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Imaging genetics of structural brain connectivity and neural integrity markers

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Abstract

We review studies that have used diffusion imaging (DI) and magnetic resonance spectroscopy (MRS) to investigate genetic associations. A brief description of the measures obtainable with these methods and of some methodological and interpretability limitations is given. The usefulness of DI and MRS in defining intermediate phenotypes and in demonstrating the effects of common genetic variants known to increase risk for psychiatric manifestations on anatomical and metabolic phenotypes are reviewed. The main focus is on schizophrenia where the greatest amount of data has been collected. Moreover, we present an example coming from a different approach, where the genetic alteration is known (the deletion that causes Williams syndrome) and the DI phenotype can shed new light on the function of genes affected by the mutation. We conclude that, although these are still early days of this type of research and many findings remain controversial, both techniques can significantly contribute to the understanding of genetic effects in the brain and the pathophysiology of psychiatric disorders.

Keywords

diffusion tensor imaging; spectroscopy; genes; psychiatric disorders

Rationale

As other reviews in this issue will expand on in more detail, there is a strong rationale for imaging genetics. The characterization of genetic mutations or common variants that impact on protein expression or function provides a natural experimental laboratory in order to understand the function of such proteins. Since imaging is the only tool that allows the description of brain phenotypes in vivo non-invasively, imaging genetics is critical to our understanding of normal and pathological anatomy and function, especially when in the case of psychiatric disorders. In this review, we will focus on diffusion imaging (DI) and magnetic resonance spectroscopy (MRS).

Due to space limitations, it is impossible to review all studies contributing to this field. We will focus on schizophrenia, where we will explore studies showing whether measures derived from either methodology have intermediate phenotype status (i.e. are phenotypes present in patients and to a lesser degree in unaffected individuals at genetic risk for

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developing the disorder) and investigations of common genetic variants (single nucleotide polymorphisms: SNPs) in genes known to increase risk for the disorder. We will also mention Williams syndrome as an example of a well defined genetic alteration (a hemideletion) that can provide information on the specific genes affected by the mutation. We will also mention a couple of examples from genes implicated in other neuropsychiatric diseases (i.e. mood disorders, attention deficit hyperactivity disorder [ADHD] and Alzheimer disease).

Measures/Advantages

Diffusion Imaging

Diffusion imaging (DI) - a method increasingly used to study the structural integrity of white matter tracts and implicitly brain connectivity- yields quantitative measures such as fractional anisotropy (FA), mean, longitudinal and radial diffusivity, and principal eigenvector directionality that reflect the microscopic organization of tissue. We refer the reader to (Basser and Pierpaoli, 1996; Mori and Zhang, 2006; Pierpaoli and Basser, 1996) to understand how these quantities are derived, and we will give a brief explanation of the issues involved in interpreting them. There is a great potential for diffusion imaging to become a tool to investigate the effect of genes on white matter with broad implications for the advancement of both anatomical and functional knowledge, however some cautions are necessary.

The main quantities studied so far are fractional anisotropy (FA) and mean diffusivity (Pierpaoli and Basser, 1996). Trace and apparent diffusion coefficient (ADC) are related to mean diffusivity and subject to similar interpretation. FA represents an index of the directional bias of water diffusion, generally caused by the presence of multiple obstacles to diffusion coherently oriented in space. This is the case in white matter, where axon membranes and myelin impede water diffusion in the direction perpendicular to the tracts, and where FA will be high. FA will be low in areas of where diffusion is isotropic, i.e. regions of the brain where barriers to diffusion do not exist (e.g. in cerebrospinal fluid: CSF) or are not coherently oriented in space (e.g. gray matter). A special case of the latter, are areas of white matter with crossing fibers. High FA is usually associated with high myelination and preserved structural integrity of white matter, however there are notable exceptions because a change in the “architecture” of crossing fibers may have occurred (e.g. Pierpaoli et al., 2001). Mean diffusivity (MD) is the total amount of diffusivity and its increase can indicate destruction of tissue and atrophy, however it also may be dependent on T2 values of the tissue. It is also used as an index of cellularity in tumors, where low MD in the tumor reflects a higher number of cells. Other less frequently used measures such as mode (Ennis and Kindlmann, 2006) or skewness (Pierpaoli and Basser, 1996) can help detect areas of crossing fibers.

