forbes NF, Carrick LA, McIntosh AM, Lawrie SM. 2009. Working memory in

schizophrenia: a meta-analysis. *Psychological Medicine* **39:**889-905. doi:

10.1017/s0033291708004558.

Fukasawa M, Aoki M, Yamada K, Iwayama-Shigeno Y, Takao H, Meerabux J, Toyota T,

Nishikawa T, Yoshikawa T. 2004. Case-control association study of human netrin G1

gene in Japanese schizophrenia. *Journal of Medical and Dental Sciences* **51:** 121-128.

Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. 2015. Non-

adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature.

Nature **525:**372-375. doi: 10.1038/nature15256.

Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BWM, Willemsen MH,

Kwint M, Janssen IM, Hoischen A, Schenck A, Leach R, Klein R, Tearle R, Bo T, Pfundt

R, Yntema HG, de Vries BBA, Kleefstra T, Brunner HG, Vissers LELM, Veltman JA.

2014. Genome sequencing identifies major causes of severe intellectual disability. *Nature*

511:344-347. doi: 10.1038/nature13394.

Glascher J, Tranel D, Paul LK, Rudrauf D, Rorden C, Hornaday A, Grabowski T, Damasio

H, Adolphs R. 2009. Lesion Mapping of Cognitive Abilities Linked to Intelligence.

Neuron **61:**681-691. doi: 10.1016/j.neuron.2009.01.026.

Green SA, Simoes-Costa M, Bronner ME. 2015. Evolution of vertebrates as viewed from the

crest. *Nature* **520:**474-482. doi: 10.1038/nature14436.

Guilloux JP, Gaiteri C, Sibille E. 2010. Network analysis of positional candidate genes of

schizophrenia highlights ... more than ... myelin-related pathways. *Molecular Psychiatry*

15:786-788. doi: 10.1038/mp.2009.68.

Gulsuner S and McClellan JM. 2014. *De Novo* Mutations in Schizophrenia Disrupt Genes

Co-Expressed in Fetal Prefrontal Cortex. *Neuropsychopharmacology* **39:**238-239. doi:

10.1038/npp.2013.219.

Haier RJ, Karama S, Colom R, Jung R, Johnson W. 2014. A comment on "Fractionating

Intelligence" and the peer review process. *Intelligence* **46:**323–332. doi:

10.1016./j.intell.2014.02.007.

Hampshire A, Highfield RR, Parkin BL, Owen AM. 2012. Fractionating Human Intelligence.

Neuron **76:**1225-1237. doi: 10.1016/j.neuron.2012.06.022.

Hawrylycz M, Miller JA, Menon V, Feng D, Dolbeare T, Guillozet-Bongaarts AL, Jegga

AG, Aronow BJ, Lee C-K, Bernard A, Glasser MF, Dierker DL, Menche J, Szafer A,

Collman F, Grange P, Berman KA, Mihalas S, Yao Z, Stewart L, Barabási A-L, Schulkin

J, Phillips J, Ng L, Dang C, Haynor DR, Jones A, Van Essen DC, Koch C, Lein E. 2015.

Canonical genetic signatures of the adult human brain. *Nature Neuroscience* **18:**1832-

1844. doi: 10.1038/nn.4171.

Hemani G, Shakhbazov K, Westra H-J, Esko T, Henders AK, McRae AF, Yang J, Gibson G,

Martin NG, Metspalu A, Franke L, Montgomery GW, Visscher PM, Powell JE. 2014.

Detection and replication of epistasis influencing transcription in humans. *Nature*

508:249–253. doi: 10.1038/nature13005.

Hyman SE. 2014. How Far Can Mice Carry Autism Research? *Cell* **158:**13-14. doi:

10.1016/j.cell.2014.06.032.

Iossifov I, O'Roak BJ, Sanders SJ, Ronemus M, Krumm N, Levy D, Stessman HA,

Witherspoon KT, Vives L, Patterson KE, Smith JD, Paeper B, Nickerson DA, Dea J,

Dong S, Gonzalez LE, Mandell JD, Mane SM, Murtha MT, Sullivan CA, Walker MF,

Waqar Z, Wei L, Willsey AJ, Yamrom B, Lee Y, Grabowska E, Dalkic E, Wang Z, Marks

S, Andrews P, Leotta A, Kendall J, Hakker I, Rosenbaum J, Ma B, Rodgers L, Troge J,

Narzisi G, Seungtai Y, Schatz MC, Kenny Y, McCombie WR, Shendure J, Eichler EE,

State MW, Wigler M. 2014. The contribution of *de novo* coding mutations to autism

spectrum disorder. *Nature* **515:**216–221. doi: 10.1038/nature13908.

Irimia M, Weatheritt RJ, Ellis JD, Parikshak NN, Gonatopoulos-Pournatzis T, Babor M,

Quesnel-Vallières M, Tapial J, Raj B, O'Hanlon D, Barrios-Rodiles M, Sternberg MJE,

Cordes SP, Roth FP, Wrana JL, Geschwind DH, Blencowe BJ. 2014. A Highly Conserved

Program of Neuronal Microexons Is Misregulated in Autistic Brains. *Cell* **159:**1511-1523.

doi: 10.1016/j.cell.2014.11.035.

Ishigaki S, Masuda A, Fujioka Y, Iguchi Y, Katsuno M, Shibata A, Urano F, Sobue G, Ohno

K. 2012. Position-dependent FUS-RNA interactions regulate alternative splicing events

and transcriptions. *Scientific Reports* **2**: A529. doi: 10.1038/srep00529.

Iskow RC, Gokcumen O, Abyzov A, Malukiewicz J, Zhu Q, Sukumara AT, Pai AA, Mills

RE, Habegger L, Cusanovich DA, Rubel MA, Perry GH, Gerstein M, Stone AC, Gilade Y,

Lee C. 2012. Regulatory element copy number differences shape primate expression

profiles. *Proceedings of the National Academy of Sciences of the United States of America.*

109*:*12656–12661. doi: 10.1073/pnas.1205199109.

