# A STUDY OF MINOR PHYSICAL ANOMALIES IN TWIN PAIRS AGE 5-12 YEARS: A PREDICTOR OF BEHAVIORAL VARIATION?

by

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Minor physical anomalies (MPA) are defined as unusual morphological features found in less than 4% of the general population, but with no serious medical or cosmetic significance to the bearer. An increase in MPA has been associated with irritability in newborns, hyperactivity, and adult-onset schizophrenia and bipolar disorders. In our study, we wish to determine whether minor physical anomalies serve as a predictor for behavioral variation and whether certain regions of the body are more likely to manifest anomalies related to behavioral problems. To determine the combination of MPA most predictive of behavioral variation, we performed a meta-analysis of existing literature examining the relationship between schizophrenia and MPA. Additionally, we sought to determine the heritability of this trait in a twin design. Twin pairs were recruited from Twinsburg, Ohio during the 2005 annual Twin's Day Festival and from the Pittsburgh Registry of Infant Multiplets (PRIM). The only inclusion criterion was that twin pairs were between 5 and 12 years of age. The Stroop Task and the Continuous Performance test were administered to assess attention and impulsiveness in the twin pairs. A 15-20 minute assessment for minor physical anomalies using an expanded version of the standardized Waldrop Physical Anomaly Scale was performed by two investigators. We determined, via meta-analysis, the subset of MPA that is most predictive of schizophrenia. Using a twin design, we estimated the intraclass correlations and heritability of these MPA in a set of 50 twin pairs. We determined that MPA may not be useful as predictors for behavioral variations but may be more useful in specific psychotic populations, specifically schizophrenia. This study has implications for public health because research into the biological etiology of MPA could identify risk factors that would enable early detection and prevention of later psychosis.

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## ACKNOWLEDGEMENTS

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# 1.0 INTRODUCTION

The effectiveness of treatment of a disorder frequently depends on its early detection. This may be also true for behavioral and mental disorders such as schizophrenia, which are poorly understood and the diagnosis of which is delayed. Minor physical anomalies (MPA)--variations in normal phenotypes that are found in less than 4% of the population—have beeen found to be associated, to a various degree, with behavioral dysfunction and schizophrenia. These relationships between somatic and behavioral and psychiatric traits may have common foundation in developmental deviations, which can be in part due to shared genetic causes. The elucidation of these relationships may thus inform prevention and treatment.

The main goal of this study was to evaluate the association of MPA with schizophrenia and characteristics of mental functioning, and estimate the heritability of these traits. The following specific aims were pursued.

**Aim 1**: To evaluate, using meta-analysis, the relationship between minor physical anomalies (MPA) and schizophrenia.

**Hypothesis 1.1**: The risk for schizophrenia is associated with an increased number of MPA.

**Hypothesis 1.2**: This association is particulary prominent for MPA in the craniofacial region.

**Aim 2:** To determine if specific MPA are associated with behavioral characteristics in children and could potentially serve as a predictor for risk assessment.

**Hypothesis 2.1:** An increase in MPA is associated with lower performance scores on the behavioral assessments.

**Hypothesis 2.2:** MPA in the craniofacial regions have a higher correlation with the behavioral scores

**Aim 3:** To estimate heritability of MPA and behavioral characteristics, using a twin sample including Pittsburgh Registry of Infant Multiplets (PRIM),
**Hypothesis 3:** The traits under study have a significant heritability.

To accomplish these aims, a meta-analysis on the extant literature on MPA and schizophrenia was conducted. This work also included registration of multiparous births in a major maternity hospital (Magee-Womens Hospital), recruitment of mothers in a twin registry (PRIM), and assessment of twins for MPA and neuropsychological characteristics with subsequent analysis of these data.

