ON-LINE APPENDIX: STATISTICAL ANALYSIS ON THE GROUP LEVEL

The 2 measures for clival hypoplasia (Ba-Es, Ba-Xs) and the measure for basioccipital steepness (the Welcher angle) are highly correlated, as shown in the correlation matrix table (On-line Table 3). We orthogonalized the data using factor analysis.

We chose to analyze the factors instead of the original data because the high correlations suggest a clear common component. Indeed, the first factor (principal component analysis, correlation-based) is a weighted average of all measures, and adding the data reduces the noise. The first factor explained 86% of the total variance. This factor (factor 1) has a positive correlation with Ba-Es and Ba-Xs and a negative correlation with the Welcher angle. Uncorrelated second and third factors could also contain information, though this is unlikely in view of the small amount of variance explained by these factors. Indeed, there was no significant difference between patients with CHARGE syndrome and healthy controls in these transformed measures (results not shown).

We modeled the first factor with nonlinear regression analysis on the normal data. Our measures were dependent on age, as shown by the highly significant linear and quadratic components of age (On-line Table 4), showing that our measures were dependent on age. *P* values are not corrected for multiple testing. We then applied the regression equation to all data and computed the observed minus predicted "residue" value for all data. The result is shown in the On-line Figure. Most of the patients with CHARGE syndrome showed a reduced value in factor 1.

After this correction for age, the difference between controls and patients with CHARGE syndrome can be tested without interfering bias due to the difference in the age distribution of controls and patients with CHARGE syndrome with a 2-sample *t* test. Despite some patients with CHARGE syndrome having clearly normal values, as a group they had significantly lower values ($P < 0.001$) for factor 1. This means that patients with CHARGE syndrome, as a group, had a smaller clivus (Ba-Es and Ba-Xs) and a larger Welcher angle.

On-line Table 1: Lists of clinical criteria of Blake et al, Verloes, and Hale et al for CHARGE syndrome

Major Criteria	Minor Criteria	Inclusion Rule
Blake et al[5]		
Coloboma or microphthalmia	Cardiovascular malformations	Typical
Choanal atresia or stenosis	Tracheo-esophageal defects	4 Major or
Characteristic external ear anomaly, or middle/inner ear anomalies, or mixed deafness	Genital hypoplasia or delayed puberty	3 Major +3 minor
Cranial nerve dysfunction	Cleft lip/palate	
	Developmental delay	
	Growth retardation	
	Characteristic face	
Verloes[6]		
Ocular coloboma	Heart or esophagus malformation	Typical
Choanal atresia	Malformation of the middle or external ear	3 Major or
Hypoplastic semicircular canals	Rhombencephalic dysfunction, including sensorineural deafness	2 Major +2 minor
		Partial
	Hypothalamo-hypophyseal dysfunction	2 Major +1 minor
	Mental retardation	Atypical
		2 Major +0 minor or
		1 Major +3 minor
Hale et al[7]		
Coloboma	Cranial nerve dysfunction, including hearing loss	2 Major + any No. of minor
Choanal atresia or cleft palate	Dysphagia/feeding difficulties	
Abnormal external, middle or inner ears, including hypoplastic semicircular canals	Structural brain abnormalities	
	Developmental delay/ID/autism	
Pathogenic *CHD7* variant	Hypothalamo-hypophyseal dysfunction (gonadotropin or growth hormone deficiency) and genital anomalies	
	Heart or esophagus malformation	
	Renal anomalies skeletal/limb anomalies	

Note:—ID indicates intellectual disability.