Johnson MR, Shkura K, Langley SR, Delahaye-Duriez A, Srivastava P, Hill WD, Rackham

OJL, Davies G, Harris SE, Moreno-Moral A, Rotival M, Speed D, Petrovski S, Katz A,

Hayward C, Porteous DJ, Smith BH, Padmanabhan S, Hocking LJ, Starr JM, Liewald DC,

Visconti A, Falchi M, Bottolo L, Rossetti T, Danis B, Mazzuferi M, Foerch P, Grote A,

Helmstaedter C, Becker AJ, Kaminski RM, Deary IJ, Petretto E. 2015. Systems genetics

identifies a convergent gene network for cognition and neurodevelopmental disease.

Nature Neuroscience **online**. doi: 10.1038/nn.4205.

Jordan DM, Frangakis SG, Golzio C, Cassa CA, Kurtzberg J, Task Force for Neonatal

Genomics, Davis EE, Sunyaev SR, Katsanis N. 2015. Identification of *cis*-suppression of

human disease muattions by comparative genomics. *Nature.* **524:**225-229. doi:

10.1038/nature14497.

Jung RE and Haier RJ. 2007. The Parieto-Frontal Integration Theory (P-FIT) of

intelligence: Converging neuroimaging evidence. *Behavioral and Brain Sciences* **30:**135-

187. doi: 10.1017/s0140525x07001185.

Kim J, Ghim J-W, Lee JH, Jung MW. 2013. Neural Correlates of Interval Timing in Rodent

Prefrontal Cortex. *Journal of Neuroscience* **33:**13834-13847. doi: 10.1523/jneurosci.1443-

13.2013.

Kim S, Burette A. Chung HS, Kwon SK, Woo J, Lee HW, Kim K, Kim H, Weinberg RJ,

Kim E. 2006. NGL family PSD-95-interacting adhesion molecules regulate excitatory

synapse formation. *Nature Neuroscience* **9:**1294-1301. doi: 10.1038/nn1763.

Kimura M. 1983. The neutral theory of molecular evolution. Cambridge University Press.

King IF, Yandava CN, Mabb AM, Hsiao JS, Huang HS, Pearson BL, Calabrese JM, Starmer J, Parker JS, Magnuson T, Chamberlain SJ, Philpot BD, Zylka MJ. 2013. Topoisomerases facilitate transcription of long genes linked to autism. *Nature* **501:**58-62. doi: 10.1038/nature12504.

Konopka G, Bomar JM, Winden K, Coppola G, Jonsson ZO, Gao FY, Peng S, Preuss TM, Wohlschlegel JA, Geschwind DH. 2009. Human-specific transcriptional regulation of CNS development genes by FOXP2. *Nature* **462:**213-217. doi: 10.1038/nature08549.

Konopka G, Friedrich T, Davis-Turak J, Winden K, Oldham MC, Gao F, Chen L, Wang G-Z, Luo R, Preuss TM, Geschwind DH. 2012. Human-Specific Transcriptional Networks in the Brain. *Neuron* **23:**601-617. doi: 10.1016/j.neuron.2012.05.034.

Krause J, Lalueza-Fox C, Orlando L, Enard W, Green RE, Burbano HA, Hublin J-J, Hänni C, Fortea J, de la Rasilla M, Bertranpetit J, Rosas A, Pääbo S. 2007. The derived *FOXP2* variant of modern humans was shared with neandertals. *Current Biology* **17:**1908-1912. doi: 10.1016/j.cub.2007.10.008.

Lagier-Tourenne C, Polymenidou M, Hutt KR, Vu AQ, Baughn M, Huelga SC, Clutario KM, Ling S-C, Liang TY, Mazur C, Wancewicz E, Kim AS, Watt A, Freier S, Hicks GG, Donohue JP, Shiue L, Bennett CF, Ravits J, Cleveland DW, Yeo GW. 2012. Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. *Nature Neuroscience* **15:**1488-1497. doi: doi:10.1038/nn.3230.

Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, Perlis RH, Mowry BJ, Thapar A, Goddard ME, Witte JS, Absher D, Agartz I, Akil H, Amin F, Andreassen OA, Anjorin A, Anney R, Anttila V, Arking DE, Asherson P, Azevedo MH, Backlund L, Badner JA, Bailey AJ, Banaschewski T, Barchas JD, Barnes MR, Barrett TB, Bass N, Battaglia A,

Bauer M, Bayés M, Bellivier F, Bergen SE, Berrettini W, Betancur C, Bettecken T,

Biederman J, Binder EB, Black DW, Blackwood DHR, Bloss CS, Boehnke M, Boomsma

DI, Breen G, Breuer R, Bruggeman R, Cormican P, Buccola NG, Buitelaar JK, Bunney

WE, Buxbaum JD, Byerley WF, Byrne EM, Caesar S, Cahn W, Cantor RM, Casas M,

Chakravarti A, Chambert K, Choudhury K, Cichon S, Cloninger CR, Collier DA, Cook E

H, Coon H, Cormand B, Corvin A, Coryell WH, Craig DW, Craig IW, Crosbie J, Cuccaro

ML, Curtis D, Czamara D, Datta S, Dawson G, Day R, De Geus EJ, Degenhardt F,

Djurovic S, Donohoe GJ, Doyle AE, Duan JB, Dudbridge F, Duketis E, Ebstein RP,

Edenberg HJ, Elia J, Ennis S, Etain B, Fanous A, Farmer AE, Ferrier IN, Flickinger M,

Fombonne E, Foroud T, Frank J, Franke B, Fraser C, Freedman R, Freimer NB, Freitag C

M, Friedl M, Frisén L, Gallagher L, Gejman PV, Georgieva L, Gershon ES, Geschwind

DH, Giegling I, Gill M, Gordon SD, Gordon-Smith K, Green EK, Greenwood TA, Grice

DE, Gross M, Grozeva D, Guan WH, Gurling H, De Haan L, Haines JL, Hakonarson H,

Hallmayer J, Hamilton SP, Hamshere ML, Hansen TF, Hartmann AM, Hautzinger M,

Heath AC, Henders AK, Herms S, Hickie IB, Hipolito M, Hoefels S, Holmans PA,

Holsboer F, Hoogendijk WJ, Hottenga JJ, Hultman CM, Hus V, Ingason A, Ising M,

Jamain S, Jones EG, Jones I, Jones L, Tzeng JY, Kähler AK, Kahn RS, Kandaswamy R,

