

# Evaluation of a structural polymorphism in the ankyrin repeat and kinase domain containing 1 (ANKK1) gene and the activation of executive attention networks

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The specificity of genetic effects on brain activation is a central issue in understanding how molecular actions at the synapse relate to anatomic patterns of brain activity. In an effort to understand the basis for the specificity of gene-associated brain activity, we explore a well-studied genetic polymorphism, Taq1A, which lies downstream of the DRD2 gene in the protein-encoding region of a neighboring gene, ANKK1, which is not expressed in the brain. We utilize the attention network test and find that carriers of the A1 allele show gene-associated functional activation in an anatomically specific, dopamine-rich region of the brain comprising the anterior cingulate gyrus, a finding partially consistent with prior data from functional imaging genetics. A review of the patterns of expression for ANKK1 and DRD2 and the extent of linkage disequilibrium between the two genes sheds light on additional criteria for the selection of candidate genes in imaging-genetic studies.

The function of cognitive networks can be increasingly understood in terms of anatomy and circuitry. With progress in this area, it has become possible to query how molecular events may underpin neural network dynamics. In pursuing this aim, we have previously reported our efforts to link the brain networks underlying attention to the study of individual differences (Fossella et al., 2002). The attention network test (ANT) measures individual differences in efficiency in each of the largely independent networks of alerting, orienting, and executive control that carry out attention (Fan, McCandliss, Sommer, Raz, & Posner, 2002). Initial studies using the ANT found that measures of the three networks were reliable and were, for the most part, independent. Functional magnetic resonance imaging (fMRI) studies have shown that the networks activated by the test were similar to those found in previous studies using individual components of the test (Fan, McCandliss, Fossella, Flombaum, & Posner, 2005) and were consistent with other evidence on neural areas involved in aspects of attention (Corbetta, Kincade, Ollinger, McAvoy, & Shulman, 2000; Mesulam, 1981; Posner & Petersen, 1990).

A preliminary twin study using healthy adult twins (Fan, Wu, Fossella, & Posner, 2001) found that the efficiency of the executive network was highly heritable ( $h_F^2 = .89$ ), but lower heritabilities were observed for alerting and median reaction time ( $h_F^2 = .18$  and  $.16$ , respectively). The heritabilities of these cognitive measures suggest that candidate gene association studies on executive attention are appropriate.

Following on this result, initial candidate gene studies conducted by our group have probed whether certain genes might influence specific aspects of attentional performance more than others. This hypothesis stems from pharmacological evidence implicating dopamine, norepinephrine, and acetylcholine in different attentional processes. For example, studies showing that activation of mesocortical dopaminergic neurons via apomorphine enhances activity in the prefrontal cortex (Geraud, Arne-Bes, Guell, & Bes, 1987; McCulloch, Savaki, McCulloch, Jehle, & Sokoloff, 1982) suggest that dopaminergic modulation is expected to influence executive mechanisms for resolving conflict among thoughts, feelings, and responses (Diamond, 1996). Similarly, pharmacological studies with alert monkeys have related the brain's norepinephrine system, whose cell bodies are located in the locus coeruleus, to the ability to maintain an alert state (Davidson & Marrocco, 2000). Finally, acetylcholine has been shown to specifically modulate the orienting of attention (Davidson

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& Marrocco, 2000). To this end, some genetic evidence has been found that associates variation in dopaminergic genes to cognitive performance on executive tasks and acetylcholinergic genes to attentional tasks involving the orienting of attention (Greenwood, Fossella, & Parasuraman, 2005).

With these behavioral genetic results in hand, we sought to further examine the specificity of the relationship between genetic variation and cognitive function through the use of functional neuroimaging. Reported findings in the emerging imaging genetics literature show that certain genes can demonstrate associations not only with specific cognitive operations, but also with somewhat specific patterns of brain activation when subjects are performing certain tasks (Goldberg & Weinberger, 2004). Our fMRI-based imaging-genetic data showed that DRD4 and MAOA, candidate genes with broad domains of gene expression, had relatively specific effects on activation in the anterior cingulate gyrus (Fan, Fossella, Sommer, Wu, & Posner, 2003). Building on this result, a structural MRI study showed that the DRD4 gene was associated with cortical structure and that the DAT1 gene influenced subcortical structure (Durstun et al., 2005). This result seems in harmony with the known cortical expression of the DRD4 gene and high subcortical expression of the DAT1 gene. Indeed, the relationship between anatomical regions of gene expression and of associated brain activity is central to understanding, and dissecting, the specificity of genetic effects on cognition.