Analysis of DI quantities varies widely, from region of interest (ROI) analysis (including the use of tractography, which has been applied rarely to imaging genetics) to voxel-based methods of analysis. The reader should be aware of the pitfalls associated with each (e.g. Jones et al., 2005; Smith et al., 2006).

DI acquisition is fairly complex and, unfortunately, most of the literature does not conform to standards of excellence in the acquisition of the images (Smith et al., 2007) and quality control procedures are rarely described. There are also limitations of interpretability to the measures obtained with DI. Often, multiple biological processes may explain the same finding (for example FA reductions can be interpreted as reduced myelin, reduced number of axons or increases in crossing fibers), and histological studies are often not available to support or diminish an interpretation made on the basis of *in vivo* data.

MRS—MRS is the only method that allows detection of certain brain metabolites *in vivo*, therefore, it too can have a critical role in gene effect discovery. We will discuss here only ^1H spectroscopy because of space limitations. The most commonly studied peaks (which usually include two or more metabolites) are N-acetyl-aspartate (Naa), Choline (Cho), and creatine (Cre). Naa is a molecule produced exclusively in neurons, possibly implicated in fatty acids and steroid synthesis in myelination processes and maintenance of osmotic equilibrium within neurons (for an extensive review see Moffett et al., 2007), and likely linked to glutamate metabolism (Petroff et al., 2002) due to similar energy requirements in the production of these aminoacids. Naa decreases when there is tissue destruction, and reversibly so when the neuronal tissue is suffering (De Stefano et al., 1998) and therefore non-specifically reflects the general state of health of neurons. Moreover, it has been correlated with synaptophysin (Lentz et al., 2005), a marker of synaptic integrity. 10–15% of the Naa peak, especially when acquired at short times of echo, is constituted by N-acetyl-aspartyl-glutamate (NAAG), an aminoacid that can function as an inhibitory neurotransmitter for N-methyl-d-aspartate (NMDA) and as an agonist at metabotropic glutamate receptors (Coyle and Tsai, 2004). Separation of the signal coming from this specific metabolite remains difficult methodologically and no studies of genetic regulation of NAAG are available. The Cho peak contains various metabolites that reflect membrane metabolism. An increase in this peak has been observed in multiple sclerosis where dramatic membrane rearrangement occurs (Narayana, 2005). Cre is a peak that appears to be relatively stable in the face of pathology and has therefore been used mainly as a reference value for other metabolites (Urenjak et al., 1993). Other metabolic peaks of interest are Glx, which combines glutamate (Glu), glutamine (Gln) and a small amount of γ -aminobutyric acid (GABA). When this peak is measured with a time of echo (TE) of 80 ms, it expresses mainly glutamate (Schubert et al., 2004).

In the MRS field, methodological variation in time of echo of the acquisition, signal-to-noise ratio (SNR), and lack of standardized procedures for quality control are common. The biggest problem relates to the subjectivity of assessment of spectral quality (Kreis, 2004). Even measures such as Cramer-Rao lower bounds, a frequently used metric of spectral fitting error, are not foolproof as they will not apply where the data deviate drastically from the model to be fitted and they represent a minimum possible error. The values chosen for analysis are also variable and include ratios between metabolites or to water. The latter have gained credence in the field as “absolute” values but should be viewed with caution especially in voxels that include CSF at high fields where prolongation of T1 values make saturation of the signal an issue. All this conspires to make replication of a study a rarity in the field. This review will focus almost exclusively on Naa, but interpretation of the results is limited due to the polymorphous and partly understood function of this metabolite.

Imaging genetics and neuronal integrity as assessed by DI and MRS

Schizophrenia

Intermediate Phenotype studies—We will refer the reader to (Meyer-Lindenberg and Weinberger, 2006; Tan et al., 2008) for description of this strategy to demonstrate genetic effects in psychiatric disorders. Despite the presence of two papers showing substantial heritability of FA in several brain regions of normal twins (Chiang et al., 2009; Pfefferbaum et al., 2001), definition of a clearly identifiable DI phenotype in schizophrenia is lacking. There have been a multitude of studies, with many disparate findings (Kanaan et al., 2005) and recent attempts to find common ground among these (Ellison-Wright and Bullmore, 2009) are not unequivocal due to methodological limitations of meta-analyses in imaging space. One widely accepted pathophysiological hypothesis of schizophrenia highlights altered functional and structural connectivity, for instance between the frontal and the temporal lobe (Bullmore et al., 1998; Friston and Frith, 1995; Meyer-Lindenberg et al.,

