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A Novel Approach to Dysmorphology to Enhance the Phenotypic Classification of Autism Spectrum Disorder in the Study to Explore Early Development

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Abstract

The presence of multiple dysmorphic features in some children with autism spectrum disorder (ASD) might identify distinct ASD phenotypes and serve as potential markers for understanding causes and prognoses. To evaluate dysmorphology in ASD, children aged 3–6 years with ASD and non-ASD population controls (POP) from the Study to Explore Early Development were evaluated using a novel, systematic dysmorphology review approach. Separate analyses were conducted for non-Hispanic White, non-Hispanic Black, and Hispanic children. In each racial/ethnic group, ~ 17% of ASD cases were Dysmorphic compared with ~ 5% of POP controls. The ASD–POP differential was not explained by known genetic disorders or birth defects. In future epidemiologic

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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studies, subgrouping ASD cases as Dysmorphic vs. Non-dysmorphic might help delineate risk factors for ASD.

Keywords

Autism spectrum disorder; Birth defects; Dysmorphic features; Dysmorphology; Genetic disorders; Morphologic anomalies; Phenotypic classification; Race/ethnicity

Introduction

Dysmorphology is the study of atypical development of physical features. Atypical physical features can be separated into two categories: major and minor morphologic anomalies (Hennekam et al. 2013). Major anomalies, also known as birth defects, are those with significant medical, surgical, or cosmetic consequences; they are individually uncommon, but altogether are present in approximately 3% of newborns in the United States (Centers for Disease Control and Prevention 2008; Egbe 2015). Minor anomalies, also known as dysmorphic features, do not have significant medical, surgical, or cosmetic implications. Some minor anomalies may be either present or absent (e.g., ear pit). Other categories of minor anomalies are measurement abnormalities and descriptive traits. Measurement abnormalities are structures with significantly higher or lower measurements compared to age-specific population means and standard deviations (e.g., tall stature, microcephaly, short philtrum). Descriptive traits are physical features with a continuum of variation in the general population that are considered dysmorphic at the extremes of the range of variability; these can be challenging to define (e.g., prominent tragus, hypoplastic alae nasi, prominent Cupid's bow). Each minor anomaly occurs in approximately < 4% of the general population (Marden et al. 1964; Méhes 1983; Leppig et al. 1987; Aase 1990; Merks et al. 2003).

Many single gene disorders and chromosomal anomalies, as well as some syndromes of unknown cause, have specific associated major and minor morphologic anomalies that define their phenotypes. Similarly, exposure to various teratogenic agents during pregnancy —for example, certain medications, infections, maternal conditions, dietary imbalances, toxins, and chemicals—can cause recognizable phenotypes composed of major and minor morphologic anomalies. Thus, multiple dysmorphic features, sometimes in conjunction with major anomalies, may be markers for underlying aberrant developmental processes. In fact, while 15–40% of otherwise healthy term infants may have one or two dysmorphic features, it is uncommon for individuals to have multiple dysmorphic features unless there has been an underlying genetic condition or gestational exposure that affected prenatal development (Marden et al. 1964; Hook 1971; Leppig et al. 1987; Merlob et al. 1985).

Autism spectrum disorder (ASD) defines a behavioral phenotype characterized by impairments in communication skills and social interactions along with restricted and repetitive behaviors and interests (American Psychiatric Association 2013). ASD has an appreciable genetic basis, based on high heritability in twin studies and numerous single gene disorders and chromosomal anomalies with increased risks for ASD (Muhle et al. 2004; Miller et al. 2005; Abrahams and Geschwind 2008; Bill and Geschwind 2009; Ronald

and Hoekstra 2011 ; Rosti et al. 2014; Sandin et al. 2014; Robert et al. 2017). In addition, epidemiologic studies have identified environmental factors that increase the risk for ASD, with some potentially acting through teratogenic mechanisms during pregnancy to affect brain development, including maternal metabolic syndrome and obesity, and certain prenatal infections and medication use (reviewed in Karimi et al. 2017). Given both the genetic and environmental causes of ASD and the general observation that children with multiple dysmorphic features often have underlying genetic conditions or gestational exposures that affected prenatal development, we hypothesized that the presence of multiple dysmorphic features in some children with ASD might identify distinct ASD phenotypes and serve as potential markers for understanding causes and prognoses.

Prior studies have investigated dysmorphic features in relation to ASD. Most early studies compared children with autistic disorder to control groups using a list of 16 physical anomalies (the Minor Anomaly Scale) developed by Waldrop et al. (1968). This scale was based on a list of 16 features originally selected by Goldfarb and Botstein (1956) and described in an unpublished manuscript on organic connections in childhood schizophrenia. Studies using the Minor Anomaly Scale found that children with autistic disorder had higher mean physical anomaly scores than controls (Mnukhin and Isaev 1975; Steg and Rapoport 1975; Walker 1977; Campbell et al. 1978; Links 1980; Links et al. 1980; Gualtieri et al. 1982). While these early studies compared the presence of particular dysmorphic features in children with autistic disorder to controls, they were limited to children with symptoms at the severe end of the ASD spectrum, and only 16 features were evaluated.

More recently, Miles and Hillman (2000) evaluated dysmorphic features in 94 individuals who fulfilled the diagnostic criteria for autistic disorder by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition and the Childhood Autism Rating Scale. They defined the following five categories of individuals based on an assessment of 200 dysmorphic features: (1) Minimal (≤ 3 dysmorphic features); (2) Mild (> 3 and < 6 dysmorphic features); (3) Moderate (≥ 6 dysmorphic features); (4) Severe (≥ 6 dysmorphic features along with a major morphologic anomaly); and (5) Syndrome (presence of an autism-associated genetic syndrome, such as fragile X syndrome, tuberous sclerosis, Sotos syndrome, or supernumerary isodicentric chromosome 15). For classification purposes, the Minimal category was defined as “phenotypically normal,” the Mild category was defined as “equivocal,” and the Moderate and Severe categories were defined as “phenotypically abnormal.” Their analysis showed that among the 94 individuals with autistic disorder, 26% were phenotypically abnormal. Miles et al. (2005) subsequently expanded their analysis to 260 individuals with autistic disorder. The classifications described in the original publication (Miles and Hillman 2000) were redefined as follows: phenotypically normal = non-dysmorphic, equivocal = equivocal, and phenotypically abnormal = dysmorphic. The proportion of individuals with autistic disorder classified as dysmorphic was 16%. Additionally, individuals classified as dysmorphic or those with microcephaly (head circumference ≤ 2nd percentile) irrespective of their dysmorphology classification, were further defined as having “complex” autism (18% of individuals with autistic disorder) while the remaining non-dysmorphic individuals were defined as “essential” autism.

Miles et al. (2008) subsequently modified the classification approach so that clinicians without extensive training in dysmorphology could classify individuals with autistic disorder. Using classification and regression tree analysis, this methodology relied on 12 body regions, coded as either normal or abnormal, to differentiate individuals with autistic disorder as either dysmorphic (complex autism) or non-dysmorphic (essential autism). Validation assessments indicated that this simplified classification method, defined as the autism dysmorphology measure (ADM), had 81–82% sensitivity and 95–99% specificity compared to the classifications obtained through the more rigorous approaches of Miles and Hillman (2000) and Miles et al. (2005).

The classification approaches described by Miles et al. (2005, 2008) have subsequently been used in other studies to characterize clinical and genetic differences between individuals with essential and complex autism (Stoelb et al. 2004; Angkustsiri et al. 2011; Tammimies et al. 2015; Flor et al. 2017; Zachariah et al. 2017). While most studies utilized the Miles et al. (2005) or the Miles ADM approaches, Wong et al. (2014) developed a different subgroup classification scheme based on dysmorphology for patients with ASD, with the goals of improving etiologic assessments and aiding in determining prognoses. The authors performed a retrospective, chart review study in China—medical records of 1261 patients with ASD from a single hospital were evaluated for any physical abnormalities recorded by pediatricians, developmental pediatricians, or child neurologists. Patients with diagnoses of tuberous sclerosis or certain specified syndromes—Williams, Rett, fragile X, Down, Dravet, Crouzon, Stickler, Kabuki, Angelman, and Sotos syndromes—were excluded. After exclusions, those patients with at least one “physical abnormality, measurement abnormality or observed descriptive feature or malformation” were classified as dysmorphic while those with no recorded physical abnormalities were defined as non-dysmorphic. Through this analytic approach, the proportion classified as dysmorphic was 10.8%.

Although the classification approaches described by Miles et al. (2005, 2008) were developed specifically to differentiate between essential and complex autism among individuals with autistic disorder, some studies have applied the algorithm to controls without autistic disorder to determine the proportion of dysmorphic individuals among that group as well. Angkustsiri et al. (2011), using the Miles et al. (2005) approach, classified the following as dysmorphic: 17.4% of 149 children with ASD and 5.4% of 112 controls who were typically-developing. Zachariah et al. (2017) used the Miles ADM to classify the following Indian children as dysmorphic: 26.9% of 26 children with autistic disorder and 10.0% of 140 controls without autistic disorder.

Previous classification approaches based on dysmorphic features (Miles et al. 2005, 2008 ; Wong et al. 2014) were developed exclusively utilizing single site, clinic-based patient populations of individuals with autistic disorder and did not utilize control groups of individuals without ASD to define the basis for identifying descriptive traits as dysmorphic. In addition, the classification approaches were developed utilizing individuals who were primarily White (86% White, 7% biracial, 5% Black, 1.5% Asian in Miles et al. 2005) or Chinese (Wong et al. 2014). Furthermore, as children grow, physical features and the extent of dysmorphology change (examples described in Allanson 1989; Cole and Hughes 1994; Braddock et al. 2007; Cung et al. 2015). As a result, assessments of cohorts with broad age

ranges may not adequately describe the dysmorphic features of the ASD population at specific ages. The mean age of individuals (primarily children) in the classification approach of Miles et al. (2005) was 9 years, and in Wong et al. (2014), the ages ranged from < 1 year to 32.8 years at time of diagnosis. Therefore, there are potential limitations for utilizing these classification methods to define ASD subgroups of dysmorphic individuals from broader non-clinic-based populations that may have higher proportions of other racial and ethnic groups. In addition, the validity of using these approaches among controls without ASD to define a dysmorphic subgroup is unknown.