## 2.0 PITTSBURGH REGISTRY OF INFANT MULTIPLETS AND TWIN DATA COLLECTION

The Pittsburgh Registry of Infant Multiplets (PRIM) is a research registry that originated in 1996 (IRB#0410086). The goal of the registry is to include all multiple births occurring at Magee-Womens Hospital, a tertiary care center located in Pittsburgh, Pennsylvania that provides obstetrical care to Allegheny and other surrounding counties. The specific aim of the registry is to build a large population base in the Pittsburgh area of twins and other higher order births that can serve as a resource for studying the relationship between genetic and environmental influences on behavior.

In order to identify individuals for potential recruitment, three wings of the hospital, designated for labor and delivery suites, are targeted. After mothers deliver, a congratulatory tag is placed outside of her door. Baby boys are given blue tags and baby girls are given pink tags. Any multiple birth would be identified by more than one tag either of the same of different colors. Once a mother of a multiple birth is identified, a nurse or other health care provider approaches the mother and asks if she agrees to be approached regarding the registry. If she agrees, the mother is given a detailed explanation of the goals and purpose of the registry.

My role as the study coordinator for the registry involves first identifying individuals for potential recruitment from three wings of the hospital, designated for labor and delivery suites. After mothers deliver, a congratulatory tag is placed outside of her door. Baby boys are given blue tags and baby girls are given pink tags. Any multiple birth would be identified by more than one tag either of the same or different colors. Once a mother of a multiple birth is identified, a nurse or other health care provider approaches the mother and asks if she agrees to be approached by myself regarding the registry. If she agrees, the mother is given a detailed explanation of the goals and purpose of the registry.

If the mother gives her permission to enroll, she is asked to sign a consent form that reviews the information described to her. She is then asked for general information including her name, date of birth, and contact information along with information regarding her pregnancy (newborns' names, date of delivery, birth weights, Apgar scores, and pregnancy complications). Once consent is given, a letter reviewing the registry, a brochure reiterating the goals of the registry and a copy of the consent form are sent to the mother. The demographic and pregnancy information is then entered into a Microsoft Access database. Another copy of their consent form is given to Magee-Womens Hospital Medical Records Department to be filed with the mother's medical records. To date, there are currently over 625 families enrolled into the registry ranging from a few days old to ten years of age. More recently, a connection with the North Pittsburgh Mothers of Multiples (NPMOM) has allowed for participation to be extended to mothers of twins who were not born at Magee-Womens Hospital. An article about PRIM was placed in the NPMOM newsletter with contact information for mothers that were interested in enrolling their twins. In return, each mother who is enrolled at Magee-Womens Hospital is asked if they would like information regarding twin clubs in the Pittsburgh area and a copy of the NPMOM newsletter is given to them.

The investigators associated with the registry who are listed on the consent form have access to their information and may to contact the parents of the twins for particular studies. When a study is conducted, registry members are sent an invitation to participate. Each study is voluntary and they may decide to decline a study if they wish. The participants are assured of the confidentiality of the registry and may withdraw from the registry at any time.

## 3.0 STUDY 1. MINOR PHYSICAL ANOMALIES AND BEHAVIOR

Minor physical anomalies (MPA) are defined as unusual morphological features that are found in less than 4% of the general population and have no serious medical or cosmetic significance to an individual (Jones, 1988). The diagnostic use of MPA has been reviewed in several publications. Pinsky (1985) has termed minor anomalies as "informative morphogenetic variants." Aase (1981) has referred to them as "microsigns." Approximately 15-30% of newborns have one MPA and have a 3% risk for an associated major anomaly. Less than 1% of newborns have three or more anomalies and these newborns are at a 20% risk for a major malformation (Marden, Smith, and McDonald, 1964). In the field of dysmorphology, MPA have been assessed for several decades with various implications. When several specific MPA are seen together in an individual, they can serve are external markers for underlying genetic disorders (Hoyme, 1993). These minor anomalies can result from malformations or structural defects that arise from intrinsically abnormal development, deformations or abnormal structures caused by non-disruptive mechanical forces applied to a normally forming structure, or disruptions which are defects that arise from the destruction of a once normally formed structure (Spranger et al., 1982).