2001; Weinberger et al., 1992). Although this hypothesis is likely to apply to synaptic connections at a microscopic ultrastructural level that cannot be captured by DI methodology, it is also true that communication via long distance white matter tracts may be affected by subtle myelination deficits. Various evidence has emerged implicating genes that are mainly expressed in oligodendrocytes (e.g. Georgieva et al., 2006; Peirce et al., 2006) and that could affect myelination (partly reflected in FA measures) in schizophrenia risk. Also genes such as neuregulin 1 (NRG1) and ErbB4 (Norton et al., 2006; Silberberg et al., 2006; Stefansson et al., 2002) have been associated with schizophrenia and are involved in the formation of major neural pathways during development. Risk variants within these genes may modify the normal architecture of white matter tracts and possibly produce FA, MD and fiber tract directionality changes. Regional specificity is not necessarily predictable for these proposed pathophysiological mechanisms and genetic heterogeneity may constitute one of the sources for the disparate findings in schizophrenia.

A recent study addressing this issue (Munoz Maniega et al., 2008) found that high risk individuals and patients with schizophrenia shared decreased FA in the anterior limb of the internal capsule, which could then constitute an intermediate phenotype. However, a smaller study (Hoptman et al., 2008) did not demonstrate regions of altered anisotropy overlapping between patients and high risk individuals (see Table 1 for more details on DI and MRS intermediate phenotype studies in schizophrenia).

Narr et al. (2009) showed that mean diffusivity was increased in the bilateral temporal lobes and superior temporal gyri, even after covarying for CSF volume, in patients with schizophrenia compared to their age matched controls. This difference was also present in the first degree unaffected relatives, therefore constituting an intermediate phenotype in schizophrenia. In a smaller study of patients and high risk individuals, another group (DeLisi et al., 2006) found a different regional pattern of heritability. Measures of cortical mean diffusivity are difficult to evaluate in the absence of rigorous control of partial volume effects with CSF, so these results await replication.

Compared with DI studies, a somewhat clearer picture related to intermediate phenotypes emerges for MRS studies (see Table 1 for details). The prior hypothesis for these studies had been laid out by the initial studies by Bertolino et al. (1998;1996), showing reduced Naa in the dorsolateral prefrontal cortex and in the hippocampal formation in patients with schizophrenia as compared to normal controls. The largest intermediate phenotype study to date (Callicott et al., 1998), found hippocampal reductions of Naa/Cre in 47 patients and 60 unaffected siblings as compared to 66 healthy controls. About 25% of the siblings had a psychiatric diagnosis, however, hippocampal Naa/Cre ratios were still significantly lower in the sibling group as compared to the controls even after those data points were removed. Moreover, substantial heritability of low hippocampal Naa/Cre was found (relative risk of 8.8 for the average of left and right hippocampi, 3.6 after dropping sibling pairs with psychiatric diagnoses) on a qualitative measure of Naa/Cre reduction.

In the anterior cingulate, Naa/Cho ratios were reduced in offspring of patients with schizophrenia without psychosis compared to matched normal controls (Keshavan et al., 1997), but this early finding was recently contradicted by two negative studies (Purdon et al., 2008; Yoo et al., 2009). Reductions of Naa, Cre and Cho in the left thalamus were also proposed as a possible intermediate phenotype (Yoo et al., 2009). Data from a small group of family members of patients with schizophrenia was recently published, showing that dorsolateral prefrontal Naa/Cho in non-psychotic family members of patients with schizophrenia were intermediate between controls and patients, but not statistically significantly different from those of controls (Block et al., 2000; Wobrock et al., 2008). This

result is in line with findings by Callicott et al. (1998) in the much larger study mentioned above.

An interesting alternative approach consisting of the examination of twins discordant for schizophrenia was recently published (Lutkenhoff et al., 2008). In this study, medial temporal lobe metabolite levels (Cho, Cre and especially Naa), were increased in patients with schizophrenia, but unchanged from controls in their unaffected twins, therefore constituting a “state” variable. These results are clearly discordant with those of Callicott et al. (1998) and with the general consensus of the literature accumulated on 1.5T scanners (Steen et al., 2005). There are several important methodological differences between the studies, the main ones being the use of a 3T scanner and quantification with reference to water. Although these findings await further replication in larger studies, it is possible that a biological basis exists for these discrepancies, especially when considering alterations in the T2 of water (Aydin et al., 2007; Flynn et al., 2003; MacKay et al., 2006; Pfefferbaum et al., 1999) and metabolites (Tunc-Skarka et al., 2009) that have been reported in schizophrenia.

