Influence of DARPP-32 genetic variation on BOLD activation to happy faces

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Abstract

Dopaminergic pathways play a crucial role in reward processing, and advanced age can modulate its efficiency. DARPP-32 controls dopaminergic function and is a chemical nexus of reward processing. In 61 younger (20–30 years) and older adults (65% $\pm$ 74 years), we examined how blood–oxygen-level dependent (BOLD) activation to emotional faces, vary over genotypes at three single nucleotide polymorphism (SNPs), coding for DARPP-32 (rs879606; rs907094; rs3764352). We also assessed age-magnification of DARPP-32 effects on BOLD activation. We found that major homozygote G, T or A genotypes, with higher cortical expression of DARPP-32, higher dopamine receptor efficacy, and greater bias toward positive cues, had increased functional connectivity in cortical–subcortical circuits in response to happy faces, engaging the dorsal prefrontal cortex (DLPFC), fusiform gyrus (FG) and the midbrain (MB). Local BOLD response to happy faces in FG, and MB was age-dependent, so that older carriers of the major G, T or A alleles showed lesser activation than minor genotypes. These genetic variants of DARPP-32 did not modulate BOLD response to angry faces, or engagement of the inferior occipital gyrus, to happy or angry faces. Taken together our results lend support for a potential role of DARPP-32 genetic variants in neural response to potential reward triggering cues.

Key words: DARPP-32; happy faces; dopamine; reward; PPP1R1B; SNP

Introduction

Neuroimaging genetics assesses the impact of genetic variation on brain function and connectivity. Dopamine (DA) signaling plays an important role in neuro-cognition. Specifically, processing of social stimuli, i.e. emotional faces, is associated with DA activity, and genetic proxies thereof (Meyer-Lindenberg et al., 2007; Rympa et al., 2015). A well-studied modulator of DA signaling is 32-kDa, DA and cAMP-regulated neuronal phosphoprotein (DARPP-32), which is encoded by the gene PPP1R1B. The DARPP-32 protein modulates cellular excitability and synaptic plasticity(Gould and Manji, 2005; Karunakaran et al., 2016), and has been associated with emotion and memory processing (Meyer-Lindenberg et al., 2007; Curcić-Blake et al., 2012; Persson et al., 2017). DARPP-32 is expressed in regions targeted by the midbrain DA projections, i.e. the striatum (Gould and Manji, 2005) and amygdala (Ouimet et al., 1992), prefrontal and medial temporal lobe (Albert et al., 2002; Kunii et al., 2014; Narita et al., 2010), and localized to neurons containing DA D1 and D2 receptors (Nishi et al., 1997). DARPP-32 mediates DA signaling in part through chemical regulation of protein kinase A (PKA) and protein phosphatase 1 (PP-1) (Hemmings et al., 1984). The major alleles G, T and A at three single nucleotide polymorphisms (SNPs) in PPP1R1B (rs879606, rs907094 and rs3764352,

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respectively) confer higher protein expression of DARPP-32, conveying greater DA efficacy (Meyer-Lindenberg et al., 2007; Kun et al., 2014). Recently, genotypes with higher expression of DARPP-32 showed greater activation of fronto-striatal (Meyer-Lindenberg et al., 2007), and fronto-medial (Curcić-Blake et al., 2012) circuits, and greater gray matter integrity of the dorsolateral prefrontal cortex (DLPFC) (Persson et al., 2017). Such findings warrants further investigation of the specific cortical/subcortical functional networks of emotion face processing that are potentially mediated by DARPP-32. Of importance for the present study is that DARPP-32 regulates reward circuits (Gould and Manji, 2005), and recognition of happy faces may convey social reward (Tronick et al., 1978; Strathearn et al., 2008). Functional magnetic resonance imaging (fMRI) studies show that happy faces activates DA generating areas as the the midbrain (MB) (Strathearn et al., 2008; Fusar-Poli et al., 2009), but also regions innervated by DA projections, as the the DLPCF, fusiform gyrus (Keightley et al., 2007; Fusar-Poli et al., 2009; Rypma et al., 2015) (but see reference Gauthier and Tarr, 2016), amygdala and striatum(Monk et al., 2008; Fusar-Poli et al., 2009). MB DA neurons fire in response to reward signaling cues, and the MB projects to the cortex via the striatum, but also directly by mesocortical pathways (Bayer and Glimcher, 2005). Meso-cortical signals are of importance for cognitive functions of the DLPFC, and is involved in emotional processing(Mattay et al., 2002; Leh et al., 2010). Findings from fMRI suggest that activity in DLPFC is associated with phasic MB signal, which further supports the presence of direct meso-cortical paths, without basal ganglia regulating access to DLPFC (D’Ardenne et al., 2012). DARPP-32 controls DA function and is a chemical nexus of reward processing (Gould and Manji, 2005). Summing up, the associations of DA mediating PPP1R1B polymorphisms with functional cortical/subcortical networks of emotional face perception is yet far from understood, and remains to be further specified.