Keller MC, Kennedy JL, Kenny E, Kent L, Kim Y, Kirov GK, Klauck SM, Klei L,

Knowles JA, Kohli MA, Koller DL, Konte B, Korszun A, Krabbendam L, Krasucki R,

Kuntsi J, Kwan P, Landen M, Långström N, Lathrop M, Lawrence J, Lawson WB,

Leboyer M, Ledbetter DH, Lee PH, Lencz T, Lesch KP, Levinson DF, Lewis CM, Li J,

Lichtenstein P, Lieberman JA, Lin DY, Linszen DH, Liu CY, Lohoff FW, Loo SK, Lord

C, Lowe JK, Lucae S, MacIntyre DJ, Madden PAF, Maestrini E, Magnusson PKE, Mahon

PB, Maier W, Malhotra AK, Mane SM, Martin CL, Martin NG, Mattheisen M, Matthews

K, Mattingsdal M, McCarroll SA, McGhee KA, McGough JJ, McGrath PJ, McGuffin P,

McInnis MG, McIntosh A, McKinney R, McLean AW, McMahon FJ, McMahon WM,

McQuillin A, Medeiros H, Medland SE, Meier S, Melle I, Meng F, Meyer J, Middeldorp

CM, Middleton L, Milanova V, Miranda A, Monaco AP, Montgomery GW, Moran JL,

Moreno-De-Luca D, Morken G, Morris DW, Morrow EM, Moskvina V, Muglia P,

Mühleisen TW, Muir WJ, Muller-Myhsok B, Murtha M, Myers RM, Myin-Germeys I,

Neale MC, Nelson SF, Nievergelt CM, Nikolov I, Nimgaonkar V, Nolen WA, Nöthen

MM, Nurnberger JI, Nwulia EA, Nyholt DR, O'Dushlaine C, Oades RD, Olincy A,

Oliveira G, Olsen L, Ophoff RA, Osby U, Owen MJ, Palotie A, Parr JR, Paterson AD,

Pato CN, Pato MT, Penninx BW, Pergadia ML, Pericak-Vance MA, Pickard BS, Pimm J,

Piven J, Posthuma D, Potash JB, Poustka F, Propping P, Puri V, Quested DJ, Quinn EM,

Ramos-Quiroga JA, Rasmussen HB, Raychaudhuri S, Rehnström K, Reif A, Ribasés M,

Rice JP, Rietschel M, Roeder K, Roeyers H, Rossin L, Rothenberger A, Rouleau G,

Ruderfer D, Rujescu D, Sanders AR, Sanders SJ, Santangelo SL, Sergeant JA, Schachar R,

Schalling M, Schatzberg AF, Scheftner WA, Schellenberg GD, Scherer SW, Schork NJ,

Schulze TG, Schumacher J, Schwarz M, Scolnick E, Scott LJ, Shi JX, Shilling PD, Shyn

SI, Silverman JM, Slager SL, Smalley SL, Smit JH, Smith EN, Sonuga-Barke EJS, St

Clair D, State M, Steffens M, Steinhausen HC, Strauss JS, Strohmaier J, Stroup TS,

Sutcliffe JS, Szatmari P, Szelinger S, Thirumalai S, Thompson RC, Todorov AA, Tozzi F,

Treutlein J, Uhr M, van den Oord EJCG, Van Grootheest G, Van Os J, Vicente AM,

Vieland VJ, Vincent JB, Visscher PM, Walsh CA, Wassink TH, Watson SJ, Weissman

MM, Werge T, Wienker TF, Wijsman EM, Willemsen G, Williams N, Willsey AJ, Witt

SH, Xu W, Young AH, Yu TW, Zammit S, Zandi PP, Zhang P, Zitman FG, Zöllner S,

International Inflammatory Bowel Disease Genetics Consortium (IIBDGC), Devlin B,

Kelsoe JR, Sklar P, Daly MJ, O'Donovan MC, Craddock N, Sullivan PF, Smoller JW,

Kendler KS, Wray NR. 2013. Genetic relationship between five psychiatric disorders

estimated from genome-wide SNPs. *Nature Genetics* **45:**984-994. doi: 10.1038/ng.2711.

Lesnick TG, Papapetropoulos S, Mash DC, Ffrench-Mullen J, Shehadeh L, de Andrade M,

Henley JR, Rocca WA, Ahlskog JE, Maraganore DM. 2007. A genomic pathway

approach to a complex disease: Axon guidance and Parkinson disease. *PLoS Genetics*

3:984-995. doi: 10.1371/journal.pgen.0030098.

Lin S, Lin Y, Nery JR, Urich MA, Breschi A, Davis CA, Dobin A, Zaleski C, Beer MA,

Chapman WC, Gingeras TR, Ecker JR, Snyder MP. 2014. Comparison of the

transcriptional landscapes between human and mouse tissues. *Proceedings of the National*

Academy of Sciences of the United States of America **111:**17224-17229. doi:

10.1073/pnas.1413624111.

Lips ES, Cornelisse LN, Toonen RF, Min JL, Hultman CM, the International Schizophrenia

Consortium, Holmans PA, O'Donovan MC, Purcell SM, Smit AB, Verhage M, Sullivan

PF, Visscher PM, Posthuma D. 2012. Functional gene group analysis identifies synaptic

gene groups as risk factor for schizophrenia. *Molecular Psychiatry* **17:**996-1006. doi:

10.1038/mp.2012.37.

Liu XL, Somel M, Tang L, Yan Z, Jiang X, Guo S, Yuan Y, He L, Oleksiak A, Zhang Y, Li

N, Hu YH, Chen W, Qiu ZL, Pääbo S, Khaitovich P. 2012. Extension of cortical synaptic

development distinguishes humans from chimpanzees and macaques. *Genome Research*

22:611-622. doi: 10.1101/gr.127324.111.

Maier R, Moser G, Chen G-B, Ripke S, Cross-disorder Working Group of the Psychiatric

Genomics Consortium, Coryell W, Potash JB, Scheftner WA, Shi J, Weissman MM,

Hultman CM, Landén M, Levinson DF, Kendler KS, Smoller JW, Wray NR, Lee SH.

2015. Joint Analysis of Psychiatric Disorders Increases Accuracy of Risk Prediction for

Schizophrenia, Bipolar Disorder, and Major Depressive Disorder. *The American Journal*

of Human Genetics **96:**283-294. doi: 10.1016/j.ajhg.2014.12.006.

