9. Yano T. The energy-transducing NADH: quinone oxidoreductase, complex I. Mol Aspects Med 2002;23:345–368.
10. King MP, Attadi G. Mitochondria-mediated transformation of human rho(0) cells. Methods Enzymol 1996;264:313–334.
11. Loeffen JL, Smeitink JA, Trijbels JM, et al. Isolated complex I deficiency in children: clinical, biochemical and genetic aspects. Hum Mutat 2000;15:123–134.
12. Li YY, Hengstenberg C, Maisch B. Whole mitochondrial genome amplification reveals basal level multiple deletions in mtDNA of patients with dilated cardiomyopathy. Biochem Biophys Res Commun 1995;210:211–218.
13. Andrews RM, Kubacka I, Chinnery PF, et al. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 1999;23:147.
14. Nijtmans LG, Henderson NS, Holt IJ. Blue Native electrophoresis to study mitochondrial and other protein complexes. Methods 2002;26:327–334.
15. Bai Y, Attardi G. The mtDNA-encoded ND6 subunit of mitochondrial NADH dehydrogenase is essential for the assembly of the membrane arm and the respiratory function of the enzyme. EMBO J 1998;17:4848–4858.
16. Cardol P, Matagne RF, Remacle C. Impact of mutations affecting ND mitochondria-encoded subunits on the activity and assembly of complex I in Chlamydomonas. Implication for the structural organization of the enzyme. J Mol Biol 2002;319: 1211–1221.
17. Carelli V, Ghelli A, Bucchi L, et al. Biochemical features of mtDNA 14484 (ND6/M64V) point mutation associated with Leber's hereditary optic neuropathy. Ann Neurol 1999;45: 320–328.
18. Corona P, Antozzi C, Carrara F, et al. A novel mtDNA mutation in the ND5 subunit of complex I in two MELAS patients. Ann Neurol 2001;49:106–110.
19. Kirby DM, Kahler SG, Freckmann ML, et al. Leigh disease caused by the mitochondrial DNA G14459A mutation in unrelated families. Ann Neurol 2000;48:102–104.
20. Ravn K, Wibrand F, Hansen FJ, et al. An mtDNA mutation, 14453G→A, in the NADH dehydrogenase subunit 6 associated with severe MELAS syndrome. Eur J Hum Genet 2001; 9:805–809.

Functional Neuroimaging Provides Evidence of Anomalous Cerebral Laterality in Adults with Klinefelter's Syndrome

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This study aimed to characterize cerebral perfusion in men with Klinefelter's syndrome, known to present specific deficits in language, using ^{99m}Tc-hexamethylpropylene-amine-oxime (HMPAO) scintigraphy and Talairach normalization. While a perfusion asymmetry toward the left hemisphere was found in controls, perfusion was mostly symmetrical in Klinefelter patients in the upper temporal and lower parietal areas. Scores on verbal tests were inversely correlated with perfusion changes, providing neurobiological substrate of anomalous cerebral laterality.

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Klinefelter's syndrome (KS) is the most common sex chromosome aneuploidy with a prevalence of 1/800 phenotypic boys. The presence of one (47,XXY) or more extra X-chromosomes is responsible for decreased testosterone production and impaired spermatogenesis, clinically identified at puberty by overt features such as small testes, infertility, and gynecomastia.[1,2]

Besides endocrinological characteristics, numerous studies have evidenced extension of the disorder to cognitive and behavioral domains. In childhood, two hallmark features associate a deficit in language processing and a learning disability in reading and spelling, which affect overall school performance.[3–7] Neu-

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ropsychological tests often indicate a decrease of the verbal intellectual quotient and other verbal deficits resembling dyslexia, contrasted with quite normal performance on nonverbal or visual-spatial tests.[3–6] In adulthood, these deficits persist, at least in a subset of patients.[8–10]

Specific impairment of language skills has yielded speculation that a left-hemisphere dysfunction is involved, possibly by means of alteration in the typical pattern of language dominance.[4,9] Indeed, high-resolution magnetic resonance imaging has revealed significant reduction of left temporal gray matter volume in 10 KS adults.[11] Up-to-date, almost no functional neuroimaging data are available, except for a case report of a neuropsychiatric patient[12] in whom [^{123}I]iodoamphetamine single-photon emission computed tomography (SPECT) revealed focal hypoperfusion in the left temporal lobe.

This study was designed (1) to characterize regional cerebral blood flow (rCBF) in KS adults, compared with controls, and (2) to determine whether perfusion abnormalities in KS correlate with neurocognitive function.