Study aims and hypotheses. The goal of this study was to investigate the influence of DARPP-32 genetic variation on neural correlates of facial emotion perception, by using blood-oxygen-level dependent (BOLD) contrast imaging. We assessed (i) variation in BOLD response to happy and angry faces in major allele homozygotes (GG, TT or AA for rs879606, rs907094 and rs3764352), vs carriers of minor allele variants/minor homozygotes; and (ii) potential genotype differences in BOLD response between younger and older adults. We assumed that genetic variation does not directly cause behavioral phenotypes but rather mediates neuronal features that influence neural systems-level processing (Meyer-Lindenberg and Weinberger, 2006). We hypothesized that homozygote carriers of the major alleles (GG, TT or AA) would show greater activation of cortical/subcortical circuits, given their higher DA receptor efficacy, and the previous fMRI findings outlined above (Meyer-Lindenberg et al., 2007; Curcić-Blake et al., 2012). Further, we hypothesized that the major homozygotes would show greater BOLD response to happy faces as their higher DA level may promote bias towards positive cues (Strathearn et al., 2008; Frank and Fossella, 2011). We assessed age-differences as age-related changes emerge in parts of the DA system, with evident declines in DA receptors and transporters (Volkow et al., 1996; Volkow et al., 1996), in mid-brain nuclei (Volkow et al., 1996; Bannon and Whitty, 1997), striatum (Seeman et al., 1987), frontal and temporal cortices (Kaasinen et al., 2016) [while DA synthesis shows relative maintenance(Berry et al., 2016)], which may enhance the effect of DA regulating genotypes. Regions of interests (ROIs) in the current analysis (DLPFC, fusiform gyrus, amygdala, midbrain region and striatum) were selected for relevance for emotional face processing (Fusar-Poli et al., 2009; Ebner and Johnson, 2009; Fischer et al., 2010; Rypma et al., 2015; Fusar-Poli et al., 2009) and for their neuroanatomical basis of DA dependent circuits (Seeman et al., 1987; Bannon and Whitty, 1997; Kaasinen et al., 2016).

Materials and methods

Participants

Thirty young (20–30 years) and 31 older (65–74 years) adults (54% ♂), were recruited through local media advertisement. All participants were right-handed native Swedish speakers with normal or corrected-to-normal vision. They were all free of a history of neurological, psychiatric and cardiovascular diseases. None of the participants reported any use of psychotropic drugs. Each individual signed an informed consent after the experimental procedures were explained. They were financially compensated for the study. The study was approved by the regional ethics Committee in Stockholm, at Karolinska institutet, and was conducted in line with the declaration of Helsinki.

Genotyping

Genotyping was performed on DNA extracted from peripheral blood samples. Samples were subsequently labeled anonymously and transferred to the Mutation Analysis Facility at the Karolinska Institute, Huddinge, Sweden, for DNA extraction and genotyping. Genotyping was conducted with a single-nucleotide extension reaction, with allele detection by mass spectrometry (Sequenom MassArray® system; Sequenom, San Diego, CA). Polymerase chain reaction (PCR) and extension primers were designed using the MassArray® assay design software. The genotype success rate for the SNPs rs879606, rs907094 and rs376423 was 100%.

