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Age-Dependent Increase in Kalirin-9 and Kalirin-12 Transcripts in Human Orbitofrontal Cortex

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Abstract

KALRN (KAL) is a Rho GEF that is highly involved in regulation of the actin cytoskeleton within dendrites. There are several isoforms of the protein that arise from differential splicing of *KALRN*'s 66 exons. KAL isoforms have different functions in development. For example, overexpression of the KAL9 and KAL12 isoforms induce dendritic elongation in early development. However, in mature neurons KAL9 overexpression reduces dendritic length, a phenotype also observed in normal human aging. We therefore hypothesized that KAL9 would have increased expression with age, and undertook to evaluate the expression of individual *KALRN* exons throughout the adult lifespan. Postmortem human brain gray matter from Brodmann's area (BA) 11 and BA47 derived from a cohort of 209 individuals without psychiatric or neurodegenerative disease, ranging in age from 16–91, were analyzed for *KALRN* expression by Affymetrix exon array. Analysis of the exon array data in an isoform-specific manner, as well as confirmatory isoform-specific qPCR studies, indicated that the longer KAL9 and KAL12 isoforms demonstrated a statistically significant, but modest increase with age. The small

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magnitude of the age effect suggests that inter-individual factors other than age likely contribute to a higher degree to KAL9 and KAL12 expression. In contrast to KAL9 and KAL12, global *KALRN* expression did not increase with age. Our work suggests that global measures of *KALRN* gene expression may be misleading and future studies should focus on isoform-specific quantification.

Keywords

Kalirin; human aging; dendritic complexity; postmortem

Introduction

Kalirin has emerged as a key regulator of dendrite morphogenesis. Data from a variety of models systems including dissociated primary cortical neuron culture and knockout animal models demonstrate that kalirin plays important roles in dendritic elongation, arborization, spine formation, and spine maintenance (Penzes, Johnson et al. 2001, Penzes, Beeser et al. 2003, Deo, Cahill et al. 2012).

Kalirin (KAL) is a complicated protein with multiple isoforms arising from alternative splicing of the *KALRN* gene (Johnson, Penzes et al. 2000). Each isoform is marked by differential functions reflecting its specific protein domains (Johnson, Penzes et al. 2000, Penzes, Johnson et al. 2000, McPherson, Eipper et al. 2002). The full-length isoform, KAL12, has two guanine nucleotide exchange factor (GEF) domains, the first of which (RacGEF) serves to activate RhoG/Rac1, a signaling pathway which promotes the formation, growth, and maintenance of spines (Tolias, Duman et al. 2011, Yan, Eipper et al. 2014). The second GEF domain (RhoGEF) activates RhoA, which induces spine elimination (Tolias, Duman et al. 2011, Yan, Eipper et al. 2014). Additionally, KAL12 also has a C-terminal kinase domain (McPherson, Eipper et al. 2002). KAL9 is an alternatively spliced isoform that also contains the 2 GEF domains but lacks the kinase domain; in addition, rodent KAL9 has been shown to contain a short (5 amino acid) unique C-terminal region (Johnson, Penzes et al. 2000). KAL7 is another alternatively spliced isoform that contains a single GEF domain (RacGEF) as well as a unique C-terminal region not shared by the longer isoforms, which has been shown to be important in targeting to dendritic spines (Penzes, Johnson et al. 2001). KAL5 is the shortest of the isoforms and contains no unique regions but does contain the RacGEF domain (Johnson, Penzes et al. 2000). Table 1 summarizes the features and functions of the various isoforms.

The various KAL isoforms also demonstrate different expression patterns throughout neurodevelopment (Johnson, Penzes et al. 2000, Penzes, Johnson et al. 2000). In rodents, it has been shown that KAL9 and KAL12 are preferentially expressed early in development but by adulthood KAL7 is the predominantly expressed isoform in the cortex (McPherson, Eipper et al. 2002, Remmers, Sweet et al. 2014). In contrast, little is known about age dependent expression of KAL isoforms in human cortex, despite substantial evidence of age-related reductions in dendritic complexity and spine number (Dickstein, Kabaso, et al. 2007). Similarly, KAL isoform expression is reported to be altered in both schizophrenia and

Alzheimer disease (Hill, Hashimoto et al. 2006, Youn, Jeoung et al. 2007, Deo, Cahill et al. 2012, Murray, Kirkwood et al. 2012, Rubio, Haroutunian et al. 2012), illnesses that manifest at either extreme of the adult lifespan and that are characterized by alterations in dendritic morphology and spine density (Penzes, Buonanno et al. 2013). Given the differential effect of the various isoforms on dendritic formation and function, coupled with the isoform-specific alterations in expression during development characterized in rodents, we hypothesized that there would be age-dependent changes in KAL expression in human cortex in an isoform-specific manner. Additionally, we hypothesized that the KAL9 isoform, which has been shown to decrease dendritic length in mature neurons (Deo, Cahill et al. 2012), would increase with age.