The main finding of this study (Lutkenhoff et al., 2008), however, was that glutamate was reduced overall in patients and their twins as compared to controls, which would thus qualify for a potential intermediate phenotype. This effect was most marked in the mesial prefrontal cortex. These results are in the opposite direction of those from Purdon et al. (Purdon et al., 2008) in unaffected siblings of patients with schizophrenia. These authors found that a qualitative measure of high glutamate (median split) could segregate high risk from non-risk individuals with moderate sensitivity (71%) and specificity (67%). The finding of altered glutamate levels in patients with schizophrenia, however, is controversial, with three studies showing reductions (Lutkenhoff et al., 2008; Tayoshi et al., 2009; Theberge et al., 2003), two demonstrating no difference (Shirayama et al., 2009; Stanley et al., 1996) and two increases in chronic patients as compared to controls (Rusch et al., 2008; van Elst et al., 2005). In addition, most studies of glutamate in never treated first episode patients (Bartha et al., 1999; Bartha et al., 1997; Theberge et al., 2002) and of the combined peak of glutamate and glutamine (Glx) (Block et al., 2000; Kegeles et al., 2000; Ohrmann et al., 2008; Wood et al., 2007) have found no difference between patients with schizophrenia vs. controls. The discrepancies in the literature remain for glutamine (up in Bartha et al., 1997; Rusch et al., 2008; Stanley et al., 1996; Theberge et al., 2002; van Elst et al., 2005, unchanged in Bartha et al., 1999; Shirayama et al., 2009; Tayoshi et al., 2009, down in Theberge et al., 2003), and, in addition, the uncertainty in determination of these values are larger than for any of the other metabolites and should consequently be viewed conservatively. Larger studies of patients and individuals at risk are needed to confirm these findings.

In summary, the above MRS findings although not unequivocal, suggest that this might be a powerful tool for the characterization of intermediate phenotypes in schizophrenia. The hippocampal reduction in Naa/Cr described by Callicott et al. (1998) remains the best supported evidence because of its size.

The case for multi-modal studies in families with ADHD—An interesting example of a successful intermediate phenotype study comes from the ADHD field. We propose that a similar strategy might also well serve identification of intermediate phenotypes for schizophrenia. Casey et al. (2007) focused on a well characterized behavioural abnormality (deficit in cognitive control), characterized its physiological mechanisms (alterations in prefrontal-striatal activation with fMRI), and used DT tractography between brain regions defined with fMRI to show a substantial degree of correlation between the FA of right prefrontal tracts in parent and child dyads with ADHD with task performance and fMRI activation. The FA measures in parents and their children were also highly correlated,

indicating a substantial degree of familiarity and possibly genetic contribution. There was no such correlation in the healthy volunteer parent-child dyads, indicating that this intermediate phenotype might be illness specific. The lesson would be to move away from whole brain analysis of a not well replicable and diffuse phenotype to focus on the anatomical underpinnings of well established and functionally relevant multimodal phenotypes.

Common genetic variation in schizophrenia—The effect of common genetic variation associated with risk for schizophrenia on white matter of fronto-thalamic tracts has also emerged from studies of the association of DI phenotypes with variation in neuregulin-1 (NRG1). NRG1 is a protein with multiple functions and very complex regulation that appears to be an important growth factor and modulator of central nervous system development (Falls, 2003). In the peripheral nervous system, it has an important role in myelination (Vartanian et al., 1997), and although this may be the case also for the central nervous system (Taveggia et al., 2008), a recent investigation suggests otherwise (Brinkmann et al., 2008). Neuregulin and its receptors (ErbB4 in particular) have been shown to regulate the tangential migration of interneurons to the cortex (Flames et al., 2004) and the formation of a permissive corridor through the medial ganglionic eminence that is critical in the appropriate formation of thalamocortical connections (Lopez-Bendito et al., 2006).