Imaging protocol

Images were acquired using a 3T Siemens Magnetom Tim Trio scanner at Huddinge Hospital, Karolinska Institute, Stockholm, Sweden. Foam padding was used to fixate each participant’s head carefully in the head coil and reduce involuntary head motions. After localizer scans, two runs of 160 functional images each were acquired with a T*2-weighted echo-planar sequence; repetition time (TR) = 2500 ms, echo time (TE) = 40 ms, flip angle (FA) = 90°, field of view (FOV) = 230 mm, voxel size = 3 × 3 × 3 mm). Thirty-nine oblique axial slices were positioned parallel to the AC-PC line and acquired interleaved. T1-weighted images were used for co-registration with functional images using the following parameters: TR = 1900 ms, TE = 2.52 ms, FA = 9 degrees, FOV = 256, voxel size 1 × 1 × 1 mm. FLAIR and T1-weighted images were inspected by a radiologist for potential signs of pathology: FLAIR: TE, 89 ms; TR, 9000 ms; FA, 130°; inversion time (TI), 2500 ms; section thickness, 4.0 mm; FOV, 199 × 220 mm.

Regions of interest

ROIs were defined by the WFU PickAtlasv2.4 (Maldjian et al., 2003; Maldjian et al., 2004); (http://www.nitrc.org/projects/wfu_pickatlas/); based on the Talairach Daemon. The ROIs consisted of left and right hemispheres: DLPFC, fusiform gyr (FG), inferior occipital gyr (IOG), striatum and the midbrain (MB).
Emotional face stimuli

Face images were taken from the FACES database (Ebner et al., 2016). Forty-eight pictures of young (18–31 years) and 48 pictures of older (69–80 years) faces (different face identities) were presented, with equal numbers of neutral, happy and angry expressions displayed in young and older faces (i.e. 16 per age of face by facial expression). Stimulus presentation and response collection (accuracy and response time) were controlled using E-Prime (Schneider et al., 2002).

During the fMRI session, participants worked on a facial expression identification task (Figure 1). This task had a mixed 2 (participant age: young, older) × 2 (age of face: young, older) × 3 (facial expression: neutral, happy, angry) factorial design, with participant age as a between-subjects factor and age of face and facial expression as within-subject’s factors. As shown in Figure 1, participants were exposed to faces, one at a time. Each face was presented for 3500 ms. Participants were asked to indicate whether the displayed face showed a happy, neutral, or angry expression by pressing one of three response buttons on a button box (index finger for ‘happy’, middle finger for ‘neutral’, and ring finger for ‘angry’). Response options appeared in black on a grey background below the faces and were always presented in the same order. Between faces, a black fixation cross appeared on a grey background. The inter-stimulus interval (ISI) pseudo-randomly varied between 3000 and 4000 ms in 250 ms increments (mean ISI = 3500 ms). In one-third of the trials (48 out of 144 total trials) ‘low-level baseline events’, consisting of three black crosses on a grey background, were presented. Participants pressed one of the three buttons that they also used for indicating the facial expressions to indicate appearance of the low-level baseline trial. The presentation order of face identities was identical for each participant with facial expressions counterbalanced across participants (each participant only saw each face with one expression). Lists were pseudo-randomized with the constraints that no more than three faces and no more than two low-level baseline events were presented in a row, and no more than two faces of the same category (i.e. age, sex, facial expression) were repeated in a row. The task started with four practice trials. The actual task was split into two runs, each lasting for 8.4 min.