Methods

Subjects

All of the brain specimens were collected during autopsies conducted at the Allegheny County Office of the Medical Examiner with permission obtained from the subjects' next-of-kin. The protocol used to obtain consent was approved by the University of Pittsburgh Institutional Review Board (IRB) and Committee for Oversight of Research Involving the Dead. An independent committee of experienced clinicians made consensus DSM-IV diagnoses for each subject, using information obtained from clinical records and structured interviews with surviving relatives. These procedures were IRB approved. A total of 209 control subjects (i.e. without any DSM-IV diagnosis) were used in this study (Table 2), according to previously defined details (Seney, Chang et al. 2013).

Tissue Processing

Upon brain collection, ~2 cm coronal blocks from the right hemisphere were cut through the rostro-caudal extent of the brain and stored at -80°C . The RNA integrity (RIN) of each brain was assessed by chromatography (Agilent Bioanalyzer; Santa Clara, CA). Samples were obtained from two adjacent prefrontal cortex (PFC) regions, Brodmann areas (BA) 11 and 47. These areas were selected based on prior findings showing robust age-related changes in gene expression that were highly correlated with other PFC regions (e.g. BA9) (Erraji-Benchekroun, Underwood et al. 2005). Grey matter samples containing all 6 layers and excluding white matter were harvested from 3–4 consecutive 20 μm sections and stored in Trizol reagent.

RNA Arrays

Total RNA was extracted from frozen BA11 and BA47 samples stored in Trizol and reverse transcribed to cDNA using a mix of oligo d(T) and random hexamer priming, and were processed for microarray analysis using GeneChip Human Gene 1.1 ST from Affymetrix according to manufacturer's protocol (<http://www.affymetrix.com>). Gene expression data was extracted using Expression Console build 1.2.1.20. The normalization method is based on quantile normalization to eliminate batch effects. Data from arrays were processed by RMA method. Gene expression probes were processed at gene-level and taken in log2 scale for further analysis. After normalization, 33,297 gene-level probes remained, including *KALRN*.

Probe Mapping

A total of 62 exon probes were mapped to regions of the *KALRN* gene using the targeted genomic location provided by the manufacturer. Figure 1 illustrates the process by which from among these probes we selected those identifying regions of interest within *KALRN* for further exploration of isoform specific mRNA expression. Utilizing the UCSC Genome Browser (<http://genome.ucsc.edu/>), genomic coordinates for each probe were translated *in silico* to generate the amino acid sequence within the KAL protein that would be the expected probe target. This amino acid sequence was then cross-matched to the full-length *rattus norvegicus* (rat) KAL protein sequence from the UniProt database (<http://www.uniprot.org/>), as full-length rat *KALRN* is known to possess >99% homology to the human isoform but is more robustly curated (uniprot IDs: P97924-1, KAL 12; P97924-7, KAL 9; P97924-6, KAL 7; P97924-5, KAL 5). This cross-matching allowed the start site within the translated protein to be identified that was the target of each probe from the gene array, and this information was subsequently used to determine which KAL protein isoforms were identified by each probe. Three probes did not map to identifiable locations on the *KALRN* gene using the UCSC Genome Browser, and thirteen additional probes had discrepancies between the amino acid sequence from the UCSC Genome Browser and the KAL protein sequence available in the UniProt database, and thus these probes were excluded from further analysis. Of the 49 array probes included in the analysis, 31 had expression values above background. These probes were carried forward in subsequent analyses of age-dependent expression patterns.

qPCR Validation

All qPCR primers were designed using Primer3Plus software (<http://primer3plus.com/cgi-bin/dev/primer3plus.cgi>). qPCR primers were designed targeting regions of the gene that lead to translation of the unique C-terminal ends of the KAL5/7 and KAL9, as well as a region of the gene unique to only the full-length KAL12 isoform, to detect alterations in expression with high specificity (Table 3, rows 1–3). Primers were tested and validated using previously defined methods (Sibille, Su et al. 2007). cDNA generated from the 209 subject cohort was run in quadruplicate using primers for the KAL isoforms of interest along with 3 internal standard genes (β -Actin, Cyclophilin, GAPDH). Reactions were run on an Opticon real-time PCR machine (MJ Research, Waltham, MA, USA), using universal PCR conditions (65C–59C touch-down, followed by 35 cycles (15 s at 95C, 10 s at 59C and 10 s at 72C)). cDNA (150 pg) was amplified in 15 μ l reactions (0.3 $\times$ Sybr-green, 3 mM MgCl₂, 200 μ M dNTPs, 200 μ M primers, 0.5 unit Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA)). Results were calculated using the comparative Ct method in which the relative expression level of *KALRN* was calculated based on the geometric mean of the 3 internal standard genes.

