Neuregulin 1 and Schizophrenia: Genetics, Gene Expression, and Neurobiology

Paul J. Harrison and Amanda J. Law

Neuregulin 1 (NRG1) is a leading schizophrenia susceptibility gene. The NRG1 locus on chromosome 8p shows linkage to the disorder, and genetic association has been found between schizophrenia and various non-coding polymorphisms and haplotypes, especially at the 5′ end of the NRG1 gene, in many but not all case-control and family studies. NRG1 is a pleiotropic growth factor, important in nervous system development and functioning; roles include the modulation of neuronal migration, synaptogenesis, gliogenesis, neuron-glia communication, myelination, and neurotransmission. Understanding the neurobiology of NRG1 and its involvement in schizophrenia is challenged by the complexity of the gene, which gives rise to multiple functionally distinct isoforms, including six "types" of NRG1 defined by 5′ exon usage. Type IV and type I NRG1 may be particularly relevant to schizophrenia, with initial data showing altered expression of these isoforms in the disorder or in association with NRG1 risk alleles. We review the structure and functions of NRG1, consider the evidence for and against it being a schizophrenia susceptibility gene, and discuss mechanisms that might underlie the contribution of NRG1 to disease pathophysiology.

Key Words: Neuregulin, gene, schizophrenia, mRNA, pathophysiology

The neuregulins are a family of four genes (NRG1-4) encoding proteins that share an epidermal growth factor (EGF)-like domain, activate ErbB receptor tyrosine kinases, and play central roles in normal developmental processes, plasticity, and oncogenesis (Burden and Yarden 1997; Fischbach and Rosen 1997; Yarden and Sliwkowski 2001). Neuregulin 1 (NRG1; Holmes et al 1992) is the most well characterized member of the family, and it is known to be important in many organs, including heart, breast, and nervous system. Within the nervous system, NRG1 has diverse functions (Table 1). A psychiatric relevance of NRG1 has emerged with the increasing evidence that it is a susceptibility gene for schizophrenia. Here we review the neurobiology of NRG1, the genetic evidence linking it to schizophrenia, and consider how NRG1 might be involved in the pathophysiology of the disease.

Neurobiology of Neuregulin 1

The NRG1 Gene and Its Isoforms

The human NRG1 gene is located at chromosome 8p13. It spans approximately 1.4 megabases, has more than 20 exons and several large introns (Figure 1), and gives rise to at least 15 isoforms. All isoforms contain a core EGF domain, EGFc, encoded by exon E130, but other elements of the protein are variable. (In the absence of an agreed or definitive exon numbering system for NRG1, exons are labeled as described by Steinthorsdottir et al [2004], with the number denoting their length in nucleotides; this will vary for other species).

The NRG1 isoforms are known by a range of alternative names that reflect their original discovery (Table 2). The isoforms result from several sources of variation: 1) Alternative promoter usage leads to different "types" of NRG1 defined by their 5′ exon. Until recently, three types of NRG1 were known (types I–III), but recently transcripts containing additional 5′ exons have been found in human brain; the isoforms encoded by these transcripts have been designated types IV–VI (Steinthorsdottir et al 2004). 2) Types I and II NRG1 contain an immunoglobulin (Ig) domain, encoded by exons E178 and E122, hence their common designation as Ig-NRG1; the novel types IV and V NRG1 also come into this category. 3) Retention of exon E68 or exon E59 results in inclusion of EGFα or β variants, respectively. The NRG1s with a β-type EGF sequence are many times more potent than those with EGFα and predominate in the brain. 4) After the EGF domain, most NRG1 variants have a transmembrane domain (TMc; exon E103), sometimes preceded by a "1" stalk (exon E24); however, isoforms with a "3" stalk (exon E551) are truncated at that point, lack the TMc domain, and are synthesized as soluble isoforms (TMc-containing NRG1s without a stalk are known as "2" isoforms). 5) Beyond the TMc domain is the carboxy-terminal region. The first part, called c1-c3 (encoded by exons E127, E131, and E207), is shared by all TMc-containing NRG1s. It is followed by the carboxy tail, which can be either "a" or "b" form, encoded by exons E846 and E142 respectively; in the nervous system, the "a" tail prevails.

Many combinations of these sources of diversity are possible and give rise to a terminology for specific NRG1 isoforms. For example, "I-β2a" refers to type I NRG1 with a β-EGF domain, no stalk, and an "a" tail. Note also that the full repertoire of expressed NRG1 protein variants remains to be determined, because many of the isoforms have only been identified at the transcript level, whilst others remain hypothetical.

NRG1 Signaling and Processing

Neuregulin 1 is a ligand for ErbB receptor tyrosine kinases, binding to ErbB3 or ErbB4 receptors via its EGF domain. Neuregulin 1 does not interact directly with ErbB2, but this receptor is the preferred partner in the heterodimerization that follows NRG1-ErbB3/4 binding. After NRG1-activated ErbB dimerization, autophosphorylation of tyrosine residues in the cytoplasmic domain of the receptor creates docking sites for various adaptor proteins such as Shc, Grb2, and the regulatory subunit of phosphoinositide-3-kinase (PI3-kinase). These in turn activate the mitogen-activated protein (MAP) kinase and PI3-kinase pathways and modulate transcriptional activity in the cell. For reviews of NRG-ErbB signaling, see Yarden and Sliwkowski (2001), Murphy et al (2002), Carpenter (2003), and Citri et al (2003).

Most NRG1 isoforms are synthesized as transmembrane proproteins which then undergo proteolytic cleavage events. The proteolytic processing and release of NRG1 is controlled

From the Department of Psychiatry (PJH, AJL), University of Oxford, Warneford Hospital, Oxford, United Kingdom.

Address reprint requests to Prof. P. J. Harrison, Neurosciences Building, University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK; E-mail: paul.harrison@psych.ox.ac.uk.

Received June 30, 2005; revised November 14, 2005; accepted November 22, 2005.

0006-3223/06/$32.00
doi:10.1016/j.biopsych.2005.11.002

© 2006 Society of Biological Psychiatry

Table 1. Developmental and Other Functions of Neuregulin 1 in the Nervous System

Neuronal specification
Neuronal migration
Neuron-glial signalling
Glial development and differentiation
Synapse formation
Myelination
Modulation of synaptic transmission
Dendritic growth
Transcriptional regulation
Regulation of NMDA, $GABA_A$, and nicotinic receptors
Modulation of long-term potentiation
Hormonal control of puberty
Protection against ischaemic damage

For discussion and references, see text. GABA, gamma-aminobutyric acid; NMDA, N-methyl-D-aspartate.

Table 2. Nomenclature for Neuregulin 1 and Its Isoforms

Type	5′ Exon[a]	Characteristic Domain[b]	Alternative Terms
I	E592	Ig	Heregulin (Hrg); acetylcholine receptor-inducing factor (ARIA); Neu differentiation factor (NDF)
II	E1006	Ig	Glial growth factor (GGF); kringle-like domain isoform
III	E1160	CRD	Sensory and motor neuron-derived factor (SMDF); CRD-NRG; nARIA
IV	E187	Ig	
V	E92	Ig	
VI	E290	Not known	

"Neuregulin" is sometimes still used to refer to neuregulin 1, even though the existence of NRG2–4 makes the singular term imprecise.
[a]Terminology of Steinthorsdottir et al (2004). See Figure 1.
[b]Ig, immunoglobulin; CRD, cysteine rich domain. See Figure 2.

by the activity of cell-surface metalloproteases and is regulated in an activity-dependent manner with involvement of growth factors (Loeb 2003; Ozaki et al 2004); NRG1 expression is also enhanced by neuronal activity (Eilam et al 1998). An overview of the membrane topology and processing of NRG1 is shown in Figure 2.

