Transcriptome analysis of male–female differences in prefrontal cortical development

Molecular Psychiatry (2009) **14,** 558–561; doi:10.1038/mp.2009.5

Human neuropsychiatric disorders that involve the prefrontal cortex show gender differences in age of onset, prevalence and symptomatology. Gender dimorphisms in human-brain development may include differential cortical expression of genes on both the sex chromosomes[1,2] and on the autosomes. Here we identify gender differences in gene expression during human cortical development that may illuminate the molecular basis of gender biases in vulnerability to brain disorders.

Using an unbiased discovery driven microarray approach and targeted quantitative PCR (qPCR; Supplementary Methods) we identified and verified gender differences after surveying the expression of 55 000 transcripts in the prefrontal cortex of humans ranging in age from 1 month to 50 years (Supplementary Table S1). We found 8061 transcripts changed in expression level during development of the frontal cortex ($P<0.001$; Supplementary Table S2). Developmental changes involved genes in numerous functional groups including metabolic, synaptic and intracellular signaling. Principle component analysis revealed that age accounted for $\sim$30% of the variance (Supplementary Figure S1), whereas gender accounted for $\sim$8%, RNA integrity number (RIN) $\sim$3%, and race, pH and postmortem interval (PMI) $<$2%. Regression analysis (age and gene expression) captured most of the differences with $>$10 000 transcripts changing significantly with age ($P<0.05$). This extends previous findings from a study with older individuals that identified 540 age-regulated genes.[3]

The expression of 130 transcripts differed between males and females ($P<0.001$, false discovery rate $\sim$20% or 26 transcripts expected by chance). Twenty-five genes on the sex chromosomes ($>$40 transcripts) differed in expression between males and females (1.71% of those on X; 65.7% of those on Y; Supplementary Table S2). This is a threefold increase in the detection of Y chromosome genes from those previously identified as expressed in adult human frontal cortex indicating that the inclusion of younger ages improves sensitivity.[4,5] NLGN4Y, TTTY15 and PCDH11Y showed the highest expression in the youngest males, and thus may escape detection in adulthood. Five transcripts (EIFIAY, CYorf15A, CYorf15B, SMCY, TMSB4Y) increased expression during postnatal life in males and six (DDX3Y, HSFY1, RPS4Y1, USP9Y, UTY, ZFY) showed constant elevation in the male cortex (Supplementary Table S3; Supplementary Figure S2). qPCR of several selected genes confirmed the large gender difference in expression and also confirmed the increase in expression (DDX3Y) and decrease in expression (PCDH11Y) across age in the males (Figure 1a).

Eleven X chromosome genes showed gender dimorphic expression: ASMTL, CD99, DDX3X, EIF2S3, HDH1A, OFD1, PCDH11X, SMCX, USP9X, WBP5 and XIST (Supplementary Table S3). Five of these are X homologs of Y genes that show increased expression in males. The 100 times higher female expression of XIST, a gene responsible for inactivating portions of the X chromosome, is the largest gender-specific change detected, and replicates previous findings.[4,5] One gene, PCDH11X, was increased in the cortex of males as compared to females (Supplementary Figure S2) as previously reported.[4] However, the PCDH11 microarray probe sets are not chromosome specific. qPCR confirmed the gender difference and developmental decrease in pan PCDH11 expression ($R=0.649$; $P<0.0001$; Figure 1a). The specific PCDH11X was similar in males and females at each age. Thus, the high PCDH11Y expression in young male brains may explain the higher overall PCDH11 in developing males. Our results suggest that PCDH11X is not compensatorily upregulated in females.