Overall the imaging studies with NRG1 focused on two SNPs: rs6994992 (SNP8NRG243177: associated with differential type IV mRNA expression in normal and schizophrenia postmortem data (Law et al., 2006) and rs35753505 (SNP8NRG221533: the only SNP individually associated with schizophrenia in the original report (Stefansson et al., 2002). McIntosh et al. (2008) first described an association between reduced FA in the anterior limb of the internal capsule and the risk allele (T) at rs6994992. Winterer et al. (2008) explored rs35753505-SNP8NRG221533, finding that FA was reduced in the white matter underlying medial pre-frontal cortex in risk allele (C) carriers. This occurred in the absence of significant changes in brain volume, as assessed with voxel based morphometry (VBM) of T1-weighted images. A recent study (Sprooten et al., 2009), conducted on a small number of individuals (n=34), showed that the two NRG1 SNPs studied were in linkage disequilibrium, belonged to the same haplotype, and were both associated with lower FA in the anterior thalamic radiation in the left hemisphere, as defined by tractography.

Further data on the effect of rs35753505 were reported by Wang et al. (2009) in a restricted portion of the anterior cingulum bundle in patients with schizophrenia and controls of Chinese ethnicity. Their findings in patients had inverse directionality to those reported by Winterer et al. (2008) for the same SNP in normal controls, but the two studies remain difficult to compare due to the different combination of genotypes reported, and to the fact that the allele that increases risk for schizophrenia may change with ethnic background (Tosato et al., 2005). Moreover, effects of treatment in patients with schizophrenia are not well characterized in DI studies.

Another study focused on genetic variation in ErbB4 (Konrad et al., 2009). Also this gene has been associated with schizophrenia risk (Nicodemus et al., 2006; Norton et al., 2006; Silberberg et al., 2006) and SNP rs839523 tagged the schizophrenia risk haplotype block and had the highest association with schizophrenia of all investigated ErbB4 SNPs. The authors (Konrad et al., 2009) found a reduction of FA in risk allele (G) carriers for rs839523 in the temporal lobe, especially on the left side, in a region where fibers from different tracts intersect each other (including ILF/IFO, uncinate and arcuate fasciculi). The results were independent of any volumetric change measured with T1-weighted imaging. This study stands out because of the significant negative correlation of FA in the left temporal lobe with reaction time during a visual oddball task, a meaningful functional measure that is also

altered in schizophrenia. While the initial NRG1 studies mentioned above (McIntosh et al., 2008; Sprooten et al., 2009; Winterer et al., 2008) may all be compatible with the regionality of the intermediate phenotype study of Munoz-Maniega et al. (2008), the ErbB4 study, seems to point to a different brain region mediating genetic influences on FA.

A further study deserves mention in this section, although the association of the gene studied (catechol-o-methyl-transferase: COMT) with schizophrenia is controversial (Williams et al., 2007). Given the importance of cognitive dysfunction to schizophrenia, and the association of COMT with cognitive abilities (Dickinson and Elvevag, 2009; Egan et al., 2001), however, we think that the paper by Li et al. (2009a) is relevant. They found that in 80 healthy controls and 15 individuals with mental retardation significant correlations between IQ and FA in the bilateral basal frontal lobe and the hippocampal formation were mediated by COMT val158met genotype, with val homozygotes accounting for the entire effect. FA and IQ were not significantly correlated to each other in the met carriers. This study was adequately sized and quite powerful in conception. Val homozygotes are thought to have low synaptic cortical dopamine levels and reduced dopaminergic cell firing (reviewed in Tunbridge et al., 2006). In this situation, the degree of myelination of the white matter of the hippocampal formation and prefrontal cortex may be a critical factor in determining IQ, while if the cortex is adequately supplied with dopamine, the myelination of the white matter fibers may not be as essential. Other interpretations are possible, however. The generalizability of this finding to Caucasian populations remains to be established.

The MRS field has focused on different risk genes for schizophrenia. Callicott et al. (2005) studied associations of SNPs in DISC1, implicated in risk for schizophrenia by several investigations (e.g. Blackwood et al., 2001; Devon et al., 2001; Millar et al., 2000) with MRS measures in the hippocampal formation. DISC1 is critical to hippocampal formation development (Austin et al., 2004) and its highest expression is found in this area of the brain (Miyoshi et al., 2003). Callicott et al. (2005) found trend reductions of Naa/Cr associated with the risk allele of a SNP (rs821616) responsible for a Ser704Cys substitution. The Ser alleles were also associated with increased risk for schizophrenia, reduced hippocampal gray matter volume, excessive hippocampal activation during fMRI of a working memory task and reduced mnemonic performance.