Two main signaling strategies exist for NRG1, paracrine and juxtacrine. These depend on the region of the protein beyond the EGF domain and on the mode of presentation of NRG1 to the ErbB receptor (Falls 2003a, 2003b). Cleavage of Ig-NRG1 between the EGF and TMc domains releases a soluble N-terminal fragment (NTFs) containing the EGF and Ig domains (Figure 2A). The NTFs diffuses through the extracellular space and acts in a paracrine fashion to activate ErbB receptors on neighboring cells, via the EGF domain. The Ig domain binds proteoglycans at the

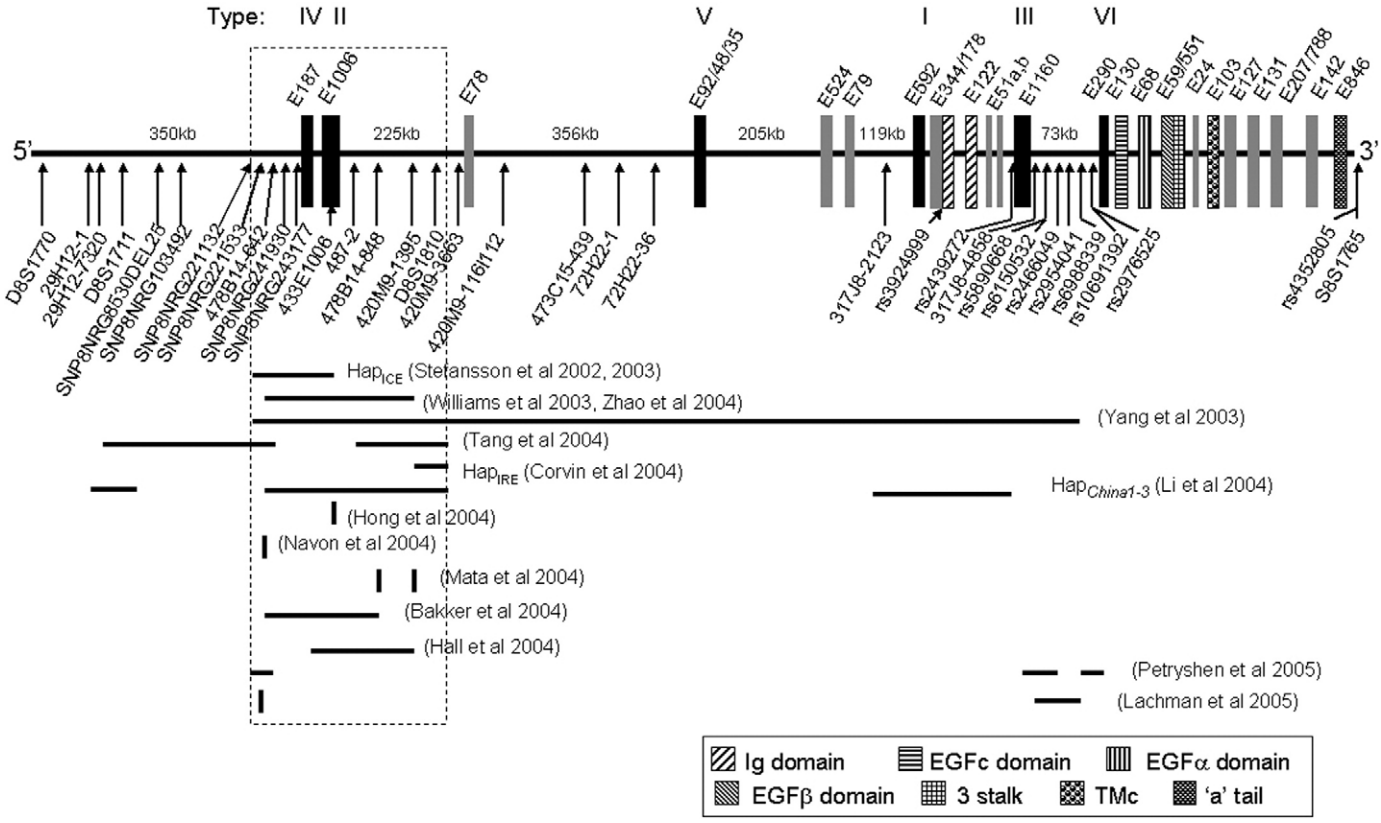


Figure 1. Human neuregulin 1 gene structure. Exons are represented as vertical bars, numbered as per Steinthorsdottir et al (2004). The 5′ exons, which define the "types" of NRG1, are in black, with the corresponding Roman numeral above. Exons giving rise to key functional domains are patterned, per the box; other exons are in grey. Additional non-coding exons reported by Steinhorsdottir et al (2004) are omitted. The gene is drawn roughly to scale, with the length of non-coding regions greater than 50kb shown. Below the gene are the polymorphisms that define the 5′ and 3′ limits of markers used in each schizophrenia study and that define the 5′ and 3′ ends of the various risk haplotypes thus identified. Horizontal lines denote the extent of each risk haplotype. The dashed rectangle shows the region wherein most positive associations have been found. For further details see Table 3. The figure is constructed largely from information in Steinthorsdottir et al (2004) and from Falls (2003b) and Petryshen et al (2005).

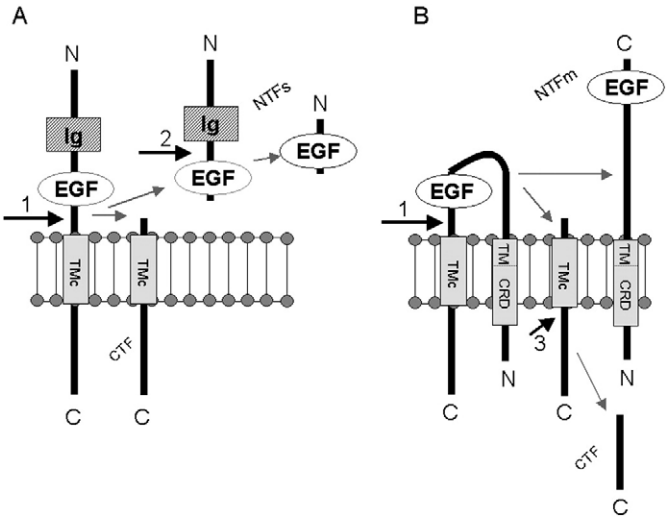


Figure 2. Neuregulin 1 membrane topology and processing. **(A)** Neuregulin 1 isoforms containing the Ig domain (Ig-NRG1; classically types I and II, also the novel types IV and V; Table 2) are predicted to have a single transmembrane domain, TMc, with an extracellular N terminus and a cytoplasmic C terminus. Proteolytic cleavages (arrows 1 and 2) produce soluble N terminal fragments (NTFs) that interact via the epidermal growth factor (EGF) domain with ErbB receptors on adjacent cells, and a C terminal fragment (CTF). **(B)** Unlike Ig-NRG1, type III NRG1 (CRD-NRG1; Table 2) spans the membrane twice, with both N and C termini being intracellular. Note that the cysteine rich domain (CRD) overlaps with the first transmembrane domain, as indicated by the dashed line. After proteolytic cleavage, both resulting proteins remain membrane-bound, with the EGF-containing protein (NTFm) available for juxtacrine signaling. The CTF of type III NRG1 can be released into the cytoplasm, but the details of the cleavage event (arrow 3) are unclear. The CTF of Ig-NRG1s might also undergo the same cleavage. The orientation and processing of type VI NRG1 is not known, because it lacks both Ig and CRD encoding exons. For further details, see text, also Falls (2003a), Loeb (2003), and Bao et al (2003).

cell surface and in the extracellular matrix in a mechanism thought to sequester NRG1 to the synapse and potentiate ErbB receptor stimulation (Li and Loeb 2001). A further cleavage (labeled "2" in Figure 2A) is proposed to release an EGF fragment lacking the Ig domain (Falls 2003b). The second mode of signaling is used by membrane-bound type III NRG1 (Figure 2B). It has two transmembrane domains, so the effect of cleavage is not the release of NTFs but the generation of a membrane-bound NTFm (Falls 2003a, 2003b; Wang et al 2001). The juxtacrine nature of this signaling implies that type III NRG1 predominantly activates ErbB receptors in direct contact with the NRG1-expressing cell.

The preceding descriptions emphasize the central role of the EGF domain in mediating the actions of NRG1 upon ErbB receptors, and this remains the classical, "forward" mode of NRG1 signaling; however, the C terminal fragment (CTF) of NRG1 is also functional, forming complexes with cytoplasmic proteins including LIMK I, a protein kinase that is concentrated in synapses and that modulates the actin cytoskeleton and synaptic plasticity (Meng et al 2003). This might represent a "reverse" mode of signaling, in which ErbB acts as the ligand and NRG1 as the receptor; bi-directional signaling of this kind is established for the related ephrins and Eph receptors. There is also evidence that the CTF of NRG1 can be released from the membrane, perhaps by γ-secretase cleavage, and allow retrograde signaling to the nucleus where it regulates transcription of apoptotic regulators and other genes (Figure 2B; Bao et al 2003).