The dopaminergic candidate genes that we selected for our behavioral and imaging-genetic studies were chosen because they (1) have been repeatedly associated with disorders in which attention is disrupted, (2) are pharmacologically related to the executive attention network, and (3) have been biochemically characterized so that each allelic class is associated with a difference in biochemical activity or expression level. These three criteria were useful for screening from a pool of many possible dopaminergic candidate genes in the literature. However, a closer look at the structural and functional imaging-genetic data raises new questions concerning their interpretation. Why, for example, is the gene-associated activity in relatively specific brain regions, when the task is known to activate multiple nodes in a widely distributed neural network? Also, why are areas of associated brain activity found, such as the insula and cerebellum, that do not express the MAOA or DRD4 genes? It is important to begin to understand factors that mediate the interaction between *regions of gene expression* and *regions of functional activation*. A focus on understanding this basic question should yield findings that point to mechanisms that link synaptic physiology with patterns of brain activity and morphometry.

Relationships between where a gene is expressed and where it exerts an effect during an imaging-genetic study are complex. First, it should be noted that many of the genes used in imaging-genetic studies are widely expressed. Recent imaging-genetic studies on 5HTT and COMT illustrate this point. A query of gene expression

data in UniGene, a public-access repository of genomic data ([www.ncbi.nlm.nih.gov/UniGene/](http://www.ncbi.nlm.nih.gov/UniGene/)), showed wide-ranging expression patterns of both 5HTT (brain, bone, colon, muscle, lung, placenta, and pancreas) and COMT (brain, bladder, bone, cervix, heart, kidney, liver, lung, ovary, prostate, skin, stomach, and other areas). Within the brain, 5HTT is expressed in a very restricted anatomical manner—namely, in serotonergic cells projecting from the median and dorsal raphe nuclei, in the suprallemniscal cell group, and in the oral pontine nucleus (Austin, Bradley, Mann, & Blakely, 1994). Patterns of brain activity associated with a 5HTT promoter length polymorphism include the basolateral amygdala and the prefrontal cortex (PFC; Heinz et al., 2005). Although these brain regions do not express large quantities of 5HTT, these broad anatomical areas are likely candidates for gene-associated effects on brain activity, given that serotonin modulates synaptic activity of prefrontal–amygdala connections. The COMT gene shows a broad pattern of expression among cortical and subcortical neurons in the human brain. Imaging-genetic studies show that functional activity within areas of the dorsolateral prefrontal cortex (DLPFC) is associated with structural variants of COMT when subjects perform tasks involving working memory (Egan et al., 2001). Despite the high levels of expression of COMT in the striatum, gene-associated brain activation is mainly found in the DLPFC. This is consistent, however, with findings showing that COMT activity accounts for less than 15% of total dopamine turnover in the striatum but greater than 60% in the PFC (Sesack, Hawrylak, Matus, Guido, & Levey, 1998).

In the cases of both 5HTT and COMT, existing evidence from microcircuitry and synaptic physiology allows a straightforward interpretation of the link between molecular function and gene-associated patterns of brain activity. 5HTT and COMT are therefore exemplary candidate genes for imaging-genetic studies. Recent findings on the brain-derived neurotrophic factor (BDNF), which is expressed in the hippocampus and shows gene-associated brain activity in that region, also serve as a tangible link between molecular function and patterns of brain activity (Hariri et al., 2003). As the number of candidate genes rapidly outpaces detailed synaptic physiology studies, however, it will be ever more critical to select candidate genes carefully.

In the normal course of our continuing investigations of dopaminergic regulation of executive attention, we have turned to an investigation of the dopamine D2 receptor gene (DRD2), an exceptional candidate gene according to current selection criteria. A member of the G-protein-coupled superfamily of receptors, DRD2 is expressed both on presynaptic dopaminergic neurons and postsynaptic sites throughout the mesocorticolimbic circuitry, including temporal, frontal, and cingulate cortex (Usiello et al., 2000). Stimulation of D2 receptors leads to inhibition of dopamine-synthesizing enzymes such as tyrosine hydroxylase and aromatic L-amino acid decarboxylase in striatum, whereas blockade of D2 receptors has the op-

posite effect (Sealfon & Olanow, 2000). One of the most commonly studied genetic polymorphisms is a TaqIA polymorphism that arises from a C/T single-nucleotide polymorphism (SNP) located 10,541 base pairs downstream of the termination codon in the 3'-flanking region. Since the polymorphism was originally discovered as a restriction fragment length polymorphism, before the actual sequence of the two alleles was known, the two alleles at this site are historically referred to as the "A1" and "A2" alleles.