Markowitz DA, Curtis CE, Pesaran B. 2015. Multiple component networks support working

memory in prefrontal cortex. *Proceedings of the National Academy of Sciences of the*

United States of America **112:**11084-11089. doi: 10.1073/pnas.1504172112.

Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, Reynolds AP,

Sandstrom R, Qu HZ, Brody J, Shafer A, Neri F, Lee K, Kutyavin T, Stehling-Sun S,

Johnson AK, Canfield TK, Giste E, Diegel M, Bates D, Hansen RS, Neph S, Sabo PJ,

Heimfeld S, Raubitschek A, Ziegler S, Cotsapas C, Sotoodehnia N, Glass I, Sunyaev SR,

Kaul R, Stamatoyannopoulos JA. 2012. Systematic Localization of Common Disease-

Associated Variation in Regulatory DNA. *Science* **337:**1190-1195. doi:

10.1126/science.1222794.

McLean CY, Reno PL, Pollen AA, Bassan AI, Capellini TD, Guenther C, Indjeian VB, Lim

XH, Menke DB, Schaar BT, Wenger AM, Bejerano G, Kingsley DM. 2011. Human-

specific loss of regulatory DNA and the evolution of human-specific traits. *Nature*

471:216-219. doi: 10.1038/nature09774.

McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N, Cowley AP, Lopez R. 2013.

Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Research* **41:**597-600.

doi: 10.1093/nar/gkt376.

Meerabux JMA, Ohba H, Fukasawa M, Suto Y, Aoki-Suzuki M, Nakashiba T, Nishimura S,

Itohara S, Yoshikawa T. 2005. Human netrin-G1 isoforms show evidence of differential

expression. *Genomics* **86:**112–116. doi: 10.1016/j.ygeno.2005.04.004.

Miller JA, Horvath S, Geschwind DH. 2010. Divergence of human and mouse brain

transcriptome highlights Alzheimer disease pathways. *Proceedings of the National*

Academy of Sciences of the United States of America **107:**12698-12703. doi:

10.1073/pnas.0914257107.

Nakashiba T, Ikeda T, Nishimura S, Tashiro K, Honjo T, Culotti JG, Itohara, S. 2000.

Netrin-G1: a Novel Glycosyl Phosphatidylinositol-Linked Mammalian Netrin That Is

Functionally Divergent from Classical Netrins. *The Journal of Neuroscience* **20:**6540-

6550.

Nakashiba T, Nishimura S, Ikeda T, Itohara S. 2002. Complementary expression and neurite

outgrowth activity of netrin-G subfamily members. *Mechanisms of Development* **111:**47-

60. doi: 10.1016/S0925-4773(01)00600-1.

Nakaya T, Alexiou P, Maragkakis M, Chang A, Mourelatos Z. 2013. FUS regulates genes

coding for RNA-binding proteins in neurons by binding to their highly conserved introns.

RNA **19:**498-509. doi: 10.1261/rna.037804.112.

Nectoux J, Girard B, Bahi-Buisson N, Prieur F, Afenjar A, Rosas-Vargas H, Chelly J,

Bienvenu T. 2007. *Netrin G1* mutations are an uncommon cause of atypical Rett

syndrome   with   or   without   epilepsy.   *Pediatric   Neurology*   **37:**270-274.   doi:

10.1016/j.pediatrneurol.2007.06.002.

Nishimura-Akiyoshi S, Niimi K, Nakashiba T, and Itohara S. 2007. Axonal netrin-Gs

transneuronally determine lamina-specific subdendritic segments. *Proceedings of the*

National Academy of Sciences of the United States of America **104:**14801-14806. doi:

10.1073/pnas.0706919104.

O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C,

Smith JD, Turner EH, Stanaway IB, Vernot B, Malig M, Baker C, Reilly B, Akey JM,

Borenstein E, Rieder MJ, Nickerson DA, Bernier R, Shendure J, Eichler EE. 2012a.

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

mutations. *Nature* **485:**246-250. doi: 10.1038/nature10989.

O'Roak BJ, Vives L, Fu WQ, Egertson JD, Stanaway IB, Phelps IG, Carvill G, Kumar A,

Lee C, Ankenman K, Munson J, Hiatt JB, Turner EH, Levy R, O'Day DR, Krumm N, Coe

BP, Martin BK, Borenstein E, Nickerson DA, Mefford HC, Doherty D, Akey JM, Bernier

R, Eichler EE, Shendure J. 2012b. Multiplex Targeted Sequencing Identifies Recurrently

Mutated Genes in Autism Spectrum Disorders. *Science* **338:**1619-1622. doi:

10.1126/science.1227764.

Ohno S. 1970. Evolution by Gene Duplication. Springer, New York.

Ohtsuki T, Horiuchi Y, Koga M, Ishiguro H, Inada T, Iwata N, Ozaki N, Ujike H, Watanabe

Y, Someya T, Arinami T. 2008. Association of polymorphisms in the haplotype block

spanning the alternatively spliced exons of the *NTNG1* gene at 1p13.3 with schizophrenia

in Japanese populations. *Neuroscience Letters* **435:**194-197. doi:

10.1016/j.neulet.2008.02.053.

Pamplona GSP, Neto GSS, Rosset SRE, Rogers BP, Salmon CEG. 2015. Analyzing the

association between functional connectivity of the brain and intellectual performance.

Frontiers in Human Neuroscience **9:**A61. doi: 10.3389/fnhum.2015.00061.

Pan Y, Fang M, Shen L, Wang L, Lv Y, Xi Z, Chen D, Li C, Wang X. 2010. Abnormal

expression of netrin-G2 in temporal lobe epilepsy neurons in humans and a rat model.

Experimental Neurology **224:**340-346. doi: 10.1016/j.expneurol.2010.04.001.

Parikshak NN, Gandal MJ, Geschwind DH. 2015. Systems biology and gene networks in

neurodevelopmental and neurodegenerative disorders. *Nature Reviews Genetics* **16:**441-

458. doi: 10.1038/nrg3934.