Our group (Egan et al., 2004) found reduced Naa/Cr in the prefrontal cortex of individuals homozygote for the A allele of rs6465084 in the glutamate metabotropic receptor 3 (GRM3) gene, also implicated in risk for schizophrenia by several association studies (e.g. Egan et al., 2004; Fujii et al., 2003; Marti et al., 2002). The MRS result was subsequently confirmed at higher field (Marenco et al., 2006) in healthy volunteers. Although the location of reduced Naa was not the region associated with intermediate phenotype status in schizophrenia, the findings fit with what we know of the critical role of GRM3 in the physiology of the prefrontal cortex (Egan et al., 2004; Tan et al., 2007) and of the location of expression of novel splice variants in schizophrenia (Sartorius et al., 2008). For this gene, there is also some evidence of association with obstetric complications (Nicodemus et al., 2008), which might explain in part the discordance with the regional distribution of intermediate phenotype studies.

Common variation in genes associated with mood disorders, aging and Alzheimer disease—A similar approach has been followed in the investigation of Alzheimer disease, where asymptomatic populations with specific genetic risk structure have been selected (because of specific mutations in presenilin-1 and amyloid precursor protein: Ringman et al., 2007) or because of carrying one or more copies of the Apoε4 allele (Honea et al., 2009; Nierenberg et al., 2005; Persson et al., 2006; Smith et al., 2008). From this literature, reduced FA in the medial temporal lobe, fornix and perisplenial portions of

the cingulum bundle have emerged as possible phenotypes of interest, possibly mediating some of the effects of aging and Alzheimer disease. The MRS literature on ApoE4 (Bartres-Faz et al., 2002; Enzinger et al., 2003; Kantarci et al., 2002; Laakso et al., 2003) is conflicting and has some methodological limitations, and therefore remains inconclusive.

The effects of variation at the val66met locus of the brain derived neurotrophic factor (BDNF) gene have also been studied. This common variant causes a substitution of a valine for a methionine in the protein and causes alterations in the post-translational processing of the BDNF protein affecting its intra-cellular trafficking and activity-dependent secretion (Egan et al., 2003). BDNF plays an important role in the maintenance and development of neural networks (Kuczewski et al., 2009) and in neural mechanisms associated with learning (Lu and Martinowich, 2008), and is thought to have relevance for depression and possibly schizophrenia (Rybakowski, 2008). Egan et al. (Egan et al., 2003) characterized biological associations in met allele carriers such as reduced episodic memory performance and excessive hippocampal activation with fMRI of working memory. They also found that Naa/Cr in the left hippocampal formation was reduced in healthy individuals with at least one met allele at the BDNF val66met locus as compared to val homozygotes. This result was recently confirmed on a higher field magnet in a group of healthy volunteers (Stern et al., 2008). A recent investigation (Gallinat et al., 2009), however, found that hippocampal Cr was decreased and Naa/Cr was increased in met carriers as compared to val homozygotes, an effect opposite to what was expected based on our prior findings. In addition, Naa in the anterior cingulate was significantly increased in met carriers (Gallinat et al., 2009). Several factors, including acquisition methodology, genetic background, recruitment procedures, and possibly other sample characteristics (such as intellectual abilities) may have contributed to these differences. Further studies are needed to understand these discrepancies and their implication for the biology of mental illness.

Other studies of interest have addressed the effects of a common variant (known as L for long and S for short) of the promoter region in the serotonin transporter gene (5-HTTLPR), implicated in risk for depression and anxious personality traits (Caspi et al., 2003; Greenberg et al., 2000; Lesch et al., 1996; Mazzanti et al., 1998; Schinka et al., 2004) both with DI and MRS. The S allele (producing less 5-HTT and associated with anxious traits) was associated with lower the FA in the anterior uncinate fasciculus (Pacheco et al., 2009) and reduced Naa in the left hippocampus (Gallinat et al., 2005) compared with L homozygotes. Moreover, there was a negative correlation between hippocampal Naa and trait anxiety scores. The authors interpret this in the context of serotonin's role in supporting neurogenesis and hippocampal response to stress (Czeh et al., 2001).