DRD2 is extremely well suited for behavioral and imaging-genetic studies, since it is a key player in dopaminergic modulation of synaptic function whose expression and synaptic function is well characterized via a multitude of electrophysiological and imaging methods. For example, PET studies using [<sup>11</sup>C]raclopride (Jonsson et al., 1999; Pohjalainen et al., 1998), [<sup>18</sup>F]FDOPA (a radiolabeled analog of the dopamine precursor L-DOPA; Laakso et al., 2005), and <sup>18</sup>F-deoxyglucose (Noble, Gottschalk, Fallon, Ritchie, & Wu, 1997) reveal a convergence of evidence surrounding the A1 allele of the TaqIA polymorphism. The PET studies show that the A1 allele is associated with reduced D2 autoreceptor density in vivo in human striatum (as measured by [<sup>11</sup>C]raclopride) and a corresponding decrease in autoreceptor function (as measured by [<sup>18</sup>F]FDOPA), accompanied by reduced mean relative glucose metabolic rate in the medial PFC and other brain regions. The suggestion that lower D2-receptor expression leads to decreased autoreceptor function is supported by an association of the DRD2 TaqIA A1 allele (TaqA1) with higher levels of urinary homovanillic acid (Ponce et al., 2004). Together, these data suggest that A1-allele carriers show poor dopaminergic function.

One disconcerting feature of the TaqIA RFLP, however, is that it lies 10 kb downstream of the coding region of DRD2. Recently, Neville, Johnstone, and Walton (2004) explored the possibility that the polymorphism might fall within the protein-encoding region of a neighboring gene or regulatory region. They determined that the TaqIA SNP occurs in exon 8 of a gene named *ankyrin repeat and kinase domain containing 1* (ANKK1), resulting in a glu713-to-lys (E713K) substitution within the 11th ankyrin repeat of that gene. More intriguing still, gene expression studies have *not* yet been able to detect ANKK1 in the developing or adult brain. Expression studies have found ANKK1 mRNA expressed at low levels in the placenta and spinal cord (Neville et al., 2004). As cognitive and imaging-genetic studies continue to probe the specificity of genetic effects on behavior and brain function, the conundrum posed by the DRD2-ANKK TaqA1 polymorphism warrants closer inspection.

In an ongoing effort to understand general principles that relate patterns of gene expression to patterns of associated brain activity, this particular discrepancy involving a well-studied candidate gene should be resolved. As part of our efforts to search for and understand the specificity of genetic effects on cognition, we present an ANT evaluation of the relationship of this genetic variant to activation of the executive attention network.

## METHOD

### Subjects

The subjects in our study were recruited from the New York area only. Candidates with a history of psychopathology and/or taking medication were excluded. The subjects consisted of 15 right-handed normal adults (mean age = 27.2 years, *SD* = 5.7, range: 18–36 years; 8 female, 7 male) who performed the ANT while being scanned in an event-related fMRI experiment.

### The Attention Network Test

The details of the ANT used in our behavioral genetic study were illustrated by Fan and colleagues (Fan et al., 2002; Fan et al., 2001). The stimuli consisted of a row of five visually presented horizontal black lines with arrowheads pointing left or right, against a gray background. A single arrow subtended 0.58° of visual angle, and the contours of adjacent arrows or lines were separated by 0.06° of visual angle. The stimuli (one central arrow plus four flankers) subtended a total 3.27° of visual angle. The target was a leftward or rightward arrowhead at the center. To introduce a conflict resolution component, the central arrow was flanked on either side by congruent or incongruent stimuli: two arrows in the same direction as the target (congruent condition) or in the opposite direction (incongruent condition). The subjects' task was to identify the direction of the centrally presented arrow by pressing one key for the left direction and another key for the right. To introduce an attentional orienting component to the task, the row of five arrows was presented in one of two locations outside the point at which the subject was fixating, either 1.06° above or below the fixation point. To measure the alerting or/and orienting benefits, there were three warning conditions: no cue (baseline), center cue (alerting), and orienting cue (alerting plus orienting). In this article, we will only report the conflict effect, which reflected executive control of attention. The conflict effect was calculated on the basis of two measurements, response time (RT) and error rate. For RTs, the ratio score of the conflict effect was calculated as the RT of the incongruent condition minus the RT of the congruent condition divided by the mean RT. Only the RTs of correct responses were included for the calculation of these scores. For the error rate, conflict was calculated as the error rate of the incongruent condition minus the error rate of the congruent condition.