Petersen TN, Brunak S, von Heijne G, Nielsen H. 2011. SignalP 4.0: discriminating signal

peptides from transmembrane regions. *Nature Methods* **8:**785-786. doi:

10.1038/nmeth.1701.

Pollard KS, Salama SR, King B, Kern AD, Dreszer T, Katzman S, Siepel A, Pedersen JS,

Bejerano G, Baertsch R, Rosenbloom KR, Kent J, Haussler D. 2006. Forces shaping the

fastest evolving regions in the human genome. *PLoS Genetics* **2:**1599-1611. doi:

10.1371/journal.pgen.0020168.

Power RA, Steinberg S, Bjornsdottir G, Rietveld CA, Abdellaoui A, Nivard MM,
Johannesson M, Galesloot TE, Hottenga JJ, Willemsen G, Cesarini D, Benjamin DJ,
Magnusson PKE, Ullén F, Tiemeier H, Hofman A, van Rooij FJA, Walters GB,
Sigurdsson E, Thorgeirsson TE, Ingason A, Helgason A, Kong A, Kiemeney LA,
Koellinger P, Boomsma DI, Gudbjartsson D, Stefansson H, Stefansson K. 2015. Polygenic
risk scores for schizophrenia and bipolar disorder predict creativity. *Nature Neuroscience*
18:953-955. doi: 10.1038/nn.4040.

Preuss TM. 2012. Human brain evolution: From gene discovery to phenotype discovery.
Proceedings of the National Academy of Sciences of the United States of America
109:10709-10716. doi: 10.1073/pnas.1201894109.

Prosselkov P, Hashimoto R, Polygalov D, Kazutaka O, Zhang Q, McHugh TJ, Takeda M,
Itohara S. 2015. Cognitive Endophenotypes of modern and extinct hominins associated
with NTNG gene paralogs. *BioRxiv,* doi: 10.1101/034413.

Rada-Iglesias A, Bajpai R, Prescott S, Brugmann SA, Swigut T, Wysocka J. 2012.
Epigenomic Annotation of Enhancers Predicts Transcriptional Regulators of Human
Neural Crest. *Cell Stem Cell* **11:**1-16. doi: 10.1016/j.stem.2012.07.006.

Reddy LF, Lee J, Davis MC, Altshuler L, Glahn DC, Miklowitz DJ, Green MF. 2014.
Impulsivity and Risk Taking in Bipolar Disorder and Schizophrenia.
Neuropsychopharmacology **39:**456-463. doi: 10.1038/npp.2013.218.

Richiardi J, Altmann A, Milazzo A-C, Chang C, Chakravarty MM, Banaschewski T, Barker
GJ, Bokde ALW, Bromberg U, Büchel C, Conrod P, Fauth-Bühler M, Flor H, Frouin V,
Gallinat J, Garavan H, Gowland P, Heinz A, Lemaître H, Mann KF, Martinot J-L, Nees F,
Paus T, Pausova Z, Rietschel M, Robbins TW, Smolka MN, Spanagel R, Ströhle A,
Schumann G, Hawrylycz M, Poline J-B, Greicius MD, IMAGEN consortium. 2015.
Correlated gene expression supports synchronous activity in brain networks. *Science*

348:1241-1244. doi: 10.1126/science.1255905.

Riga D, Matos MR, Glas A, Smit AB, Spijker S, Van den Oever MC. 2014. Optogenetic

dissection of medial prefrontal cortex circuitry. *Frontiers in Systems Neuroscience* **8**:A230.

doi: 10.3389/fnsys.2014.00230.

Rogelj B, Easton LE, Bogu GK, Stanton LW, Rot G, Curk T, Zupan B, Sugimoto Y, Modic

M, Haberman N, Tollervey J, Fujii R, Takumi T, Shaw CE, Ule J. 2012. Widespread

binding of FUS along nascent RNA regulates alternative splicing in the brain. *Scientific*

Reports **2**:A603. doi: 10.1038/srep00603.

Romero E, Ali C, Molina-Holgado E, Castellano B, Guaza C, Borrell J. 2007.

Neurobehavioral and immunological consequences of prenatal immune activation in rats.

Influence   of   antipsychotics.   *Neuropsychopharmacology*   **32:**1791-1804.   doi:

10.1038/sj.npp.1301292.

Scharf JM, Yu D, Mathews CA, Neale BM, Stewart SE, Fagerness JA, Evans P, Gamazon E,

Edlund CK, Service SK, Tikhomirov A, Osiecki L, Illmann C, Pluzhnikov A,

Konkashbaev A, Davis LK, Han B, Crane J, Moorjani P, Crenshaw AT, Parkin MA, Reus

VI, Lowe TL, Rangel-Lugo M, Chouinard S, Dion Y, Girard S, Cath DC, Smit JH, King

RA, Fernandez TV, Leckman JF, Kidd KK, Kidd JR, Pakstis AJ, State MW, Herrera LD,

Romero R, Fournier E, Sandor P, Barr CL, Phan N, Gross-Tsur V, Benarroch F, Pollak Y,

Budman CL, Bruun RD, Erenberg G, Naarden AL, Lee PC, Weiss N, Kremeyer B, Berrío

GB, Campbell DD, Silgado JCC, Ochoa WC, Restrepo SCM, Muller H, Duarte AVV,

Lyon GJ, Leppert M, Morgan J, Weiss R, Grados MA, Anderson K, Davarya S, Singer H,

Walkup J, Jankovic J, Tischfield JA, Heiman GA, Gilbert DL, Hoekstra PJ, Robertson

MM, Kurlan R, Liu C, Gibbs JR, Singleton A for the North American Brain Expression

Consorium, Hardy J for the UK Human Brain Expression Database, Strengman E, Ophoff

RA, Wagner M, Moessner R, Mirel DB, Posthuma D, Sabatti C, Eskin E, Conti DV,

Knowles JA, Ruiz-Linares A, Rouleau GA, Purcell S, Heutink P, Oostra BA, McMahon

WM, Freimer NB, Cox NJ, Pauls DL. 2013. Genome-wide association study of Tourette's

syndrome. *Molecular Psychiatry* **18:**721-728. doi: 10.1038/mp.2012.69.

Seiradake E, Coles CH, Perestenko PV, Harlos K, McIlhinney RAJ, Aricescu AR, Jones EY.