NRG1 Functions in the Nervous System

Complementing the complexity of its gene structure, expression profile, and signaling pathways, NRG1 has a diverse array of functions. Many pertain to neurodevelopment, but others emphasize the importance of NRG1 in subsequent aspects of neural functioning and plasticity (Table 1). It is beyond the scope of this article to review this large literature (see Buonanno and Fischbach 2001, Falls 2003a, 2003b, and Ozaki 2001). Instead, we mention a few studies that have been published since these reviews, selected to illustrate areas of current research into the neurobiology of NRG1 that might be of relevance to schizophrenia.

Flames et al (2004) showed that NRG1 controls migration of cortical gamma-aminobutyric acid (GABA)ergic interneurons. Local expression of type III NRG1 creates a permissive pathway for migration from the lateral ganglionic eminence, whereas cortical expression of soluble Ig-NRG1 establishes a chemoattractive gradient that directs the neurons towards the cortex. Interference with NRG1/ErbB signaling (in conditional mutant embryos) decreased the density of interneurons in the postnatal cortex. Flames et al postulated that alterations in the expression of different NRG1 isoforms, perhaps in conjunction with NRG1 sequence variation, could contribute to the neurodevelopmental pathology of GABAergic interneurons seen in schizophrenia (Lewis et al 2005).

There is good evidence for oligodendrocyte and myelin abnormalities in schizophrenia (Davis et al 2003) but little understanding of its origins or its relationship to the neuronal and synaptic pathology (Harrison 1999). In this regard, recent studies show that NRG1 plays a major role signaling between axons and Schwann cells (the peripheral counterpart of oligodendrocytes). Michailov et al (2004) found that expression of type III NRG1 regulates myelination and proposed that NRG1 signals axonal calibre to ensure appropriate amounts of myelin formation. Involvement of type III NRG1 in myelin ensheathment is corroborated by the data of Taveggia et al (2005). The work of Esper and Loeb (2004) supports a key role for NRG1 in axonal-glial signaling and highlight the bidirectional nature of this process, with NRG1 release by axons being determined both by neuronal activity and by growth factors released by glia.

Another focus of research has been the regulation of N-methyl-D-asparate (NMDA) receptors by NRG1 (Buonanno and Fischbach 2001). One point of interaction is via the plasticity-associated protein, post-synaptic density-95 (PSD-95); PSD-95 is associated with ErbB4 and NMDA receptors at excitatory synapses (Garcia et al 2000) and enhances NRG1 signaling by facilitation of ErbB4 dimerization (Huang et al 2000). Two recent studies have advanced understanding of this topic. Bao et al (2004) reported that the retrograde translocation of the NRG1 CTF to the nucleus (Figure 2B) is increased by synaptic activity and leads to enhanced transcription of PSD-95 via binding of the CTF to the transcription factor Eos. Gu et al (2005) provide direct evidence that NRG1 regulates NMDA receptor function in the adult brain, with exposure to NRG1 decreasing NMDA receptor currents and channel activity in cortical pyramidal neurons, at least in part by increasing receptor internalization. Both reports note the potential relevance of their data for the NMDA receptor dysfunction postulated to occur in schizophrenia (Coyle et al 2003).

Most studies of NRG1 have been carried out in rodents or in cell lines, and many have focused on the neuromuscular junction rather than the central nervous system. As a result, little is known about NRG1 in human brain, information that will be essential to elucidate the role of NRG1 in schizophrenia. Extending preliminary data (Chaudhury et al 2003), Law et al (2004) provided the

first description of NRG1 messenger RNA (mRNA) and protein distribution in adult human brain. Expression of NRG1 mRNA and protein was observed in all regions studied and in several cell types. These data highlight that involvement of NRG1 in schizophrenia need not be due solely to its developmental functions but might occur later in life. Ongoing studies are characterizing the developmental profile of human brain NRG1 expression and the distribution of individual NRG1 isoforms. The latter is necessary because the "pan" probe and antibody used by Law et al (2004) targeted parts of the molecule common to most NRG1 subtypes, and it is known in rodents that NRG1 isoforms are expressed in a regionally and cell type-specific manner (Kerber et al 2003; Meyer et al 1997). Moreover, given the emerging data that changes in NRG1 expression in schizophrenia are also isoform selective (see below), this level of detail becomes essential.

These and other recent findings (e.g., Gerecke et al 2004; Gierdalski et al 2005; Kwon et al 2005; Lai and Feng 2004; Roysommuti et al 2003; Schmid et al 2003; Shyu et al 2004) together illustrate that NRG1 has roles in the nervous system that are 1) isoform-specific, 2) occur in the mature as well as the developing organism, 3) affect structure as well as function, 4) involve glia and neurons (and their interactions), and 5) include both acute and longer-term effects. Moreover, virtually all the processes influenced by NRG1 (Table 1) overlap with pathways and processes already implicated in schizophrenia (Corfas et al 2004). At one level, this correspondence suggests that, from a neurobiological perspective, NRG1 is a deceptively strong candidate gene for the disorder. Conversely, its candidacy might indeed be deceptive, reflecting the fact that NRG1 is pleiotropic and that all pathophysiological hypotheses about schizophrenia are weak. Either way, biological plausibility means little without direct evidence for genetic association. We now review the data that link NRG1 to schizophrenia, before considering mechanisms that might underlie the association.

Neuregulin 1 as a Schizophrenia Gene

The 8p Locus

Chromosome 8p, especially a 30cM region around 8p21.1-22, has been implicated as a locus harboring one or more schizophrenia genes by several linkage studies (Blouin et al 1998; Brzustowicz et al 1999; Gurling et al 2001; Kendler et al 1996; Levinson et al 1996; Liu et al 2005; Pulver et al 1995; Stefansson et al 2002). As with all other putative schizophrenia loci, there have also been equivocal and negative findings (DeLisi et al 2000; Kaufmann et al 1998; Kunugi et al 1996; Shaw et al 1998; Sklar et al 2004; Williams et al 2003a); importantly, however, the two meta-analyses of schizophrenia genome scans identified 8p as a disease locus with a high probability. Badner and Gershon (2002) reported an overall p value of $< 2 \times 10^{-4}$, whereas Lewis et al (2003), with a more conservative approach, found $p < .05$ for both permutation tests used. These data in total make NRG1—and all other genes in the vicinity—a positional candidate for schizophrenia.

Association Studies of NRG1 With Schizophrenia

The study of Stefansson et al (2002) was a landmark article (Harrison and Owen 2003). In the Icelandic population they found linkage of schizophrenia to chromosome 8p and then used linkage disequilibrium mapping of the region to demonstrate association of variants within the NRG1 gene locus with the disease. The authors identified a NRG1 haplotype (Hap_{ICE}) in the 5′ region of the gene (Figure 1) that doubled the risk of schizophrenia. Notably, the findings were soon closely replicated in a Scottish sample (Stefansson et al 2003), since when a number of other case-control and family-based association studies were reported. Table 3 summarizes all the fully published data and sufficiently informative abstracts of which we are aware (as of October 2005). A simple vote count from Table 3 reveals 17 positive reports (i.e., those in which at least one polymorphism or haplotype was associated with schizophrenia, in at least one of the samples) and 4 negative ones. In this crude respect, 80% of studies support a genetic association of NRG1 with schizophrenia, leading to a broad—though not unanimous—consensus that NRG1 is probably a schizophrenia susceptibility gene. For example, recent reviews describe the evidence as "strong" (Owen et al 2005) and as "convincing but not yet compelling" (Tosato et al 2005).

The "case in favor" of NRG1 as a schizophrenia gene is bolstered by several other factors: first, by the prior evidence for linkage to 8p (albeit the NRG1 locus lies some 10–15cM centromeric to the linkage peak at 8p21.1-22); second, by the relevance to the disease process of the known functions of NRG1 (Table 1; Corfas et al 2004); third, by the schizophrenia-like phenotype seen in NRG1 mutant mice (Falls 2003b; Stefansson et al 2002); and fourth, by the findings that NRG1 expression is altered in schizophrenia and related to the risk polymorphisms, as described below. Despite the relatively consistent genetic findings plus these other considerations, however, caution must be exercised before accepting NRG1 as a bona fide schizophrenia gene, for two cogent reasons.