### Functional Magnetic Resonance Imaging

We used event-related fMRI to study the changes in brain activity of these attentional networks. In each trial, a fixation cross first appeared in the center of the screen. At the same time, depending on the condition, an asterisk cue either was (cued condition) or was not (no-cue condition) presented for 200 msec. After a variable duration (drawn from a set of discrete times from 300 to 11,800 msec approximating an exponential distribution, with a mean interval of 2,800 msec), the target and flankers were presented until the subject responded with a buttonpress or until 1,700 msec had elapsed. After the subject made a response, the target and flankers disappeared immediately and a posttarget fixation cross appeared for a variable duration. The duration between the onset of the target and the start time of the next trial was also variable (a set of discrete times from 3,000 to 15,000 msec, with a mean of 6,000 msec, exponentially distributed). We isolated brain activity associated with the subtraction of the congruent condition from the incongruent condition for the measurement of the conflict effect.

Magnetic resonance imaging was carried out using a GE Signa 3T scanner. Blood oxygenation level dependent (BOLD) functional images were collected using a T2\*-weighted gradient echoplanar imaging (EPI) sequence (TE = 35 msec, TR = 2,000 msec, flip angle = 80°) with an in-plane resolution of 3.44 × 3.44 mm (64 × 64 matrix, 220 × 220 mm field of view). Twenty-four 5-mm slices (1 mm skipped between slices) were acquired along the AC-PC plane as determined by the midsagittal section. In-plane structural

scans were collected using a T1-weighted sequence in the same orientation as the functional sequences to provide detailed anatomic images aligned to the functional scans. A high-resolution structural MRI sequence was also acquired for the purpose of normalization. Distortion in EPI images was corrected on the basis of the estimated parameters of the phase map.

Statistical parametric mapping (SPM) was conducted using SPM99 software (developed by Wellcome Department of Cognitive Neurology in London). A timing correction procedure was used to correct differences in image acquisition time between slices using sinc interpolation. Spatial alignment was performed to realign the time series of images acquired from the same subject using a least-squares approach and a six-parameter (rigid body) spatial transformation. All volumes from each subject were realigned to the first volume. EPI images were registered to each subject's T1 in-plane images and then to the high-resolution images. The high-resolution images were normalized to a standard template supplied with the SPM software that approximates the space described in Jezzard and Balaban's (1995) atlas. Then the transformation parameters were applied to the EPI images. Voxels were resampled with a  $2 \times 2 \times 2$  mm<sup>3</sup> voxel size. An  $8 \times 8 \times 12$  mm full-width-at-half-maximum Gaussian kernel was used to smooth the EPI images. For the statistical analysis, high-pass filtering was applied to the time series of EPI images to remove the low-frequency drift in EPI signal. The global changes in signal intensity were removed by proportional scaling. Statistical analysis was then conducted with general linear modeling. Regressors were created by convolving a train of delta functions that represented the individual trials with the base functions, which were a synthetic hemodynamic response function, composed of two gamma functions, and its derivative. Six realignment parameters were used as covariates. A random-effects analysis was carried out to make inferences to the population, with the resultant parameter estimates for each contrast from each subject as the input. The thresholds for the random-effects model were  $p \leq .01$  for the height and  $k \geq 80$  voxels for the extent. The brain activity related to conflict was defined as the contrast of the incongruent condition minus the congruent condition.

To examine whether genetic variations might contribute to differences in brain activation, the interaction between conditions (incongruent vs. congruent) and genotypic groups (A2/A2 vs. A1/A2 and A1/A1) was tested. Region-of-interest analysis was conducted to get the activation values (the resultant parameter estimates for the contrast) of the 15 subjects for the activated anterior cingulate cortex (ACC) cluster based on the results of a *t* test. ANCOVA was then conducted for control of the potential confounding variables of gender, age, conflict effect calculated using the RT ratio scores, and conflict effect based on error rate.