Mapping the BOLD signal

Data from this event-related fMRI study were analyzed using Statistical Parametric Mapping (SPM5; Wellcome Department of Imaging Neuroscience, London, UK). Pre-processing included slice timing correction, motion correction, co-registration of functional images to the participant’s anatomical scan, spatial normalization, and smoothing [9-mm full-width half maximum (FWHM) Gaussian kernel]. Spatial normalization used a study-specific template brain composed of the average of the young and older participants’ T1w structural image. After normalization to standard space, functional images had dimensions of 53 × 63 × 46 with 3 mm isotropic voxels. Following preprocessing, first-level condition-specific effects of emotional faces were estimated for each individual using linear contrasts (happy face > + + +; angry face > + + +). Individual contrast images were then used in second-level random effects models accounting for scan-to-scan and participant to-participant variability using one-sample t-tests. BOLD parameter estimates exhibiting a main effect of task (i.e., differential effects of emotional face vs eyes at fixation cross) were extracted using SPM5, effectively yielding a weighted mean of contrast estimates over each region, where atypical voxels were down weighted. BOLD signal contrast between the emotional faces and fixation at three crosses are presented in Table 1, for the ROIs including DLPFC, FG, and MB regions. Activations for striatum or amygdala did not reach significance. Figure 2 presents a visual illustration for the activation data in DLPFC. Brain activation estimates were extracted by averaging BOLD signal (% signal change) from an 8-mm sphere around the peak voxel for each ROI. We used a threshold of .001, and including 10 or more contiguous voxels. These values were then used for plotting the results in Sigmaplot, and to perform latent variable analyses in the Mplus software (Muthén & Muthén, 1998–2011).

Statistical analyses

As shown in Table 1, BOLD activation in the striatum did not meet the threshold for significance and were therefore omitted from further analyses.

For all other regions we estimated latent difference scores in latent factors for BOLD response to happy and angry faces accounting for activation in response to eye fixation on the baseline stimuli (+ + +). For each ROI, we created a latent factor that was represented by several indicators of BOLD estimates, and a latent difference score carrying change in BOLD signal, going from the baseline stimuli to exposure to happy (or angry) faces. See references Coman et al., 2013; Persson et al., 2014; Persson et al., 2016 for further information about latent difference scores.
To investigate connectivity over cortical-subcortical pathways, we investigated inter-correlations across our ROIs. We examined inter-correlations between BOLD activation over the factor ROIs, for the total sample as well as for each allelic group. In a series of prediction models, we added age and sex, or level of education (\(P<0.05\)) correction for false discovery rate (FDR). We corrected for the false discovery rate (FDR) using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995), in addition to the chi-squared test with its degrees of freedom (df). We corrected for the false discovery rate (FDR) using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995), with the critical level denoted \(a'\) and the nominal significance level of \(a = 0.05\).

### Results

#### Descriptive statistics

Descriptive statistics are presented in Table 2. The genotype distribution of rs879606 (\(\chi^2 = 0.143, P = 0.704\)) and rs907094/ rs3764352 (\(\chi^2 = 0.01, P = 0.931\)), conformed to Hardy–Weinberg equilibrium among the participants. The allelic variants in rs879606 and rs907094/rs3764352 did not differ by means of age, sex, or level of education (\(P > 0.05\)). Correlations for the variables of interest are presented in Table 3.

### The latent difference score models with common factors

As mentioned in the statistical analyses section, we measured latent change in BOLD activation between the baseline stimuli (+ +), and exposure to happy and angry faces respectively, by constructing a latent difference score.

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**Table 1. Results of ROI analyses: peak activation in response to emotional faces**