First, amongst the positive studies, many different alleles and haplotypes have been associated with schizophrenia (Figure 1 and Table 3). No one variant has been uniformly implicated. Thus, the evidence must be viewed as inconclusive and the likelihood of false positive findings and publication bias noted; however, the majority of studies, particularly of Caucasians, find association to the region around the first two exons of the gene (see dashed rectangle in Figure 1), as originally reported by Stefansson et al (2002), providing a degree of convergent evidence that this is the critical area of the gene vis-à-vis schizophrenia. In contrast, some Chinese populations show association to more 3′ markers (in the vicinity of the 5′ exons of types III and VI NRG1), a difference that might reflect allelic heterogeneity. Moreover, there are marked population differences in NRG1 allele and haplotype frequencies that could contribute substantially to the variability in results (Gardner et al 2006). A meta-analysis of NRG1 association studies, taking ethnicity into account, will help clarify the situation.

The second caveat is that all the NRG1 polymorphisms associated with schizophrenia are non-coding, apart from 433E1006 (in the 5′ exon of type II NRG1, E1006) and rs3924999 (in the first Ig-encoding exon, E178), both of which encode an Arg-Gly substitution. The interpretation of non-coding associations is inherently less clear than for coding variants, and there are several alternative explanations (see Harrison and Weinberger 2005). These include the possibility that rare NRG1 mutations do exist but have yet to be found, or that the genetic association is actually with another gene close to or within the NRG1 locus. A more likely explanation, however, is that the current polymorphisms, or other non-coding NRG1 polymorphisms in linkage disequilibrium with them, are functional by virtue of an effect upon NRG1 gene expression. That is, the "true" NRG1 gene variants are associated with schizophrenia because they influence the transcription, splicing, mRNA degradation, or transla-

Table 3. Summary of NRG1 Genetic Association Studies in Schizophrenia

	Cases/Control Subjects	Ethnicity	SNP, MS[a]	From (5′)[b]	To (3′)[b]	Association With Schizophrenia
Case-Control Studies						
Stefansson et al 2002	402/394	Icelandic	8,3	SNP8NRG8530DEL25	420M9-1395	Yes: SNP8NRG221533 ($p = .003$), $Hap_{ICE}5$ ($p = .000008$)[c], Hap_{ICE} ($p = .0018$)[c]
Stefansson et al 2003	609/618	Scottish	5,2	SNP8NRG221132	420M9-1395	Yes: SNP8NRG221533 ($p = .00006$), SNP8NRG241930 ($p = .002$), SNP8NRG243177 ($p = .0008$). $Hap_{ICE}5$ ($p = .00003$)[c], $Hap_{ICE}7$ ($p = .0003$)[c]
Williams et al 2003b	573/618	British/Irish	1,2	SNP8NRG221533	420M9-1395	Yes: haplotype ($p = .04$, one-tailed; $p = .02$ in familial subset)[d]
Iwata et al 2004	607/515	Japanese	5,2	SNP8NRG221132	420M9-1395	No
Tang et al 2004	540/279	Han Chinese	0,12	D8S1770	72H22-1	Yes: 487-2 ($p = .02$), 478B14-848 ($p = .0014$), D8S1810 ($p = .01$), 420M9-3663 ($p = .01$), 4-marker haplotype ($p = .00003$)[e], 5-marker haplotype ($p = .0003$)[e]
Zhao et al 2004	369/299	Han Chinese	3,2	SNP8NRG221132	420M9-1395	Yes: 5-marker haplotype ($p = .006$)[f], 2-marker haplotype ($p = .006$)[f]
Li et al 2004	298/334	Han Chinese	3,22	D8S1770	S8S1765	Yes: 29H12-1 ($p = .0004$), 478B14-642 ($p = .035$), 317J8-4858 ($p = .03$), Hap_{China1} ($p = .000006$)[g], Hap_{China2} ($p = .003$)[g]
Corvin et al 2004	243/222	Irish	3,17	D8S1770	72H22-36	Yes: 478K14-72458 ($p = .039$), 473C15-533 ($p = .036$), Hap_{IRE} ($p = .013$)[h]
Hong et al 2004	228/295	Han Taiwanese	1,0	rs3924999		No
Kampman et al 2004	94/395	Finnish	1,0	SNP8NRG221533		No
Navon et al 2004	60/130	Ashkenazi	5,0	SNP221132	433E1006	Yes: SNP8NRG221533 ($p < .03$); haplotype ($p = .052$)[i]
Mata et al 2004	103/106 + 53/68[j]	Spanish	0,2	478B14-848	420M9-1395	Yes: 478B14-848 ($p < .00005$, $p < .02$)[j], 420M9-1395 ($p < .06$, $p < .03$)[j]
Bakker et al 2004	130 + 130[k]/585	Dutch	1,2	SNP8NRG221533	478B14-848	Yes: SNP8NRG221533 ($p < .01$). In non-deficit cases: SNP8NRG221533 ($p < .0004$), 478B14-848 ($p < .05$); 2-marker haplotype ($p < .0001$)[k]
Lachman et al 2005	141/142 + 177/164	African-American and Caucasian	1,3	SNP8NRG221533	rs10691392	Yes, in African Americans: rs6150532 ($p = .02$) and SNP8NRG221533 (p not stated), and 3 marker haplotype ($p < .05$)[l]
Family Studies						
Yang et al 2003	246 trios	Han Chinese	3,0	SNP8NRG221533	rs2954041	Yes: SNP221533 ($p = .004$), rs3924999 ($p = .003$), rs2954041 ($p = .0003$), haplotype ($p < .00001$)[m]
Zhao et al 2004	351 trios	Han Chinese	3,2	SNP8NRG221132	420M9-1395	Yes: 5-marker haplotype ($p = .005$), 2-marker haplotype ($p = .02$)
Li et al 2004	184 trios	Han Chinese	3,22	D8S1770	S8S1765	Yes: 478B14-642 ($p = .025$), 487-2 ($p = .02$), 420M9-1395 ($p = .02$), D8S1810 ($p = .037$), 317J8-2123 ($p = .025$), 317J8-4858 ($p = .001$); Hap_{China2} ($p = .0047$)[g], Hap_{China3} ($p = .00004$)[n]
Hall et al 2004	210 + 233 trios	USA, Afrikaner	3,2	SNP8NRG221533	420M9-1395	Yes in South African sample: 3-marker haplotype[o]
Hong et al 2004	221 trios	Han Taiwanese	1,0	rs3924999		Yes: Gln38 allele over-transmitted ($p = .05$)
Thistleton et al 2004	270 high-density families	Irish	3,4	SNP8NRG221533	420M9-116I112	No
Petryshen et al 2005	321/242 + 111 trios	Portugese	43,2	SNP8NRG103492	rs4352805	Yes: SNP8NRG221132 ($p = .03$)[p], SNP8NRG241930 ($p = .05$); Hap2 ($p < .05$)[q], Hap7 ($p < .05$)[q], Hap9 ($p < .05$)>[m]

[a]Number of single nucleotide polymorphisms (SNP) and microsatellites (MS) tested for association.
[b]Most 5′ and most 3′ marker (for location, see Figure 1).
[c]$Hap_{ICE}5$ ("core haplotype"): SNP8NRG221132, SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, 433E1006; $Hap_{ICE}7$: $Hap_{ICE}5$ plus 478B14-848, 420M9-1395.
[d]Haplotype: SNP8NRG221533, 478B14-848, 420M9-1395.
[e]Four-marker haplotype: 29H12-7320, D8S1711, 29H12-121L21, 478B14-642; five-marker haplotype: 487-2, 478B14-848, 420M9-1395, D8S1810, 420M9-3663.
[f]Five-marker haplotype: SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, 478B14-848, 420MP9-1395; two-marker haplotype: 478B14-848, 420MP9-1395.
[g]Hap_{China1}: 29H12-1, D8S1711; Hap_{China2}: 478B14-642, 487-2, 420M9-1395, D8S1810.
[h]Hap_{IRE}: 420M9-1395, D8S1810.
[i]SNPs comprising haplotype not stated in abstract.
[j]Original sample and replication sample. The two p values in right-hand column refer to these respectively.
[k]130 cases with, and 130 without, deficit syndrome. Haplotype: SNP8NRG221533, 478B14-848.
[l]Haplotype: rs5890668, rs6150532, rs10691392.
[m]Haplotype: SNP8NRG221533, rs3924999 (Arg38 allele), rs2954041.
[n]Hap_{China3}: 317J8-2123, 317J8-1, 317J8-2, 317J8-4858.
[o]Three-marker haplotype: SNP8NRG243177, 478B14-848, 420M9-1395. p value not apparent.
[p]Presented as $p = .015$, one-tailed, in report.
[q]Hap2: SNP8NRG221132, SNP8NRG221533; Hap7: rs2439272, rs2466058, rs2466049; Hap9: rs6988339, rs2975498, rs2919382, rs2976525.

tion of NRG1. The functional consequences in turn arise from the resultant change in the quantity, proportions, or distribution of NRG1 gene products. Other diseases and genes provide many precedents for the proposal that the mechanism of association is altered expression (see Harrison and Weinberger 2005); relevant examples include dysbindin in schizophrenia (Bray et al 2005), ubiquilin in Alzheimer's disease (Bertram et al 2005), and α-synuclein in Parkinson's disease (Singleton et al 2004). Empirical evidence that it also applies to NRG1 in schizophrenia is beginning to emerge, as discussed later.