Statistical Analysis

Analysis of Global *KALRN* expression array data—A random intercept model (RIM) with variable selection described in our previous paper (Wang, Lin et al. 2012) was applied to microarray data and exon data to assess the association between age and both global *KALRN* and *KALRN* exon expression. Technical factors (pH, RIN, and PMI) and

geographical factors (sex and race) were considered as potential cofactors for variable selection in the analysis. Among five cofactors, up to three were selected to adjust confounding effects, where the variable selection procedure was based on Bayesian information criterion. This resulted in selection of PMI and pH as cofactors for analysis of BA 11 and PMI as the only cofactor for analysis of BA 47 in the analysis of global *KALRN* expression. P-values were derived via permutation analysis (null distribution of the test statistic was generated through permuting sample label 1,000 times). Multiple testing corrections for analyses of exon expression were considered through controlling the false discovery rate by Benjamini-Hochberg procedure. To test the difference between regressions in BA11 and BA47, a full regression model including age effect, all covariates, and brain region effect was used. This produced an interaction term between age and brain region effect and reflects if there is a region-wise age trajectory difference. Calculations of confidence intervals for the correlation of amino acid start number with age effect for individual exons used the Fisher r to z transformation.

Analysis of Isoform Specific qPCR—For qPCR data analysis, RIM was also applied to assess the association between age and KAL isoform expression, as described above for the Global *KALRN* analysis. After consideration of each cofactor for each isoform, only RIN remained as significant, and only for KAL9, and thus this variable was considered in the KAL9 model. However, we observed that substantial variation, including univariate and bivariate outliers, was present in the plots of qPCR data versus age, reducing confidence in the conclusions of this analysis. Therefore, we applied robust regression to provide more accurate slope estimates of qPCR data, with cross-validation to estimate R^2 (Yohai 1987, Renaud and Victoria-Feser 2010). Bootstrap confidence intervals for the slope estimates were calculated (Yohai 1987). Correlations with bootstrap confidence intervals were calculated using a bivariate generalization of M-estimators (Goldberg and Iglewicz 1992), which handles the inclusion of multivariate outliers automatically. Multiple testing corrections were considered through controlling the false discovery rate by Benjamini-Hochberg procedure.

Results

KALRN expression changes are age-dependent in BA47

Using a general linear model fit, *KALRN* expression (adjusted for covariates) was plotted against age. Based on RIM analysis, a small but significant decrease in expression with age is seen in BA47 ($df = 102.5$, $\beta = -.0013$, $p=.029$, 95 % CI: $(-2.375e-03, -1.316e-04)$). The age-dependence in BA11 is not significant ($df = 102.5$, $\beta = .00039$, $p=.526$, 95% CI: $(-8.248e-04, 1.614e-03)$). The difference in the relationship between age and residual in BA11 and BA47 is small but statistically significant ($p=0.047$, 95% CI: 0.003, 0.455). Figure 2 illustrates the overall age effect for each brain region.

Age-dependent *KALRN* expression is isoform-specific

Probes were mapped to the KAL protein and this information was used to further stratify into isoform-specific locations (Table 4). Probes that mapped to a sequence identified within the KAL protein and were expressed above background level (as defined by a difference

greater than 20% in signal over the array background signal) were carried forward for further analysis, leaving N=31 probes. Initial analysis of the data revealed a significant correlation between age-dependent expression and exon location within the *KALRN* gene; expression levels appeared to increase in an age-dependent manner as exon number within the gene increased. In BA11, there was a significant correlation between the amino acid starting location of the probe and its increasing age-dependent expression ($r^2=0.621$, $p = 1.4e-07$, 95% CI: 0.602, 0.892). Similarly, a significant correlation was also seen in BA47 ($r^2=0.453$, $p = 3.3e-05$, 95% CI: 0.419, 0.829). Age-dependent *KALRN* probe expression is shown in Figure 3 as a function of probe location within the protein plotted against the age effect value of its expression. Inspection of Figure 3 reveals that multiple probes targeting KAL9 and KAL12 had significantly increased expression as subject age increased. In contrast, multiple probes targeting regions shared amongst all isoforms had significantly reduced expression as subject age increased. These findings were consistent across brain regions, although a greater total number of probes with significant age associations was seen in BA11.

To confirm these observations, isoform-specific qPCR studies were performed in BA11. Using robust regression, a line was fitted to the data points which are shown as relative expression (expression of the KAL isoform of interest normalized to the geometric mean of 3 internal standard genes) compared to age (Figure 4). Both KAL9 and KAL12 transcripts show a modest, statistically significant increase with age (Figure 4, panels B&C). The expression of the internal standard genes was also analyzed for age effect and was found to have no age dependent change (data not shown: β -actin, cyclophilin, GAPDH p-values for linear regression versus age were 0.8, 0.6, 0.87, respectively). However, the large spread of the data points in Figure 4 B&C suggests that overall age contributes much less to individual KAL9 and KAL12 expression compared to unaccounted for inter-individual factors. The slope of the fitted line represents change per year. For KAL9, the increase in expression between ages 20 and 80 equates to a 0.1% increase and for KAL12 this equates to a 0.6% increase. KAL5/7 shows no age-dependent change in expression (Figure 4, panel A).