Here we describe the development of the quantitative methods used to characterize and classify children based on dysmorphology in the Study to Explore Early Development (SEED). This methodology utilizes a large non-clinic-based sample of children with ASD in a narrow age range and a comparison group of population controls without ASD from three racial/ethnic groups: non-Hispanic White (NHW), non-Hispanic Black (NHB), and Hispanic. The dysmorphology assessments, reviews, and analyses that we performed allow us to characterize racial/ethnic-specific dysmorphology among the SEED ASD cohort, identify the dysmorphic proportion of children with ASD in each racial/ethnic group relative to the respective population control groups without ASD, and assess for differences in these proportions based on race/ethnicity and sex. We also assess the effects from known single gene disorders, chromosomal anomalies, and major morphologic anomalies of unknown cause on the proportions of children with ASD or population controls who are dysmorphic.

Methods

SEED is a multi-site case–control study of genetic and environmental risk factors for ASD. Details of the SEED protocol and methodology were previously published (Schendel et al. 2012).

Study Subjects

SEED enrollment and study methods were conducted at six study sites in California, Colorado, Georgia, Maryland, North Carolina, and Pennsylvania. Children and their caregivers (98% biologic mothers) were enrolled when the child was aged 2–5 years. Eligible children were born in 2003–2006 and lived in the respective site's study area, both at birth and at study enrollment. Additional enrollment criteria included an English-speaking (all sites) or Spanish-speaking (two sites) caregiver who was responsible for the child since age 6 months and able to provide legal consent. Children for the ASD case group were primarily identified from multiple special education and clinical sources that provide services to children with developmental disabilities. Potential ASD case children had special education or International Classification for Disease codes indicative of autism/ASD or other developmental disabilities that are typically precursors or co-occurring diagnoses in children eventually diagnosed with ASD. SEED ascertainment of ASD cases was intentionally broad for the types of disabilities included for children potentially eligible for the ASD case group in order to identify yet undiagnosed cases of ASD in young children who had come to the attention of a healthcare provider or school as having a developmental delay. Children for the general population control group (POP group) were identified via random samples of

birth certificates within each site's defined geographic study area. To ensure that ASD cases and POP controls were from the same study base, sources for the ASD group at each site included most major public school special education programs and large clinical sources serving children with ASD in the study area; thus, children sampled for the POP group would have likely been served at one of the respective site's ASD data sources had they been identified as having ASD.

Although children were initially identified as potentially being eligible for a given study group, the final study group classification was determined from standardized research developmental assessments. Upon enrollment, all children were screened for possible autism characteristics through their caregiver's completion of the Social Communication Questionnaire (SCQ). Children with SCQ scores ≥ 11 were designated as potential ASD cases regardless of how they were initially identified. Additionally, all children with a previous ASD diagnosis or autism special education classification were designated as potential ASD cases regardless of their SCQ scores. Study personnel skilled in administering developmental assessments subsequently evaluated all enrolled children in person. Children in the potential ASD group were administered the Autism Diagnostic Observation Schedule (ADOS) and their caregivers were administered the Autism Diagnostic Interview-Revised (ADI-R). Final inclusion in the ASD case group was based on the ADOS and ADI-R scores. For children ascertained from birth certificates, those who had SCQ scores < 11 and those who had SCQ scores ≥ 11 but based on the ADOS and ADI-R scores did not meet the criteria for classification as an ASD case, received a final classification of POP. These methods assured that children in the case group fulfilled inclusion based on ADOS and ADI-R results, and children in the POP group did not have ASD. Comorbid conditions, such as attention-deficit/hyperactivity disorder, seizure disorder, internalizing behaviors and externalizing behaviors, were not exclusions for enrollment in SEED. SEED also included a third study group of children with other (non-ASD) developmental disabilities, but children in this group are not included in this analysis as dysmorphology assessment and review focused primarily on children with a final classification of ASD or POP. As part of SEED data collection, parents reported their races/ethnicities on a caregiver interview, and child race/ethnicity was defined based on parental reported races/ethnicities (DiGuiseppi et al. 2016).

Dysmorphology Assessment

Each child underwent a systematic dysmorphology assessment immediately following the initial developmental assessment or at a second in-person assessment. Research assistants at each site were trained by clinical geneticists to perform a systematic dysmorphology assessment. The clinical geneticist provided quality control for parts 1–5 of the assessment by obtaining reliability with the research assistant in obtaining in-person measurements, performing the systematic examination and recording the findings, and obtaining photographs and digital hand scans. Each research assistant was also supervised by an on-site clinician. The "SEED Physical and Dysmorphology Examination Training Manual" utilized by research assistants and on-site clinicians contains 204 pages of detailed information on general dysmorphology, and descriptions of the procedures for performing all aspects of the dysmorphology assessment, the required training procedures for research

assistants, and the quality assurance/quality control procedures to be followed to maintain quality and reliability. A copy of the manual is available upon request.

The dysmorphology assessment consisted of six parts: (1) in-person measurements of the child and available parents; (2) systematic examination of the child by visual inspection and under Woods lamp; (3) a standard series of photographs of the child, along with additional photographs of unusual findings; (4) digital scans of the ventral (palm) surface of both hands; (5) filling out a Dysmorphology Examination Form (DEF) with the collected data from the examination; and (6) after the in-person assessment, performing a set of measurements from photographs and hand scans, recording those measurements on the DEF, and determining and recording percentiles for all measurements obtained.

Part 1—In-person measurement of the child included height using a model 214 Seca stadiometer (HealthCheck Systems, Inc.), weight using a MedWeigh MS-3200 digital high capacity portable scale (HealthCheck Systems, Inc.) calibrated prior to each use with a standard weight, and head circumference using a clinical ¼″ wide fiberglass tape measure. Parental heights and head circumferences were similarly obtained; no other measurements or components of the dysmorphology assessment were performed on available parents. In addition, children stood in bare feet on blank sheets of paper, lines were drawn at the ends of the great toe and the heel, and the distance between the lines was obtained with the tape measure and recorded as the foot length.

Part 2—The SEED dysmorphology assessment included only external features that are relatively easy to observe and photograph. Research assistants performed a careful visual inspection of the child for the following body regions: head, forehead, hair, face, eyes, eyebrows, nose, mouth and lips, teeth, ears, hands, feet, nails, and skin of the face, neck, chest, back, abdomen, and extremities. Skin areas were also examined under a Woods lamp #UV501 (Burton Medical). Measurements were obtained in two dimensions for all hyperpigmented (e.g. café au lait spots) or hypopigmented macules using the tape measure. Additionally, research assistants observed the child's gait. All measurements and unusual or questionable findings were recorded on the DEF. The SEED dysmorphology assessment was not a clinical exam, nor could it be used for genetic diagnosis. Based on ethical grounds and to comply with the wishes of many parents, the chest was not photographed, and the buttocks and external genitalia were not included in the dysmorphology assessment. The palate exam was also excluded given the challenges of adequate visualization in 3–6 year old children and obtaining satisfactory photographs for dysmorphology review.

Part 3—Research assistants utilized a digital camera (at least three megapixels) to obtain a standard set of photographs of the child: (1) head, anterior (face), non-smiling; (2) head, anterior (face), smiling (showing teeth); (3) head, posterior (showing hairline); (4) head, crown (showing hair whorls); (5) head, left ¾ view; (6) head, right ¾ view; (7) head, left lateral; (8) left ear, lateral; (9) head, right lateral; (10) right ear, lateral; (11) left hand, dorsal; (12) right hand, dorsal; (13) left foot, dorsal (standing); (14) left foot, dorsal (seated and dangling); (15) right foot, dorsal (standing); and (16) right foot, dorsal (seated and dangling). Additionally, skin features noted on exam, as well as all unusual or questionable findings, were photographed. Prior to obtaining face and ear photographs, square or

rectangular stickers of preset dimensions (internal measurement) were placed on the skin in the following locations: the glabella and just anterior to the left and right tragi; a face photograph without the sticker was also typically obtained. If a child was not able to hold completely still for photography, a study staff assistant, often with the assistance of a family member, helped position and hold the child for photographs.

Part 4—Research assistants performed a hand scan of the child using a digital flatbed scanner. The child's hands were placed palm-side down on the scanner bed next to a square or rectangular sticker of preset dimensions or a flat plastic ruler (internal measurement). If a child was not able to place his or her hands completely flat on the scanner, study staff, often with the assistance of a family member, held the child's palms and digits flat on the scanner bed, typically by placing their adult hand over the child's and pressing evenly over the course of scanning the hand. Hand scans were saved as digital images.

Part 5—Research assistants recorded all observations and data obtained during the in-person dysmorphology assessment on the DEF. Additionally during the in-person assessment, research assistants asked the child's caregiver the following questions and recorded the answers: "Was the child born with any problems in the structure of his/her body or organs (also known as birth defects)?"; "Has the child had any corrective surgeries, which includes surgeries to repair findings in the abdominal or genital region (such as hernias)?"; "Does the child have a clinical diagnosis of a syndrome?"; and "Has the child had a genetics evaluation, blood tests, or been seen by a genetic counselor?" Affirmative answers to the first three questions prompted follow-up questions about the types of birth defects, operations, or syndrome diagnoses; an affirmative answer to the fourth question prompted follow-up questions about the results of the genetic evaluation and tests.