### Genotyping Analysis

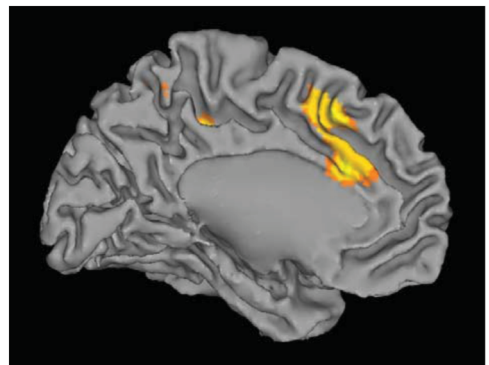
Buccal swabs were obtained via buccal cell brush from consenting subjects and were prepared as directed by the manufacturer. We used the MasterAmp Buccal Swab DNA Extraction Kit (Epicentre Biotechnologies; www.epibio.com). Yields ranged from 0.5 to 3  $\mu$ g of DNA from each buccal sample. Yields were determined spectrophotometrically by absorbance at 260 nm. Taq polymerase, PCR buffer, and dNTPs were obtained from QIAGEN (www1.qiagen.com) and used at recommended concentrations for a 20- $\mu$ l PCR reaction. PCR reactions and restriction digests (PCR-RFLP) were optimized for each marker and performed on a PTC-100 programmable thermal controller (MJ Research, a division of Bio-Rad Laboratories; www.mjr.com) outfitted with a heated lid for oil-free amplifications. A "touchdown" PCR cycling regimen and the addition of DMSO (10% final v:v) were used in order to automatically optimize hybridization stringency. For genotyping the DRD2 TaqA1 polymorphism, the forward primer 5'-CCGTCGACGGCTGGCCAAGTTGTCTA-3' and reverse primer 5'-CCGTCGACCCTTCTGAGTGTATCA-3' were used, as described by Jonsson et al. (1999). After digestion with TaqA1 (New England Biolabs; www.neb.com), gel electrophoresis

in LE agarose, followed by staining in ethidium bromide, was used to resolve and visualize DNA fragments. The A1 allele yielded an uncut band of 310 base pairs, and the A2 allele yielded bands of 180 and 130 base pairs.

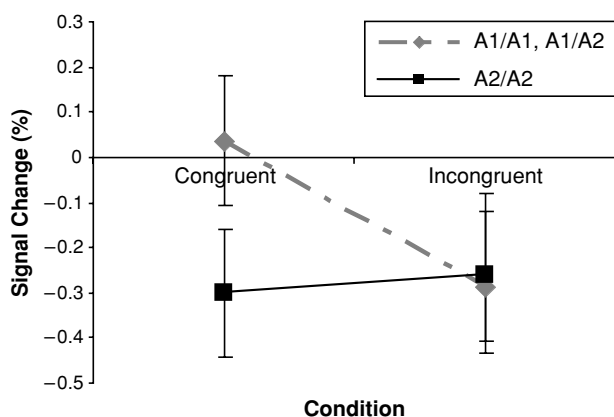
## RESULTS

For the TaqA1 polymorphism, a comparison of behavioral data for a population of A2/A2 homozygotes ( $n = 11$ ) with a population consisting of both A1/A2 heterozygotes ( $n = 3$ ) and A1/A1 homozygotes ( $n = 1$ ) revealed a significant conflict effect (incongruent minus congruent condition) for both RT [831 vs. 727 msec;  $F(1,13) = 32.15$ ,  $p < .01$ ] and accuracy [.94 vs. .95,  $SE = .0065$ ;  $F(1,13) = 11.36$ ,  $p < .01$ ]. The group differences and interactions were not significant ( $F_s < 1$ ). The lower allele frequency of the A1 allele is consistent with large population samples in which frequencies of .3 for the A1 allele and .7 for the A2 allele have been reported (see NCBI's SNP Web page, www.ncbi.nlm.nih.gov/SNP/snp\_ref.cgi?rs=1800497). The grouping of alleles was chosen simply to contrast the effects of A1 versus A2 while attempting to balance group sizes. No evidence is presently available about whether A1 or A2 is dominant. An analysis of BOLD responses in the two genotypic groups showed that a cluster of ACC activation was extracted. As shown in Figure 1, ANCOVA with ACC activation as the dependent variable and gender, age, conflict effect calculated with ratio scores, and conflict effect based on error rate as the covariate variables showed that the difference between the two DRD2 groups was significant ( $p < .01$ ).