Discussion

We characterized *KALRN* expression within the orbitofrontal cortex across the spectrum of normal human adult aging, with subsequent analysis of isoform specificity of these findings. Our exon array data demonstrate that when the gene is analyzed globally, the presumed expression may not be fully reflective of the actual pattern that results from alternative splicing of the gene. Isoform-specific qPCR supports this finding, confirming that both KAL9 and KAL12 show a modest increase with advancing age in BA11, a finding not seen when Global *KALRN* expression is assessed in BA11. It should be noted that the large variance amongst data points for KAL9 and KAL12 suggests that inter-individual factors other than age contribute to a higher degree to KAL9 and KAL12 expression, but the age-related correlations retain statistical significance in analysis of the overall cohort. Perhaps more important here is the observation that the isoform-specific quantification showed opposing results compared to the global analysis. Although age may not be the most influential factor in changes in *KALRN* expression (less than 1% for both KAL9 and

KAL12 over a 60 year span), analyses that consider only shared regions of the gene may overlook isoform-specific changes.

KAL is a Rho family GTPase that signals to the actin cytoskeleton and serves to regulate critical processes necessary for neurite outgrowth, branching, and development (Miller, Yan et al. 2013). As humans age, cortical changes include regression of the dendritic arbor and loss of complexity (Jacobs, Driscoll et al. 1997, de Brabander, Kramers et al. 1998, Hof and Morrison 2004). The underlying mechanisms for these age-related changes have yet to be fully elucidated. However, KAL9 and KAL12 are uniquely poised to regulate these processes, as their two GEF domains allow them to serve dual roles. Elegant knockdown studies in neurons of both KAL9 and KAL12 have demonstrated a critical role for these proteins in normal dendritic morphology during early development, as ablation of either isoform in immature hippocampal neurons results in decreased arborization and branching (Yan, Eipper et al. 2014). On the other hand, overexpression of KAL9 in mature neurons results in diminished dendrite length (Deo, Cahill et al. 2012), presumably due at least in part to increased activation of Rac1 (Penzes, Johnson et al. 2001, Deo, Cahill et al. 2012)). Thus, the increase in KAL9 seen with advancing age in the current study is consistent with the direction of dendritic morphological changes in the cortex associated with normal human aging. Because KAL12 also has dual GEF activity and its expression increases with age, it is possible it may also contribute to cortical dendritic alterations with aging, but as of yet the effects of increased KAL12 expression on the morphology of mature neurons has not been described. However, with such a small magnitude of age effect on KAL 9 and 12 (0.1% for KAL9 and 0.6% for KAL12), KAL expression is unlikely to explain the changes in dendritic complexity seen with normal aging.

The shorter isoforms of KAL, namely KAL5 and KAL7, lack the second GEF domain and signal primarily through Rac1 mediated pathways to facilitate spine maintenance during adulthood (Cahill, Xie et al. 2009). They localize to the postsynaptic density (PSD) through a targeting sequence contained in their unique C-terminal end, and this localization poises them for a role in integrating diverse signaling cues involved in spine maintenance (Penzes, Cahill et al. 2008). A reduction in KAL7 expression in cultured neurons results in decreased spine density and a loss of excitatory synapses (Ma, Huang et al. 2003, Xie, Srivastava et al. 2007); similarly, overexpression of KAL7 results in the opposite finding and gives rise to an increase in spiny synapse formation in cultured neurons (Penzes, Johnson et al. 2001, Ma, Huang et al. 2003, Penzes, Beeser et al. 2003, Ma, Kiraly et al. 2008). KAL7 knockout studies in mice further demonstrated evidence that loss of KAL7 is not associated with abnormal spine density during formative stages of development, but that the adult animals do display significantly decreased cortical spine density and cognitive deficits (Ma, Kiraly et al. 2008, Cahill, Xie et al. 2009, Xie, Cahill et al. 2010). Thus, a decrease in the shorter KAL isoforms could lead to an inability to properly maintain spines during adult life and present with loss of spine density, a finding that has been described in normal human aging within the cortex (de Brabander, Kramers et al. 1998). This underscores the need for isoform-specific studies to further investigate the potential role of shorter isoforms in dendritic spine changes seen in normal aging.