A careful dysmorphology exam is essential for the detection of MPA and because 71% of anomalies are present in the craniofacial area and the hands, careful attention to these areas can be helpful in diagnosing occult major anomalies (Smith and Bostian, 1964). Preauricular skin tags on the ears are commonly found in newborns and may increase the risk for hearing loss. The first and second brachial arches during embryogenesis give rise to the hillocks that combine to form the external and middle ear, as well as parts of the maxilla, mandible and neck. A preauricular tag indicates a deviation in the first and second brachial arch formation and may be related to other occult abnormal derivatives of the first and second arches, including the middle ear structures that could be malformed causing the possibility of hearing loss (Carey, 1993).

Hair shafts erupt on the fetal scalp at 16 to 18 week of embryogenesis and the orientation of the shafts is dependent on the stretch of the scalp. Hair shafts that erupt during the normal time frame will have a concentric pattern around a single posterior parietal hair whorl, which represents the point of maximal stretch in the scalp (Smith and Gong, 1973). An abnormality in brain growth can cause abnormal stretching of the scalp resulting either in the absence or hair a whorl or multiple hair whorls in unusual places. Therefore, aberrant hair whorl patterns may indicate early gestational problems in brain growth. Nevi in the craniofacial area can occasionally be markers for abnormal neuroectodermal development. In epidermal nevi syndrome, craniofacial epidermal nevi are markers for abnormalities in brain development (Baker et al., 1987). MPA found in the hands and feet serve as markers for abnormalities in embryonic morphogenesis. Palmar creases reflect the underlying movement of the hand in the embryo and are often seen in children with some decreased fetal movement (Popich and Smith, 1970).

It has been hypothesized that MPA, particularly of the mouth region, can impact behavior later in life. Orofacial structures that do not form properly can result in problems with communication, emotional expression, mastication and deglutition. Anomalies that occur in the mouth can also lead to sucking problems and feeding irregularities during the first years of life that could also affect the mother-child relationship (Selly et al., 1990). Therefore, children with oral disruptions can have difficulty with socializing and may have neurological deficits from feeding difficulties during the first few months of life.

In the past several decades, researchers have attempted to define etiological factors in childhood psychopathology with specific interest in reliable diagnostic tools, including the study of minor physical anomalies. The first significant investigation was done by Goldfarb and Botstein (1976) who found a correlation between schizophrenic and behavior disorders and an increase in the number of MPA. A high number of MPA are thought to be a primary cause of a disruption that occurs during the first trimester of development when the ectodermal germ layer of the fetus is developing. This tissue layer of the developing human embryo is also responsible for the creation of the central nervous system (CNS); therefore, central nervous system dysfunctions may be detectable by the presence of MPA The development of the CNS is parallel with the development of many of the body's organs. As a result, MPA do not directly cause behavioral deviance but rather serve as markers for some fetal disturbance of development in the

first trimester (Smith 1982). Each organ has a critical period of development where vulnerability to teratogens can result in the disruption of proper development (Moore, 1982). For example, critical hand and feet development occurs during the eight week of gestation while palate development occurs during the ninth week. It is therefore plausible that disruptions during a critical stage of development of a physical feature can cause MPA that can lead to a change in brain development that can cause other behavioral problems. In addition to teratogenic causes, a frequently cited cause for the development of MPA is neurological damage that results from either prenatal or perinatal birth complications (Laufer et al., 1957 and Steward, 1970). Other investigators have shown a significant correlation between dopamine beta-hydroxylase (DBH) in newborns and their irritability and unsocial response in infancy (Rapoport, 1974). DBH is responsible for the final step in the biosynthesis of norepinephrine. Because greater amounts are present after immobilization, stress, or work, changes in activity of this enzyme could reflect changes in the peripheral sympathetic nervous system (Weinshilboum et al., 1971). The authors suggest the possibility of minor insults that occur early in development that may produce anomalies that can have permanent influence on norepinephrine biosynthesis.