Figure 2 shows that A2/A2 homozygotes showed greater conflict-related brain activity—that is, a greater difference between the congruent and incongruent conditions in ACC—than did subjects with only one or no A2 allele. An examination of activity for the peak at BA24



**Figure 1.** Brain regions where differences in incongruent versus congruent brain activity show associations with genotype at the DRD2 TaqA1 polymorphism. The significant voxels shown reflect greater activity in A2/A2 homozygotes than in A1/A2 heterozygotes or A1/A1 homozygotes. Only a cluster of anterior cingulate cortex activation (with two local peaks) passed the threshold. The coordinates of the two peaks were  $x = -6, y = 24, z = 24$  (BA24) and  $x = 2, y = 22, z = 42$  (BA6/32) in MNI space. The total voxel number was 474, with a  $2 \times 2 \times 2$  mm<sup>3</sup> voxel size.



**Figure 2.** The main effect and interaction between conditions (incongruent vs. congruent) and genotypic groups (A2/A2 vs. A1/A2 and A1/A1) in anterior cingulate cortex.

(a spherical region of 5-mm radius) in the congruent and incongruent conditions of these two groups showed a significant condition (congruent vs. incongruent) by group (A1/A1 and A1/A2 vs. A2/A2) interaction [ $F(1,13) = 11.02, p < .01$ ]. The main effect of conflict was also significant [ $F(1,13) = 6.69, p < .05$ ], but the main effect of group was not ( $F < 1$ ). Simple comparisons indicated that only the group composed of A1/A2 heterozygotes and A1/A1 homozygotes showed a significant difference between the congruent and incongruent conditions ( $p < .01$ ). As illustrated in Figure 2, the A2/A2 group showed below-baseline ACC activation for both the congruent and incongruent conditions, and the A1 carriers showed below-baseline ACC activity for the incongruent condition but activation marginally above baseline for the congruent condition.

## DISCUSSION

PET measures of receptor availability, dopamine synthesis, and glucose metabolism show that A1-allele carriers have reduced dopaminergic function (Jonsson et al., 1999; Laakso et al., 2005; Noble et al., 1997; Pohjalainen et al., 1998). From an anatomical point of view, a genetic association with fMRI-measured activity in the anterior cingulate gyrus would be consistent with a similar dopaminergic role for TaqIA, since this brain area is highly innervated by dopaminergic fibers. Given the well-known inverted-U-shaped dependence of behavior and synaptic function on dopamine levels, it is usually problematic to predict the direction of brain activity (more or less BOLD) in response to higher or lower dopamine levels. Our findings reveal that the differences in activity in the ACC between the two genetic groups are driven largely by differences in the activation of the ACC in the non-A2/A2 genotypic group ( $n = 4$ ). This group shows a rather unexpected decrease in signal change in the incongruent condition, a condition that is often associated with higher BOLD responses. PET studies have shown

similar lower mean relative glucose metabolic rates in the medial prefrontal and other brain regions of A1 carriers, and pharmacologic blockade of dopamine D2 receptors downregulates D1-receptor-dependent brain function as measured by working memory tasks (Castner, Williams, & Goldman-Rakic, 2000). Thus, our main finding is not fully consistent with the existing literature, since there is no finding of a loss of function or activity associated with the A1 allele. However, the association with activity in a dopamine-rich prefrontal region seems consistent with a role for that allele in dopamine-dependent regulation of brain function.