Part 6—Research assistants obtained measurements from photographs and hand scans using Fetal Alcohol Syndrome Facial Photographic Analysis Software, version 1.0.0 (FAS Diagnostic and Prevention Network, University of Washington, Seattle, 2003). The stickers of preset dimensions placed on the glabella and just anterior to the tragi prior to photography of the face and ears were used to compute the measurements. The head, anterior (face), non-smiling photograph was used to obtain interpupillary distance, inner canthal distance, left and right palpebral fissure lengths, philtrum length, interalal distance, and mouth width. Lateral ear photographs were used to obtain left and right ear lengths. Lengths of the left and right index (2nd) fingers, middle (3rd) fingers, ring (4th) fingers, palms, and hands were measured from the digital hand scans. The square or rectangular sticker of preset dimensions or the flat plastic ruler placed on the scanner bed prior to scanning the hands was used to compute the measurements. Research assistants determined percentiles for the following child's measurements using ABase (Zankl and Molinari 2003) developed for the Palm OS® Emulator operating system, version 3.5 (Palm Inc., Santa Clara, CA): height, weight, head circumference, inner canthal distance, ear length, middle finger length, hand length, and foot length. Published growth charts for maternal and paternal head circumferences (Bushby et al. 1992), interpupillary distance (Feingold and Bossert 1974), palpebral fissure length (Thomas et al. 1987), philtrum length (Feingold and Bossert 1974), and palm length (Feingold and Bossert 1974) were used to develop Microsoft® Office Excel® tools for

calculating percentiles for the respective measurements, which were then used by research assistants to determine the percentiles. Body mass index (BMI) and percentile were calculated using the BMI Percentile Calculator for Children from the Centers for Disease Control and Prevention.[1]

In order to maintain quality control for measurements obtained from photographs and digital hand scans across the six SEED sites, every month a common set of images was distributed to the research assistants at each site who were responsible for obtaining measurements. Two research assistants at the Georgia SEED site, both skilled in obtaining measurements, would obtain and agree on a reliable measurement for each measured parameter. Research assistants at the other five SEED sites would independently perform the same measurements and submit the values for comparison to the Georgia SEED standard. Measurements could not differ from the standard by more than 5%. Research assistants who did not achieve the concordance threshold were retrained in obtaining measurements and retested for reliability with Georgia SEED standard measurements.

Dysmorphology Review

Seven clinical geneticists affiliated with SEED were each responsible for a specific dysmorphology review of all children in both the ASD case and POP control groups. Each clinical geneticist reviewed one of the following seven body regions: ears; eyes and eyebrows; growth and skin; head, hair, face, and neck; hands and feet; mouth, lips, and teeth; or nose and philtrum. In most cases, a single clinical geneticist reviewed all study children for the specified body region. A total of 397 potential major and minor morphologic anomalies were analyzed for each child (Table 1): 90 for ears; 62 for eyes and eyebrows; 16 for growth and skin; 68 for head, hair, face, and neck; 83 for hands and feet; 26 for mouth, lips, and teeth; and 52 for nose and philtrum. 42 reviewed features were major anomalies and the remaining 355 features were minor anomalies.

Each clinical geneticist developed a systematic Dysmorphology Review Form (DRF) that listed the features for his or her review. Since physical features often represent a continuum in the population, the DRF utilized a Likert scale to denote the “quality” of the feature being examined: 0 = normal or absent; 1 = possible or questionable; 2 = mild; 3 = moderate; and 4 = severe. When a feature was defined by a measurement, then percentile ranges, ratio ranges, or angle measurement ranges were specified for each value of the Likert scale. For example, for the feature of long ear length, Likert scores were assigned to the following percentile ranges: 0 = length < 90th percentile; 2 = length ≥ 90th percentile and < 97th percentile; 3 = length ≥ 97th percentile and < 3 standard deviations (SD) above the mean; and 4 = length ≥ 3 SD above the mean. Likert scores for features not based on a continuum, but either present or absent (e.g., question mark ear), were 0 = feature absent and 4 = feature present. Many descriptive traits comprise a continuum in the general population from mild to severe (e.g., prominent forehead). For such traits, the clinical geneticists used published consensus descriptions (such as in Allanson et al. 2009; Hall et al. 2009; Hunter et al. 2009; Hennekam et al. 2009; Carey et al. 2009; Biesecker et al. 2009) as reference material and

[1] https://www.cdc.gov/healthyweight/bmi/calculator.html.

then evaluated photographs of 50–100 children enrolled in SEED in order to develop criteria for assigning Likert scores of 0, 2, 3, and 4 to represent normal, mild, moderate, and severe, respectively.

Clinical geneticists performed their dysmorphology assessments for their respective assigned body regions by reviewing all of the data recorded in the DEF for each child, and examining all photographs and scans pertaining to the features in their DRF. Reviews were blinded—clinical geneticists did not know whether the child being reviewed was in the ASD or POP group. When a potential feature could not be ascertained (e.g., measurement could not be obtained, photograph not available, photograph not of adequate quality for review of the feature, or feature obscured, such as hair obscuring certain ear structures), no Likert score was assigned, and the dysmorphology review for the feature was recorded as "missing data." For bilateral features—ears, hands and feet, most for the eyes and eyebrows, and a few in the other body regions (cheeks, nasolabial folds, paranasal tissue, alae nasi, nares, and lips)—each side was assessed separately and received its own Likert score; the higher Likert score of the pair was specified as the overall score for that feature in the child. Results of each clinical geneticist's dysmorphology review were recorded in the respective DRF. Geneticists conducted dysmorphology reviews first for all NHW children, followed by all NHB children, then all Hispanic children.

One geneticist also evaluated all responses to the questions posed to the caregiver about birth defects or syndromes in the child and included consistent descriptions of the responses within four categories: non-chromosomal genetic syndromes (caused by nucleotide variants and other genetic sequence anomalies, trinucleotide repeat expansions, and other pathogenic changes affecting a single gene), chromosomal anomalies (caused by aneuploidies and copy number variants affecting more than a single gene), major anomalies, and minor anomalies (i.e., morphologic anomalies not among the features reviewed for the seven body regions, such as sacral dimple). The SEED protocol also included abstraction of child medical records from birth to age 3 years. These record abstractions (when available), were used to clarify and correct caregivers' descriptions of birth defects or syndromes.

Initial SEED enrollment targets specified that each site should enroll equivalent numbers of children in each group, ASD and POP, yet final sample sizes varied because of the aforementioned methodology to allow yet undiagnosed children to be classified as ASD cases and because of variable completion rates for various study components. Due to the labor-intensive aspects of dysmorphology review, and to ensure that SEED primary research questions could be addressed sufficiently, dysmorphology reviews were performed only on those children who had completed dysmorphology assessment and who had achieved study completion as defined in Bradley et al. (2018). Therefore, all NHW, NHB, and Hispanic ASD cases and POP controls who met the criteria for study completion underwent dysmorphology review.

Dysmorphology Data Analysis

Dysmorphology analyses were conducted separately for NHW, NHB, and Hispanic children; sample size limitations precluded further subdivision of Hispanic children into ancestry subgroups. The first step in dysmorphology data analysis was to determine the range of

Likert scores for each of the 397 features that corresponded to dysmorphic vs. normal variation (i.e., non-dysmorphic) in the POP group. For each feature, the children in the POP group were assigned to three categories according their Likert scores: ≥ 2, ≥ 3, and = 4. A feature was defined as dysmorphic if it occurred in ≤ 5% of the POP group. Each range of possible Likert scores (i.e., ≥ 2, ≥ 3, and = 4) was statistically evaluated to determine if it included ≤ 5% of the POP group. To achieve this, the following were calculated for each feature: the frequency of Likert scores ≥ 2, ≥ 3, and = 4, and the Bayesian shortest 95% confidence interval for each frequency.[2] Several examples are shown in Table 2. The largest range of Likert scores that included 5% or had an upper confidence limit ≤5%, was selected as the Likert score range that defined the feature as dysmorphic. Features that had Likert scores of only 0 (normal or absent) or 1 (possible or questionable) for all children in both POP and ASD groups within a race/ethnicity category were not informative to the analysis and were, thus, excluded. Additionally, a small number of features (1 for NHW and 3 for NHB children) were excluded as non-informative since the smallest frequency of Likert scores (i.e., = 4) had a lower confidence limit that was > 5%. After these exclusions, the total numbers of features available for analysis for children in the NHW, NHB, and Hispanic categories were 307, 284, and 276, respectively; features excluded from racial/ethnic-specific analyses are noted in Table 1.

A racial/ethnic-specific dysmorphology score was then calculated for each child. The dysmorphology score was defined as the number of dysmorphic features that a child had divided by the total number of features for which the child had received any Likert score, and then that fraction was multiplied by 100. The total number of features for which the child had received any Likert score was the difference between the total number of features available for analysis of children in the race/ethnicity category (e.g., 307 for NHW) and the number of features with missing data. For example, if a NHW child had five dysmorphic features and seven features with missing data, then the dysmorphology score would be calculated as $[5/(307 - 7)] \times 100 = 1.67$. Children who were missing data for more than 80 features were excluded from further analysis; after this exclusion, the highest number of features with missing data for a child was 80 for NHW, 50 for NHB, and 63 for Hispanic.

The distribution of dysmorphology scores that best described the children in the POP group in each race/ethnicity category was then identified by plotting histograms that displayed the distribution shapes and testing distribution adequacy with Kolmogorov–Smirnov and Anderson–Darling tests. The distribution shapes and the tests showed that the data from children in the POP group for each racial/ethnic category were an excellent fit for the log normal distribution (Kolmogorov–Smirnov test p value > 0.250; Anderson–Darling test p value > 0.250).

Finally, the expected values of the log normal distribution of dysmorphology scores were utilized to convert the dysmorphology score of each child in the POP group to a corresponding percentile (1st–99th percentile) of the log normal distribution. The racial/ethnic-specific log normal distributions of dysmorphology scores were similarly used to convert the dysmorphology scores of the corresponding racial/ethnic groups of children with

[2] https://www.causascientia.org/math_stat/ProportionCI.html.

ASD to percentiles. Categorized dysmorphology classifications for all children were specified for ranges of percentiles of the log normal distributions: Non-dysmorphic is ≤ 90th percentile; Equivocal is > 90th percentile and ≤ 95th percentile; and Dysmorphic is > 95th percentile. Child dysmorphology classifications are capitalized in this report (Dysmorphic, Equivocal, Non-dysmorphic) to differentiate them from how individual features are defined (dysmorphic, non-dysmorphic).

All analyses were conducted using SAS® 9.3 (SAS Institute Inc., Cary, NC). Categorical dysmorphology classifications were compared between POP and ASD, between racial/ethnic groups (NHW, NHB, and Hispanic), and between boys and girls by chi square test or Fisher's exact test. All statistical tests were two-sided, and p values < 0.05 were considered statistically significant.