In the current study, there is robust evidence of a small but statistically significant increase in KAL9 and KAL12 expression with age as shown by both exon array and qPCR data. However, this increase is not reflected in analysis of the global *KALRN* gene, and instead appears to be masked by the presence of multiple probes which are decreased with age whose target sequences are shared amongst all known KAL isoforms. Developmental studies within rodent cortex indicate that by adulthood KAL7 is the most abundantly expressed isoform (McPherson, Eipper et al. 2002), thus the probes targeting shared sequences would be expected to include an overrepresentation of KAL7 given its higher relative abundance compared to KAL5 and the longer KAL9 and KAL12 isoforms. Taken together, this would suggest that KAL7 is decreased with age to an extent sufficient to mask the increase in KAL9/KAL12. However, this is not confirmed in the isoform-specific qPCR designed to amplify the region coding the C-terminal peptide sequence shared by KAL5/KAL7. While much evidence exists supporting the role for KAL7, little is known about the alternatively spliced KAL5 (also known as KAL7). Given that KAL5 and KAL7 both share 100% homology of the 20 amino acid C-terminal region that is distinct from KAL9/12, our qPCR validation studies did not distinguish between KAL5 and KAL7. Thus, there is the potential for opposing patterns of expression between KAL5 and KAL7 that are not resolved at the transcript level within the limitations of the current study.

Furthermore, it is possible that there are additional contributors measured by the exon array, such as uncharacterized splice variants or untranslated mRNAs, whose sequences are within regions shared by the four isoforms. Nonetheless, the positive correlation with age and KAL9/12 as supported by both exon array data and qPCR, coupled with the overall decrease in *KALRN* when the global dataset is analyzed, suggests that shorter transcripts are indeed negatively correlated with age to a degree capable of masking the increase in expression of KAL9/12.

Cortical spine loss has also been associated with neuropsychiatric diseases that contain within their clinical criterion changes in cognition. Delineating the molecular contributions to alterations in dendrite morphology and spine density that occur throughout the adult lifespan is critical to understanding how aberrant processes may contribute to disease development at different stages of adulthood. As the current study has indicated, global consideration of the *KALRN* gene can be misleading as the various isoforms demonstrate differential expression patterns that can be masked when the gene expression is analyzed as a whole. Thus, given the multiple implications of *KALRN* for human neuropsychiatric diseases characterized by changes in dendritic morphology, spine density, and cognition (Remmers, Sweet et al. 2014), it becomes crucial to conduct studies investigating isoform-specific expression patterns of KAL in disease. Future studies should focus on isoform-specific expression patterns as well as further investigation into the possibility of shorter splice variants or untranslated mRNAs that may be serving as of yet uncharacterized functions.

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Figure 1.

Flow diagram outlining selection of probes from exon array for inclusion in further analyses. Probes were excluded if they were unable to be mapped to the UCSC Genome Browser, failed to map to the protein sequence following *in silico* translation, or lacked significant expression values above background on the array. Remaining probes were then subject to statistical analyses for age-dependent expression patterns.

Figure 2.

Global *KALRN* expression versus age in BA47 and BA11. *KALRN* expression was plotted using a general linear model fit. To demonstrate the age-dependency, we kept the age-effect in the model and subtracted the effects of covariates (PMI, pH, RIN, sex and race) from the *KALRN* expression value, as residual values, on the Y axis with age on the x axis to demonstrate age-dependence. Independent regional analyses based on RIM showed a significant age-dependent association only within BA47 (A) BA47, $df = 102.5$, $\beta = -.0013$, $p=.029$, 95% CI: $-2.375e-03$, $-1.316e-04$ (B) BA11, $df = 102.5$, $\beta = .00039$, $p=.526$, 95% CI: $-8.248e-04$, $1.614e-03$

Figure 3.

Alterations in *KALRN* exon expression occur in an isoform-specific manner. Age effect represents the beta value from linear regression analysis for each probe. This age effect was plotted against the starting amino acid number within the protein for each probe sequence for all probes whose array expression was above background. A simplified representation of the length of each isoform is placed above the graph, with the representative lines corresponding to the amino acids included within each isoform. Filled in markers represent probes which had a statistically significant age effect; a positive value for age effect indicates increased expression with age, whereas a negative value indicates a decreased expression with age. Dashed lines indicate the N-terminus of KAL5 (far left) or C-termini of the remaining isoforms. The correlations between amino acid start number and age effect were significant, revealed by decreased age-dependent expression of multiple probes targeting regions shared by all isoforms and increased age-dependent expression of multiple probes targeting regions unique to the longer isoforms, KAL9 and KAL12. (A) BA47 ($r^2=0.453$, $p = 3.3e-05$, 95% CI: 0.419, 0.829) (B) BA11 ($r^2=0.621$, $p = 1.4e-07$, 95% CI: 0.602, 0.892)

Figure 4.