The transcripts of 58 autosomal genes were differentially expressed in males as compared to females (Supplementary Table S4). Many of these genes (10/58) act as transcription factors or have zinc finger motifs which can also be involved in transcription (6/58).[6] However, as many transcripts encode both general and specific transcription factors, the 16 transcripts found to be changed according to gender do not represent any particular functional (GO) category. Many genes also encoded proteins involved in intracellular signaling (11/58); however, again these proteins are involved in diverse pathways from G-proteins to tyrosine kinases. Gender differences occurred in eight heat shock proteins (HSPs), which are induced in response to cellular stress and involved in protein folding and chaperoning. qPCR confirmed gender differences in 4/6 HSP mRNAs (Figure 1b): HSP90A1A expression was 19-fold higher in infant females than in infant males ($P<1\times10^{-6}$), and more than 5-fold higher than in all other males ($P<1\times10^{-5}$). Similarly, DNAJB1 expression in infant females was 12-fold higher than in infant males ($P<1\times10^{-5}$), and more than 6-fold higher than in all other males ($P<0.001$). HSPH1 and HSP90AA1 were threefold and twofold higher, respectively, in infant females relative to infant males ($P<1\times10^{-5}$ and $P<0.01$, respectively).

Dramatic developmental changes were identified in the human transcriptome that involve numerous categories of genes and which impact most aspects of cell function in prefrontal cortex. Significant gender-specific changes in gene expression were identified and verified in the prefrontal cortex and demonstrate that the human male and female brains differ at the molecular level especially in infancy. The