2011. Structural basis for cell surface patterning through NetrinG–NGL interactions. The

EMBO Journal **30:**4479-4488. doi: 10.1038/emboj.2011.346.

Sanders SJ, He X, Willsey AJ, Ercan-Sencicek AG, Samocha KE, Cicek AE, Murtha MT,

Bal VH, Bishop SL, Dong S, Goldberg AP, Jinlu C, Keaney III JF, Klei L, Mandell JD,

Moreno-De-Luca D, Poultney CS, Robinson EB, Smith L, Solli-Nowlan T, Su MY, Teran

NA, Walker MF, Werling DM, Beaudet AL, Cantor RM, Fombonne E, Geschwind DH,

Grice DE, Lord C, Lowe JK, Mane SM, Martin DM, Morrow EM, Talkowski ME,

Sutcliffe JS, Walsh CA, Yu TW, Autism Sequencing Consortium, Ledbetter DH, Martin

CL, Cook EH, Buxbaum JD, Daly MJ, Devlin B, Roeder K, State MW. 2015. Insights into

Autism Spectrum Disorder Genomic Architecture and Biology from 71 Risk Loci. *Cell*

87:1215–1233. doi: 10.1016/j.neuron.2015.09.016.

Shucard JL, Lee WH, Safford AS, Shucard DW. 2011. The Relationship Between

Processing Speed and Working Memory Demand in Systemic Lupus Erythematosus:

Evidence From a Visual N-Back Task. *Neuropsychology* **25:**45-52. doi:

10.1037/a0021218.

Song M, Zhou Y, Li J, Liu Y, Tian L, Yu C, Tianzi, J. 2008. Brain spontaneous functional

connectivity and intelligence. *Neuroimage* **41:**1168-1176. doi:

10.1016/j.neuroimage.2008.02.036.

Skeide MA and Friederici AD. 2015. Response to Bornkessel-Schlesewsky *et al.* - towards

a nonhuman primate model of language? *Trends in Cognitive Sciences.* **9:**483. doi:

10.1016/j.tics.2015.05.011.

Song YS, Lee H-J, Prosselkov P, Itohara S, Kim E. 2013. Trans-induced cis interaction in the

tripartite NGL-1, netrin-G1 and LAR adhesion complex promotes development of

excitatory synapses. *Journal of Cell Science* **126:**4926-4938. doi: 10.1242/jcs.129718.

Soto F, Watkins KL, Johnson RE, Schottler F, Kerschensteiner D. 2013. NGL-2 Regulates

Pathway-Specific Neurite Growth and Lamination, Synapse Formation, and Signal

Transmission in the Retina. *Journal of Neuroscience* **33:**11949-11959. doi:

10.1523/jneurosci.1521-13.2013.

Spellman T, Rigotti M, Ahmari SE, Fusi S, Gogos JA, Gordon JA. 2015. Hippocampal–

prefrontal input supports spatial encoding in working memory. *Nature* **522:**309-314. doi:

10.1038/nature14445.

Steentoft C, Vakhrushev SY, Joshi HJ, Kong Y, Vester-Christensen MB, Schjoldager KTBG,

Lavrsen K, Dabelsteen S, Pedersen NB, Marcos-Silva L, Gupta R, Bennett EP, Mandel U,

Brunak S, Wandall HH, Levery SB, Clausen H. 2013. Precision mapping of the human *O*-

GalNAc glycoproteome through SimpleCell technology. *Embo Journal* **32:**1478-1488.

doi: 10.1038/emboj.2013.79.

Stepanyan A, Zakharyan R, Boyajyan A. 2013. The netrin G1 gene rs628117 polymorphism

is   associated   with   ischemic   stroke.   *Neuroscience   Letters*   **549:**74-77.   doi:

10.1016/j.neulet.2013.05.066.

Stewart SE, Yu D, Scharf JM, Neale BM, Fagerness JA, Mathews CA, Arnold PD, Evans

PD, Gamazon ER, Osiecki L, McGrath L, Haddad S, Crane J, Hezel D, Illman C,

Mayerfeld C, Konkashbaev A, Liu C, Pluzhnikov A, Tikhomirov A, Edlund CK, Rauch

SL, Moessner R, Falkai P, Maier W, Ruhrmann S, Grabe HJ, Lennertz L, Wagner M,

Bellodi L, Cavallini MC, Richter MA, Cook EH, Kennedy JL, Rosenberg D, Stein DJ,

Hemmings SMJ, Lochner C, Azzam A, Chavira DA, Fournier E, Garrido H, Sheppard B,

Umana P, Murphy DL, Wendland JR, Veenstra-VanderWeele J, Denys D, Blom R,

Deforce D, Van Nieuwerburgh F, Westenberg HGM, Walitza S, Egberts K, Renner T,

Miguel EC, Cappi C, Hounie AG, do Rosário MC, Sampaio AS, Vallada H, Nicolini H,

Lanzagorta N, Camarena B, Delorme R, Leboyer M, Pato CN, Pato MT, Voyiaziakis E,

Heutink P, Cath DC, Posthuma D, Smit JH, Samuels J, Bienvenu OJ, Cullen B, Fyer AJ,

Grados MA, Greenberg BD, McCracken JT, Riddle MA, Wang Y, Coric V, Leckman JF,

Bloch M, Pittenger C, Eapen V, Black DW, Ophoff RA, Strengman E, Cusi D, Turiel M,

Frau F, Macciardi F, Gibbs JR, Cookson MR, Singleton A for the North American Brain

Expression Consorium, Hardy J for the UK Human Brain Expression Database, Crenshaw

AT, Parkin MA, Mirel DB, Conti DV, Purcell S, Nestadt G, Hanna GL, Jenike MA,

Knowles JA, Cox N, Pauls DL. 2013. Genome-wide association study of obsessive-

compulsive disorder. *Molecular Psychiatry* **18:**788-798. doi: 10.1038/mp.2012.85.

Stolfi A, Ryan K, Meinertzhagen IA, Christiaen L. 2015. Migratory neuronal progenitors

arise from the neural plate borders in tunicates. *Nature* **527:**371-374. doi:

10.1038/nature15758.