| MNI | Region | BA | Hemisphere | t     | P     | p, FDR |
|-----|--------|----|------------|-------|-------|--------|
|     |        |    |            |       |       |        |
| x   | y      | z  |            |       |       |        |
| −42 | −54    | −18 | FG         | 37    | L     | 18.88  |
| 42  | −51    | −18 | FG         | 37    | L     | 20.97  |
| −30 | −84    | −9 | FG         | 19    | L     | 17.90  |
| 33  | −81    | −12| FG         | 19    | R     | 19.50  |
| 36  | −90    | −3 | IOG        | 18    | R     | 18.41  |
| −42 | 27     | 24 | MFG        | 9     | L     | 6.92   |
| 51  | 33     | 21 | MFG        | 9     | R     | 9.28   |
| 36  | 3      | 48 | MFG        | 6     | R     | 7.48   |
| −45 | 6      | 33 | IFG        | 6     | L     | 7.61   |
| 45  | 12     | 27 | IFG        | 6     | R     | 10.50  |
| 9   | −27    | −6 | MB         | 9     | R     | 5.05   |
| −6  | −30    | −3 | MB         | 9     | L     | 5.16   |
| 21  | −3     | −15| AM         | R     |       | 3.54   |
| −42 | −54    | −18| FG         | 37    | L     | 18.13  |
| 42  | −51    | −18| FG         | 37    | R     | 17.55  |
| 33  | −81    | −12| FG         | 19    | R     | 16.58  |
| 36  | −90    | −3 | IOG        | 18    | R     | 13.34  |
| −45 | 27     | 24 | MFG        | 46    | L     | 9.01   |
| 51  | 30     | 21 | MFG        | 46    | R     | 11.56  |
| 39  | 30     | 0  | IFG        | 45    | R     | 8.55   |
| −6  | −27    | −3 | MB         | 9     | R     | 6.34   |
| 6   | −30    | −3 | MB         | 9     | R     | 5.41   |
| 9   | −12    | −3 | MB         | 9     | R     | 3.59   |

AM, amygdala; BA, Brodmann’s area; FDR, correction for false discovery rate; FG, fusiform gyrus; IFG, inferior frontal gyrus; IOG, inferior occipital gyrus; MB, midbrain; MNI Montreal imaging institute coordinate space height threshold; MFG, middle frontal gyrus; L, left; R, right.
The carriers of rs879606 GG or rs907094/rs3764352 TT genotypes involved in happy face processing is present in persons with heterozygotes and minor homozygotes. These findings displayed to happy faces, across the MB and cortical regions, compared to A for rs3764352), showed positive inter-correlated BOLD response 2A, major homozygotes with rs879606 G or rs907094 T alleles (or To investigate connectivity over cortical–subcortical pathways in Connectivity over cortical-subcortical pathways by genotype groups. The following three factors were constructed with indicators of peak activation in response to happy faces (h) within each ROI (Table 1). For DLPCFh, five bilateral peak activation estimates from the middle frontal gyrus (MFG) and inferior frontal gyrus (IFG) were used as factor indicators. For the FG, four bilateral peak values were used to specify a latent factor, and two peaks from left and right hemispheres constructed the factor constituting MBh.

The following factors were specified for BOLD activation data to angry faces (a). The DLPCFa factor was represented by three peak estimates from MFG and IFG. For FGa we used unilateral BOLD estimates from four peaks; and for MBa we used two activation peaks from the left and right hemispheres. For the IOG (BA 18, right hemisphere), peak engagement to emotional faces (happy and angry faces respectively) were specified as single indicator latent difference scores.

All factor correlations exceeded 0.50. The models met the aforementioned thresholds for good fit to the data over several criteria for good fit to the data (CFI > 0.95; SRMR; < 0.08; RMSE; < 0.08).

Connectivity over cortical-subcortical pathways by genotype groups. To investigate connectivity over cortical–subcortical pathways in response to emotional stimuli, we assessed inter-correlations across our ROIs. As presented in Supplementary Tables 1A and 2A, major homozygotes with rs879606 G or rs907094 T alleles (or A for rs3764352), showed positive inter-correlated BOLD response to happy faces, across the MB and cortical regions, compared to heterozygotes and minor homozygotes. These findings displayed that greater intrinsic connectivity in cortical–subcortical circuits involved in happy face processing is present in persons with genotypes exhibiting greater DA receptor efficacy(Kuni et al., 2014). As seen in Supplementary Tables 1B and 2B, a more homogenous pattern of correlations, engaging several of the ROIs, were observed across the four allelic groups concerning BOLD engagement to angry faces (us eyes at fixation cross).

The effects of age and genotype on region-specific activation. As we found no effects of sex (all P’s > 0.05), we excluded this covariate from further analyses for the sake of model parsimony. As presented in Table 4, we observed no main effects of age, once genetic variation, and age × gene interactions were accounted for. The carriers of rs879606 GG or rs907094/rs3764352 TT genotypes showed greater activation when viewing happy faces in the DLPCF, than heterozygotes and minor allele homozygotes (see Figure 3A and B).