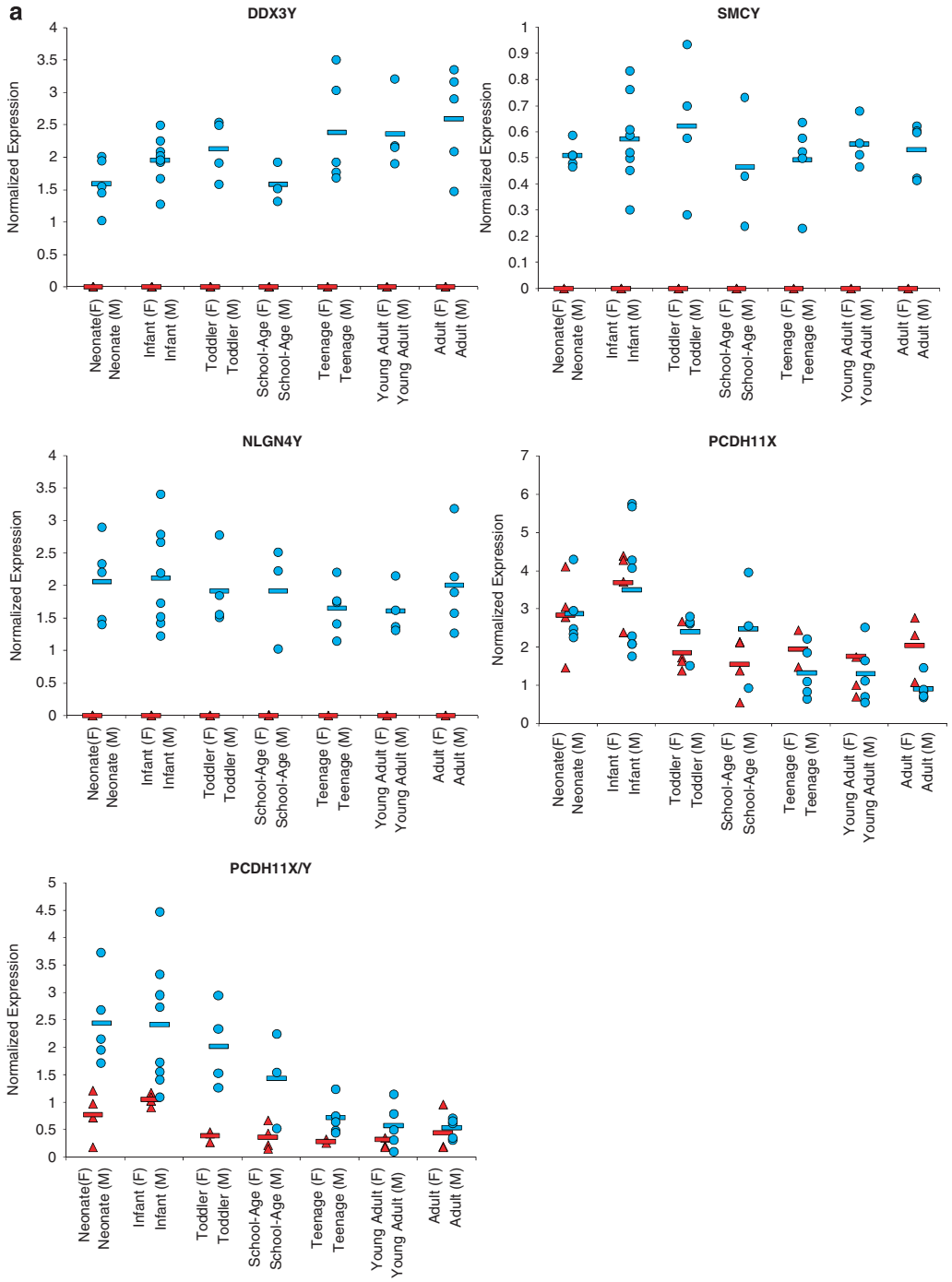

Figure 1 Target gene expression level as a ratio of the geometric mean of four housekeeper mRNAs (*y* axis) shown for each age group (*x* axis) with males (M, blue) and females (F, red). (**a**) Quantitative RT-PCR analysis of age- and gender-dependent expression changes for selected genes on the sex chromosomes. The qPCR also confirmed the increase in expression of DDX3Y and the decrease in expression of PCDH11Y across age in the males. Bars represent the group mean. (**b**) Quantitative RT-PCR analysis of age-dependent expression changes for selected autosomal genes involved in chaperone pathways. Gender differences in expression of HSP90A1A, HSP90AA1, HSPH1 and DNAJB1 by microarray were confirmed by qPCR, with increased expression specifically seen in infant females (F, red) as compared to males (M, blue). Bars represent the group mean.

Y-chromosome transcripts, PCHD11Y and NLGN4Y that were more highly expressed in infant males are prime candidates for influencing early male-specific development of cortical brain cells. In contrast, HSP mRNAs were elevated in infant females and may contribute to alterations in cortical development or influence the response to stress or trauma in the early life of females.

