ON-LINE APPENDIX

Image Processing

Data Processing of Volumetric Images. SPM8 25 was used for VBM preprocessing and statistical analysis. The protocol consisted of the following: a study-specific gray matter template was built from the 65 segmented native images affine-registered to the International Consortium for Brain Mapping-152 gray matter template. The native segmented images were then nonlinearly normalized onto this template, introducing a "modulation" for distortions due to the nonlinear component of the transformation by dividing each voxel of each registered gray matter volume image by the Jacobian of the warp field. The modulated normalized gray matter volume images were then smoothed with an isotropic Gaussian kernel with a ς of 3 mm.

For statistical analyses, we used a parametric *t* test as implemented by SPM8, by using age as a covariate of no interest. Results for gray matter were considered significant for $P < .05$, family wise error–corrected.

All 3D T1-weighted data were also processed by FreeSurfer to derive a quantitative estimate of cortical thickness, sulcation, and curvature. The sulcation conveys information on how far a particular surface vertex point is from a hypothetical "midsurface," which exists between the gyri and sulci. The curvature conveys information on the curvature (not distance) at a specific vertex point. The sharper the curve, the higher the value (positive or negative) is. The color conveys the sign and is just an arbitrary choice. FreeSurfer processing for volumetric T1-weighted images included the following: brain extraction, removal of nonbrain tissue by using a hybrid watershed/surface deformation procedure, automated spatial transformation and intensity normalization, tessellation of the GM/WM boundary and automated topology correction, and surface deformation following intensity gradients to optimally place GM/WM and GM/CSF borders at the location where the greatest shift in intensity defines the transition to the other tissue class. Image output from each stage of FreeSurfer was visually inspected. Cortical thickness, sulcation, and curvature were estimated for the whole brain.

Results were considered significant for $P < .05$, false discovery rate–corrected.

Data Processing of DTI (Tract-Based Spatial Statistics). All DTI data were preprocessed by the FMRIB Diffusion Toolbox within FSL.[26] First, the diffusion-weighted volumes were aligned to their corresponding non-diffusion-weighted (B0) image with an affine transformation to minimize image distortion from eddy currents and to reduce simple head motion. Then, nonbrain tissue and background noise were removed from the B0 image by using the Brain Extraction Tool. After these steps, the diffusion tensor for each voxel was estimated by the multivariate linear fitting algorithm, and the tensor matrix was diagonalized to obtain its 3 pairs of eigenvalues (L1, L2, L3) and eigenvectors. Maps of fractional anisotropy, mean diffusivity, axial diffusivity (first eigenvalue), and radial diffusivity (average of the second and third eigenvalue) were then generated.

Whole-brain analysis of FA images was performed by using tract-based spatial statistics. In brief, FA maps of all subjects were first realigned to a common target and then the aligned FA volumes were normalized to the Montreal Neurological Institute standard space (MNI 152). Thereafter, the registered FA images were averaged to generate a cross-subject mean FA image; then the mean FA image was applied to create a mean FA skeleton, which represented the main fiber tracts and the center of all fiber tracts common to the group. The mean FA skeleton was further thresholded by an FA value of 0.2, to exclude peripheral tracts where there was significant intersubject variability and/or partial volume effects with gray matter. Following the thresholding of the mean FA skeleton, the aligned FA data of each participant were projected onto the mean skeleton to create a skeletonized FA map, by searching the area around the skeleton in the direction perpendicular to each tract, finding the highest local FA value, and then assigning this value to the corresponding skeletal structure.

For group comparisons concerning FA and diffusivity values, the skeletonized data were fed into the voxelwise statistical analysis, which was based on nonparametric permutation testing (5000 permutations) by using the threshold-free cluster enhancement[27] method to account for multiple comparison correction across space. Age and sites of MR imaging acquisition were entered into the analysis as covariates.