Real-time qPCR of isoform-specific *KALRN* transcripts in BA11. All samples were normalized to the geometric mean of β -actin, cyclophilin, and GAPDH. A robust regression model was fitted with inclusion of all covariates. Residual values of relative expression after considering all covariates (PMI, pH, RIN, sex and race) in the model are plotted versus age. Slope estimates (solid lines) with bootstrap CI (dashed lines, 95% CI) were calculated. R^2 was estimated by cross-validation, and correlation with bootstrap CI were obtained. (A) KAL5/7, slope estimate $1.31\text{e-}05$ (bootstrap CI $-1.18\text{e-}04, 1.46\text{e-}04$), $p=.812$, $q=0.812$, cross-validated $R^2=-0.018$, correlation with bootstrap CI $(-0.142, 0.151)$ (B) KAL9, slope estimate $5.73\text{e-}06$ (bootstrap CI $3.38\text{e-}07, 1.05\text{e-}05$), $p=0.04$, $q=0.060$, cross-validated $R^2 = 0.007$, correlation with bootstrap CI $(0.028, 0.285)$ (C) KAL12, slope estimate $1.43\text{e-}04$ (bootstrap CI $4.90\text{e-}05, 2.36\text{e-}04$), $p= 0.004$, $q=0.012$, cross-validated $R^2 = 0.024$, correlation with bootstrap CI $(0.047, 0.322)$

Table 1

Summarization of the structure and function of the various KAL isoforms.

	KAL5	KAL7	KAL9	KAL12
Domains (Johnson, Penzes et al. 2000, Penzes, Johnson et al. 2001, Tolias, Duman et al. 2011, Yan, Eipper et al. 2014)	RacGEF C-terminus involved in PSD targeting (20 amino acids)	RacGEF C-terminus involved in PSD targeting (20 amino acids)	RhoGEF RacGEF Unique 5 amino acid C-terminus in rodents	RhoGEF RacGEF Kinase
Signaling pathways (Tolias, Duman et al. 2011)	RacGEF→RhoG/Rac1	RacGEF→RhoG/Rac1	RacGEF→RhoG/Rac1 RhoGEF→RhoA	RacGEF→RhoG/Rac1 RhoGEF→RhoA Kinase activity
Developmental Time points (McPherson, Eipper et al. 2002)	Not measured specifically (no unique regions)	Initially lower in the cortex during early development, increases until dominant isoform by adulthood	Higher expression in rodent cortex during development, decreases in adulthood	Higher expression in rodent cortex during development, decreases in adulthood
Dendritic functions (Penzes, Johnson et al. 2001, Penzes, Beeser et al. 2003, Yan, Eipper et al. 2014)	Proposed: spine maintenance (RacGEF)	Evidenced: spine maintenance (RacGEF)	Evidenced: spine formation (RacGEF) spine elimination (RhoGEF) Dendrite elongation, branching (early development); shrinkage (later maturation)	Evidenced: spine formation (RacGEF) spine elimination (RhoGEF) Increased neurite length (kinase domain) Dendrite elongation, branching (early development); Proposed: shrinkage (later maturation)

Table 2

Demographic and technical characteristics of Human Subjects. PMI, postmortem interval; RIN, RNA integrity number.

Variable	N (%) or Mean (SD)
Age, years	50.5 (14.6)
Range	16–91
Sex	
Male	166 (79)
Female	43 (21)
Race	
Caucasian	178 (85)
African-American	31 (15)
PMI	17.2 (5.9)
Range	4.8–37.5
pH	6.7 (0.3)
Range	5.8–7.6
RIN	8.0 (0.73)
Range	5.9–9.6

Table 3

PCR primers used for subsequent analysis. Primers were designed using Primer3 Plus Software. Rows 1&2 represent primers designed to target to the unique C-termini of KAL5/7 and KAL9, respectively. Row 3 contains primers targeting a region present only in the full length isoform. Rows 4–6 contain internal standard genes.

Primer name	Forward	Reverse	Product size	Detected isoform
Unique KAL5/7	AAACCCATCATTCTAATCTA	AGTTAACCCCTCATCTTCTCT	96	5, 7
Unique KAL9	AAACCCTACTGAAACCTTAG	TAAATAGAAGTCCAGTCCAG	91	9
Unique KAL12	TGATACTGCAGTGCAAAGTCT	GTGTATGTGGCTGAGCTGTT	103	12
β-Actin	ACGGTGAAGGTGACAGCA	TTAGGATGGCAAGGGACTTC	145	
Cyclophilin	GCAGACAAGGTCCCAAAG	GAAGTCACCACCTGACAC	126	
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATG	87	

Table 4

Affymetrix Probes targeting the *KALRN* gene. Those that passed initial parameters (see Figure 1) were subjected to subsequent age-dependent analyses. Columns 7–9 provide results of age-dependent analyses in BA11 for probes expressed above background.