Williams syndrome

An illustration of how phenotypic characterization with DI can help guide understanding of genetic effects comes from the work done in Williams syndrome, a hemideletion on chromosome 7 that is associated with cognitive abnormalities (usually mental retardation and a remarkable deficit in visual construction), hypersociability and increased incidence of phobias. Other reviews in this issue will describe this syndrome in more detail. Two papers showed that anisotropy was increased in longitudinal tracts in Williams syndrome (Hoeft et al., 2007; Marenco et al., 2007). In addition, Marenco et al. (2007) showed in a small sample of participants with preserved IQ that fiber tract directionality was altered in a number of structures, with increased longitudinally oriented fibers and decreased fibers in the mediolateral orientation. This led to the hypothesis that one or more of the genes involved in the hemideletion might be involved in guiding axons over the midline or in the mediolateral orientation, and that the hypofunction of these genes resulted in the antero-posterior rearrangement of fibers. This is a mechanism that is known to occur for other genes such as Netrin-1 and Deleted in Colon Cancer (DCC) (Ren et al., 2007). This hypothesis awaits

further confirmation in short deletion syndromes that should allow the reduction of the list of candidate genes underlying this phenotype. If this hypothesis is confirmed, then new understanding will have emerged regarding the role of the involved genes.

Conclusion

Both DI and MRS can offer critical information to the field of imaging genetics. With few exceptions, however, the number of individuals reported in each study has been fairly low and very few studies have been replicated. Sometimes results from different groups are frankly contradictory and it remains unclear whether these discrepancies are due to methodological or true biological differences. The latter might also include differences in genetic background in different ethnicities.

Advances are likely to derive from the combination of knowledge acquired across different imaging methodology and from convergent approaches to genetic discovery: animal models, heritability studies, genetic syndromes and common genetic variation. So far, DI and MRS have not ventured to define the imaging correlates of copy number variation, which might contribute to schizophrenia, autism and mental retardation (e.g. (International Schizophrenia Consortium, 2008; Friedman et al., 2006; Froyen et al., 2007; Sebat et al., 2007; Szatmari et al., 2007; Walsh et al., 2008; Xu et al., 2008), however demonstration of imaging associations with rare deletions and duplications will require well characterized families and very large numbers of individuals. A similar obstacle applies to genome wide association studies, which has been attempted for fMRI (Potkin et al., 2009). Other reviews in this issue will be dealing with general strategies for imaging genetics, but we propose that breakthroughs may be expected from technological advancements in DI and MRS, which are likely to provide new information, possibly more sensitive to genetic factors (e.g. dynamic measures of the metabolism of GABA and glutamate with ^{13}C MRS: de Graaf et al., 2003; Gruetter et al., 1998; Li et al., 2009b; Rothman et al., 1992; Shen et al., 1999; Yang et al., 2009; Yang et al., 2007) or high angular resolution techniques for DI that should allow better separation of crossing fibers or the detection of the distribution of axonal size in some brain regions (Assaf et al., 2008; Tuch et al., 2002; Tuch et al., 2003).

In conclusion DI and MRS, especially when combined with behavioral measures and other imaging modalities, provide important anatomical and neurochemical information and are likely to significantly further our understanding of the effects of genes on brain development and function and of their role in psychiatric disorders.

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Table 1

Studies of intermediate phenotypes in people at increased genetic risk for schizophrenia

Reference	Subjects	Acquisition methods issues	Analysis type	Results in relatives compared to nc	Problems/comments
Diffusion imaging					
Delisi et al. 2006	15 scz, 15 first degree relatives < 30 y.o., 25 nc	(-) anisotropic voxel resolution; only one volume wit b=0; no description of QC (e.g. motion correction); (+) correction for EPI distortion	Voxel based, stat. threshold p<0.01 + cluster size=200 mm ³ (6.4x resolution)	↑ ADC in L MFG in scz and first degree relatives as compared to nc	Large differences in age between groups, some of the first degree relatives were related to each other, as were the controls
Hoptman et al. 2008	23 scz, 22 first degree relatives < 30 y.o. (14 with prodromal sx.), 37 nc	(-) anisotropic voxel resolution; only one volume with b=0; no description of QC (e.g. motion correction); NEX=7; (+) correction for EPI distortion	Voxel based, stat. threshold p<0.05-0.001 + cluster size=100 mm ³ (3.2 x resolution)	↓ FA in WM adjacent to L IFG, bilat. post. cingulate, angular gyrus. ↑ FA in L subgenual ACC and R. MFG/SFG	Large differences in age and sex between groups, some of the first degree relatives were related to each other, as were the controls
Munoz-Maniega et al. 2008	31 scz, 22 first degree relatives (none prodromal),	(+/-) somewhat anisotropic voxels, ratio of b=0 to diffusion weighted of 0.6 (optimally 0.12); (-) no QC of individual images; (+) standard correction for	Voxel based: 4 tracts analyzed with SVC (FWE) and 12 mm smoothing; ROI based: same tracts	Voxel based analysis of 4 tracts was negative, however the ROI analysis showed ↓ FA in the	Some difference in mean age of groups, IQ and handedness. (+) all first degree