The Japanese Schizophrenia Sib-Pair Linkage Group (JSSLG), Arinami T, Ohtsuki T,

Ishiguro H, Ujike H, Tanaka Y, Morita Y, Mineta M, Takeichi M, Yamada S, Imamura A,

Ohara K, Shibuya H, Ohara, K, Suzuki Y, Muratake T, Kaneko N, Someya T, Inada T,

Yoshikawa T, Toyota T, Yamada K, Kojima T, Takahashi S, Osamu O, Shinkai T,

Nakamura M, Fukuzako H, Hashiguchi T, Niwa S, Ueno T, Tachikawa H, Hori T, Asada T,

Nanko S, Kunugi H, Hashimoto R, Ozaki N, Iwata N, Harano M, Arai H, Ohnuma T,

Kusumi I, Koyama T, Yoneda H, Fukumaki Y, Shibata H, Kaneko S, Higuchi H, Yasui-

Furukori N, Numachi Y, Itokawa M, Okazaki Y. 2005. Genomewide high-density SNP

linkage analysis of 236 Japanese families supports the existence of schizophrenia

susceptibility loci on chromosomes 1p, 14q, and 20p. *American Journal of Human*

Genetics **77:**937-944. doi: 10.1086/498122.

The PsychENCODE Consortium, Akbarian S, Liu C, Knowles JA, Vaccarino FM, Farnham

PJ, Crawford GE, Jaffe AE, Pinto D, Dracheva S, Geschwind DH, Mill J, Nairn AC,

Abyzov A, Pochareddy S, Prabhakar S, Weissman S, Sullivan PF, State MW, Weng Z,

Peters MA, White KP, Gerstein MB, Senthil G, Lehner T, Sklar P, Sestan N. 2015. The

PsychENCODE project. *Nature Neuroscience* **18:**1707-1712. doi: 10.1038/nn.4156.

Thothathiri M. and Rattinger M. 2015. Ventral and dorsal streams for choosing word order

during sentence production. *Proceedings of the National Academy of Sciences* **112:**15456-

15461. doi: 10.1073/pnas.1514711112.

Ule J, Ule A, Spencer J, Williams A, Hu JS, Cline M, Wang H, Clark T, Fraser C, Ruggiu M,

Zeeberg BR, Kane D, Weinstein JN, Blume J, Darnell RB. 2005. Nova regulates brain-

specific splicing to shape the synapse. *Nature Genetics* **37:**844-852. doi: 10.1038/ng1610.

van Kuilenburg ABP, Meijer J, Mul ANPM, Hennekam RCM, Hoovers JMN, de Die-

Smulders CEM, Weber P, Mori AC, Bierau J., Fowler B, Macke K, Sass JO, Meinsma R,

Hennermann JB, Miny P, Zoetekouw L, Vijzelaar R, Nicolai J, Ylstra, B, Rubio-Gozalbo

ME. 2009. Analysis of severely affected patients with dihydropyrimidine dehydrogenase

deficiency reveals large intragenic rearrangements of *DPYD* and a de novo interstitial

deletion del(1)(p13.3p21.3). *Human Genetics* **125:**581-590. doi: 10.1007/s00439-009-

0653-6.

Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, Mill J, Cantor RM,

Blencowe BJ, Geschwind DH. 2011. Transcriptomic analysis of autistic brain reveals

convergent molecular pathology. *Nature* **474:** 380-384. doi: 10.1038/nature10110.

Wang K, Zhang H, Bloss CS, Duvvuri V, Kaye W, Schork NJ, Berrettini W, Hakonarson H,

the Price Foundation Collaborative Group. 2011. A genome-wide association study on

common SNPs and rare CNVs in anorexia nervosa. *Molecular Psychiatry* **16:**949-959.

doi: 10.1038/mp.2010.107.

Wilcox JA and Quadri S. 2014. Replication of NTNG1 association in schizophrenia.

Psychiatric Genetics **24:**266-268. doi: 10.1097/ypg.0000000000000061.

Wise SP. 2008. Forward frontal fields: phylogeny and fundamental function. *Trends in*

Neurosciences **31:**599-608. doi: 10.1016/j.tins.2008.08.008.

Wu JQ, Wang X, Beveridge NJ, Tooney PA, Scott RJ, Carr VJ, Cairns MJ. 2012.

Transcriptome Sequencing Revealed Significant Alteration of Cortical Promoter Usage

and Splicing in Schizophrenia. *PLoS One* **7**:e36351. doi: 10.1371/journal.pone.0036351.

Xu K, Schadt EE, Pollard KS, Roussos P, Dudley JT. 2015. Genomic and network patterns

of schizophrenia genetic variation in human evolutionary accelerated regions. *Molecular*

Biology and Evolution **32:**1148–1160. doi: 10.1093/molbev/msv031.

Yaguchi K, Nishimura-Akiyoshi S, Kuroki S, Onodera T, Itohara S. 2014. Identification of

transcriptional regulatory elements for *Ntng1* and *Ntng2* genes in mice. *Molecular Brain*

7:19. doi: 10.1186/1756-6606-7-19.

Yelin-Bekerman L, Elbaz I, Diber A, Dahary D, Gibbs-Bar L, Alon S, Lerer-Goldshtein T,

Appelbaum L. 2015. Hypocretin neuron-specific transcriptome profiling identifies the

sleep modulator Kcnh4a. *eLife* **4:**e08638. doi: 10.7554/eLife.08638.

Yin Y, Miner JH, Sanes JR. 2002. Laminets: Laminin- and netrin-related genes expressed in

distinct neuronal subsets. *Molecular and Cellular Neuroscience* **19:**344-358. doi:

10.1006/mcne.2001.1089.

Yoon JH, Sheremata SL, Rokem A, Silver MA. 2014. Windows to the soul: vision science as

a tool for studying biological mechanisms of information processing deficits in

schizophrenia. *Frontiers in Psychology* **4**:A681. doi: 10.3389/fpsyg.2014.00681.

Zakharyan R, Boyajyan A, Arakelyan A, Gevorgyan A, Mrazek F, Petrek M. 2011.

Functional variants of the genes involved in neurodevelopment and susceptibility to

schizophrenia in an Armenian population. *Human Immunology* **72:**746-748. doi:

10.1016/j.humimm.2011.05.018.