## 3.1 ORIGIN OF MINOR PHSYICAL ANOMALIES

The relationship between physical characteristics and behavior has intrigued man for centuries. Galen, a Roman physician and major medical authority during the Middle Ages began the physiognomy era and advocated the view that physical features could reflect inner characteristics of behavior (Griffin and Griffin, 1978). Plato, Aristotle, and Merton were also believers in physiognomy (Brandt, 1980). The Catholic Church placed a ban on this era of thinking and in 1559, the Church placed all physiognomic writing on the Index. Not long after Galen, della Porte published his theory of physiognomy that escaped the ban and became popular. His theory suggested that deviant behavior could be predicted from certain physical characteristics of the heads and hands. Although della Porte's theory was popular during his time, physiognomy lessened during the 17$^{th}$ century, however it did not completely disappear. Phrenology, the determination of character from the configuration of the skull, became more accepted in the 19$^{th}$

century. The phrenological theories were predicated on the belief that specific areas of the brain were responsible for different mental functions and irregularities of the skull could predict a correlating mental function. Cesare Lombroso is known for his work in criminal anthropology. He stated that individuals whose physical appearance differed from the normal would also differ in mental functioning. Through his studies, he related specific physical deviations, such as jaw size and facial asymmetries, to the tendency to turn to criminal behavior.

In 1866, Down described 21 specific physical anomalies that included small ears, upslanting palpebral fissures, flat nasal bridge, epicanthal folds, protruding tongue, fifth finger clinodactyly and a wide space between the first and second toes that characterized a type of mental retardation that he called "mongolism," which today is known as Down syndrome (Down, 1866). Still (1902) noted a correlation of physical anomalies in a group of children that were grouped together as "hyperactive." Years later, a correlation between physical anomalies and febrile seizures and epilepsy were reported (Majovski and Oetiinger, 1975).

Some of the early studies focused on the incidence of major as well as minor physical anomalies in normal and deviant children (Marden et al., 1964). They found that compared with normal children, children with cleft lip and/or palate had a 10% more anomalies and an 18% increase in children with a septal defect and 92% in a group of retarded children. This was one of the first studies that implied that defects in brain functioning could cause congenital anomalies and MPA serve as a marker for such behavior disorders. In the absence of an identifiable syndrome, an increase in MPA have been reported in several groups including newborns (Burg, Hart, Quinn and Rapoport, 1978; Burg, Rapoport, Bartley, Quinn and Timmons, 1980; Waldrop, Bell, McLaughlin and Halverson, 1978), school-aged children (Waldrop, Bell, and Goering, 1976; Waldrop and Goering, 1971), schizophrenic and autistic youngsters (Campbell, Geler, Small, Petti and Ferris, 1978; Goldfarb and Botstein, 1967), mentally retarded children (Firestone, Peters, River and Knights, 1978; Smith and Bostian, 1964), psychoneurotic children (Firestone and Prabhu, 1983; Steg and Rapoport, 1975), learning-disabled children (Steg and Rapoport, 1975), speech and language impaired children (Waldrop and Halverson, 1971), hyperactive children (Firestone, Lewy, and Douglas, 1976; Firestone et al., 1978, 1983; Rapoport, Quinn, and Lamprechet, 1974; Rapoport and Quinn, 1975; Waldrop and Halverson, 1971), and inhibited children (Waldrop et al., 1976; Waldrop and Goering, 1971).

## 3.2 MINOR PHYSICAL ANOMALY ASSESSMENT

Most studies of minor physical anomalies have structured their assessment on a list of anomalies that was originally developed by Goldfarb and Botstein (1967). This list comprised 21 anomalies of the head, hands and feet, and was used to study the relationship between the number of these anomalies in 29 schizophrenic, 11 nonschizophrenic behaviorally disordered children, and 76 normal children. They found that nine of these anomalies were more frequently found in the behavioral disordered children compared to the normal controls. A few years later, Waldrop and Halverson (1971) modified the original list, identifying specific minor physical anomalies that differentiated a population of schizophrenic patients from normal controls. According to their method, anomalies were rated 0, 1 or 2 according to the degree of deviation from the norm. A score of 0 meant that the child did not deviate from the norm for that particular anomaly (Appendix A).