Patients and Methods

Subjects

We studied prospectively nine KS men (18–37 years), rigorously right-handed, not specifically selected for cognitive status and naive to testosterone therapy. We also enrolled nine age-matched and right-handed male controls (18–35 years), without learning disability history or neuropsychiatric conditions. The study was approved by our Institutional Review Board, and all participants gave written consent.

Neuropsychological Assessment

KS patients were administered a 4-hour neuropsychological battery[10] (Table 1). Because assessment of some cognitive domains required the use of two or more tests, raw test scores were converted into standard equivalents (Z-scores) using raw test data from a group of 22 normal subjects.[10] This approach allowed reduction of the number of variables from 34 to 13, representing distinct functional domains.

Single Photon Emission Computed Tomography Acquisition

Brain imaging was performed within a week on a brain-dedicated camera (Headtome II, Shimadzu, Kyoto, Japan), featuring circular high-sensitivity collimator allowing a spatial resolution ranging 8 to 15mm. Supine subjects were in-

Table 1. Demographics, IQ, and Neuropsychological Performances in KS Patients

Variables	Mean ± SD	Neuropsychological tests
Age (yr)	27.8 ± 6.6	
Education (yr)	12.8 ± 1.6	
Intelligence		
VIQ	92.0 ± 12.2	Saltz–Mogel format for the WAIS-R verbal IQ
PIQ	94.1 ± 10.2	Saltz–Mogel format for the WAIS-R performance IQ
Verbal attention	−0.61 ± 1.37	Digit span raw score from the WAIS-R
Language	−0.85 ± 0.88	Boston naming test; word sequencing (noncapture errors); WRAT-R reading and spelling raw scores
Spatial/constructional ability	−0.08 ± 1.19	Copy of the Rey–Osterrieth complex figure
Information processing speed		
Verbal	−0.70 ± 1.41	Stroop test (Comalli version) parts A and B
Nonverbal	−0.37 ± 0.54	Trailmaking part A; WAIS-R digit symbol raw score[a]
Memory		
Verbal	−0.25 ± 0.72	Logical memory subtest of the WMS-R (early/delayed); Rey AVLT (5th trial, recall after interference, 30-min recall)
Nonverbal	0.04 ± 0.89	Visual reproduction subtest of the WMS-R (early/delayed); 3-min recall of the Rey–Osterrieth; CVMT total score
Executive		
Verbal	−0.86 ± 0.71	Stroop test part C; trailmaking part B[a]; verbal fluency (words summed /3 trials); auditory consonant trigrams; word sequencing (capture errors); AVLT (false positives)
Nonverbal	−0.30 ± 0.65	WCST (perseverative responses)[a]; design fluency (in 5 min); emotional situations (errors); Rey tangled lines (time/trial)
Arithmetic	−0.36 ± 0.58	WRAT-R arithmetic raw score
Motor speed	−0.47 ± 0.70	Grooved pegboard (dominant/nondominant)

[a]The clustering of tests according to verbal and nonverbal subdomains was based on our previous cognitive studies in Klinefelter and other patient populations.[10]

VIQ = Verbal Intellectual Quotient, PIQ = Performance IQ, WAIS-R = Wechsler Adult Intelligence Scale-Revised, WRAT-R = Wide Range Achievement Test-Revised, WMS-R = Wechsler Memory Scale-Revised, AVLT = Auditory Verbal Learning Test, CVMT = Continuous Visual Memory Test, WCST = Wisconsin Card Sorting Test.

jected with 740MBq ^{99m}Tc-hexamethylpropylene-amine-oxime (^{99m}Tc-HMPAO, Medi-Physics, Anaheim, CA) after 10 minutes of relative sensorial isolation. Whole-brain images were acquired 30 minutes later for the best signal-to-noise contrast, in 64 × 64 matrices.

Image Processing

Raw data were reconstructed by filtered back-projection (Butterworth order 4, cutoff 0.25 cycles/pixel), and resulting transaxial volumes were segmented with a threshold of 50 to 55% of the maximum count to extract the three-dimensional brain surfaces. These surfaces were automatically coregistered to the Talairach atlas[13] using a custom software previously validated,[14] relying on a Powell function with 12 degrees of freedom. Brain volumes were then re-sliced according to the atlas cuts (27 total), in the anterior commissural–posterior commissural plane. Spatial normalization of SPECT images into the same geometry allowed us to analyze ^{99m}Tc-HMPAO distribution with high reproducibility within 65 volumes of interest (VOIs) previously drawn on the atlas, following the cortical gyri and subcortical structures. Mean counts were calculated automatically within each VOI and normalized to the average full-brain activity; results were expressed as relative rCBF (percentage). Asymmetry scores were computed using the following formula: (left − right) × 2 / (left + right).