Genotype-Phenotype Correlations

As the evidence for NRG1 as a schizophrenia susceptibility gene has grown, studies have begun to refine the phenotype(s) with which NRG1 is most strongly associated. The Cardiff group has found that the core Hap_{ICE} risk haplotype is also associated with bipolar disorder in their British case-control population and exerts a specific effect in the subset of cases of psychosis with manic or mood incongruent psychotic features (Green et al 2005). In a Dutch study, Bakker et al (2004) found association to NRG1 for non-deficit but not deficit forms of schizophrenia. Results of these two studies are broadly compatible, in that both find association of NRG1 haplotypes to cases of psychosis characterized by relative preservation of affect and good prognosis. The Green et al (2005) data illustrate that some schizophrenia susceptibility genes might increase risk for bipolar disorder as well (Craddock and Owen 2005). Neuregulin 1 is now also implicated in the psychosis that occurs in some patients with Alzheimer's disease; Go et al (2005) report linkage of this phenotype to 8p and association to a NRG1 haplotype including two of the polymorphisms contained in Hap_{ICE}. Finally, there might be an association between NRG1 and schizotypal personality traits (Lin et al 2005). Thus, NRG1 might contribute to a range of psychosis-related phenotypes; however, whether NRG1 genotype will ever prove to demarcate a valid or clinically useful subtype of psychosis remains to be seen.

One small study suggests that NRG1 genotype (at SNP8NRG221533) is associated with therapeutic response to clozapine (Kampman et al 2004). Thus, as with COMT (Tunbridge et al 2006), NRG1 genotype might prove relevant to the treatment as well as the etiology of schizophrenia. Again, however, much stronger evidence is required before this possibility could be seriously entertained.

Interactions of NRG1 With Other Genes

There is increasing but still preliminary evidence that interactions between several of the susceptibility genes modify their individual contributions to schizophrenia risk. Such data are beginning to appear for NRG1; for example, the effect size for schizophrenia might be increased by a combination of SNPs in NRG1 and ErbB4 (Norton et al 2006) and between NRG1 and COMT $Val^{158}Met$ (D.R. Weinberger, personal communication). Gene-environment interactions are also to be anticipated whereby NRG1 variants modify the response to environmental events. Similarly, epigenetic factors might prove to modify the NRG1 genetic influence upon disease risk.

Neuregulin 1 Expression and Function in Schizophrenia

As noted earlier, an alteration in NRG1 expression, and thence NRG1 function, is the putative molecular mechanism mediating the influence of NRG1 upon schizophrenia risk. Recent data provide initial experimental support for, and refinement of, this possibility (Table 4).

Hashimoto et al (2004) studied types I–III NRG1 transcripts in the dorsolateral prefrontal cortex and found increased type I NRG1 mRNA in schizophrenia. The elevation was present relative to the other NRG1 isoforms and to three housekeeping genes. Notably, Law et al (unpublished data) replicated the finding of increased type I NRG1 mRNA in schizophrenia, in a separate and larger sample, in the hippocampus. Hashimoto et al had found that NRG1 mRNA abundance correlated with neuroleptic exposure, raising the possibility that the increase was at least partially a medication effect; however, this was not seen in the Law et al (unpublished data) study.

Law et al (unpublished data) additionally measured the novel type IV NRG1 mRNA, because the position of its 5′ exon, adjacent to the Hap_{ICE} region, suggested that this isoform might be more directly related to the genetic involvement of NRG1 in schizophrenia (Figure 1). As predicted, an SNP

Table 4. Effects of Schizophrenia and Genotype Upon NRG1 Expression

Study	mRNA Isoforms Detected	Method	Region[a]	Cases, Control Subjects	Main Findings in Schizophrenia
Fully Published					
Hashimoto et al 2004	Types I–III	qPCR	DPFC	20,19	Type I increased.
Petryshen et al 2005	Various[c]	Array, RT-PCR	Leucocytes	33,33; 19,19	Array: Increased β3-containing isoforms. RT-PCR: increased type III.
Law et al, unpublished data	Types I–IV, Pan[b]	qPCR	HC	38,53	Type I increased, and associated with SNP8NRG221132. Type IV associated with SNP8NRG243177 and Hap_{ICE}. Pan-NRG1 unchanged.
Abstracts					
Law et al 2005	Pan[b]	ISHH	CB	32,32	Decreased in Purkinje and Golgi cells.
Navon et al 2004	Types I, II	qPCR	DPFC	43,40	Increased ($p = .063$).
Dempster et al 2004	Type III	qPCR	CB	15,15	Decreased type III associated with Hap_{ICE}.
Leonard et al 2005	Types I–III	Array, qPCR	HC, fibroblasts	12,12	Unchanged in HC. Type I decreased in fibroblasts.

[a]DPFC, dorsolateral prefrontal cortex; HC, hippocampus; CB, cerebellum; NRG1, Neuregulin 1.
[b]Pan probe targeted to, or amplifying, portion of epidermal growth factor domain common to all known NRGl isoforms.
[c]The authors measured six NRG1 isoforms, using the nomenclature provided for the Affymetrix U133 gene chips used; however, the NRG1 probe sets on the array also recognize transcripts encoding other isoforms. For example, the "206343/sensory and motor neuron-derived factor (SMDF)" (i.e., type III) probe set is based on a type III-β3-encoding transcript and thus detects other β3 isoforms too (e.g., II-β3). For details see Affymetrix website (http://www.affymetrix.com/index.affx). Thus, the authors' description of increased "SMDF signal" indicates elevation of one or more β3-containing NRG1 messenger RNAs. In contrast, the primers used for the follow-up reverse transcription-polymerase chain reaction (RT-PCR) amplified a region common to, and specific for, type III NRG1s.

(SNP8NRG243177) and a four-marker haplotype from Hap_{ICE} including this SNP were associated with type IV NRG1 expression, with the risk allele(s) associated with higher transcript level. Type IV NRG1 expression was not related to other SNPs in the 5′ or 3′ region of the gene; thus, the effect of SNP8NRG243177 on type IV NRG1 was relatively specific. Moreover, the influence of the SNP on type IV NRG1 mRNA was more marked in the schizophrenia group than in the control group, suggesting that additional factors (either genetic or environmental) might be interacting with NRG1 genotype in the disease.

Apart from these preliminary hints, the functional correlates of the disease- and genotype-associated alterations in NRG1 isoform expression remain unclear: firstly, because there are no functions known to be specific to type I NRG1 (because studies have not usually distinguished it from other Ig-NRG1s), and because type IV NRG1 has not yet been characterized at all; secondly, although transgenic and knockout mice show that manipulations of the NRG1 gene can have deleterious effects (see Crone and Lee 2002; Falls 2003b), there is little information as to the functional consequences of the more subtle differences in NRG1 expression seen in schizophrenia; thirdly, it is not yet known whether the mRNA alterations summarized in Table 4 are also manifested in terms of protein expression, in part because of a lack of suitable antibodies; and fourthly, investigation of ErbB receptors and other molecules in the NRG1 pathway is required to understand the overall status of NRG1 signaling. To date, quantitative PCR studies in schizophrenia report decreased frontal cortex ErbB3 (Tkachev et al 2003) and increased ErbB4 (CYT-1 isoform; Silberberg and Navon 2005) mRNA; however, the ErbB3 finding was not replicated in an in situ hybridization analysis (Beltaifa et al 2005).