The modified Waldrop and Halverson (1971) scale focused on anomalies in the area of the head, eyes, ears, mouth, hand, and feet. Anomalies of the head consist of fine electric hair, two or more hair whorls, and head circumference out of the normal range. Electric hair is defined as very fine, usually blond hair that will not comb done (weighted 2) or fine hair that soon becomes awry after combing (weighted 1). A hair whorl, in most people, is a singular circular pattern that appears on either the right or left side of the hemisphere. Few people have more than one whorl and a few others have a line instead of a point in which the hair grows. A hair whorl anomaly is classified where there are two or more distinctive whorls or a line an inch or more in length and is weighted zero. Head circumference out of the normal range is considered an anomaly when the size is more than one standard deviation from normal (weighted 1) and greater than 1.5 standard deviations (weighted 2).

Anomalies of the eyes focus on epicanthal folds and hypertelorism. Epicanthal folds are fairly common among infants and toddlers compared to school-aged children (Gibson and Frank, 1961). An epicanthal fold is considered to be present if the subject looks straight ahead and there is any vertical skin fold completely covering (weighted 2) or partially covering (weighted 1) the lachrymal caruncle on either eye. Hypertelorism can be defined as wide-side eyes where the intercanthal distance is greater than normal. Several investigators have found racial differences in the measurement of hypertelorism (Pryor, 1969; Laestadius, Aase and Smith, 1969).

Anomalies of the ears include low-set ears, adherent ear lobes, malformed ears, asymmetrical ears, and soft and pliable ears. Low set ears, like hypertelorism, can be hard to judge without taking measurements. It has been suggested that when a straight line is drawn from the corner of the eye towards the ear lobes, if the top of the ear is at or 0.5 cm below the line, a score of 1 should be given. If the top of the ear was lower than 0.5 cm, a score of 2 was given. Adherent ear lobes are not present if any part of the lower edge of the ear extends below the lowest point of attachment. When the lower edge of the ear lobe extends towards the back of the head, this is given a weighted score of 1. When the lower edge extends upwards towards the crown of the head, a weight of two is scored. Ears are considered malformed fi they are grossly misshaped, corresponding to a score of 1. Asymmetrical ears are weighted 1 when the ears were obviously different from each other. The ears are considered soft and pliable when they are so soft that they feel jellylike when the ear is held between the thumb and first finger when moved back and form. Soft and pliable ears do not quickly spring back into place and the cartilage feels weak.

Anomalies of the mouth include a high-steeped palate, furrowed tongue, and a tongue with smooth, rough spots. A high steeped palate is weighted two when an angle, not an arch, is formed from a cross section view. A weight of one can be given when there is a narrow flat part that appears at the top of the two sides. A furrowed tongue is one that has deep grooves that are usually not along the center line. A tongue with smooth-rough spots is when there is localized thickening of the epithelium or with smooth, rough spots that should not be confused with papillae that are exaggerated in appearance due to recent consumption of certain foods.

Anomalies of the hand include a curved fifth finger and a single transverse palmar crease. A curved finger with a small degree of curvature inward towards the other fingers is weighted 1 and when the fifth finger is more drastically curved inward, a weight of 2 is given. A single transverse palmar crease, also known as a Simian crease, is an unbroken line in the palm of the hand that going more or less straight across the hand. This anomaly is seen most frequently in children with Down syndrome. A weight of 1 is given if a crease is present on either or both hands.