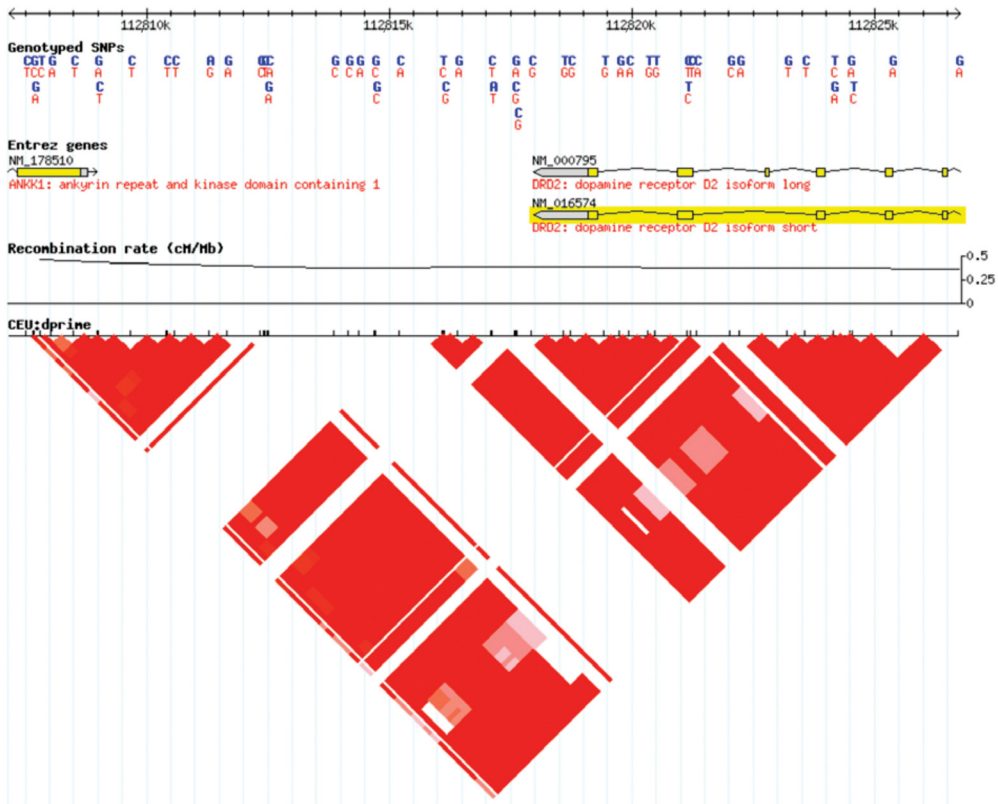
An alternative, nondopaminergic interpretation of these imaging-genetic findings may be plausible. It might be surprising for an imaging-genetic study that focused specifically on the role of ANKK1 and was naive to the DRD2-related literature to find relatively specific gene-associated functional activity in the adult brain. These findings might not be such a surprise, however, in a study that involved an ankyrin-repeat-containing gene. An overview of protein structural motifs shows that the ankyrin repeat is one of the most common protein-protein interaction motifs in nature. The repeated modules of about 33 amino acids occur in a large number of functionally diverse proteins, including transcriptional initiators, cell-cycle regulators, cytoskeletal proteins, ion transporters, and signal transducers. Since the ankyrin fold appears to show a common structure rather than a common cellular function, there is no specific sequence or structure that is universally recognized by it that might be useful in determining the importance of the TaqIA change. An electrostatic surface potential analysis of the crystal structure of the *non-ATPase subunit 6* (Nas6p) protein showed that the regions of ANK1-4 and ANK6-7 repeats have negatively charged surfaces (Padmanabhan, Adachi, Kataoka, & Horikoshi, 2004). The negatively charged residues exposed on the surface, as well as the distribution of charged amino acids in this region, are well conserved. In addition, the surface charge of these sites was suggested by Padmanabhan et al. to be functionally important for protein-protein binding. Therefore, a change in ANKK1 from a negatively charged amino acid (glutamate) to a positively charged one (lysine) is not without the possibility of a change in functional role. An attractive candidate for the important functions that ankyrin repeats can perform can be seen in the *Notch* family of single-pass transmembrane receptors that are involved in cell fate decisions early in brain development. The *Notch* intracellular domain contains seven ankyrin repeats that may be necessary for recruitment of transcriptional coactivators when the intracellular domain is translocated into the nucleus. An alanine-to-threonine substitution in the second repeat of human Notch3 is associated with CADASIL, a hereditary adult-onset condition causing stroke and dementia (Joutel et al., 1996). A similar early role for ANKK1 would likely be more difficult to detect in humans, since the domains of expression could be limited and transient. A number of straightforward gene expression and targeted mutagenesis experiments

might begin to assess more definitively the potential role of ANKK1 in the mammalian brain.

The more likely explanation for a dopaminergic interpretation would be that the TaqIA polymorphism in ANKK1 is in linkage disequilibrium (LD) with upstream polymorphisms in DRD2 and lies within a haplotype block that spans the overlap between the two genes. One very convenient way to assess this possibility would be to examine data provided by the International HapMap Project (2003), sponsored by the International HapMap Consortium. Extensive SNP analyses, from populations consisting of Yoruba in Ibadan, Nigeria; Japanese in Tokyo, Japan; Han Chinese in Beijing, China; and Utah residents with ancestry from northern and western Europe, permits pairwise analyses of linkage disequilibrium between variable sites in the genome. A quick view of public haplotype data provided by the International Haplotype Map (available online at [www.hapmap.org](http://www.hapmap.org)) in Figure 3 shows that the C/T transition TaqIA site (dbSNP rs1800497) sits

inside a well-delineated haplotype block. Though an intergenic region shows little linkage disequilibrium, strong LD does extend between the TaqIA site and DRD2. A view of phased haplotypes using the hierarchical clustering methodology tools available in the data browser on the HapMap site also shows evidence for haplotypes that encompass the TaqIA site and the DRD2 gene.