Reference	Subjects	Acquisition methods issues	Analysis type	Results in relatives compared to nc	Problems/comments
	51 nc	motion	for FA>0.2	ALIC	relatives unaffected
Narr et al. 2009	26 scz, 36 first degree relatives, 20 nc, 32 nc first deg. relatives	(+) isotropic voxel resolution; (-) no QC of individual images	ROI based. Bonferroni correction not performed for effects in relatives	↑ MD in bilat. STG (CSF accounted for statistically)	some of the first degree relatives were related to each other, but this was kept into account statistically
MRS					
Callicott et al. 1998	47 scz, 60 unaffected siblings, 66 nc	Spectroscopic imaging on a 1.5T system, long TE (272 ms),	Several ROIs. Ratios of metabolites.	↓ Naa/Cre in hippocampal formation	Some siblings affected with non-psychotic disorders.
Keshavan et al. 1997	9 scz offspring ~15 y.o., 10 nc	Single voxel on a 1.5T system, STEAM, short TE (20 ms)	Ratios of metabolites	↓ Naa/Cho in subgenual ACC	Multiple disorders in the offspring
Lutkenhoff et al. 2008	9 scz, 12 unaffected twins (11 DZ), 21 nc twins (15 DZ)	Single voxels in ACC, L frontal WM, L hippo on a 3T system, PRESS, short TE (30 ms)	LCmodel	↓ Glu in all ROIs but more so in the ACC	(-) Somewhat lax QC (SD<25%); majority of DZ twins renders the analysis of twins less useful
Purdon et al. 2008	14 scz siblings, 15 nc	Single voxel on a 3T system, STEAM, short TE (20 ms)	LCmodel	Higher proportion of subjects with ↑ Glu in ACC in scz siblings	(-) Somewhat lax QC (SD<25%); no scz group; (+) no psychopathology in scz siblings
Tibbo et al. 2004	20 scz adolescent offspring, 22 nc	Single voxel on a 3T system, STEAM, short TE (20 ms)	Ratios of metabolites	↑ Glx/Cre in R medial frontal cortex.	
Wobrock et al. 2008	22 scz, 34 scz family members, 43 nc	Single voxel on a 1.5T system, PRESS, short TE (30 ms)	Ratios of metabolites	↓ Naa/Cho in left prefrontal cortex in scz	30% of family members affected with dementia/delirium

Reference	Subjects	Acquisition methods issues	Analysis type	Results in relatives compared to nc	Problems/comments
Yoo et al. 2009	22 scz family members, 22 nc	Single voxels in ACC, L DLPFC and L thalamus, on a 1.5T system, PRESS, long TE (140 ms)	LCmodel	↓ Naa, Cre, Cho in thalamus	Not specified whether some high risk individuals had psychiatric conditions

Abbreviations: scz = patient with schizophrenia; nc = healthy volunteer; QC = quality control; (−) negative features; (+) positive ones; ADC = apparent diffusion coefficient; y.o. = years old; ROI = region of interest; MD = mean diffusivity; CSF = cerebrospinal fluid; NEX = number of excitations (when this parameter is > 1 it means that multiple acquisitions of the same image were averaged during the reconstruction process, eliminating the ability to quality control individual images); WM = white matter; L = left; R = right; bilat. = bilateral; ACC = anterior cingulate cortex (usually includes portions of the medial prefrontal cortex); IFG = inferior frontal gyrus; MFG = middle frontal gyrus; SFG = superior frontal gyrus; STG = superior temporal gyrus; sx. = symptoms; IQ = intelligence quotient; SVC = small volume correction; FWE = family-wise error correction for multiple comparisons; ALIC = anterior limb of the internal capsule; STEAM = stimulated echo acquisition mode; PRESS = point resolved spectroscopy; LCmodel = a commonly used “absolute” quantification method with reference to the water signal; SD = standard deviation (a measure of error in the fitting of spectral lines generated by LCmodel. This number is usually less than 20% in the literature); T = Tesla