Among all children receiving a dysmorphology score, approximately 74% were missing data on ≥ 1 features, so a sensitivity analysis was conducted to assess the impact of missing data on the observed results. First, the number of dysmorphic features corresponding to the 95th percentile of the dysmorphology score distributions for the POP groups (i.e., the number of dysmorphic features required to be assigned the categorical dysmorphology classification of Dysmorphic) was calculated for each race/ethnicity. For NHW, NHB, and Hispanic, these thresholds corresponded to 16, 16, and 18 features, respectively. When applying these thresholds, approximately 64% of children with missing data for ≥ 1 features could be assigned a definitive categorical dysmorphology classification of either Non-dysmorphic or Dysmorphic regardless of whether or not their features with missing data were dysmorphic or non-dysmorphic. The reduction in children with potentially uncertain dysmorphology classifications was possible because either (1) the number of dysmorphic features observed in the child was already greater than or equal to the threshold value so that the dysmorphology classification must be Dysmorphic, regardless of the number of features with missing data, or (2) the sum of the dysmorphic features observed in the child and the features with missing data was less than the threshold, so that the dysmorphology classification must be Non-dysmorphic. Multiple imputation by fully conditional specification (Rubin 1987; Little and Rubin 1987; Raghunathan et al. 2001; van Buuren 2007) was used to assess the effect of missing data in the remaining children (approximately 27% of the total sample) whose categorical classifications could change from Non-dysmorphic to Dysmorphic if the sum of dysmorphic features observed in the child and the features with missing data was greater than or equal to the threshold value. Categorical dysmorphology classification, site (CA, CO, GA, MD, NC, PA), sex (male, female), case–control status (ASD, POP), race/ethnicity (NHW, NHB, Hispanic) and Mullen score were included in the imputation model, and ten imputed datasets were created. Each imputed dataset was analyzed individually and results were then combined (Yuan 2000; Liu and De 2015).

Results

SEED ASD cases and POP controls included in the dysmorphology data analysis are described in Table 3. The male-to-female ratio of ASD cases was 4-to-1, similar to the prevalence ratio reported in population-based surveillance of ASD (Baio et al. 2018); the

male-to-female ratio of POP controls was not significantly different (p = 0.631) from the ratio reported in the 2010 U.S. population census for children aged < 10 years (Howden and Meyer 2011). The age at dysmorphology assessment was not significantly different between ASD cases and POP controls (p = 0.840). The mean age (cases and controls combined) at the time of dysmorphology assessment was approximately 5 years (mean, 61 months; range 34–84 months). Separate dysmorphology data analyses were conducted for NHW (185 POP; 310 ASD), NHB (96 POP; 117 ASD), and Hispanic (90 POP; 87 ASD) participants who had undergone dysmorphology review by the clinical geneticists. Since the dysmorphology scores of each racial/ethnic POP group were fit to the log normal distribution to develop the categorical child dysmorphology classifications of Non-dysmorphic, Equivocal, and Dysmorphic, this step defines each POP group as comprised of approximately 90% Non-dysmorphic, 5% Equivocal, and 5% Dysmorphic children. Overall, 4.6% of children in the POP group had scores > 95th percentile and, thus, classified as Dysmorphic. The proportion of Dysmorphic children was nearly four times greater for children with ASD; 17.1% had scores > 95th percentile (p < 0.001) (Table 4). Since each racial/ethnic POP group was fit to a log normal distribution and dysmorphology classifications were defined for each POP group as Non-dysmorphic (≤ 90th percentile), Equivocal (> 90th percentile and ≤ 95th percentile), and Dysmorphic (> 95th percentile), there was no statistically significant difference in the distributions of child dysmorphology classifications between the three racial/ethnic POP groups (p = 0.601) (Table 4). Interestingly, there was also no statistically significant difference in the distributions of child dysmorphology classifications between the three racial/ethnic ASD groups (p = 0.845) (Table 4).

Since the significant difference in the dysmorphology distributions between POP and ASD could be due to a higher prevalence of non-chromosomal genetic disorders and chromosomal anomalies—henceforth referred to as “genetic disorders” in this report—among children with ASD compared to children in the POP group, all children (both POP and ASD) with parent-reported genetic disorders were excluded, and the dysmorphology distributions re-evaluated. The numbers of children with ASD with reported genetic disorders were 8 (2.2%), 3 (4.5%) and 19 (21.6%) of those classified as Non-dysmorphic, Equivocal, and Dysmorphic, respectively. Among the children in the POP group, those with reported genetic disorders were 10 (3.0%), 1 (4.8%), and 1 (5.9%) for those classified as Non-dysmorphic, Equivocal, and Dysmorphic, respectively. Once excluding all children with reported genetic disorders, the difference between ASD and POP was slightly attenuated, but there was still a statistically significant difference between 4.5% of children in the POP group and 14.3% of children with ASD classified as Dysmorphic (p < 0.001) (Table 5). Similarly, the presence of major morphologic anomalies could be indicative of an unknown aberrant in utero genetic or teratogenic developmental process associated with an increased risk for ASD. Therefore, after exclusion of all children with genetic disorders, children (both POP and ASD) with major morphologic anomalies were additionally excluded, and the dysmorphology distributions re-evaluated. The difference between ASD and POP was slightly more attenuated, but there was still a statistically significant difference between approximately 4.3% of children in the POP group and 12.6% of children with ASD classified as Dysmorphic (p < 0.001) (Table 5). Finally, there was no statistically significant difference in the distributions of child dysmorphology classifications between males and

females with ASD, either before (p = 0.294) or after excluding all those with parent-reported genetic disorders (p = 0.517) (Table 6).

The sensitivity analyses for the effects of missing data on the association between case–control status and categorical dysmorphology classification showed that all of the combined p values were < 0.001 when varying the threshold number of dysmorphic features for the dysmorphology classification as Dysmorphic between − 4 below the race/ethnicity specified thresholds (i.e., 12, 12, and 14 for NHW, NHB, and Hispanic, respectively) to + 4 above the thresholds (i.e., 20, 20, and 22 for NHW, NHB, and Hispanic, respectively). Therefore, taking into account multiple scenarios under the missing at random assumption, the missing data for physical features did not affect the observed associations between case-control status and categorical dysmorphology classification.

Discussion

Our assessment of dysmorphology in a large diverse sample of children drawn from multiple clinical and education sources in select communities expands on previous studies that enrolled cases with ASD from a single clinical source. In this study, we were able to compare children with the broad ASD phenotype from three racial/ethnic groups to population controls drawn from the same communities, and thus contribute more generalizable information about the ASD phenotype than past studies. Our finding that approximately 17% of children with ASD were classified as Dysmorphic was close to the percentages in previous reports by Miles and Hillman (2000) (25.5%), Miles et al. (2005) (15.8%), Miles et al. (2008) (14.6%) and Angkustsiri et al. (2011) (17.4%), but somewhat higher than the prevalence reported by Wong et al. (2014) (10.8%) and Flor et al. (2017) (5.6%). We additionally found that the prevalence of children classified as Dysmorphic was comparable among NHW, NHB, and Hispanic children with ASD, and in all three racial/ethnic groups, children with ASD had a markedly higher chance than children in the general population control groups of being classified as Dysmorphic. The finding that there was little variation by race/ethnicity suggests that the group of genetic and environmental factors resulting in the co-occurrence of ASD and a preponderance of dysmorphic features is similar across the three racial/ethnic groups.

Overall, known genetic disorders accounted for part of the differences between cases and controls since these conditions were reported in 5.8% of SEED cases versus 3.2% of controls, although the difference did not reach significance (p = 0.072). The higher prevalence of these conditions among children with ASD was expected since ASD has a significant genetic basis, and dysmorphic features are prevalent among the numerous identified genetic disorders with an increased risk for ASD (e.g., Angelman syndrome, Down syndrome, fragile X syndrome, Phelan-McDermid syndrome, Rett syndrome, etc.). In our study, 21.6% of children with ASD classified as Dysmorphic had a known genetic disorder compared to only 2.6% of those in the combined Non-dysmorphic and Equivocal categories (p < 0.001). This finding is consistent with other studies. In the initial Miles and Hillman (2000) study, phenotypically abnormal individuals were ten times more likely to have a known genetic disorder than phenotypically normal individuals (21% vs. 2%). In the expanded Miles et al. (2005) study, no individuals with essential autism but 24% of

individuals with complex autism had a known genetic disorder or teratogenic exposure. Additionally, Tammimies et al. (2015) reported that 38% of children with complex autism had genetic conditions diagnosed by molecular testing compared to 6% of children with essential autism. We also found that part of the case-control differences in the prevalence of children classified as Dysmorphic was attributed to the higher proportion of major morphologic anomalies among children with ASD; this finding is possibly due to aberrant developmental processes resulting from unknown underlying genetic conditions or gestational exposures. After excluding those with known genetic disorders from cases and controls, major morphologic anomalies were increased among ASD cases compared to controls (18.2% vs. 8.9%, $p < 0.001$); 27.5% of children with ASD classified as Dysmorphic had one or more major morphologic anomalies compared to 16.6% of those in the combined Non-dysmorphic and Equivocal categories ($p = 0.029$). To our knowledge, no other study that assessed dysmorphic features among individuals with ASD examined the prevalence of major morphologic anomalies, independent from genetic disorders, among those categorized as Dysmorphic versus Non-dysmorphic.

Nonetheless, genetic disorders and major morphologic anomalies explained only part of the case–control differences that we observed. The prevalence of the Dysmorphic classification in a case subsample that excluded these conditions was still nearly three times the prevalence of the Dysmorphic classification among the subsample of the control group. However, we cannot rule out the possibility that some children in the Dysmorphic ASD group have an undiagnosed genetic disorder or teratogenic exposure.

One hypothesis to explain the higher prevalence of ASD among males is that females may require more genetic changes to manifest ASD. This is referred to as a multiple-threshold multifactorial liability model (Reich et al. 1975). In support of this model, studies have shown that females with ASD are often more severely affected than males, tending to have lower IQ scores and more frequent co-morbidities, such as epilepsy (Miles et al. 2005; Amiet et al. 2008; Eaves and Ho 2008). This higher “genetic load” required to manifest ASD might increase the risk for females with ASD to be Dysmorphic, compared to males. However, in our study, the proportion within each dysmorphology classification category did not differ significantly between males and females with ASD, although females with ASD were more likely than males to be Dysmorphic. However, once individuals with known genetic disorders were excluded, the female:male sex difference in those classified as Dysmorphic was attenuated. We did observe that females with ASD had a higher prevalence of known genetic disorders compared with males (12.5% vs. 4.3%, $p = 0.002$). The relatively low prevalence of ASD diagnosis among females compared to males (Werling and Geschwind 2013; Duvekot et al. 2017; Baio et al. 2018), combined with the fact that females identified with ASD are often more severely affected than males, suggests that females ultimately diagnosed with ASD may receive comprehensive genetics evaluations more often than males with ASD, thus identifying a higher proportion of females than males with ASD who have genetic disorders. Understanding the actual reasons behind this difference in the prevalence of genetic disorders will require further investigation.