Statistical Analysis

Within each VOI pair, rCBF was compared between left and right sides using paired Student's t test. Then, rCBF was compared between KS and controls using unpaired t test assuming that the fraction of cardiac output reaching the brain was similar in all participants. Finally, linear regression analysis was performed in KS patients between neurocognitive Z-scores and rCBF.

Results

Neurocognitive Profiles of Klinefelter's Syndrome Patients

Average performances on neuropsychological tests are summarized in Table 1. The lowest Z-scores involved verbal executive skills, language, and verbal processing speed, consistent with previous findings.[10] By contrast, Z-scores on nonverbal abilities were systematically higher than corresponding verbal scores, although not significantly. Except for nonverbal memory, all Z-scores were negative compared with the 22-subject reference population.

Brain Single Photon Emission Computed Tomography Perfusion Profiles

In the controls, significant leftward perfusion asymmetries occurred in 11 pairs of homologous VOIs (22 out of 65), as expected from right-handed people. Left-greater-than-right rCBF ($p < 0.05$) was found in three perirolandic gyri (precentral, middle, postcentral), four perisylvian gyri (insula, superior, middle, and transverse temporal), cuneus, superior and inferior parietal gyri, and cerebellum. In one region, the cingulate gyrus, reversal of cerebral asymmetry was found.

By contrast, rCBF was mostly symmetrical in KS patients, except for the precentral gyrus, transverse temporal gyrus and cerebellum, where leftward asymmetry persisted. As a consequence, significant rCBF increase was observed in many right hemisphere regions compared with controls (Table 2), namely the prefrontal motor areas, parietal associative areas, and temporal language areas. Reciprocally, rCBF decrease was observed in several subcortical structures, including hippocampi and cerebellum.

Correlations Between Neurocognitive Scores and Regional Cerebral Blood Flow

Linear regressions between neuropsychological scores in the 13 explored domains and rCBF in the above-mentioned VOIs (with significant perfusion changes) are presented in Table 3. As an attempt at controlling for random correlations, only r-values ≥ 0.60 were considered significant. Of interest, neuropsychological scores were inversely correlated with regions involving the right upper temporal and lower parietal lobes (where rCBF was increased), whereas positive relationships were found with the areas of decreased perfusion previously described. For example, low scores on verbal memory were associated with greater rCBF in the right superior, middle, and insular temporal gyri, and with lower rCBF in the left hippocampus. This finding may indicate that lower neuropsychological performance is associated with a rightward shift of cerebral perfusion, except for the motor speed task. We also noticed that left cerebellar rCBF decrease was interacting with the largest number of tests (see Table 3), probably because this structure is involved in a wide range of functions.

Discussion

Our study demonstrates a lack of leftward cerebral perfusion asymmetry in right-handed KS patients, further confirmed by a significant rCBF increase in many right-sided cortical regions, compared with controls. Moreover, perfusion changes include regions involved in language processing and correlate with lower performance on verbal tasks.

Language is actuated by a wide cerebral network preferentially distributed in the perisylvian cortex of the left hemisphere in 95% of right-handers. This functional asymmetry is supported by a striking anatomical asymmetry of the planum temporale.[15] Loss of this typical pattern with a shift toward the right hemisphere is seen in many left-handers and is referred to as anomalous cerebral dominance.[15] Neuroimaging studies have also suggested that cerebral organization of language differs between sexes,[16,17] thus supporting the notion that gonadal steroids do influence language lateralization.

Table 2. Areas of significantly different regional cerebral perfusion (mean ± SD) in the KS patients, compared to the normal controls