The last category, anomalies of the feet, includes a third toe that is longer than the second toe, partial syndactyly of the two middle toes, and a big gap between the first and second toes. The third toe longer than the second toe has been found to partially be a function of age, where it

fairly common in 2 ½ year-olds and more rare in elementary school children. When the toes are held in an extended position, if the second and third toes appear to be the same length, a score of 1 is given and if the third toe is obviously longer than the second toe, a score of two is given. Partial syndactyly of the two middle toes is weighted one when there is apparent webbing that extends from the lower toe joints. A weighted score of 1 can be given for a big gap between the first and second toe where a flat base across the gap of more than half the width of the second toe is present.

Waldrop and Halverson (1971) have argued that stability in the number of MPA and not the specific category of minor physical anomaly as a predictor for behavioral problems. However, this is not true for all cases. For example, a higher incidence of anomalies of the mouth have been linked to psychosis is several studies (Campbell et al., 1978; O'Callaghan et al., 1991) as well as in schizophrenic patients (Green et al., 1994). Although most researchers have adopted these measures of scoring, known as the Waldrop Physical Anomaly Scale, there are some faults that exist (Firestone and Peters, 1983). Many researchers have modified the Waldrop scale depending on their population because they believe that some anomalies occur more frequently or less frequently in some populations compared with others (Jacklin, Maccoby and Halverson, 1980). For example, fine electric hair is rarely seen in the African-American population and epicanthal folds are seen more commonly in the Asian populations. Because there are variations in the anomaly scales used by researchers, results have modified the number and type of MPA analyzed and therefore the total MPA score seen in a particular behavioral population can differ between studies. A study by Steg and Rapoport (1975) reported a mean score of 4.25 in a population of autistic children while Walker (1977) reported 5.76 mean MPA score and Links et al. (1980) reported an average of 6.24. Despite these differences in MPA scores among various studies, the interrater reliabilities within studies are generally between 0.75 and 0.90.

## 3.3 POSSIBLE MECHANISMS ASSOCIATED OF MPA ASSOCIATION WITH BEHAVIORAL/PSYCHIATRIC TRAITS

### 3.3.1 Family studies

Some minor malformations are thought to be familial and have no clinical significance. Firestone et al. (1978) supported a genetic hypothesis for the transmission of MPA. They looked at the number and type of MPA in families of hyperactive, mentally challenged, and normal control children. Parents and siblings of these children were examined for MPA quantity. Results from this investigation showed a similar number of MPA in hyperactive and mentally challenged children compared with their parents and siblings. When the authors further assessed the families with higher MPA scores, they found no reports of other family members exhibiting any type of deviant behavior. However, there was no clinical investigation or objective behavior ratings to completely accept this observation. Nevertheless, from their study, they were unable to explain why some children with a high MPA score go on to develop behavioral disorders and others do not. Firestone (1983) performed a similar study a few years later and found that hyperactive children and their family members had more MPA present compared to a group of psychoneurotic and normal control children and their family members.

### 3.3.2 Twin studies of MPA and behavior

Some studies have focused on the use of twins to demonstrate environmental components that arise causing behavioral abnormality differences within twin pairs. The consequence of some twin pregnancies is discordance in birth weight (Riese, 2001). Discordance is defined as a difference of 15% between the twin pair birth weight. Research with discordant twin pairs have shown that the smaller of the twin pair is at a risk for decreased cognitive development (Babsen, 1964). More cognitive impairments have been found in the smaller twin when they are discordant by at least 1.5 pounds (Matheny and Brown, 1971). The larger of the twin pair has been described by parents as more easygoing and more self-assured (Babson and Phillips, 1973). Additionally, the smaller twin in the pair has an increased risk of major anomalies (Blickstein and Lancet, 1988). Other research has shown that the larger twin was more irritable, more

difficult to soothe, more active while awake as well as asleep, less reactive to visual and auditory stimuli, and less reinforcing to the examiner compared to the smaller of the discordant twin pair (Reise, 1994). In 2001, Reise examined several measures of integrity including weighted MPA scores and rating of developmental status in order to determine whether there was a higher risk for twins of discordant versus nondiscordant pairs. Discordant twin pairs were found to have higher weighted minor physical anomaly scores, a higher number of MPA and lower developmental status compared to nondiscordant pairs. Additionally, both the smaller and larger twin from the discordant group had higher weighted minor physical anomalies, higher number of MPA, and poorer developmental status. There was no significant difference between neonatal temperament and other interactive measurements between the discordant and nondiscordant twin pair groups. However, there was a significant difference between extremely discordant twin pairs in which the twins were more active while awake and more difficult to soothe. A higher incidence of discordance was reported for dichorionic twins compared to monochorionic twins and twin-to-twin transfusion did not have a major impact on discordance between twin pairs.