This study has a number of strengths. In contrast to prior dysmorphology studies that relied only on populations of individuals with ASD to define dysmorphology (Miles and Hillman

2000; Miles et al. 2005, 2008; Wong et al. 2014), the dysmorphology approach that we developed and implemented for the SEED sample used population control groups without ASD to define “dysmorphic” for each feature in a quantitative fashion. This approach forced adherence to the definition of dysmorphic in spite of the challenges for even experienced clinical geneticists to decide when a descriptive trait is no longer normal variation in the population but is actually dysmorphic. Second, the use of a Likert scale to describe each feature (absent or normal, mild, moderate, severe) allowed for post-dysmorphology review determination of the dysmorphic status of each feature and accommodated potential differences between perceptions of clinical geneticists (i.e., over-calling or under-calling a feature as dysmorphic). Prior dysmorphology studies did not account for the variation of descriptive features, but merely selected an arbitrary impression by the examiner to define “yes, dysmorphic” versus “no, non-dysmorphic” for various features (Miles and Hillman 2000; Miles et al. 2005, 2008). Third, in contrast to other studies of dysmorphology (Miles and Hillman 2000; Miles et al. 2005, 2008 ; Wong et al. 2014), the clinical geneticists participating in SEED were not present at the dysmorphology assessment, but instead reviewed a standard set of photographs and measurement data on each child to perform dysmorphology reviews; thus, they were blinded to final classification (case vs. control), as well as to severity among those with ASD, which reduced potential dysmorphology bias. Fourth, our SEED dysmorphology approach afforded the opportunity to evaluate dysmorphic features in three racial/ethnic groups, taking potential racial/ethnic differences into account. Although the Wong et al. (2014) study focused exclusively on Chinese individuals, none of the other studies of dysmorphology had racial/ethnic-specific data. Fifth, in contrast to previous studies of dysmorphology in ASD (Miles and Hillman 2000 ; Miles et al. 2005, 2008; Wong et al. 2014), which were all clinic-or hospital-based, SEED utilized a community-based ascertainment approach to identify a broader representation of the ASD population. Finally, SEED included participants within a narrow age range compared to age ranges of participants in other studies. Restricting participation to a narrow age range can minimize the variation that might occur as physical features and the extent of dysmorphology change with growth of the child.

This study also has several limitations. First, SEED was designed as a population-based study, but many families identified for possible inclusion could not be located and/or contacted. However, these families likely had a higher probability of being ineligible for participation, given our inclusion criteria that families have residence (both at birth and at the time of study contact) within the defined geographic area of one of the study sites, and that caregivers be able to communicate in English (four sites) or English or Spanish (two sites). Although out-migration from the geographical areas of the study sites is a potential source of bias, the large populations of diverse racial, ethnic, and socioeconomic characteristics within the study sites and each having the advantage of close proximity to major medical centers offering a broad array of services, makes it unlikely that out-migration introduced bias in relation to the presence of dysmorphic features or genetic disorders in the enrolled children. Second, the SEED dysmorphology assessment did not include a comprehensive, systematic genetic evaluation, so SEED participants had significant variation in prior genetic testing and evaluation, from none to quite extensive. Therefore, the prevalence of reported genetic disorders among SEED participants with ASD

(5.8%) and POP controls (3.2%) are likely underestimates of the true prevalence of genetic disorders in these groups. Third, although children enrolled in the ASD and POP groups were not matched for any characteristic, either one-on-one or by frequency, the SEED sample included (1) male-to-female ratios of primarily preschoolage children that matched the ratios within the underlying populations of children with ASD and children in the general population and (2) children within a relatively narrow age range of 34–84 months at the time of dysmorphology assessment whose age distributions were not significantly different between ASD cases and POP controls. Moreover, the SEED sample allowed for stratified analyses by both sex and race/ethnicity, which as previously described, is a notable strength compared to past studies. Nonetheless, as with many epidemiologic comparisons, the potential for unmeasured confounding cannot be entirely dismissed. Fourth, in light of the young age of children participating in the study, and because milder forms of ASD might not be recognized until later in childhood or even into adolescence and adulthood, those at the milder end of the spectrum, who may in fact have a different risk for being Dysmorphic, might not be as well represented in the study. Therefore, the results of our study should not be generalized to older children and adults. Finally, some physical features were a challenge for the clinical geneticists to review from photographs, particularly when there was a blurred image, suboptimal lighting, inadequate zoom, a non-standard camera angle, lack of one or more standard photographs (e.g., no open mouth photo to evaluate the teeth), or an obscured image (e.g., hair covering parts of the ear). Therefore, some physical features and some participants (if multiple photographs had issues) had more missing data than others. Although missing data had the potential to affect the validity of the dysmorphology results, our sensitivity analyses and multiple imputations showed no significant effect of missing data on the observed results. In addition, although in-person dysmorphology assessments might have resulted in less missing data, they run the high risk of dysmorphology bias based on knowing the final classification status.

Conclusion

Within the SEED, a novel approach was developed for systematic comparison of dysmorphic features between children with ASD and population controls without ASD. Approximately 17% of children with ASD had a significantly higher number of dysmorphic features and, hence, a classification as Dysmorphic, compared with approximately 5% of population controls. Findings were similar across NHW, NHB, and Hispanic racial/ethnic groups and between males and females. Differences in the proportions classified as Dysmorphic between children with ASD and population controls were due in part, but not entirely accounted for, by known conditions typically associated with dysmorphic features (genetic disorders and major morphologic anomalies). This is the first dysmorphology study among children with ASD in a diverse U.S. population that includes comprehensive, systematic comparisons to racial/ethnic-specific population control groups without ASD. Future analyses of the SEED sample that stratify ASD cases as Dysmorphic versus Non-dysmorphic might help uncover etiologic risk factors for ASD.

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References

Aase JM (1990). The physical examination in dysmorphology. In M Aase J (Ed.), Diagnostic dysmorphology (pp. 33–42). New York: Plenum Medical Book Co.

Abrahams BS, & Geschwind DH (2008). Advances in autism genetics: On the threshold of a new neurobiology. Nature Reviews Genetics, 9(5), 341–355.

Allanson JE (1989). Time and natural history: The changing face. Journal of Craniofacial Genetics and Developmental Biology, 9(1), 21–28. [PubMed: 2793999]

Allanson JE, Cunniff C, Hoyme HE, McGaughran J, Muenke M, & Neri G (2009). Elements of morphology: Standard terminology for the head and face. American Journal of Medical Genetics Part A, 149A(1), 6–28. [PubMed: 19125436]

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). Arlington: American Psychiatric Publishing.

Amiet C, Gourfinkel-An I, Bouzamondo A, Tordjman S, Baulac M, Lechat P, … Cohen D (2008). Epilepsy in autism is associated with intellectual disability and gender: Evidence from a meta-analysis. Biological Psychiatry, 64, 577–582. [PubMed: 18565495]

Angkustsiri K, Krakowiak P, Moghaddam B, Wardinsky T, Gardner J, Kalamkarian N, … Hansen RL (2011). Minor physical anomalies in children with autism spectrum disorders. Autism, 15(6), 746–760. [PubMed: 21610186]

Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, … Dowling NF (2018). Prevalence of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network. Morbidity and Mortality Weekly Report Surveillance Summaries, 67(6), 1–23.

Biesecker LG, Aase JM, Clericuzio C, Gurrieri F, Temple IK, & Toriello H (2009). Elements of morphology: Standard terminology for the hands and feet. American Journal of Medical Genetics Part A, 149A(1), 93–127. [PubMed: 19125433]

Bill BR, & Geschwind DH (2009). Genetic advances in autism: Heterogeneity and convergence on shared pathways. Current Opinion in Genetics & Development, 19(3), 271–278. [PubMed: 19477629]

Braddock SR, Henley KM, & Maria BL (2007). The face of Joubert syndrome: A study of dysmorphology and anthropometry. American Journal of Medical Genetics Part A, 143A(24), 3235–3242. [PubMed: 18000967]

Bradley CB, Browne EN, Alexander AA, Collins J, Dahm JL, DiGuiseppi CG, … Daniels JL (2018). Demographic and operational factors predicting study completion in a multisite case-control study of preschool children. American Journal of Epidemiology, 187(3), 592–603. [PubMed: 29506195]

Bushby KM, Cole T, Matthews JN, & Goodship JA (1992). Centiles for adult head circumference. Archives of Disease in Childhood, 67(10), 1286–1287. [PubMed: 1444530]

Campbell M, Geller B, Small AM, Petti TA, & Ferris SH (1978). Minor physical anomalies in young psychotic children. American Journal of Psychiatry, 135(5), 573–575. [PubMed: 645950]

Carey JC, Cohen MM Jr., Curry CJR, Devriendt K, Holmes LB, & Verloes A (2009). Elements of morphology: Standard terminology for the lips, mouth, and oral region. American Journal of Medical Genetics Part A, 149A(1), 77–92. [PubMed: 19125428]

Centers for Disease Control and Prevention. (2008). Update on overall prevalence of major birth defects—Atlanta, Georgia, 1978–2005. Morbidity and Mortality Weekly Report, 57(1), 1–5. [PubMed: 18185492]

Cole TR, & Hughes HE (1994). Sotos syndrome: A study of the diagnostic criteria and natural history. Journal of Medical Genetics, 31(1), 20–32. [PubMed: 7512144]

Cung W, Freedman LA, Khan NE, Romberg E, Gardner PJ, Bassim CW, … Stewart DR (2015). Cephalometry in adults and children with neurofibromatosis type 1: Implications for the pathogenesis of sphenoid wing dysplasia and the “NF1 facies”. European Journal of Medical Genetics, 58(11), 584–590. [PubMed: 26360873]

DiGuiseppi CG, Daniels JL, Fallin DM, Rosenberg SA, Schieve LA, Thomas KC, … Schendel DE (2016). Demographic profile of families and children in the Study to Explore Early Development (SEED): Case-control study of autism spectrum disorder. Disability and Health Journal, 9(3), 544–551. [PubMed: 26917104]

Duvekot J, van der Ende J, Verhulst FC, Slappendel G, van Daalen E, Maras A, & Greaves-Lord K (2017). Factors influencing the probability of a diagnosis of autism spectrum disorder in girls versus boys. Autism, 21(6), 646–658. [PubMed: 27940569]

Eaves LC, & Ho HH (2008). Young adult outcome of autism spectrum disorders. Journal of Autism and Developmental Disorders, 38, 739–747. [PubMed: 17764027]

Egbe AC (2015). Birth defects in the newborn population: Race and ethnicity. Pediatric Neonatology, 56(3), 183–188.