Affymetrix Probe ID	Genomic coordinates (from Affymetrix database)	Amino Acid target sequence (<i>in silico</i> translation from UCSC genome browser)	Amino Acid start number (Uniprot, rat identifier P97924-1)	KAL isoform(s) mapped	Probe raw expression value (log2 scale) in BA11	Relative expression above background (Y/N)	β value (SD) in BA11 (i.e. "age effect")	t value in BA11	p-value (q value) in BA11
8082166	chr3:123813708-123813732 (+)	WYLWYLRL	9	7, 9, 12	6.76	N			
8082167	chr3:123946867-123946891 (+)	SDVLPILK	33	7, 9, 12	5.87	N			
8082168	chr3:123953763-123953787 (+)		Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082169	chr3:123983371-123983395 (+)	LVTVLASV	95	7, 9, 12	10.00	Y	-0.001 (0.001)	-1.24	0.221 (0.434)
8082170	chr3:123987806-123987830 (+)		Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082171	chr3:124017677-124017701 (+)	SRRLIDEH	335	7, 9, 12	10.56	Y	9.86E-05 (0.001)	0.0863	0.929 (0.973)
8082172	chr3:124044955-124044979 (+)	QSHTEIGV	Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082173	chr3:124048761-124048785 (+)	ALDERSTI	445	7, 9, 12	7.62	Y	-0.001 (0.002)	-0.683	0.502 (0.718)
8082174	chr3:124053132-124053156 (+)	SEMQDLEL	478	7, 9, 12	6.90	N			
8082175	chr3:124066034-124066058 (+)	HTGVGKSL	563	7, 9, 12	7.66	Y	-0.0002 (0.002)	-0.147	0.881 (0.963)
8082176	chr3:124103791-124103815 (+)	LEVRIQDF	Discrepancy between UniProt sequence and <i>in silico</i> translation						

Affymetrix Probe ID	Genomic coordinates (from Affymetrix database)	Amino Acid target sequence (<i>in silico</i> translation from UCSC genome browser)	Amino Acid start number (UniProt, rat sequence identifier P97924-1)	KAL isoform(s) mapped	Probe raw expression value (log2 scale) in BA11	Relative expression above background (Y/N)	β value (SD) in BA11 (i.e. "age effect")	t value in BA11	p-value (q value) in BA11
8082177	chr3:124114157-124114181 (+)	PPSLGEP	712	5, 7, 9, 12	7.02	Y	-0.005 (0.003)	-1.78	0.078 (0.213)
8082178	chr3:124117578-124117602 (+)	SSSISHIE	734	5, 7, 9, 12	9.29	Y	-0.003 (0.001)	-2.43	0.018 (0.094)
8082179	chr3:124132332-124132356 (+)	DAWNEDLL	786	5, 7, 9, 12	9.45	Y	-0.002 (0.0009)	-2.49	0.015 (0.094)
8082180	chr3:124141781-124141805 (+)		Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082181	chr3:124149512-124149536 (+)	EQCLQLRH	905	5, 7, 9, 12	9.47	Y	-0.004 (0.002)	-2.11	0.04 (0.140)
8082182	chr3:124153242-124153266 (+)	WIRNGESM	963	5, 7, 9, 12	7.54	Y	-0.002 (0.002)	-0.992	0.331 (0.568)
8082183	chr3:124157812-124157836 (+)	YDADAIR	Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082184	chr3:124160847-124160871 (+)	PAAEIDHV	Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082185	chr3:124165054-124165078 (+)	NVSMPSV	Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082186	chr3:124165634-124165658 (+)	WTLKKRRL	1111	5, 7, 9, 12	11.25	Y	0.002 (0.001)	1.61	0.113 (0.274)
8082187	chr3:124174056-124174080 (+)	GEFYLSTH	Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082188	chr3:124175491-124175515 (+)	LADSFVEK	1186	5, 7, 9, 12	9.15	Y	0.0003 (0.002)	0.161	0.870 (0.963)
8082189	chr3:124180779-124180803 (+)	ASLSDREVK	1247	5, 7, 9, 12	9.56	Y	0.001 (0.001)	1.16	0.253 (0.468)
8082190	chr3:124181431-124181455 (+)	RDLHECLE	1290	5, 7, 9, 12	8.28	Y	0.002 (0.001)	1.20	0.240 (0.459)
8082191	chr3:124193541-124193565 (+)	EHIFGNI	1317	5, 7, 9, 12	7.69	Y	-0.004 (0.002)	-2.36	0.022 (0.104)
8082191	chr3:124193541-124193565 (+)	QLPEDVGH	1344	5, 7, 9, 12	9.25	Y	-0.001 (0.001)	-1.34	0.188 (0.408)