In order to determine whether the effects of the TaqIA variant are due to the function of ANKK1 or DRD2, it will be necessary for future studies to evaluate the extent of LD between the TaqIA site and other variable sites within DRD2. Typically, the small population sizes used in imaging-genetic studies allow insufficient statistical power to detect LD of associated markers with other loci or evidence of population admixture. This limitation is especially true in our present population of 15 subjects. To address this limitation, we are currently evaluating the extent of LD within a larger population of 300 subjects for whom behavioral genetic data on executive attention



**Figure 3.** Haplotype map ([www.hapmap.org](http://www.hapmap.org)) data showing genetic structure for the genomic region flanking the TaqIA site and the 3' regions of the ANKK1 and DRD2 genes. Yellow boxes indicate exons, and light areas immediately adjacent to them indicate 3' untranslated regions. The TaqIA C/T transition (dbSNP rs1800497) is located at position 112,808.5 kb (approximately the 5th SNP from the left) in the 3' coding region of ANKK1. The middle plot shows the average recombination rate across the genomic region, and the red boxes illustrate the extent of linkage disequilibrium (LD) across the region, as described at [www.hapmap.org](http://www.hapmap.org). LD is nonrandom association between alleles in the population. The LD plotter generates a pairwise plot of marker-to-marker LD values on the current view. The ticks on the line above the red boxes denote the markers with respect to their physical location on the chromosome segment, and the boxes represent the marker pair relationship, which is plotted *between* the two markers. The intensity of the red color is based on the raw score for that marker pair.

are available. We will further explore in our population the extent of haplotype blocks, which are regions of the genome showing little historical recombination. Our future experimental studies will utilize the “tagged SNPs” (tSNPs) rs2734849, rs2734848, and rs1800497 (the TaqIA site) that define a cluster of haplotypes in the vicinity of ANKK1, as well as the tSNPs rs1554929, rs2734841, and rs2734837 that define a cluster of haplotypes in the vicinity of DRD2. Interestingly, the TaqIA site emerges as a tSNP when using the Tagger algorithm (de Bakker et al., 2005). The use of tSNPs and haplotype association, if applied as a standard approach by many research teams, has the advantage of minimizing discrepancies in results across studies using different populations, allowing disparate data to be reconciled and subjected to meta-analyses.

In our prior genetic studies of dopaminergic function and executive attention, the candidate genes we selected for our behavioral and imaging-genetic studies were chosen mainly because they are associated with disorders in which attention is disrupted and are biochemically characterized, so that each allelic class is associated with a difference in biochemical activity or expression level. These criteria were useful for screening from a pool of many possible dopaminergic candidate genes in the literature. However, a closer look at our structural and functional imaging-genetic data raised new questions concerning interpretation of those data. As new imaging genetic data accumulate, additional criteria for candidate gene selection will emerge. These new criteria should assist in understanding factors that mediate the interaction between regions of gene expression and regions of functional activation.

The conundrum posed by the TaqIA polymorphism ANKK1 gene highlights the need to select candidate polymorphisms more carefully in the course of study design. If it is shown, ultimately, that linkage disequilibrium with DRD2 cannot explain gene-associated functional activity in the adult brain, more extensive studies on the biology of ANKK1 will then be warranted. If LD is generally strong between TaqIA and DRD2, though, such studies would seem unnecessary. In either case, updating our candidate gene selection process with additional criteria could be of value. First, it might be useful to have evidence concerning the expression pattern of the candidate gene in the developing and adult human brain. If the hypothesized areas of gene-associated functional activation do not overlap with the gene's known expression pattern, an investigator might have difficulty interpreting the findings from a cellular or synaptic perspective. Additionally, it could be useful to query public genome resources such as HapMap for information on the extent of LD within and outside the candidate gene of interest. The addition of tSNPs within candidate genes would also support the meta-analysis of imaging-genetic data across studies using different populations.

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