Zhang B, Gaiteri C, Bodea LG, Wang Z, McElwee J, Podtelezhnikov AA, Zhang CS, Xie T,

Tran L, Dobrin R, Fluder E, Clurman B, Melquist S, Narayanan M, Suver C, Shah H,

Mahajan M, Gillis T, Mysore J, MacDonald ME, Lamb JR, Bennett DA, Molony C, Stone

DJ, Gudnason V, Myers AJ, Schadt EE, Neumann H, Zhu J, Emilsson V. 2013. Integrated

Systems Approach Identifies Genetic Nodes and Networks in Late-Onset Alzheimer's

Disease. *Cell* **153:**707-720. doi: 10.1016/j.cell.2013.03.030.

Zhang F, Shugart YY, Yue W, Cheng Z, Wang G, Zhou Z, Jin C, Yuan J, Liu S, Xu Y. 2015.

Increased Variability of Genomic Transcription in Schizophrenia. *Scientific Reports*

5:e17995. doi: 10.1038/srep17995.

Zhang Q, Goto H, Akiyoshi-Nishimura S, Prosselkov P, Sano C, Matsukawa H, Yaguchi K,

Nakashiba T, Itohara S. 2016. Diversification of behavior and postsynaptic properties

by netrin-G presynaptic adhesion family proteins. *Molecular Brain* **9**:e6. doi:

10.1186/s13041-016-0187-5.

Zhao M, Kong L, Qu H. 2014. A systems biology approach to identify intelligence quotient

score-related genomic regions, and pathways relevant to potential therapeutic treatments.

Scientific Reports **4**:A4176. doi: 10.1038/srep04176.

Zhou H, Hu S, Matveev R, Yu Q, Li J, Khaitovich P, Jin L, Lachmann M, Stoneking M, Fu

Q, Tang K. 2015. A Chronological Atlas of Natural Selection in the Human Genome

during the Past Half-million Years. *BioRxiv*. doi: 10.1101/018929.

Zhu Y, Yang H, Bi Y, Zhang Y, Zhen C, Xie S, Qin H, He J, Liu L, Liu Y. 2011. Positive

association between *NTNG1* and schizophrenia in Chinese Han population. *Journal of*

Genetics **90:**499-502.

Figure 1. *NTNG* paralogs complementation within neurological disorders and brain transcriptome. (A-1, A-2) Reported cognitive disorders associations for *NTNG1* and *NTNG2*. *denotes rather an indirect association via a direct interaction with the research target. (B-1, B-2) RNA-seq of the STG of healthy (circle) and SCZ (cross) human subjects. The original dataset was produced by (**Wu et al., 2012**), accession number E-MTAB-1030 on ArrayExpress (**ST1a**) and reprocessed as described in **SM**. Five *NTNG1* and four *NTNG2* transcripts, consistently expressed across all 16 human samples are shown. Two samples (one healthy and one SCZ) have been omitted due to unsatisfactory quality of reads and expression profiling (**ST1b**). For the SNPs calling by SAMtools see **ST1c**. Data are presented as a mean RPKM±SEM. (C) Total number of the assembled transcripts across all samples for both paralogs (see **ST1d** for the completely reconstructed transcriptome). Dash-outlined are co-spliced exon clusters.

A(1-4). Predominantly synchronous paralogs expression classifiers

B. Predominantly asynchronous and mixed paralogs expression classifier

C. Anti-phasic (complementary) paralogs expression classifier

Figure 2. *NTNG* paralogs expression dynamics classification (A-C) in the human brain across the life span. A (1-4): further subdivision of the classifier. RNA-seq data are from the BrainSpan (www.brainspan.org) presented as a mean±SEM. TCx = temporary neocortex; OCx = occipital neocortex; PCx = parietal neocortex; LGE = lateral ganglionic eminence; CB(C) = cerebellum(cortex); DTH = dorsal thalamus; URL = upper (rostral) rombic limb; pcw = post-conception week. Two data points (MD, 12-13 pcw, and mPFC, 16-19 pcw) for *NTNG1* expression were omitted as they were 6-7 times different from the mean of other replicas. All processed brain samples are listed in **ST2**.

Figure 3. Human *NTNG* paralogs DNA and protein sequence comparisons and "structure-function" rule incongruency. (A) Identical gene structures with different sizes of introns. RNA-seq data from **Figure 1B** were used to precisely deduce the exon/intron junction boundaries. The sizes of exons 1, 10 and introns (1-2) are not indicated due to observed among the splice transcripts lengths variability (see **ST1a** for details). Arrows indicate location of CNS = conserved non-coding sequences underwent accelerated evolution in human compare to mice (mCNS) and chimpanzee (chCNS), as per **Prabhakar et al., 2006**; and ASC = anthropoid-specific constrained regions in human compare to marmoset (maASC), as per **del Rosario et al., 2014**. (B) Identical exonal composition of the longest *NTNG* encoded RNA paralog transcripts and corresponding proteins with relatively high percent of identity among them dependent on the included/excluded Ukd domain (**B-2**) encoded by the exons 6 and 7 (**B-1**). Notably, the protein sequence represents higher percent of the paralogs difference than encoded it DNA. The matrices were obtained by GeneJockey II (Biosoft). (C) Protein alignments for the longest human *NTNG* encoded proteins, Netrin-G1m and Netrin-G2b, with Loops I-III highlighting binding sites for their cognate post-synaptic binding partners NGL-1 (*Lrrc4c*) and NGL-2 (*Lrrc4*), respectively, as determined by **Seiradake et al. (2011)**. Arrow indicates a putative secretory cleavage site location, as calculated by SignalP (**Petersen et al., 2011**), the blue rectangle delineates the area of the lowest identity (3'-domain LE1+Ukd domain); ω – denotes a point of putative GPI-attachment, as predicted by Big-PI (**Eisenhaber et al., 2000**). PSD-95 interaction site via the SH3-binding domain (**Arbuckle et al., 2010**, as determined for mice Netrin-G2) overlaps with the Loop III NGL-2 binding surface. Two stars indicate a modern human (T346A) and a hominin-specific (S371A/V) amino acid substitutions (**Prosselkov et al., 2015**). (D) Identical structural motif of the Netrin-G1/NGL1 and Netrin-G2/NGL2 complexes as per **Seiradake et al. (2011)**. The figure's reproduction is covered by the Creative Commons license.