Feingold M, & Bossert WH (1974). Normal values for selected physical parameters: An aid to syndrome delineation. Birth Defects: Original Articles Series, 10(13), 1–16.

Flor J, Bellando J, Lopez M, & Shul A (2017). Developmental functioning and medical co-morbidity profile of children with complex and essential autism. Autism Research, 10(8), 1344–1352. [PubMed: 28474389]

Goldfarb W, & Botstein A (1956). Physical stigmata in schizophrenic children. Unpublished manuscript. Brooklyn, NY: Henry Ittleson Center for Child Research.

Gualtieri CT, Adams A, Shen CD, & Loiselle D (1982). Minor physical anomalies in alcoholic and schizophrenic adults with hyperactive and autistic children. American Journal of Psychiatry, 139(5), 640–643. [PubMed: 7072852]

Hall BD, Graham JM Jr., Cassidy SB, & Opitz JM (2009). Elements of morphology: Standard terminology for the periorbital region. American Journal of Medical Genetics Part A, 149A(1), 29–39. [PubMed: 19125427]

Hennekam RC, Biesecker LG, Allanson LG, Hall JG, Opitz JM, Temple IK … Elements of Morphology Consortium. (2013). Elements of morphology: General terms for congenital anomalies. American Journal of Medical Genetics Part A 161A(11), 2726–2733. [PubMed: 24124000]

Hennekam RC, Cormier-Daire V, Hall JG, Mèhes K, Patton M, & Stevenson RE (2009). Elements of morphology: Standard terminology for the nose and philtrum. American Journal of Medical Genetics Part A, 149A(1), 61–76. [PubMed: 19152422]

Hook EB (1971). Some general considerations concerning monitoring: Application to utility of minor defects as markers. In Hook. EB Janerich. DT Porter IH (Eds.), Monitoring, birth defects, and environment (pp. 177–197). New York: Academic Press.

Howden LM, & Meyer JA (2011). Age and sex composition: 2010. 2010 census briefs Washington, DC: U.S. Department of Commerce, Economics and Statistics Administration, U.S. Census Bureau.

Hunter A, Frias JL, Gillesen-Kaesbach G, Hughes H, Jones KL, & Wilson L (2009). Elements of morphology: Standard terminology for the ear. American Journal of Medical Genetics Part A, 149A(1), 40–60. [PubMed: 19152421]

Karimi P, Kamali E, Mousavi SM, & Karahmadi M (2017). Environmental factors influencing the risk of autism. Journal of Research in Medical Sciences, 22, 27. [PubMed: 28413424]

Leppig KA, Werler MM, Cann CI, Cook CA, & Holmes LB (1987). Predictive value of minor anomalies. I. Association with major malformations. Journal of Pediatrics, 110(4), 531–537. [PubMed: 3559800]

Links PS (1980). Minor physical anomalies in childhood autism. Part II. Their relationship to maternal age. Journal of Autism and Developmental Disorders, 10(3), 287–292. [PubMed: 6927655]

Links PS, Stockwell M, Abichandani F, & Simeon J (1980). Minor physical anomalies in childhood autism. Part I. Their relationship to pre-and perinatal complications. Journal of Autism and Developmental Disorders, 10(3), 273–285. [PubMed: 6927654]

Little RJA, & Rubin DB (1987). Statistical analysis with missing data (p. 278). New York: Wiley.

Liu Y, & De A (2015). Multiple imputation by fully conditional specification for dealing with missing data in a large epidemiologic study. International Journal of Statistics in Medical Research, 4(3), 287–295. [PubMed: 27429686]

Marden PM, Smith DW, & McDonald MJ (1964). Congenital anomalies in the newborn infant, including minor variations. Journal of Pediatrics, 64, 357–371. [PubMed: 14130709]

Méhes K (1983). General characterization of minor malformations: Epidemiology in the newborn populations. In Méhes K (Ed.), Minor malformations in the neonate (pp. 17–20). Budapest: Akadémiai Kiadó.

Merks JH, van Karnebeek CD, Caron HN, & Hennekam RC (2003). Phenotypic abnormalities: Terminology and classification. American Journal of Medical Genetics Part A, 123A(3), 211–230. [PubMed: 14608641]

Merlob P, Papier CM, Klingberg MA, & Reisner SH (1985). Incidence of congenital malformations in the newborn, particularly minor abnormalities. Progress in Clinical and Biological Research, 163C, 51–55. [PubMed: 3991654]

Miles JH, & Hillman RE (2000). Value of a clinical morphology examination in autism. American Journal of Medical Genetics, 91(4), 245–253. [PubMed: 10766977]

Miles JH, Takahashi TN, Bagby S, Sahota PK, Vaslow DF, Wang RE, … Farmer JE (2005). Essential versus complex autism: Definition of fundamental prognostic subtypes. American Journal of Medical Genetics Part A, 135A(2), 171–180.

Miles JH, Takahashi TN, Hong J, Munden N, Flournoy N, Braddock SR, … Farmer JE (2008). Development and validation of a measure of dysmorphology: Useful for autism subgroup classification. American Journal of Medical Genetics Part A, 146A(9), 1101–1116. [PubMed: 18383511]

Miller MT, Strömland K, Ventura L, Johansson M, Bandim JM, & Gillberg C (2005). Autism associated with conditions characterized by developmental errors in early embryogenesis: A mini review. International Journal of Developmental Neuroscience, 23(2–3), 201–219. [PubMed: 15749246]

Mnukhin SS, & Isaev DN (1975). On the organic nature of some forms of schizoid or autistic psychopathy. Journal of Autism and Childhood Schizophrenia, 5(2), 99–108. [PubMed: 1174123]

Muhle R, Trentacoste SV, & Rapin I (2004). The genetics of autism. Pediatrics, 113(5), e472–e486. [PubMed: 15121991]

Raghunathan TE, Lepkowski JM, Van Hoewyk J, & Solenberger P (2001). A multivariate technique for multiply imputing missing values using a sequence of regression models. Survey Methodology, 27(1), 85–95.

Reich R, Cloninger CR, & Guze SB (1975). The multifactorial model of disease transmission: I. Description of the model and its use in psychiatry. British Journal of Psychiatry, 127, 1–10. [PubMed: 1139078]

Robert C, Pasquier L, Cohen D, Fradin M, Canitano R, Damaj L, … Tordjman S (2017). Role of genetics in the etiology of autistic spectrum disorder: Towards a hierarchical diagnostic strategy. International Journal of Molecular Sciences 18(3), e618. [PubMed: 28287497]

Ronald A, & Hoekstra RA (2011). Autism spectrum disorders and autistic traits: A decade of new twin studies. American Journal of Medical Genetics Part B, Neuropsychiatric Genetics, 156B(3), 255–274.

Rosti RO, Sadek AA, Vaux KK, & Gleeson JG (2014). The genetic landscape of autism spectrum disorders. Developmental Medicine & Child Neurology, 56(1), 12–18. [PubMed: 24116704]

Rubin DB (1987). Multiple imputation for nonresponse in surveys (p. 258). New York: Wiley.

Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, & Reichenberg A (2014). The familial risk of autism. Journal of the American Medical Association, 311(17), 1770–1777. [PubMed: 24794370]

Schendel DE, Diguiseppi C, Croen LA, Fallin MD, Reed PL, Schieve LA, … Yeargin-Allsopp M (2012). The Study to Explore Early Development (SEED): A multisite epidemiologic study of autism by the Centers for Autism and Developmental Disabilities Research and Epidemiology (CADDRE) network. Journal of Autism and Developmental Disorders, 42(10), 2121–2140. [PubMed: 22350336]

Steg JP, & Rapoport JL (1975). Minor physical anomalies in normal, neurotic, learning disabled, and severely disturbed children. Journal of Autism and Childhood Schizophrenia, 5(4), 299–307. [PubMed: 1243135]

Stoelb M, Yarnal R, Miles J, Takahashi TN, Farmer JE, & McCathren RB (2004). Predicting responsiveness to treatment of children with autism: A retrospective study of the importance of physical dysmorphology. Focus on Autism and Other Developmental Disabilities, 19(2), 66–77.

Tammimies K, Marshall CR, Walker S, Kaur G, Thiruvahindrapuram B, & Lionel AC (2015). Molecular diagnostic yield of chromosomal microarray analysis and whole-exome sequencing in children with autism spectrum disorder. Journal of the American Medical Association, 314(9), 895–903. [PubMed: 26325558]

Thomas IT, Gaitantzis YA, & Frias JL (1987). Palpebral fissure length from 29 weeks gestation to 14 years. Journal of Pediatrics, 111(2), 267–268. [PubMed: 3612403]

Van Buuren S (2007). Multiple imputation of discrete and continuous data by fully conditional specification. Statistical Methods in Medical Research, 16(3), 219–242. [PubMed: 17621469]

Waldrop MF, Pederson FA, & Bell RQ (1968). Minor physical anomalies and behavior in preschool children. Child Development, 39(2), 391–400. [PubMed: 4172079]

Walker HA (1977). Incidence of minor physical anomaly in autism. Journal of Autism and Childhood Schizophrenia, 7(4), 165–176. [PubMed: 194879]

Werling DM, & Geschwind DH (2013). Understanding sex bias in autism spectrum disorder. Proceedings of the National Academy of Sciences of the United States of America, 110(13), 4868–4869. [PubMed: 23476067]

Wong VCN, Fung CKY, & Wong PTY (2014). Use of dysmorphology for subgroup classification on autism spectrum disorder in Chinese children. Journal of Autism and Developmental Disorders, 44(1), 9–18. [PubMed: 23666520]

Yuan YC (2000). Multiple imputation for missing data: Concepts and new developments, SAS technical report no. P267–25 (p. 11). Rockville, MD: SAS Institute, Inc.