Finally, it should be noted that NRG1 expression differences found between groups of genetically unselected cases and control subjects (such as the type I mRNA increases mentioned previously) cannot be explained fully by disease-associated genetic variants, given that only a minority of subjects carry the risk alleles (e.g., in the original NRG1 study, only approximately 14% of cases carried the Hap_{ICE} haplotype). The same caveat applies to the decreased mRNA levels of other susceptibility genes reported in schizophrenia (e.g., dysbindin [Weickert et al 2004], RGS4 [Mirnics et al 2001], and PPP3CC [Eastwood et al 2005]). The fact that expression of a susceptibility gene is seemingly altered comparably in patients who do not have risk alleles in the gene as in those who do is unexplained other than to assume that the changes in the "no risk alleles" category are secondary to other genetic, epigenetic, or environmental factors. In turn, the apparent commonality of effect on expression is one indication of the postulated interaction and functional convergence between the genes (Harrison and Weinberger 2005). Future studies will need to address more closely the distinction between direct and indirect influences on NRG1 expression.

Summary

There is now substantial but not incontrovertible evidence that genetic variation in NRG1 is associated with schizophrenia (Table 3). Proving this beyond all reasonable doubt will be intrinsically difficult, for two main reasons—which apply not only to NRG1 but to other putative susceptibility genes for psychiatric disorders (Colhoun et al 2003; Harrison and Weinberger 2005; Paige et al 2003). The first reason relates to the genetic architecture. Schizophrenia is a complex genetic disorder with multiple risk genes of small effect (e.g., for NRG1, estimated odds ratios are mostly below 1.5), none of which are necessary or sufficient, and which might influence different components of the phenotype. Moreover, even within a single gene like NRG1, allelic heterogeneity might well exist. In addition, the influence of NRG1 genetic variation must ultimately be considered in the context of epistasis with other susceptibility genes and potential interactions with epigenetic and environmental factors. The second reason concerns the nature of the associated genetic variants and the implications for the mechanism of their effect. If, as the present data indicate, the variants are non-coding, it is not a trivial matter to identify the specific risk-conferring alleles and haplotypes and then to demonstrate their molecular and cellular consequences and show that the latter really are the biological explanation for the association.

These factors together conspire to make unequivocal (dis) proof of NRG1 as a schizophrenia gene a daunting task. To address them, further case-control and family-based association studies are essential; they must be large in terms of sample size, the number of NRG1 polymorphisms assayed, and the extent of phenotypic and demographic data collected. At the same time, the neurobiology of NRG1 and the correlates of the associated genetic variants need detailed investigation, with human tissues as well as animal models and in vitro approaches. Research in these various domains should be an iterative process, in which emerging genetic and biological evidence complement and inform each other.

At present, one can speculate that NRG1 modifies schizophrenia risk by virtue of a change in the relative expression of one or more isoforms that in some cases results from NRG1 genetic variation, whereas in others it is downstream of other pathogenic factors. Altered expression in turn perturbs some aspect of the manifold functions of NRG1 and its pathways, ultimately impacting upon the characteristics of the neural circuits that underlie schizophrenia. Advancing this plausible but frustratingly vague speculation will not be easy, for conceptual and for technical reasons. The tantalizing possibility, however, that the research will result in confirmation of the first unequivocal schizophrenia gene, and discovery of how it operates, provides ample incentive to persevere.

AJL is a Medical Research Council Career Development Fellow. Our work is supported by the Stanley Medical Research Institute, Medical Research Council, Wellcome Trust, and the National Alliance for Research on Schizophrenia and Depression. We thank many people for fruitful NRG1 discussions, notably Daniel Weinberger, Andres Buonanno, Steven Carroll, Doug Falls, Klaus-Armine Nave, Lorna Role, and Cyndi Shannon Weickert. We are also grateful to an anonymous reviewer for helpful comments and advice, especially regarding Table 4.

Badner JA, Gershon ES (2002): Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 7:405–411.

Bakker SC, Hoogendoorn MLC, Selten J-P, Verduijn W, Pearson PL, Sinke RJ, Kahn RS (2004): Neuregulin-1: Genetic support for schizophrenia subtypes. *Mol Psychiatry* 9:1061–1063.

Bao J, Lin H, Ouyang Y, Lei D, Osman A, Kim T-W, et al (2004): Activity-dependent transcription regulation of PSD-95 by neuregulin-1 and Eos. *Nat Neurosci* 7:1250–1258.

Bao J, Wolpowitz D, Role LW, Talmage DA (2003): Back signaling by the Nrg-1 intracellular domain. *J Cell Biol* 161:1133–1141.

Beltaifa S, Law AJ, Hyde TM, McClintock B, Herman MM, Harrison PJ, et al (2005): Expression levels and cellular localization of ErbB receptor mRNAs in the dorsolateral prefrontal cortex in schizophrenia [Abstract]. *J Neuropathol Exp Neurol* 64:437.

Bertram L, Hiltunen M, Parkinson M, Ingelsson M, Lange C, Ramasamy K, et al (2005): Family-based associations between Alzheimer's disease and variants in *UBQLN1*. *N Engl J Med* 352:884–894.

Blouin JL, Dombroski BA, Nath SK, Lasseter VK, Wolyniec PS, Nestadt G, et al (1998): Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. *Nat Genet* 20:70–73.

Bray NJ, Preece A, Williams NM, Moskvina V, Buckland PR, Owen MJ, O'Donovan MC (2005): Haplotypes at the dystrobrevin binding protein 1 (*DTNBP1*) gene locus mediate risk for schizophrenia through reduced *DTNBP1* expression. *Hum Mol Genet* 14:1947–1954.

Brzustowicz LM, Honer WG, Chow EWC, Little D, Hogan J, Hodgkinson K, Bassett AS (1999): Linkage of familial schizophrenia to chromosome 13q32. *Am J Hum Genet* 65:1096–1103.

Buonanno A, Fischbach GD (2001): Neuregulin and ErbB receptor signaling pathways in the nervous system. *Curr Opin Neurobiol* 11:287–296.

Burden S, Yarden Y (1997): Neuregulins and their receptors: A versatile signaling module in organogenesis and oncogenesis. *Neuron* 18:847–855.

Carpenter G (2003): ErbB-4: Mechanism of action and biology. *Exp Cell Res* 284:66–77.

Chaudhury AR, Gerecke KM, Wyss M, Morgan DG, Gordon MN, Carroll SL (2003): Neuregulin-1 and ErbB4 immunoreactivity is associated with neuritic plaques in Alzheimer disease brain and in a transgenic model of Alzheimer disease. *J Neuropathol Exp Neurol* 62:42–54.

Citri A, Skaria KB, Yarden Y (2003): The deaf and the dumb: The biology of ErbB-2 and ErbB-3. *Exp Cell Res* 284:54–65.

Colhoun HM, McKeigue PM, Davey Smith G (2003): Problems of reporting genetic associations with complex outcomes. *Lancet* 361:865–872.

Corfas G, Roy K, Buxbaum JD (2004): Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. *Nat Neurosci* 7:575–580.

Corvin AP, Morris DW, McGhee K, Schwaiger S, Scully P, Quinn J, et al (2004): Confirmation and refinement of an "at-risk" haplotype for schizophrenia suggests the EST cluster, Hs.97362, as a potential susceptibility gene at the Neuregulin-1 locus. *Mol Psychiatry* 9:208–212.

Coyle JT, Tsai G, Goff D (2003): Converging evidence of NMDA receptor hypofunction in the pathophysiology of schizophrenia. *Ann N Y Acad Sci* 1003:318–327.

Craddock N, Owen MJ (2005): The beginning of the end for the Kraepelinian dichotomy. *Br J Psychiatry* 186:364–366.

Crone SA, Lee K-F (2002): Gene targeting reveals multiple essential functions of the neuregulin signaling system during development of the neuroendocrine and nervous systems. *Ann N Y Acad Sci* 971:547–553.

Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, et al (2003): White matter changes in schizophrenia: Evidence for myelin-related dysfunction. *Arch Gen Psychiatry* 60:443–456.

DeLisi LE, Shaw S, Crow TJ, Shields G, Smith AB, Larach VW, et al (2000): Lack of evidence for linkage to chromosomes 13 and 8 for schizophrenia and schizoaffective disorder. *Am J Med Genet (Neuropsychiat Genet)* 96:235–239.