The rs879606 SNP explained 10% of the variance in BOLD activation to happy faces in the DLPCF, and 7.25% of the variance in BOLD response to happy faces in DLPCF was attributed to the rs907094/rs3764352 SNPs. No other main effects of the SNPs on BOLD response to happy faces were observed (P > 0.05), despite a trend-like effect for FG (P = 0.078, x2 = 0.020). We observed no main effects of the SNPs on BOLD response to angry faces, contrasted with eye fixation at the crosses (P > 0.05).

Interaction effects of age and genotype on regional activation. Age moderated the genetic effect on BOLD response to happy faces in the FG, so that older adults, possessing two rs879606 G alleles (P = 0.005, x2 = 0.020), and rs907094/rs3764352 TT, respectively, showed lesser activation than heterozygotes and minor homozygotes, although the effect for the latter SNPs was trend like as the effect did not survive statistical correction (P = 0.041, x2 = 0.020). An additive effect of age on genetic variation in BOLD response in the MB (happy faces) was also observed (P < 0.003) (see Figure 4B; Table 4B). Decomposition of the interaction term further showed that the genetic effects were particularly pronounced in the older age group (Ps were < 0.020; x2 = 0.020, see Figure 4A and B).

Discussion

We report that BOLD response to happy faces varied as a function of genetic variation in PPP1R1B coding for DARPP-32, while no relationship was found for angry faces. This is the first report to show that major homozygotes of the SNPs rs879606, rs907094 and rs3764352 (i.e. G, T or A homozygotes) exhibit increased cortical-subcortical functional connectivity, as well as increased local DLPCF activation to positive facial cues, compared to heterozygotes and minor allele homozygotes. That G–T–A haplotype was previously associated with more full-length DARPP-32 and less tDARPP-32 in DLPCF (Meyer-Lindenberg et al., 2007; Kuni et al., 2014) which lends further support for this finding. Moreover, genetic effects on BOLD response in FG, and the MB to happy faces were particularly pronounced in older adults, so that the major allele homozygotes, with higher DA efficiency, showed lesser activation than carriers of the minor alleles. These results suggest that genetic variation in coding for DARPP-32, mediates BOLD response to cues tapping social reward, and that these effects are selectively age-magnified in the FG and MB regions.

The observed genetic effects emerged selectively for happy facial cues that potentially convey social reward. Thus, those homozygotes for G–T–A alleles with more full-length DARPP-32 showed increased cortical-subcortical connectivity between DLPCF, FG and MB in addition to enhanced local activation of the DLPCF, when viewing happy faces, compared to heterozygotes and minor homozygotes. Adding to the strength of our results have previous reports found similar enhanced activation in DLPCF striatal, and DLPCF medial temporal lobe circuits in G–T–A homozygotes(Meyer-Lindenberg et al., 2007; Curtic-Blake et al., 2012). Our results add new value by pointing out the relevance of DARPP-32 in neural processing of cues conveying social reward, which is interesting given the role of DARPP-32 in relay ing reward signaling pathways (Gould and Manji, 2005), and the relevance for the engaged regions for processing emotional and reward-related information (Siessmeier et al., 2006; Haber and Knutson, 2010).
(A, B) The bar charts illustrate the effect of DARPP-coding genetic variants (rs879606; rs907094; 3764352), on bold oxygen level (BOLD) response to happy faces (vs. eye fixation at crosses). The rs907094 are 3764352 single nucleotide polymorphisms are in complete linkage disequilibrium (LD), and results are therefore collapsed over the groups. Homozygote GG or TT (and AA for rs 3764352) genotypes are marked in light gray in A and B. BOLD change is indexed by the mean expected value of latent difference scores derived from the estimated means of the models while taking into account the effects of covariates. The error bars represent standard errors of the means.