Cerebral regions	KS	Controls	*p* value
Areas of increased perfusion			
L superior frontal gyrus	99.0 ± 2.8	94.2 ± 3.0	0.003
R superior frontal gyrus	98.7 ± 2.8	94.2 ± 2.9	0.004
R middle frontal gyrus	99.3 ± 2.9	96.2 ± 2.6	0.01
L medial frontal gyrus	104.0 ± 2.0	101.0 ± 2.9	0.02
R post-central gyrus	103.4 ± 2.6	100.6 ± 2.2	0.03
R inferior parietal lobule	106.9 ± 3.8	103.0 ± 3.3	0.03
R supramarginal gyrus	107.2 ± 3.8	100.7 ± 2.9	0.0009
R angular gyrus	102.2 ± 4.4	97.7 ± 2.6	0.02
R superior temporal gyrus	103.7 ± 1.8	100.3 ± 2.3	0.003
R middle temporal gyrus	104.8 ± 1.8	101.8 ± 1.6	0.002
R insula	108.8 ± 1.9	105.5 ± 3.7	0.03
Areas of decreased perfusion			
R cingulate gyrus	103.8 ± 1.9	105.8 ± 1.6	0.03
L hippocampus	93.8 ± 3.0	97.0 ± 2.4	0.03
R hippocampus	94.0 ± 2.5	97.3 ± 2.4	0.01
Fornix	89.1 ± 5.5	95.0 ± 3.2	0.01
L cerebellar hemisphere	103.5 ± 3.3	107.3 ± 3.8	0.04
Cerebellar vermis	107.5 ± 4.4	113.3 ± 4.0	0.01
Asymmetry scores			
Inferior parietal lobule	−0.015 ± 0.031	0.030 ± 0.019	0.002
Superior temporal gyrus	0.004 ± 0.017	0.030 ± 0.026	0.02
Middle temporal gyrus	0.006 ± 0.012	0.025 ± 0.017	0.01

L = left, R = right, KS = Klinefelter's syndrome. The *p* values are corrected for multiple comparisons using the post hoc Bonferroni/Dunn test.

Besides hormonal influence, alteration of the language-related asymmetry can be provoked by conditions such as long-standing left-hemisphere lesions, dyslexia, and stuttering. In dyslexics, failure to activate left posterior temporal and inferior parietal regions during phonological tasks was described during $H_2{}^{15}O$ imaging.[18] Concurrent hyperactivation of the left inferior frontal cortex has yielded the hypothesis of a disconnection between anterior and posterior language areas, further sustained by magnetoencephalography.[19]

Table 3. Regression Analysis between Scores on Neuropsychological Tests and Regional Cerebral Perfusion

Neuropsychological test	Cerebral regions	r-coefficient	*p* value
Verbal skills			
Verbal attention	R superior temporal gyrus	−0.73	0.04
Verbal memory	R superior temporal gyrus	−0.80	0.01
	R middle temporal gyrus	−0.74	0.02
	R insula	−0.86	0.003
	L hippocampus	+0.70	0.04
Verbal executive	R insula	−0.67	0.05
	L cerebellar hemisphere	−0.69	0.04
Nonverbal skills			
Spatial/constructional	R hippocampus	+0.82	0.006
	L cerebellar hemisphere	+0.78	0.01
Visual memory	R supramarginal gyrus	−0.77	0.01
	L cerebellar hemisphere	+0.72	0.03
Arithmetic	R angular gyrus	−0.70	0.04
	R cingulate gyrus	+0.78	0.01
	R hippocampus	+0.74	0.02
	L cerebellar hemisphere	+0.81	0.008
Motor speed	R post-central gyrus	+0.95	0.001
	R inferior parietal lobule	+0.94	0.001
	R angular gyrus	+0.77	0.04

Positive r-values indicate positive relationship, while negative r-values indicate inverse relationship.

Given the small population studied, *p* values are not corrected for multiple comparison and are given for information only. L = left, R = right.

Whereas controls show sharp activation in the left temporo-occipital region 180 milliseconds after word presentation, dyslexics show delay or failure to activate this area entirely, demonstrating either an inability to achieve early operations of word-form perception or inefficient immediate phonological extraction.[19]

Dyslexic-like language difficulties are the mainstay in KS children[6,9]; additional neurocognitive profiles may emerge in adulthood, including preferential impairment of nonverbal functions.[10] Our data demonstrate that verbal deficits (attention, memory, executive) in adults correlate with rCBF elevation in the right upper-temporal lobe and angular gyrus, these regions being possibly considered as the mirrored language areas. It may be remarked that rCBF disturbances were found at baseline and not during activation tasks: whether perfusion shift (loss of leftward asymmetry) is the cause or the consequence of language impairment remains to be determined, as it may reflect inner verbal activation or resting metabolic state. Nonverbal functions were impaired to a lesser extent with relatively higher Z-scores, compared with verbal (see Table 1). Motor speed score was proportional to right parietal rCBF (see Table 3), indicating that perfusion shift would be ambivalently associated with lower performance on verbal tasks and relatively higher performance on nonverbal tasks. Such dissociation is thought to be more specific in KS children than in adults. Perfusion studies of KS children would be of highest interest to confirm our findings, if ethical considerations allow them.