Affymetrix Probe ID	Genomic coordinates (from Affymetrix database)	Amino Acid target sequence (<i>in silico</i> translation from UCSC genome browser)	Amino Acid start number (UniProt, rat sequence identifier P97924.1)	KAL isoform(s) mapped	Probe raw expression value (log2 scale) in BA11	Relative expression above background (Y/N)	β value (SD) in BA11 (i.e. "age effect")	t value in BA11	p-value (q value) in BA11
8082192	chr3:124196157-124196181 (+)	EHAGTFFD	1379	5, 7, 9, 12	9.53	Y	0.0001 (0.001)	0.120	0.902 (0.963)
8082193	chr3:124201671-124201695 (+)	HGLANSIS	1393	5, 7, 9, 12	8.07	Y	0.0003 (0.002)	0.164	0.867 (0.963)
8082194	chr3:124207111-124207135 (+)	LSVPPKAN	1439	5, 7, 9, 12	9.71	Y	0.001 (0.002)	0.687	0.499 (0.718)
8082195	chr3:124209635-124209659 (+)	RHLFLFEI	1488	5, 7, 9, 12	5.90	N			
8082196	chr3:124210205-124210229 (+)	DPCKFALW	1532	5, 7, 9, 12	8.11	Y	-0.002 (0.001)	-1.68	0.097 (0.255)
8082197	chr3:124211608-124211632 (+)	QEWIKNIR	1561	5, 7, 9, 12	8.28	Y	-0.002 (0.002)	-1.27	0.210 (0.434)
8082198	chr3:124215187-124215211 (+)	GDGSSQPD	1611	5, 7, 9, 12	9.77	Y	-0.0002 (0.001)	-0.186	0.851 (0.963)
8082199	chr3:124238634-124238658 (+)	Unable to be translated							
8082200	chr3:124281900-124281924 (+)	SVEMDCFF	1706	9, 12	5.48	N			
8082201	chr3:124303711-124303735 (+)		Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082202	chr3:124351283-124351307 (+)		Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082203	chr3:124352722-124352746 (+)	EPDEESHT	1821	9, 12	8.94	Y	0.005 (0.002)	2.98	0.004 (0.038)
8082204	chr3:124356061-124356085 (+)	SLLAARQA	1848	9, 12	7.68	Y	0.005 (0.002)	2.51	0.014 (0.094)
8082205	chr3:124369656-124369680 (+)		Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082206	chr3:124374481-124374505 (+)	SLEGSSYR	1934	9, 12	6.19	N			
8082207	chr3:124376372-124376396 (+)	DYVKDLGI	1971	9, 12	6.24	N			
8082208	chr3:124376624-124376648 (+)	HQIYDWHK	1994	9, 12	5.74	N			
		RLAQLFIK							

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8082209	chr3:124377320-124377344 (+)	PRSEYIVA	2017	9, 12	7.65	Y	0.003 (0.002)	1.41	0.164 (0.385)
8082210	chr3:124378238-124378262 (+)	LTLSDFLI	2041	9, 12	6.34	N			
8082211	chr3:124379773-124379797 (+)	FLRYSEKA	2064	9, 12	7.45	Y	0.002 (0.002)	1.02	0.316 (0.568)
8082212	chr3:124380722-124380746 (+)	VPKRCNDM	2088	9, 12	7.89	Y	0.004 (0.002)	2.14	0.035 (0.140)
8082213	chr3:124385391-124385415 (+)	VFLFEQIV	2138	9, 12	5.09	N			
8082214	chr3:124385940-124385964 (+)		Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082215	chr3:124390516-124390540 (+)	VVLQAANA	2228	9, 12	6.55	N			
8082216	chr3:124393242-124393266 (+)	SPIEYQRK	2317	9, 12	6.77	N			
8082217	chr3:124397058-124397082 (+)	SMAYIKDY	2397	9, 12	8.38	Y	0.008 (0.002)	4.95	1.59E-05 (0.0005)
8082218	chr3:124398337-124398361 (+)	LRLMRKRA	2443	12	5.54	N			
8082219	chr3:124412654-124412678 (+)	DLDPNTSM	2453	12	5.43	N			
8082220	chr3:124413319-124413343 (+)	LNPNIQIE	2507	12	7.97	Y	0.007 (0.001)	5.08	1.59E-05 (0.0005)
8082221	chr3:124415075-124415099 (+)	NSSATYTT	2549	12	7.46	Y	0.005 (0.002)	2.61	0.011 (0.088)
8082222	chr3:124416516-124416540 (+)	STSATVKV	Discrepancy between UniProt sequence and <i>in silico</i> translation						
8082223	chr3:124418780-124418804 (+)	SSTGNCTI	2624	12	7.29	Y	0.005 (0.002)	2.74	0.007 (0.067)
8082224	chr3:124420920-124420944 (+)	SPGCPYQF	2670	12	3.57	N			
8082225	chr3:124431811-124431835 (+)	ENFDSAYT	2693	12	7.98	Y	0.009 (0.002)	4.69	3.17E-05 (0.0005)
8082226	chr3:124436105-124436129 (+)	IHKATRQDV	2755	12	5.30	N			
8082227	chr3:124439503-124439527 (+)	GRLLDYLM	Unable to be translated						

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8082228	chr3:124439827-124439851 (+)	Unable to be translated							