On-line Table 2: Pearson correlation matrix

	Ba-Es	Ba-Xs	Welcher
Ba-Es	1.000000		
Ba-Xs	0.976623	1.000000	
Welcher	−0.702594	−0.692526	1.000000

On-line Table 3: Patient characteristics and measurements

			Molecular Diagnosis		Clinical Information		Clivus			Basioccipital		
Patient	Sex	Age at Evaluation	Mutation	Type	Symptoms[a]	Blake/Verloes	Morphology	Ba-Es (mm) (SD)	Ba-Xs (mm) (SD)	Welcher Angle (degrees) (SD)	Basioccipital Hypoplasia	Basilar Invagination
1	F	3 Days	7344_7345del	Frameshift	CHAE	+/+	Abnormal	4 (−6.3)	5 (−5.9)	147 (+3.5)	+	+
2	M	10 Days	1480C>T	Nonsense	HE	+/?	Abnormal	3 (−6.9)	2 (−7.5)	146 (+3.3)	+	+
3	M	12 Days	1820_1821insTTGT	Frameshift	CAGE	+/+	Abnormal	4 (−6.3)	4 (−6.5)	141 (+2.1)	+	−
4	M	1 Mo	5680_5681del	Frameshift	CHRE	+/+	Normal	8 (−4.0)	9 (−3.7)	134 (+0.4)	−	−
5	F	1 Mo	7879C>T	Nonsense	CHRE	+/+	Abnormal	2 (−8.8)	2 (−7.1)	176 (+13.5)	+	+
6	F	4 Mo	5222G>C	Missense	CHGE	?/+	Abnormal	13 (−1.1)	13 (−1.6)	135 (+0.6)	+	−
7	F	4 Mo	7106del	Frameshift	CHE	+/+	Abnormal, extra synchondrosis	15 (+0.1)	17 (+0.5)	139 (+1.6)	−	−
8	M	6 Mo	2238 + 1G>A	Splice site	CRGE	+/+	Abnormal	7 (−4.6)	6 (−5.8)	134 (+0.4)	+	−
9	M	6 Mo	6857dup	Frameshift	ARE	+/+	Abnormal, extra synchondrosis	17 (−1.2)	15 (−1.2)	125 (−1.5)	+	−
10	M	1 Yr	3202-?_8994?del	Deletion	CGE	−/?	Abnormal	5 (−8.9)	5 (−6.3)	140 (+1.4)	+	−
11	F	1 Yr 1 Mo	3340A>T	Splice site	RGE	−/a	Abnormal, extra synchondrosis	18 (−0.6)	19 (+0.7)	132 (−0.1)	+	−
12	M	1 Yr 2 Mo	1714C>T	Nonsense	RGE	−/a	Abnormal	5 (−8.9)	7 (−5.3)	134 (+0.3)	+	−
13	M	1 Yr 8 Mo	5535–7G>A	Splice site	GE	−/a	Abnormal	13 (−4.0)	11 (−3.9)	147 (+2.7)	+	n/e
14	M	1 Yr 10 Mo	1480C>T	Nonsense	CARGE[b]	+/+	Abnormal, extra synchondrosis	3 (−10)	4 (−6.8)	140 (+1.4)	+	+
15	M	2 Yr	7160C>A	Nonsense	CHAE	+/+	Abnormal	4 (−9.2)	5 (−6.6)	158 (+5.3)	+	+
16	F	2 Yr 10 Mo	3973T>G	Missense	CRGE	?/+	Abnormal, extra synchondrosis	17 (−1.7)	16 (−1.3)	146 (+3.3)	−	−
17	F	2 Yr 10 Mo	7769del	Frameshift	E	−/?	Normal	23 (+1.8)	18 (−0.3)	124 (−0.5)	−	−
18	M	5 Yr 7 Mo	4779del	Frameshift	HE	−/a	Abnormal	4 (−8.0)	5 (−6.0)	146 (+5.5)	+	−
19	M	7 Yr 3 Mo	2442 + 5G>C	Splice site	ARGE[b]	+/a	Abnormal, unilateral extra synchondrosis	24 (+0.4)	22 (+0.4)	127 (+0.4)	−	−
20	M	10 Yr 2 Mo	2505T>A	Nonsense	HARGE	+/+	Abnormal	11 (−4.4)	11 (−5.4)	137 (+2.0)	+	n/e
21	M	10 Yr 3 Mo	5564dupC	Frameshift	HARE[b]	−/?	Abnormal	4 (−6.3)	4 (−8.1)	144 (+3.0)	+	+
22	M	10 Yr 4 Mo	6157C>T	Nonsense	CRE	−/?	Normal	23 (−1.1)	21 (−1.5)	132 (+1.2)	−	−
23	F	16 Yr 2 Mo	4015C>T	Nonsense	CARGE[b]	+/?	Abnormal, extra synchondrosis	26 (+0.1)	24 (−0.1)	137 (+1.6)	−	−