Whether the neurocognitive phenotype of KS adults is a direct consequence of low testosterone stimulation remains controversial because androgen deficiency does not appear until after puberty.[7,9] Moreover, patients presenting with other types of supernumerous sex chromosome aneuploidies (47,XXX, 47,XYY) have opposing gonadal dysfunction but remarkably overlapping neurocognitive profiles.[5,7] A genetic contribution from the pseudoautosomal locus appears the most plausible explanation.[9,20] The hypothesis of an alteration in gene dosage in this locus is further supported by the finding that patients with 45,X0 Turner syndrome demonstrate a mirrored pattern of cognitive deficits, with remarkable preservation of verbal skills contrasted with severe impairment of visual-spatial skills.[3]

Conclusion

This preliminary study is important for the global understanding of neurocognitive disabilities associated with Klinefelter's syndrome, but further studies using task-specific brain activation imaging are justified. Perfusion SPECT may already be useful for monitoring anomalous language dominance in KS, particularly if testosterone therapy proves useful for reversal of cognitive symptoms.

References

1. Klinefelter HF, Reifenstein EC, Albright F. Syndrome characterized by gynecomastia, aspermatogenesis without a-Leydigism and increased secretion of follicle-stimulating hormone. J Clin Endocrinol Metab 1942;2:615–622.
2. Jacobs PA, Strong JA. A case of human intersexuality having possible XXY sex-determining mechanism. Nature 1959;183: 302–303.
3. Pennington BF, Bender B, Puck M, et al. Learning disabilities in children with sex chromosome anomalies. Child Dev 1982; 53:1182–1192.
4. Netley C, Rovet J. Hemispheric lateralization in 47,XXY Klinefelter's syndrome boys. Brain Gogn 1984;3:10–18.
5. Stewart DA, Bailey JD, Netley CT, et al. Growth and development from early to midadolescence of children with X and Y chromosome aneuploidy: the Toronto study. Birth Defects Orig Artic Ser 1986;22:119–182.
6. Graham JMJ, Bashir AS, Stark RE, et al. Oral an written language abilities of XXY boys: implications for anticipatory guidance. Pediatrics 1988;81:795–806.
7. Geschwind DH, Boone KB, Miller BL, Swerdloff RS. Neurobehavioral phenotype of Klinefelter syndrome. Ment Retard Dev Disabil Res Rev 2000;6:107–116.
8. Porter ME, Gardner HA, DeFeudis P, et al. Verbal deficits in Klinefelter (XXY) adults living in the community. Clin Genet 1988;33:246–253.
9. Geschwind DH, Gregg J, Boone K. Klinefelter's syndrome as a model of anomalous cerebral laterality: testing gene dosage in the X chromosome pseudoautosomal region using a DNA microarray. Dev Genet 1998;23:215–229.
10. Boone KB, Swerdloff RS, Miller B, et al. Neuropsychological profiles of adults with Klinefelter syndrome. J Int Neuropsycol Soc 2001;7:446–456.
11. Patwardhan AJ, Eliez S, Bender B, et al. Brain morphology in Klinefelter syndrome. Extra X chromosome and testosterone supplementation. Neurology 2000;54:2218–2223.
12. Miyamoto A, Kitawaki K, Koida H, Nagao K. MRI and SPECT of Klinefelter's syndrome with various neuropsychiatric symptoms: a case report. Jpn J Psychiatry Neurol 1993;47: 863–867.
13. Talairach J, Tournoux P. Co-planar stereotaxic atlas of the human brain. Stuttgart, New York: Thieme Verlag Medical Publishers, 1988.
14. Itti L, Chang L, Mangin JF, et al. Robust multimodality registration for brain mapping. Hum Brain Mapp 1997;5:3–17.
15. Steinmetz H, Volkmann J, Jancke L, Freund HJ. Anatomical left-right asymmetry of language-related temporal cortex is different in left- and right-handers. Ann Neurol 1991;29: 315–319.
16. Shaywitz BA, Shaywitz SE, Pugh KR, et al. Sex differences in the functional organization of the brain for language. Nature 1995;373:607–609.
17. Gur R, Mozley L, Mozley P, et al. Sex differences in regional cerebral glucose metabolism during a resting state. Science 1995;267:528–531.
18. Rumsey JM, Andreason P, Zametkin AJ, et al. Failure to activate the left temporoparietal cortex in dyslexia. An oxygen 15 positron emission tomographic study. Arch Neurol 1992;49: 527–534.
19. Salmelin R, Service E, Kiesila P, et al. Impaired visual word processing in dyslexia revealed with magnetoencephalography. Ann Neurol 1996;40:157–162.
20. Crow TJ. Pseudoautosomal locus for the cerebral dominance gene. Lancet 1989;2:339–340 (Letter).