Zachariah SM, Oomen SP, Padankatti CS, Grace H, & Glory L (2017). Dysmorphism in non-syndromic autism: A cross-sectional study. Indian Pediatrics, 54(7), 560–562. [PubMed: 28159942]

Zankl A, & Molinari L (2003). Abase—A tool for the rapid assessment of anthropometric measurements on handheld computers. American Journal of Medical Genetics Part A, 121A(2), 146–150. [PubMed: 12910494]

Table 1

Features included in dysmorphology reviews, classified as either descriptive trait (d), major morphologic anomaly (M), major morphologic anomaly with variability (Mv), measurement abnormality (s), minor anomaly (m), or minor anomaly with variability (mv)

Ears				
Ear length and position				
Long ear (s)	Short ear (s)	Low-set ear (mv)	Posteriorly-rotated ear (s)[b]	Anteriorly-rotated ear (s)[b,c]
Ear structure				
Anotia (M)[a,b,c]	Microtia, 1st degree (M)[a,b,c]	Microtia, 2nd degree (M)[a,b,c]	Microtia, 3rd degree (M)[a,b,c]	Shell ear (m)
Cupped ear (mv)[b,c]	Crumpled ear (m)[a,b,c]	Cryptotia (mv)[a,c]	Lop ear (mv)[a,b,c]	Satyr ear (mv)[a,c]
Protruding ear (d)				
Antihelix				
Antihelical shelf (m)	Absent antihelix (m)[a,b,c]	Hypoplastic antihelical stem (d)	Additional antihelical crus (m)	Broad antihelical stem (d)
Serpiginous stem of antihelix (mv)	Inferior antihelical stem extension (mv)	Cleft antihelical stem (m)[a,c]	Prominent inferior crus (d)	Hypoplastic inferior crus (d)
Absent inferior crus (m)[a,b,c]	Hypoplastic superior crus (d)	Absent superior crus (m)		
Antitragus				
Absent antitragus (m)	Hypoplastic antitragus (d)[b]	Prominent antitragus (d)[c]	Narrow antitragus (d)	Broad antitragus (d)
Bifid antitragus (mv)	Antitragal dimple (mv)[c]			
Crus helix				
Absent crus helix (m)[b,c]	Hypoplastic crus helix (d)	Prominent crus helix (d)	Crus helix connection to antihelix (mv)	Horizontal crus helix (m)
Serpiginous crus helix (m)	Crimped crus helix (mv)			
Concha				
Additional conchal fold (mv)	Hypoplasia of cavum concha (d)			
Helix				
Cleft helix (m)[a,b,c]	Crimped helix (mv)	Darwinian notch (mv)	Darwinian tubercle (mv)	Discontinuous ascending helical root (m)[a,b,c]
Squared superior helix (mv)	Overfolded helix (d)	Localized overfolding of helix (mv)	Minimal helical folding (d)[c]	Localized underfolding of helix (mv)
Cauda helicis tubercle (mv)[c]	Question mark ear (m)[a,c]			
Ear Lobe				

Ears				
Large ear lobe (s)	Small ear lobe (s)	Absent lobulus (m)[b]	Elongated ear lobe (s)	Shortened ear lobe (s)
Fluted ear lobe (mv)	V-shaped ear lobe (mv)	Fleshy ear lobe (mv)	Extension of helix onto ear lobe (mv)	Attached ear lobe (mv)
Ear lobe crease (mv)	Cleft ear lobe (mv)	Ear lobe nodule (mv)	Uplifted ear lobe (d)	Forward-facing ear lobe (d)
Incisura				
Absent incisura (m)	Protruding incisura (mv)	Open incisura (mv)	V-shaped incisura (mv)	Inverted V-shaped incisura (mv)
Narrow incisura width (d)	Wide incisura width (d)			
Tragus				
Absent tragus (m)[b]	Hypoplastic tragus (d)	Prominent tragus (d)	Bifid tragus (mv)[a,b]	
Ear pit/tag				
Posterior helical ear pit (mv)[a,b,c]	Auricular pit (mv)	Preauricular pit (mv)	Pretragal ectopia (mv)[a,b,c]	Preauricular tag (mv)
Auricular tag (mv)[a,b,c]	Postauricular tag (mv)[a,b,c]			
Eyes and eyebrows				
Eye measurements				
Telecanthus (s)	Decreased inner canthal distance (s)	Widely spaced eyes (hypertelorism) (s)	Closely spaced eyes (hypotelorism) (s)	Long palpebral fissure (s)
Short palpebral fissure (s)				
Eyelid				
Blepharophimosis (mv)[a,b,c]	Ectropion (d)	Ptosis (mv)	Ablepharon (M)[a,b,c]	Ankyloblepharon (Mv)[a,b,c]
Blepharochalasis (mv)[a,b,c]	Entropion (d)[c]	Epiblepharon (m)[a,b,c]	Hooding (partial epiblepharon) (mv)	Fullness upper eyelid (mv)
Cleft (mv)[c]	Infra-orbital crease (mv)	Infra-orbital fold (mv)[c]	Coloboma (Mv)[a,b,c]	
Palpebral fissure				
Downslanted palpebral fissure (mv)	Upslanted palpebral fissure (mv)	Cryptophthalmos (M)[a,b,c]	Almond shaped palpebral fissure (mv)[a,b,c]	
Canthus				
Epicanthus (mv)	Epicanthus inversus (mv)[a,c]			
Eye structure and position				
Small-appearing eye (d)	Large-appearing eye (d)[b,c]	Proptosis (d)[b]	Deeply set eye (d)	Scleral show (wide eye) (mv)[c]

Ears				
Esotropia (mv)	Exotropia (mv)	Epibulbar dermoid (Mv)[a,b,c]		
Eyebrow				
Synophrys (mv)	Prominent supraorbital ridge (d)	Underdeveloped supraorbital ridge (d)[b,c]	Thick (d)	Split (mv)
Wraparound (d)	Medial flare (d)	Central flare (d)	Lateral flare (d)	Broad (d)[a,b]
Highly arched (d)	Horizontal (m)[b,c]	Sparse (whole eyebrow) (d)	Sparse (partial eyebrow) (d)	Sculpted (d)
Eye lashes				
Absent (m)[a,b,c]	Long (d)[a,b,c]	Prominent (d)	Sparse (d)[a,b]	
Iris				
Brushfield spot (mv)[a,b,c]	Stellate iris (mv)[a,b,c]	Lisch nodule (mv)[a,b,c]	Colobomata (Mv)[a,b,c]	Lester's sign (cloverleaf iris) (mv)[a,b,c]
Cataract (Mv)[b,c]	Heterochromia (mv)[a,b,c]			
Tear duct				
Lacrimal punctum, absence (M)[a,b,c]	Lacrimal punctum, ectopic (mv)[a,b,c]			
Growth and skin				
Growth parameters				
Tall stature (s)	Short stature (s)	Overweight (s)	Underweight (s)	Macrocephaly (s)
Microcephaly (s)	Elevated BMI (s)[b]	Low BMI (s)		
Skin				
Café-au-lait spot (mv)	Hypopigmented macule (mv)	Hyperpigmented macule (mv)	Hypopigmentation following lines of Blashko (mv)	Hyperpigmentation following lines of Blashko (mv)[c]
Neveus flammeus (mv)[b]	Excessive facial hair (mv)[a,b,c]	Generalized hypertrichosis (mv)[b,c]		
Hands and feet				
Hand and feet measurements				
Long hand (s)	Long palm (s)[b]	Long middle finger (s)	Short hand (s)	Short palm (s)
Short middle finger (s)	2nd finger longer than 3rd finger (s)[b]	Decreased 2nd to 4th finger ratio (s)	Long foot (s)	Short foot (s)
Hand and palm				
Absent hand (M)[a,b,c]	Split hand (M)[a,b,c]	Trident hand (d)[a,b,c]	Abnormal palm (d)	Abnormal crease (mv)

Ears				
Single transverse crease (mv)				
Hands and feet				
Thumb				
A bsent thumb (M)[a,b,c]	Hypoplastic or truncated thumb (Mv)[b,c]	Short-appearing thumb (d)	Long-appearing thumb (d)	Broad thumb (d)
Triphalangeal thumb (mv)[b]	Preaxial thumb polydactyly (Mv)[b,c]	Bifid thumb (Mv)[a,b,c]	Small thenar eminence (d)[a,b,c]	Adducted thumb (mv)[a,b,c]
Proximally-placed thumb (mv)	Clubbed thumb (d)[b,c]			
Finger				
Absent finger (M)[a,b,c]	Hypoplastic or truncated finger (Mv)[a,b,c]	Short-appearing finger (d)	Long-appearing finger (d)	Slender finger (d)[c]
Tapered finger (d)	Broad finger (d)	Finger clinodactyly (mv)	Finger camptodactyly (mv)	Finger clubbing (d)[c]
Overlapping fingers (mv)[b,c]	Cutaneous finger syndactyly (mv)	Postaxial polydactyly (finger) (Mv)		
Finger nail				
Absent finger nail (M)[a,b,c]	Small finger nail (d)	Dysplastic finger nail (mv)[c]	Narrow finger nail (d)[a,b,c]	
Foot and position				
Absent foot (M)[a,b,c]	Split foot (M)[a,c]	Wide foot (d)	Pes planus (d)	Rocker bottom foot (mv)[a,c]
Metatarsus adductus (Mv)[a]	Varus foot (Mv)	Sandle gap (mv)	Plantar crease (mv)[a,b]	
Great toe				
Absent great toe (M)[a,b,c]	Hypoplastic or truncated great toe (Mv)[a,b,c]	Short-appearing great toe (d)	Broad great toe (d)	Long-appearing great toe (d)
Preaxial toe polydactyly $_{(Mv)}$[a,b,c]	Bifid great toe (Mv)[a,b,c]	Great toe and 2nd toe overlap (mv)		
2nd–5th toe				
Absent toe (M)[a,b,c]	2nd toe as long/longer than great toe (mv)	2nd and 3rd toes as long/longer than great toe (mv)	3rd toe longer than 2nd toe (mv)	Hallux valgus (mv)
Overlapping toes (mv)	Cutaneous toe syndactyly (mv)	Postaxial polydactyly (toe) (Mv)[a,b,c]	Proximally displaced 4th toe (mv)[a]	Proximally displaced 5th toe (mv)
Hypoplastic or truncated toe (Mv)[b,c]	Short-appearing toe (d)	Long-appearing toe (d)	Slender toe (d)[c]	Broad toe (d)
Toe clinodactyly (mv)	Toe camptodactyly (mv)			
Toe nail				
Absent toe nail (M)[b,c]	Small toe nail (d)[b]	Dysplastic toe nail (mv)	Narrow toe nail (d)[a,b,c]	