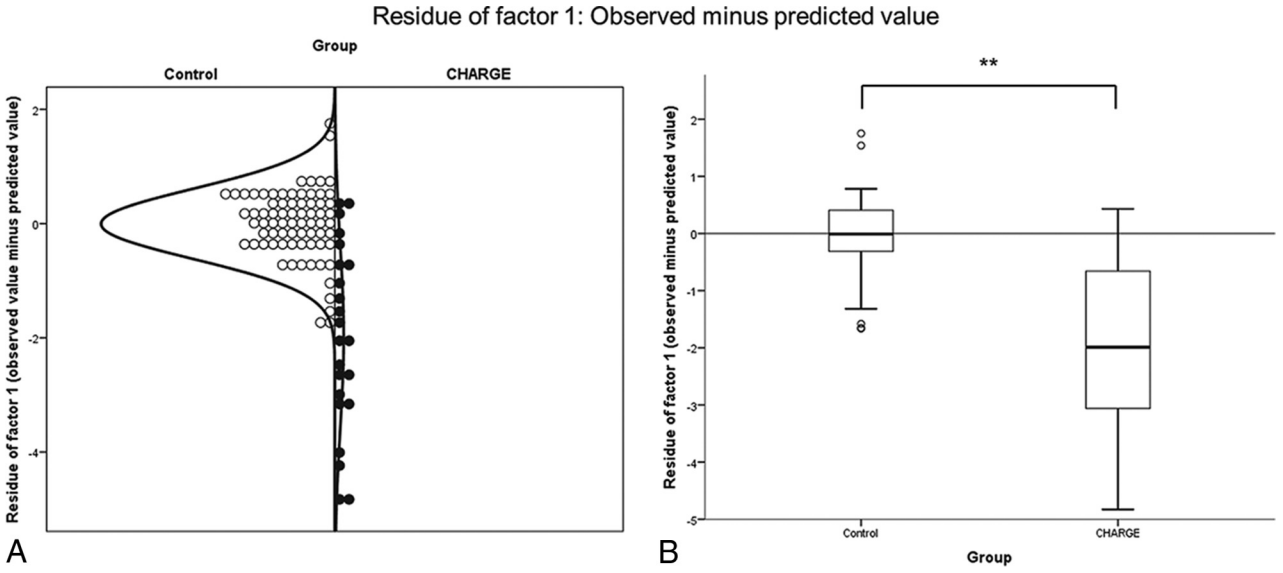
Note:—+ indicates meets criteria for typical CHARGE; ?, insufficient information; −, does not meet criteria; a, meets criteria for atypical CHARGE; n/e, examination was not possible.

[a] C indicates coloboma; H, congenital heart defect; A, atresia of choanae or bilateral cleft lip/palate (marked by [b]); R, retardation of growth or development; G, genitourinary defects or hypogonadotropic hypogonadism; E, ear anomalies or deafness.

On-line Table 4: Linear and quadratic component of age and modeling of first factor with nonlinear regression

Effect	Coefficient	Std Error	Std Coefficient	Tolerance	*T*	*P* (2-Tailed)
CONSTANT	−1.337657	0.227851	0.000000		−5.87075	<.001
AGE_MONT	0.034869	0.007086	1.417818	0.078934	4.92105	<.001
AGE_MONT*AGE_MONT	−0.000112	0.000037	−0.879080	0.078934	−3.05116	.003

Note:—Std indicates standard; AGE_MONT, age in months (linear term); AGE_MONT*AGE_MONT, age in months * age in months (quadratic term).



ON-LINE FIGURE. Residue of factor 1. Plots of the residue (observed minus predicted value) of the first factor from the principal component analysis. *A*, Scatterplot. *White dots* represent controls; *black dots*, patients. A normal curve has been fitted to both plots. *B*, Boxplot. The residue of factor 1 is significantly lower for patients with CHARGE syndrome. *Double asterisks* indicate $P < .001$.