Dempster EL, Mill JS, Murray R, Collier DA (2004): Expression analysis of five schizophrenia candidate genes in post-mortem cerebellum samples using quantitative real time PCR [abstract]. *Am J Med Genet (Neuropsychiat Genet)* 130B:146.

Eastwood SL, Burnet PWJ, Harrison PJ (2005): Decreased hippocampal expression of the susceptibility gene PPP3CC and other calcineurin subunits in schizophrenia. *Biol Psychiatry* 57:702–710.

Eilam R, Pinkas-Kramarski R, Ratzkin BJ, Segal M, Yarden Y (1998): Activity-dependent regulation of Neu differentiation factor/neuregulin expression in rat brain. *Proc Natl Acad Sci U S A* 95:1888–1893.

Esper RM, Loeb JA (2004): Rapid axoglial signaling mediated by neuregulin and neurotrophic factors. *J Neurosci* 24:6218–6227.

Falls DL (2003a): Neuregulins: Functions, forms, and signaling strategies. *Exp Cell Res* 284:14–30.

Falls DL (2003b): Neuregulins and the neuromuscular system: 10 years of answers and questions. *J Neurocytol* 32:619–647.

Fischbach GD, Rosen KM (1997): ARIA: A neuromuscular junction neuregulin. *Annu Rev Neurosci* 20:429–458.

Flames N, Long JE, Garratt AN, Fischer TM, Gassmann M, Birchmeier C, et al (2004): Short- and long-range attraction of cortical GABAergic interneurons by Neuregulin-1. *Neuron* 44:251–261.

Garcia RA, Vasudevan K, Buonanno A (2000): The neuregulin receptor ErbB-4 interacts with PDZ-containing proteins at neuronal synapses. *Proc Natl Acad Sci U S A* 97:3596–3601.

Gardner M, Gonzales-Neira A, Lao O, Calafell F, Bertranpetit J, Comas D (2006): Extreme population differences across Neuregulin 1 gene, with implications for association studies. *Mol Psychiatry* 11:66–75.

Gerecke KM, Wyss JM, Carroll SL (2004): Neuregulin-1. β induces neurite extension and arborization in cultured hippocampal neurons. *Mol Cell Neurosci* 27:379–393.

Gierdalski M, Sardi SP, Corfas G, Juliano SL (2005): Endogenous neuregulin restores radial glia in a (ferret) model of cortical dysplasia. *J Neurosci* 25:8498–8504.

Go RCP, Perry RT, Weiner H, Bassett SS, Blacker D, Devlin D, Sweet RA (2005): Neuregulin-1 polymorphism in late onset Alzheimer's disease families with psychosis. *Am J Med Genet (Neuropsychiat Genet)* 1398:28–32.

Green EK, Raybould R, Macgregor S, Gordon-Smith K, Heron J, Hyde S, et al (2005): Operation of the schizophrenia susceptibility gene, neuregulin 1, across traditional diagnostic boundaries to increase risk for bipolar disorder. *Arch Gen Psychiatry* 62:642–648.

Gu Z, Jiang Q, Fu AKY, Ip NY, Yan Z (2005): Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. *J Neurosci* 25:4974–4984.

Gurling HMD, Kalsi G, Brynjolfson J, Sigmundsson T, Sherrington R, Mankoo BS, et al (2001): Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia on chromosomes 11q23.3-24 and 20q12.1-11.23. *Am J Hum Genet* 68:661–673.

Hall D, Gogos JA, Karayiorgou M (2004) The contribution of three strong candidate schizophrenia susceptibility genes in demographically distinct populations. *Genes Brain Behav* 3:240–248.

Harrison PJ (1999): The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain* 122:593–624.

Harrison PJ, Owen MJ (2003): Genes for schizophrenia? Recent findings and their pathophysiological implications. *Lancet* 361:417–419.

Harrison PJ, Weinberger DR (2005): Schizophrenia genes, gene expression, and neuropathology: On the matter of their convergence. *Mol Psychiatry* 10:40–68.

Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR (2004): Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. *Mol Psychiatry* 9:299–307.

Holmes WE, Sliwkowski MX, Akita RW, Henzel WJ, Lee J, Park JW, et al (1992): Identification of heregulin, a specific activator of p185erbB2. *Science* 256:1205–1210.

Hong CJ, Huo S, Liao D, Lee K, Wu H, Tsai S (2004): Case-control and family-based association studies between the *neuregulin 1* (Arg38Gln) polymorphism and schizophrenia. *Neurosci Lett* 366:158–161.

Huang YZ, Won S, Ali DW, Wang Q, Tanowitz M, Du QS, et al (2000): Regulation of neuregulin signaling by PSD-95 interacting with ErbB4 at CNS synapses. *Neuron* 26:443–455.

Iwata N, Suzuki T, Ikeda M, Kitajima T, Yamanouchi Y, Inada T, Ozaki N (2004): No association with the neuregulin 1 haplotype to Japanese schizophrenia. *Mol Psychiatry* 9:126–127.

Kampman O, Anttila S, Ari I, Saarela M, Rontu R, Mattila KM, et al (2004): Neuregulin genotype and medication response in Finnish patients with schizophrenia. *Neuroreport* 15:2517–2520.

Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD, et al (1998): NIMH Genetics Initiative Millennium Schizophrenia Consortium. Linkage analysis of African-American pedigrees. *Am J Med Genet* 81:282–289.

Kendler KS, MacLean CJ, O'Neill FA, Burke J, Murphy B, Duke F, et al (1996): Evidence for a schizophrenia vulnerability locus on chromosome 8p in the Irish study of high-density schizophrenia families. *Am J Psychiatry* 153:1534–1540.

Kerber G, Streif R, Schwaiger F, Kreutzberg G, Hager G (2003): Neuregulin-1 isoforms are differentially expressed in the intact and regenerating adult rat nervous system. *J Mol Neurosci* 21:149–165.

Kunugi H, Curtis D, Valalda HP, Nanko S, Powell JF, Murray RM, et al (1996): A linkage study of schizophrenia with DNA markers from chromosome 8p21-22 in 25 multiplex families. *Schizophr Res* 22:61–68.

Kwon O-B, Longart M, Vulhorst D, Hoffman DA, Buonanno A (2005): Neuregulin-1 reverses long-term potentiation at CA1 hippocampal synapses. *J Neurosci* 12:9378–9383.

Lachman H, Pedrosa E, Ye K, Nolan K, Saito T (2005): Analysis of polymorphisms in AT-rich domains of neuregulin 1 gene in schizophrenia [Abstract]. *Am J Med Genet (Neuropsychiat Genet)* 138B:87.

Lai C, Feng LY (2004): Neuregulin induces proliferation of neural progenitor cells via PLC/PKC pathway. *Biochem Biophys Res Commun* 319:603–611.

Law AJ, Weickert CS, Hyde TM, Kleinman JE, Harrison PJ (2004): Neuregulin-1 (NRG-1) mRNA and protein in the adult human brain. *Neuroscience* 127: 125–136.

Law AJ, Weickert CS, Hyde T, Kleinman JE, Harrison PJ (2005): Alterations of neuregulin 1 and ErbB4 expression in the cerebellum in schizophrenia [Abstract]. *Schizophr Bull* 31: program no 114241.

Leonard S, Mexal S, Berger R, Freedman R (2005): Neuregulin-1 expression is decreased in primary skin fibroblasts of schizophrenic subjects [Abstract]. *Schizophr Bull* 31: program no114870.

Levinson DF, Wildenauer DB, Schwab SG, Albus M, Hallmayer J, Lerer B, et al (1996): Additional support for schizophrenia linkage on chromosomes 6 and 8: A multicenter study. *Am J Med Genet* 67:580–594.

Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, et al (2003): Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73:34–48.

Lewis DA, Hashimoto T, Volk DW (2005): Cortical inhibitory neurons and schizophrenia. *Nature Rev Neurosci* 6:312–324.

Li Q, Loeb JA (2001): Neuregulin-heparan-sulfate proteoglycan interactions produce sustained erbB receptor activation required for the induction of acetylcholine receptors in muscle. *J Biol Chem* 276:38068–38075.

Li T, Stefansson H, Gudfinnsson E, Cai G, Liu X, Murray RM, et al (2004): Identification of a novel neuregulin 1 at-risk haplotype in Han schizophrenia Chinese patients, but no association with Icelandic/Scottish risk haplotype. *Mol Psychiatry* 9:698–705.