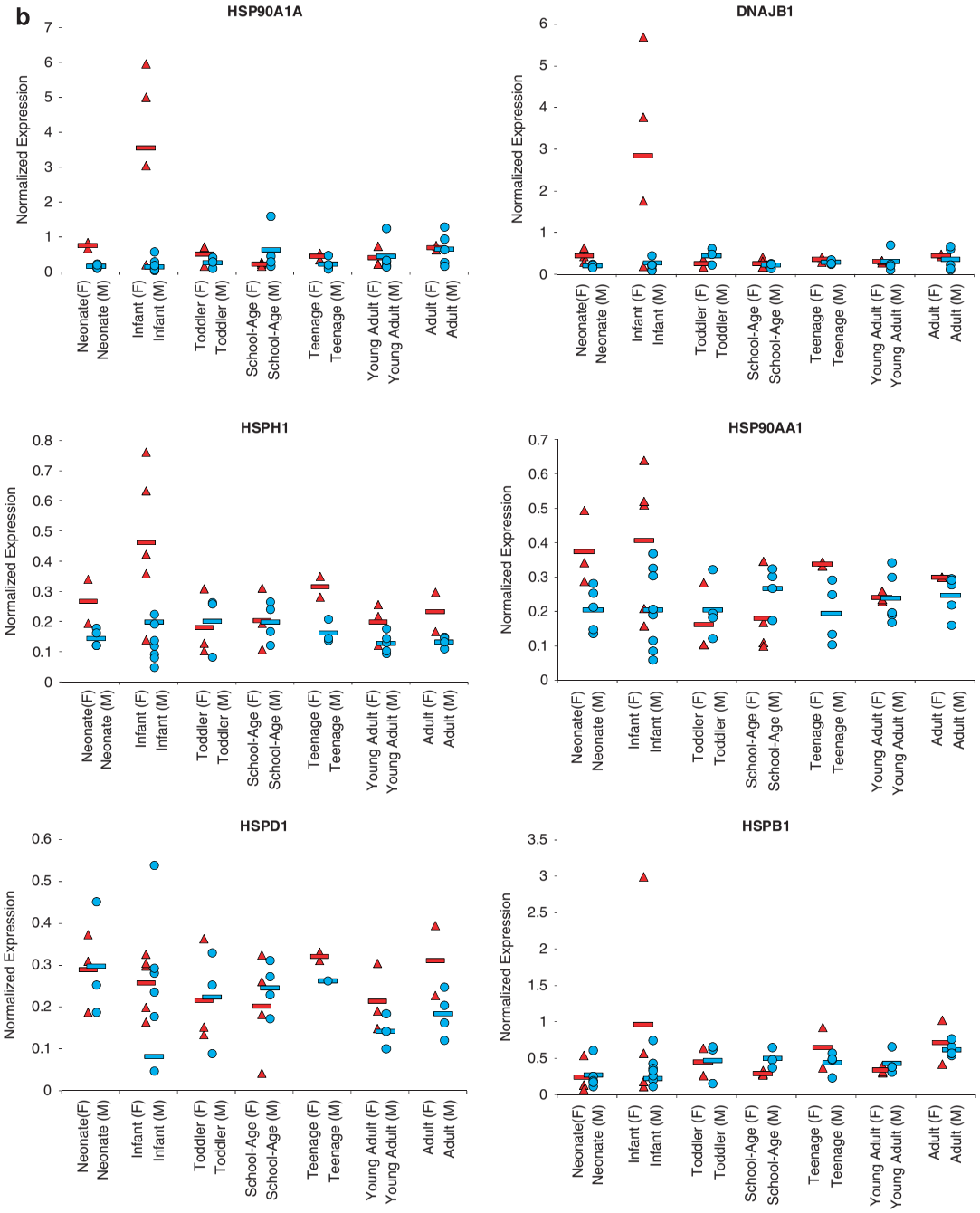

Figure 1 Continued.

CS Weickert[1,2], M Elashoff[3], AB Richards[1], D Sinclair[2], S Bahn[4], S Paabo[5], P Khaitovich[5,6] and MJ Webster[7]

[1]*MiNDS Unit, CBDB, NIMH, IRP, Bethesda, MD, USA;* [2]*Schizophrenia Research Institute (SRI), University of New South Wales, Prince of Wales Medical Research Institute, Sydney, Australia;* [3]*Cardiodx, Palo Alto, CA, USA;* [4]*Institute of Biotechnology, University of Cambridge, Cambridge, UK;* [5]*Department of Evolutionary Genetics, Max Plank Institute for Evolutionary Anthropology, Leipzig, Germany;* [6]*Institute for Computational Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, China and* [7]*Stanley Medical Research Institute, 9800 Medical Center Drive, Rockville, MD, USA*

E-mail: websterm@stanleyresearch.org

References

1 Carruth LL, Reisert I, Arnold AP. *Nat Neurosci* 2002; **5**: 933–934.
2 Rinn JL, Rozowsky JS, Laurenzi IJ, Petersen PH, Zou K, Zhong W *et al. Dev Cell* 2004; **6**: 791–800.

3 Erraji-Benchekroun L, Underwood MD, Arango V, Galfalvy H, Pavlidis P, Smyrniotopoulos P *et al. Biol Psychiatry* 2005; **57**: 549–558.
4 Galfalvy HC, Erraji-Benchekroun L, Smyrniotopoulos P, Pavlidis P, Ellis SP, Mann JJ *et al. BMC Bioinformatics* 2003; **4**: 37.
5 Vawter MP, Evans S, Choudary P, Tomita H, Meador-Woodruff J, Molnar M *et al. Neuropsychopharmacology* 2004; **29**: 373–384.
6 Hinoi E, Balcar VJ, Kuramoto N, Nakamichi N, Yoneda Y. *Prog Neurobiol* 2002; **68**: 145–165.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