Table 4. Standardized parameter estimates of covariates effects on BOLD activation to happy faces (vs. eye fixation)

|                      | DLPFCh | FGh | MBh | IOGh | DLPFCh | FGa | MBa | IOGa | Age | rs879606 | rs907094/rs376432 |
|----------------------|--------|-----|-----|------|--------|-----|-----|------|-----|----------|-------------------|
| Age                  | −0.005 (0.220) | 0.383 (0.219) | 0.0487 (0.233)* | −0.107 (0.166) |
| rs879606             | 0.476 (0.189)** | 0.345 (0.195) | 0.288 (0.214) | 0.150 (0.190) |
| Age × rs879606       | −0.401 (0.241) | −0.655 (0.233)** | −0.745 (0.253)** | −0.290 (0.217) |
| Age                  | −0.129 (0.186) | 0.213 (0.171) | −0.407 (0.200)* | −0.114 (0.158) |
| rs907094/rs376432    | 0.458 (0.173)** | 0.130 (0.164) | 0.263 (0.200) | 0.129 (0.167) |
| Age × rs907094/rs376432 | −0.362 (0.224) | −0.433 (0.216)* | −0.717 (0.239)** | −0.285 (0.210) |

BOLD, Blood oxygen level dependent signal; h, happy faces; DLPFC, dorsolateral prefrontal cortex, response to happy faces (h); FG, fusiform facial area, response to happy faces; MB, midbrain nuclei, response to happy faces; IOG, inferior occipital gyrus, response to happy faces; DLPFCa (BA 45, 46), dorsolateral prefrontal cortex, response to angry faces (a); FGa (BA 37, 19), fusiform gyrus, response to angry faces; MBa, midbrain nuclei, response to angry faces; IOGa (BA 18), inferior occipital gyrus, response to angry faces; Age (0 = younger, 1 = older; older adults: 65-74 years; (younger adults: 20-30 years)); rs879606 (1 = GG, 0 = AG, AA); rs907094/rs376432 (1 = TT, 0 = CC, CT).

Significant correlations are in bold face: **P ≤ 0.01, *P ≤ 0.05.

aIn complete linkage disequilibrium.
On the other hand, there was no association between these SNPs and BOLD response to angry faces. Previous reports have shown associations between the allelic variants studied herein, and activation to negative emotional face stimuli. Variations in results between studies may, at least in part, emerge from differences in task design and sample characteristics. Previous activation studies differ in task design from the current report by a component of associative emotional learning (Meyer-Lindenberg et al., 2007; Curcic-Blake et al., 2012), that the current report lack. Further, the samples in the mentioned studies involved younger, and middle aged individuals (Pezawas et al., 2005; Meyer-Lindenberg et al., 2007; Curcic-Blake et al., 2012), while the current study comprise both younger and older adults. Emotional stimuli, i.e. faces, have induced greater FPC activation compared with neutral stimuli in older adults compared to younger adults (Nashiro et al., 2012), which is well in line with the findings from the simple correlations (Table 3), where covariates were not accounted for. Our models, addressing both age, genotype, and their interaction, overall suggest that the two age-groups differed significantly in response to happy faces, only when genotype differences were taken into account. Our findings emphasize the need to further address genotype variation in relation to age differences in brain activity during emotion processing, and older adults’ enhanced learning from positive feedback, and greater focus on positive information (positivity effect) (Mathes, 2012, 2016), may be fruitful to further address in the context of genotype differences in future reports.

We did not observe significant activations in the striatum, despite previously reported evidence of engagement of this region in processing reward(Knutson and Gibbs, 2007) and happy facial cues(Fusar-Poli et al., 2009). However, recent work shed light on selective circuits including the MB and DLPFC, when combining BOLD fMRI with transcranial magnetic stimulation (TMS) (D’Ardenne et al., 2012). Tentatively our results suggests that engagement of regions in the meso-cortical DA pathway, with immediate projections from MB to FPC(Robbins, 2010), rather than nigrostriatal, or mesolimbic pathways, relates to DA-associated genetic variation in BOLD response to reward triggering cues. Another explanation could be that the striatal level of activation was inhibited by DLPFC, acting as a suppressor because it has been shown that while DLPFC is activated to emotional stimuli, striatum is deactivated (Meyer-Lindenberg et al., 2007). However, it is difficult to rule out the possibility that the lack of striatal effect is due to the signal averaging within the striatum, which at least washes out some possible effects. Presence of physiological noise in this subcortical region from ventricular fluids is also very challenging in single-echo data which potentially also could have influenced the results. Future studies including TMS, and fMRI could further elucidate potential causalities of such relations.