Lin H-F, Liu Y-L, Liu C-M, Hung S-I, Hwu H-G, Chen WI (2005): Neuregulin 1 gene and variations in perceptual aberration of schizotypal personality in adolescents. *Psychol Med* 35:1589–1598.

Liu CM, Hwu, HG, Fann C, Lin CY, Liu YL, Ou-Yang WC, et al (2005): Linkage evidence of schizophrenia to loci near neuregulin 1 gene n chromosome 8p21 in Taiwanese families. *Am J Med Genet (Neuropsychiat Genet)* 134B:79–83.

Loeb JA (2003): Neuregulin: An activity-dependent synaptic modulator at the neuromuscular junction. *J Neurocytol* 32:649–664.

Mata I, Arranz MJ, Staddon S, Beperet M, Lopez-Ilundain JM, Murray RM, Kerwin RW (2004): Association study of neuregulin 1 gene and schizophrenia in a population isolate from northern Spain [Abstract]. *Am J Med Genet (Neuropsychiat Genet)* 130B:83.

Meng Y, Zhang Y, Tregoubov V, Falls DL, Jia Z (2003): Regulation of spine morphology and synaptic function by LIMK and the actin cytoskeleton. *Rev Neurosci* 14:233–240.

Meyer D, Yammai T, Garratt A, Riethmacher-Sonnenberg E, Kane D, Theill LE, et al (1997): Isoform-specific expression and function of neuregulin. *Development* 124:3575–3586.

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, et al (2004): Axonal neuregulin-1 regulates myelin sheath thickness. *Science* 304:700–703.

Mirnics K, Middleton FA, Stanwood GD, Lewis DA, Levitt P (2001): Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia. *Mol Psychiatry* 6:293–301.

Murphy S, Krainock R, Tham M (2002): Neuregulin signaling via erbB receptor assemblies in the nervous system. *Mol Neurobiol* 25:67–77.

Navon R, Shamir E, Zerah G, Swartz M, Kaganovich M, Korostishevsky M (2004): Association and expression analysis of neuregulin-1 region: Implication to its role in susceptibility to schizophrenia [abstract]. *Am J Med Genet (Neuropsychiat Genet)* 130B:86.

Norton N, Moskvina V, Morris DW, Bray NJ, Zammit S, et al (2006): Evidence that interaction between Neuregulin 1 and its receptor erbB4 increases susceptibility to schizophrenia. *Am J Med Genet (Neuropsychiat Genet)* 144B:96–101.

Owen MJ, Craddock N, O'Donovan MC (2005): Schizophrenia: Genes at last? *Trends Genetics* 21:519–525.

Ozaki M (2001): Neuregulins and the shaping of synapses. *The Neuroscientist* 7:146–154.

Ozaki M, Itoh K, Miyakawa Y, Kishida H, Hashikawa T (2004): Protein processing and releases of neuregulin-1 are regulated in an activity-dependent manner. *J Neurochem* 91:176–188.

Paige GP, George V, Go RC, Page PZ, Allison DB (2003): "Are we there yet?": Deciding when one has demonstrated specific genetic causation in complex diseases and quantitative traits. *Am J Hum Genet* 73:711–719.

Petryshen TL, Middelton FA, Kirby A, Aldinger KA, Purcell S, Tahl AR, et al (2005): Support for involvement of neuregulin 1 in schizophrenia pathophysiology. *Mol Psychiatry* 10:366–374.

Pulver AE, Lasseter VK, Kasch L, Wolyniec P, Nestadt G, Blouin J-L, et al (1995): Schizophrenia: A genome scan targets chromosomes 3p and 8p as potential sites of susceptibility genes. *Am J Med Genet* 60:252–260.

Roysommuti S, Carroll SL, Wyss JM (2003): Neuregulin-1β modulates *in vivo* entorhinal-hippocampal synaptic transmission in adult rats. *Neuroscience* 121:779–785.

Schmid RS, McGrath B, Berechid BE, Boyles B, Marchionni M, Sestan N, Anton ES (2003): Neuregulin 1-erbB2 signaling is required for the establishment of radial glia and their transformation into astrocytes in cerebral cortex. *Proc Natl Acad Sci U S A* 100:4251–4256.

Shaw SH, Kelly M, Smith AB, Shields G, Hopkins PJ, Loftus J, et al (1998): A genome-wide search for schizophrenia susceptibility genes. *Am J Med Genet* 81:364–376.

Shyu W-C, Lin S-Z, Chiang M-F, Yang H-I, Thajeb P, Li H (2004): Neuregulin-1 reduces ischaemia-induced brain damage in rats. *Neurobiol Aging* 25: 935–944.

Silberberg G, Navon R (2005): The involvement of ErbB4 with schizophrenia: association and expression studies [Abstract]. *Am J Hum Genet Neuropscyhiatr Genet* 138B:130.

Singleton A, Myers A, Hardy J (2004): The law of mass action applied to neurodegnerative disease: A hypothesis concerning the etiology and pathogenesis of complex diseases. *Hum Mol Genet* 13: R123–R126.

Sklar P, Pato MT, Kirby A, Petryshen TL, Medeiros H, Carvalho C, et al (2004): Genome-wide scan in Portugese Island families identifies 5q31-5q35 as a susceptibility locus for schizophrenia and psychosis. *Mol Psychiatry* 9:213–218.

Stefansson H, Sarginson J, Kong A, Yates P, Steinthorsdottir V, Gudfinnsson E, et al (2003): Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. *Am J Hum Genet* 72:83–87.

Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, et al (2002): *Neuregulin 1* and susceptibility to schizophrenia. *Am J Hum Genet* 71:877–892.

Steinthorsdottir V, Stefansson H, Ghosh S, Birgisdottir B, Bjornsdottir S, Fasquel AC, et al (2004): Multiple novel transcription initiation sites for *NRG1*. *Gene* 342:97–105.

Tang JX, Chen WY, He G, Zhou J, Gu NF, Feng GY, He L (2004): Polymorphisms within 5′ end of the Neuregulin 1 gene are genetically associated with schizophrenia in the Chinese population. *Mol Psychiatry* 9:11–12.

Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, et al (2005): Neuregulin-1 Type III determines the ensheathment fate of axons. *Neuron* 47:681–694.

Thiselton DL, Webb BT, Neale BM, Ribble R, O'Neill FA, Walsh D, et al (2004): No evidence for linkage or association of neuregulin-1 (NRG1) with disease in the Irish study of high-density schizophrenia families (ISHDSF). *Mol Psychiatry* 9:777–783.

Tkachev D, Mimmack M, Ryan MM, Wayland M, Freeman T, Jones PB, et al (2003): Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet* 362:798–805.

Tosato S, Dazzan P, Collier D (2005) Association between the neuregulin 1 gene and schizophrenia: A systematic review. *Schizophr Bull* 31:613–617.

Tunbridge EM, Harrison PJ, Weinberger DR (2006): Catechol-o-methyltransferase, cognition and schizophrenia: Val^{158}Met and beyond. *Biol Psychiatry* (XX:XX–XX).

Wang JY, Miller SJ, Falls DL (2001): The N-terminal region of neuregulin isoforms determines the accumulation of cell surface and released neuregulin ectodomain. *J Biol Chem* 276:2841–2851.

Weickert CS, Straub RE, McClintock BW, Matsumoto M, Hashimoto M, Hyde TM, et al (2004): Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. *Arch Gen Psychiatry* 61:544–555.

Williams NM, Norton N, Williams H, Ekholm B, Hamshere ML, Lindblom Y, et al (2003a): A systematic genomewide linkage study in 353 sib pairs with schizophrenia. *Am J Hum Genet* 73:1355–1367.

Williams NM, Preece A, Spurlock G, Norton N, Williams HJ, Zammit S, et al (2003b): Support for genetic variation in neuregulin 1 and susceptibility to schizophrenia. *Mol Psychiatry* 8:485–487.

Yang JZ, Si TM, Ruan Y, Ling YS, Han YH, Wang XL, et al (2003): Association study of neuregulin 1 gene with schizophrenia. *Mol Psychiatry* 8:706–709.

Yarden Y, Sliwkowski MX (2001): Untangling the ErbB signaling network. *Nature Rev Mol Cell Biol* 2:127–137.

Zhao X, Shi Y, Tang J, Tang R, Yu L, Gu N, et al (2004): A case control and family based association study of the neuregulin1 gene and schizophrenia. *J Med Genet* 41:31–34.