Abnormal temporal and prefrontal cortical gray matter thinning in psychopaths

Molecular Psychiatry (2009) **14,** 561–562; doi:10.1038/mp.2009.12

Psychopathy is a complex clinical condition conceptualized as a combination of personality features, including shallow affect and egocentricity, as well as behavioral features including impulsivity and poor behavioral controls.[1] It has been hypothesized that deficits in the fronto-temporal neural circuit, particularly the ventromedial prefrontal cortex, dorsolateral prefrontal cortex, medial temporal regions and cingulate cortex, may play crucial roles in the neurobiological bases of psychopathy.[2,3] Prefrontal impairments, specifically, have been linked to poor decision making, whereas deficits in the temporal regions have been argued to contribute to the lack of emotion and social dysfunction in psychopathic individuals. We employed a cortical pattern-matching method,[4] to investigate localized differences in cortical gray matter thickness in psychopaths compared with that in non-psychopathic controls. We also examined whether the abnormality in cortical thickness is associated differentially with the four distinct facets of psychopathy (namely, Interpersonal, Affective, Lifestyle and Antisocial[1]).

Regional variations of cortical thickness were examined in 27 psychopaths and 32 normal controls recruited from the community. The demographic, cognitive and diagnostic characteristics of these individuals were obtained,[5] and two groups did not differ in age, gender, ethnicity, handedness, or substance abuse (all $P>0.2$). For each participant, structural magnetic resonance imaging data were collected using a 1.5T Philips S15/ACS scanner (repetition time:echo time = 34 ms:12.4 ms; voxel size = $0.9 \times 0.9 \times 1.7$ mm).[5] Cortical thickness was measured with high spatial resolution at homologous anatomical regions across individuals, using earlier-detailed cortical pattern-matching algorithms.[4] The General Linear Model implemented with R (http://www.r-project.org/), examined group differences when controlling for whole brain volumes. Linear regression analyses were used to determine the degree and direction of relationships between psychopathy and cortical gray matter thickness, across the entire sample of 59 individuals. Permutation analyses with a threshold of 0.05 were used to confirm the significance of multiple comparisons carried out at high spatial density. Regions-of-interest for frontal and temporal cortices were obtained from a probabilistic atlas to permute effects specific to these regions.

Psychopaths showed significant cortical gray matter thinning in the right frontal and temporal cortices (permutation corrected $P=0.036$, $P=0.017$, respectively) compared with that in non-psychopathic controls (see Figure 1a, for uncorrected results). Furthermore, in the right hemisphere, reduced cortical thickness was associated significantly with increased Affective facet scores (permutation corrected $P=0.034$), but not with Interpersonal ($P=0.09$), Lifestyle ($P=0.23$), or Antisocial facet scores of psychopathy ($P=0.09$). The left hemisphere cortical thickness/psychopathy facet scores associations were all statistically non-significant (all $P>0.08$). The negative correlations for the Affective facet score were significant for both the temporal and the frontal cortices' regions-of-interest (permutation corrected $P=0.018$, $P=0.045$, respectively), and observed prominently in the right middle frontal gyrus, right rectal gyrus, posterior cingulate gyrus and medial temporal cortex (including the parahippocampal gyrus and temporal pole) in the uncorrected statistical maps (Figure 1b).

Our findings of significant gray matter thinning within the right frontal and temporal cortices, suggest that the structural correlates of psychopathy may be linked to the emotional deficits characterizing psychopaths. The finding of right hemisphere pathology in psychopaths is consistent with observations from lesion studies, showing that damage to the right hemisphere impairs the ability for effective recognition, especially for negative facial expression,[6,7] similar to dysfunctions documented in psychopaths.[1]

Our findings of cortical thinning within frontal and temporal regions are also in agreement with earlier studies that show frontal and temporal gray matter volume reductions in psychopathic individuals,[5,8] and support earlier hypotheses that both regions and/or associated disturbances of fronto-temporal circuitry play an important role in the underlying neuropathology of psychopathy. Damage to the dorsolateral and ventromedial prefrontal cortices has been shown to disrupt normal decision making,[9] although damage to the medial temporal cortex critically impairs processing for the emotional and social context of information.[10] Thus, these findings