DARPP-32 is likely to affect neuronal functions that influence neural systems–level processing, as DARPP-32 coding SNPs also influences other DA-dependent mental processes such as attention, and cognitive control (Meyer-Lindenberg et al., 2007; Li et al., 2013; Hammerer et al., 2013). Level of DARPP-32, regulated by genotype, may exert secondary influence on neural activity by modulation of DA input to neurons. This in turn affects hemodynamic metabolism that manifest a notable shift in BOLD signal. Of interest for the present results is recent findings highlighting a link between blood oxygenation and DA D1 activity in response to facial cues (Knutson and Gibbs, 2007; Rypma et al., 2015). Of relevance to these findings are also the observed associations between DA release and BOLD activity during reward-related learning (Schott et al., 2008), as distributed DA mediated reward circuitry is known to overlap with the face related neural pathways (Schultz, 2007, 2010; Rypma et al., 2015).

Older adults possessing major homozygote G-T-A alleles (or haplotypes) showed lesser activation of the bilateral FG, and MB than the minor allelic variants. Age-related changes in DARPP-32 expression may further act on the effects, as tDARPP-32 expression increase in the medial temporal lobes with advanced age (Kunii et al., 2014) that may attenuate DA, as tDARPP-32 lacks the Thr 34 phosphorylation site and PP-1 inhibitory domain that are critical for DA signaling (Kunii et al., 2014). The observed genotype effect could be an indication of such age-related shifts in DARPP-32, that modifies BOLD response in the FG and MB regions which both are crucial for face-perception (Haxby et al., 2001; Hanson et al., 2004), and cortical DA projections (Bayer and Glimcher, 2005).

Another explanation that may affect the BOLD response is that older age per se causes less-than-optimal DA function, and disruption of monoaminergic connections, exerting secondary...
influence on the genetic effects. It should be noted, however, that age-related changes may occur in various transmitter systems that interacts with DARPP-32 (Svenningsson et al., 2004). Summing up, these results could be interpreted in the context of the resource modulation hypothesis (Lindenberger, 2008), which suggest that genetic influence on cognitive functions are amplified in late adulthood, when functional and neurotransmitter brain resources undertakes decline (Lindenberger, 2008, 2014).

The current results should be interpreted in the context of several limitations. First, the reported findings suffer from limited generalizability due to the non-random recruitment procedures, relying on a sample of convenience. Another key limitation is the lack of statistical power in the current report. However, in this small sample, we had sufficient power to detect small genetic effects on BOLD response to happy faces in the DLFFC, ranging from 7 to 10% [moderate: >10%, Cohen, (1992)], and small to marginal effect sizes of correlation between the cortical and subcortical regions (Cohen, 1992). Another limitation is the candidate gene approach because many genes may contribute to heritability in neural correlates of emotional face perception. The sample size of the current report was too small, however, for a thorough investigation of simultaneous effects of additional genes and their interactions. Taken together, the findings reported herein warrants further replication to increase the generalizability of the results. The specific biochemical underpinning needs to be elucidated by future studies applying a multimodal imaging protocols that incorporate magnetic resonance spectroscopy and positron emission tomography (PET) indices of DARPP-32, and DA, in addition to genetics.

In conclusion, we observed both localized and more global network-oriented BOLD effects of genetic variants regulating DARPP-32 levels. These effects on BOLD response to facial cues, that potentially conveys social reward, were such that genotypes associated with greater level of functional DARPP-32 showed greater neuronal activation. Further, advanced age magnified the genetic effect on BOLD response to happy cues in the fusiform gyri and the midbrain area.

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Author contributions
N.P.: research questions, study design, statistical analyses and interpretation of data, drafting the manuscript, editing and revising the manuscript; H.F.: editing, critical revision planning research; NCE: editing, critical revision; C.L.: editing critical revision.

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