On-line Table 1: Main clinical and demographic features in KS

Subject No.	Age (yr)	HH	Olfactory Function	Offspring	Obesity (BMI < 30)	Handedness	Kidney US	MRI Abnormalities	Other Abnormalities
1	45	Yes	Anosmia	No	Yes	Left	NA	bOB aplasia	MM
2	18	Yes	Anosmia	No	Yes	Left	Normal	bOB aplasia	
3	44	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	
4	34	Yes	Anosmia	1 Child	No	Right	Normal	bOB aplasia	
5	55	Yes	Hyposmia	No	No	Right	NA	bOB hypoplasia	
6	16	Yes	Anosmia	No	No	Left	Left RA	bOB aplasia	Ichthyosis, MM
7	36	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	MM
8	32	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	
9	16	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	
10	32	Yes	Anosmia	No	No	Right	NA	bOB hypoplasia	MM
11	20	Yes	Anosmia	No	No	Right	Normal	bOB hypoplasia	MM
12	47	Yes	Anosmia	No	Yes	Right	Left RA	bOB hypoplasia	MM
13	48	Yes	Anosmia	No	No	Right	Normal	bOB hypoplasia	
14	46	Yes	Anosmia	1 Child	No	Right	Normal	bOB hypoplasia; small posttraumatic lesions	
15	14	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	
16	17	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	
17	29	Yes	Anosmia	No	No	Right	Normal	bOB hypoplasia	Dental dysplasia
18	36	Yes	Anosmia	1 Child	No	Right	Normal	bOB aplasia	
19	23	Yes	Anosmia	No	No	Bilateral	Normal	bOB hypoplasia	
20	30	Yes	Anosmia	No	No	Left	Normal	bOB aplasia	Dental dysplasia, polydactyly, pes cavus
21	24	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	MM
22	19	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	
23	22	Yes	Anosmia	No	Yes	Right	Normal	bOB aplasia	
24	22	Yes	Anosmia	No	Yes	Right	Normal	bOB aplasia	Strabismus
25	25	Yes	Anosmia	No	No	Right	Normal	rOB aplasia, lOB hypoplasia, partial corpus callosum agenesis	Strabismus, cleft lip
26	31	Yes	Anosmia	No	No	Right	Normal	rOB aplasia, lOB hypoplasia	
27	17	Yes	Hyposmia	No	No	Right	Normal	bOB hypoplasia	Strabismus
28	18	Yes	Anosmia	No	No	Left	Left RA	bOB aplasia	Strabismus
29	28	Yes	Anosmia	No	Yes	Left	Normal	bOB aplasia	
30	43	Yes	Anosmia	No	No	Left	Normal	bOB aplasia	Ichthyosis, MM
31	40	Yes	Anosmia	No	No	Right	Normal	bOB hypoplasia	Ichthyosis
32	43	Yes	Anosmia	2 Children[a]	No	Right	Normal	bOB aplasia	MM
33	32	Yes	Anosmia	No	No	Right	NA	bOB aplasia, schwannoma VIII	
34	20	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	
35	32	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	
36	31	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	
37	22	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	Coloboma
38	23	Yes	Anosmia	No	No	Right	Normal	bOB aplasia	Bilateral neurosensorial hearing loss, Moebius syndrome
39	40	Yes	Anosmia	No	No	Right	Normal	bOB hypoplasia	Bilateral neurosensorial hearing loss, cleft lip
40	49	Yes	Anosmia	No	No	Right	Normal	bOB hypoplasia, diffuse white matter alterations (multiple sclerosis–like)	
41	42	Yes	Anosmia	No	No	Right	Normal	lOB aplasia, rOB hypoplasia	MM
42	40	Yes	Anosmia	No	No	Right	NA	bOB aplasia	MM
43	38	Yes	Anosmia	No	No	Bilateral	NA	bOB aplasia	MM
44	29	Yes	Anosmia	No	No	Right	NA	bOB aplasia	MM
45	9	Yes	Anosmia	No	No	Right	NA	bOB hypoplasia	

Note:—HH indicates hypogonadotropic hypogonadism; BMI, body mass index; NA, not available; US, ultrasound; RA, renal agenesis; MM, mirror movements; rOB, right olfactory bulb; lOB, left olfactory bulb; bOB, bilateral olfactory bulbs.

[a] Intrauterine insemination with washed capacitated sperm cells.