Ears				
Head, hair, face, and neck				
Head shape				
Brachycephaly (d)	Dolichocephaly (d)	Flat occiput (d)	Prominent occiput (d)	Plagiocephaly/asymmetric skull (mv)
Trigonocephaly (mv)[a,c]	Turricephaly (d)	Cloverleaf skull (Mv)[a,b,c]		
Forehead				
Broad forehead (d)	Narrow forehead (d)	Prominent forehead (d)	Sloping forehead (mv)	Vertical forehead crease (mv)[b]
Frontal bossing (mv)[b]	Depressed glabella (mv)[c]	Metopic depression (mv)[a,b,c]	Prominent metopic ridge (mv)	
Hair				
High anterior hairline (d)	Temporal balding (d)[c]	Low anterior hairline (d)	Widow's peak (mv)	Frontal upsweep (mv)
> 2 hair whorls (m)[a,b,c]	Abnormally positioned hair whorl (d)	Low posterior hairline (d)	Sparse scalp hair (d)	
Face				
Asymmetric face R < L (d)[b]	Asymmetric face R > L (d)	Broad face (d)	Coarse face (d)	Flat face (mv)
Long face (d)	Narrow face (d)	Round face (d)	Short face (d)[b]	Square face (mv)
Triangular face (mv)				
Maxilla				
Prominent cheekbone (d)[a,b,c]	Underdeveloped cheek bone (d)[a,b,c]	Full cheek (d)	Sunken cheek (mv)[c]	Malar flattening (d)
Malar prominence (d)[c]	Prominent nasolabial fold (d)[a,b,c]	Underdeveloped nasolabial fold (d)	Midface protrusion (d)	Midface retrusion (d)
Premaxillary prominence (d)	Premaxillary underdevelopment (d)[b,c]			
Mandible				
Broad jaw (d)[a,b,c]	Narrow jaw (d)[a,b,c]	Cleft mandible (M)[a,b,c]	Micrognathia (d)	Retrognathia (mv)
Prognathism (mv)[c]				
Chin				
Broad chin (d)[b,c]	Chin dimple (mv)[b]	Horizontal chin crease (mv)	H-shaped chin crease (mv)[a,b,c]	Vertical chin crease (mv)[b,c]
Pointed chin (mv)	Short chin (d)	Tall chin (d)		
Neck				
Broad neck (d)[a]	Long neck (d)[b]	Short neck (d)[a,b,c]	Neck webbing (mv)[a,c]	Redundant nuchal skin (mv)[a,b,c]
Mouth, lips, and teeth				

Ears				
Mouth measurements and appearance				
Wide mouth (s)	Narrow mouth (s)	Upturned corners of mouth (mv)	Downturned corners of mouth (mv)	Perioral hyperpigmentation (mv)
Upper lip				
Cleft lip (Mv)[a,b]	Thin or thick upper vermillion (d)	Everted upper vermillion (d)	Tented upper vermillion (mv)	Absent Cupid's bow (m)
Exaggerated Cupid's bow (d)				
Lower lip				
Thin or thick lower vermillion (d)	Everted lower vermillion (d)	Lip freckling (mv)		
Gums and teeth				
Gum hypertrophy (d)	Open bite (mv)	Supernumerary tooth (mv)[c]	Missing tooth (mv)	Dental crowding/overlap ping (mv)
Diastemia (d)	Widely-spaced teeth (d)	Peg-shaped tooth (mv)	Dysplastic tooth (mv)	Yellowed tooth (mv)
Brown tooth (mv)	Mottled tooth (mv)			
Nose and philtrum				
Nose				
Short nose (d)	Long nose (d)	Narrow nose (d)	Broad/wide nose (d)	Bulbous nose (d)
Prominent nose (s)	Bifid nose (Mv)[b,c]	Proboscis (M)[a,b,c]		
Nasal bridge				
Narrow nasal bridge (d)	Broad/wide nasal bridge (d)	Depressed nasal bridge (d)	Prominent nasal bridge (d)	Absent nasal bridge cartilage (M)[a,b,c]
Nasal ridge				
Narrow nasal ridge (d)	Broad/wide nasal ridge (d)	Depressed nasal ridge (d)	Prominent nasal ridge (d)	Concave nasal ridge (mv)
Convex nasal ridge (mv)[c]				
Nasal tip				
Narrow nasal tip (d)	Broad/wide nasal tip (d)	Depressed nasal tip (d)	Prominent nasal tip (d)	Overhanging nasal tip (mv)[b]
Deviated nasal tip (mv)[a,b,c]	Bifid nasal tip (mv)[b]			
Nasal base				
Narrow nasal base (d)	Broad/wide nasal base (d)			
Columella				
Short columella (d)	Broad/wide columella (d)	Low columella insertion (mv)	High columella insertion (mv)	Low hanging columella (mv)

Ears				
Ala nasum				
Thick ala nasum (d)	Underdeveloped ala nasum (d)	Cleft ala nasum (mv)[c]		
Naris and paranasal tissue				
Narrow naris (d)[c]	Enlarged naris (d)	Supernumerary naris (Mv)[a,b,c]	Anteverted nares (mv)	Single naris (M)[a,b,c]
Fullness of paranasal tissue (d)[a]				
Philtrum measurement				
Long philtrum (s)	Short philtrum (s)			
Philtrum				
Broad/wide philtrum (d)	Narrow philtrum (d)	Deep philtrum (d)	Smooth philtrum (d)	Tented philtrum (mv)
Malaligned philtral ridges (mv)	Midline raphe (mv)[a,c]	Midline sinus (m)[a,b,c]		

[a]Features excluded from analyses of non-Hispanic White children

[b]Features excluded from analyses of non-Hispanic Black children

[c]Features excluded from analyses of Hispanic children

Table 2

Examples of defining dysmorphic versus normal population variation (non-dysmorphic) for physical features among POP controls

Likert score range	Frequency among POP (%)	Lower CL (%)	Upper CL (%)	Range that defines dysmorphic
Example 1: long ear[a]				
≥ 2	22.4	17.0	29.0	No
≥ 3	10.4	6.8	15.7	No
= 4	1.7	0.6	4.7	Yes[b]
Example 2: ptosis[a]				
≥ 2	4.8	2.6	8.9	Yes[b]
≥ 3	2.7	1.2	6.1	No
= 4	0.0	0.0	2.1	No
Example 3: cutaneous toe syndactyly[a]				
≥ 2	10.3	6.5	15.3	No
≥ 3	2.2	1.0	5.8	Yes[b]
= 4	0.0	0.0	1.6	No

POP children without ASD from the general population, *CL* confidence limit

[a]Example is from the non-Hispanic White POP controls

[b]Largest range of Likert scores that includes 5% within the confidence interval or has an upper confidence limit ≤ 5%

Table 3

ASD cases and POP controls included in the dysmorphology data analysis

Characteristic	ASD, N = 514 N (%)	POP, N = 371 N (%)
Race/ethnicity		
Non-Hispanic White	310 (60.31)	185 (49.86)
Non-Hispanic Black	117 (22.76)	96 (25.88)
Hispanic	87 (16.93)	90 (24.26)
Sex		
Male	418 (81.32)	198 (53.37)
Female	96 (18.68)	173 (46.63)
Age at dysmorphology assessment		
< 48 months	31 (6.03)	22 (5.93)
48 to < 60 months	184 (35.80)	126 (33.96)
≥ 60 months	299 (58.17)	223 (60.11)

ASD autism spectrum disorder, *POP* children without ASD from the general population, *N* number

Table 4

Comparing categorical dysmorphology classifications between POP and ASD groups

Race/ethnicity	Group	Total (N)	Non-dysmorphic (%)	Equivocal (%)	Dysmorphic (%)	p Value
All	POP	371	89.76	5.66	4.58	< **0.001**
All	ASD	514	69.84	13.04	17.12	
NHW	POP	185	89.73	7.03	3.24	0.601
NHB	POP	96	89.58	4.17	6.25	
Hispanic	POP	90	90.00	4.44	5.56	
NHW	ASD	310	69.03	13.87	17.10	0.845
NHB	ASD	117	69.23	13.68	17.09	
Hispanic	ASD	87	73.56	9.20	17.24	

POP children without ASD from the general population, *ASD* autism spectrum disorder, *N* number, *NHW* non-Hispanic White, *NHB* non-Hispanic Black

Statistically significant p values are shown in bold font

Table 5

Comparing categorical dysmorphology classifications between POP and ASD groups after excluding participants with known conditions typically associated with dysmorphic features

Race/ethnicity	Group	Total (N)	Non-dysmorphic (%)	Equivocal (%)	Dysmorphic (%)	p Value
Genetic disorders excluded						
All	POP	359	89.97	5.57	4.46	< **0.001**
All	ASD	484	72.52	13.22	14.26	
Major morphologic anomalies additionally excluded						
All	POP	327	90.21	5.51	4.28	< **0.001**
All	ASD	396	75.76	11.62	12.62	

POP children without ASD from the general population, *ASD* autism spectrum disorder, *N* number Statistically significant p values are shown in bold font

Table 6

Comparing categorical dysmorphology classifications between males and females with ASD

Sex	Group	Total (N)	Non-dysmorphic (%)	Equivocal (%)	Dysmorphic (%)	p Value
All included						
Male	ASD	418	71.29	12.68	16.03	0.294
Female	ASD	96	63.54	14.58	21.88	
Genetic disorders excluded						
Male	ASD	400	73.50	12.50	14.00	0.517
Female	ASD	84	67.86	16.67	15.47	

ASD autism spectrum disorder, *N* number