### 3.3.3 Developmental instability and MPA

Developmental stability is the ability of a genotype to undergo stable development of a phenotype under any given environmental condition (Thornhill, 1997). Developmental instability, or deviations from developmental stability, can be measured in two ways: by major developmental errors such as birth defects and various minor anomalies and by a concept called fluctuating asymmetry. Fluctuating asymmetry (FA) is defined as nondirectional variation between left and right sides of bilateral characters, which can cause developmental instability (Kowner, 2001). FA, at the most basic level, serves as a marker for viability and there has been a positive relationship between bilateral symmetry and survival in various organisms. Since bilaterally symmetrical traits are encoded by the same genes, FA arises from environmental stressors or stressors that occur from a hostile genetic environment within the genome that lowers developmental homeostasis. There is an array of environmental and genetic sources that can affect FA. The state of a mother's health including her level of FA, her blood pressure, or illness such as diabetes, can affect her child's developmental stability. Women who are obese,

have infectious diseases, consume alcohol, and smoke can cause prenatal stress to her child and can cause developmental instability (Kieser, Groeneveld and Silvia, 1997).

Thoday (1958) asserted that the degree of FA in an organism could determine a buffering capacity for that organism by looking at the structural differences causing asymmetry in a normally bilateral structure which resulted in asymmetry due to developmental accident. Specific patterns of malformations allow geneticists to correctly diagnose malformation syndromes and can lead to accurate counseling for families about recurrence risk and prognosis. In the practice of clinical genetics and dysmorphology, FA is not commonly used as a screening marker for assessing syndromes but rather the presence of MPA is used to assess the degree of developmental instability in the embryo. The heritability of FA to estimate the heritability of developmental stability using parent-offspring pairs has not replicated any statically significant results and there was no detectable additive genetic variance for FA (Leamy, 1997).

Several investigators have found a relationship between FA and MPA. Green, Bracha, Satz, and Christenson (1994) showed a relationship between MPA in patients with schizophrenia and increased dermatoglyphic FA. Yeo, Gangestad, and Daniels (1993) examined the relationship between extreme handedness and developmental instability in a population of students. They found that students with extreme right or left-handedness exhibited a greater level of instability compared to other students with an ordinary level of hand preference. A study that performed MRI imaging of the brain showed that body asymmetry was correlated with deviation from the mean directional asymmetry of cerebral hemisphere size, planum temporal, and grey matter (Thoma, 1996). Researchers have also looked at the relationship of FA to mental well-being (Shackelford and Larsen, 1997) and depression (Martin, Manning and Dowrisk, 1999). A relationship to facial asymmetry and one's emotional state was examined by Shackelford and Larsen (1997) who found that men with facial asymmetry were more depressed, emotionally unstable, and impulsive compared to more symmetrical men. Asymmetrical women experienced more muscle soreness and were also more impulsive compared with symmetrical women.

As research regarding developmental instability continues, FA will hopefully offer further information into genomic and environmental stresses in the human population. The concept of FA is important because of its potential in developing monitoring tools for detecting individuals and populations under stress, enabling isolation and reduction of these stresses and improvement of developmental resistance.

## 3.4 META-ANALYSIS

The current research in MPA has focused on the relationship between the number and type of MPA associated with schizophrenia to help understand the neurodevelopmental etiological hypothesis for developing schizophrenia. Because minor physical anomalies are thought to be a direct index of prenatal damage, the association between an increase in MPA and early onset schizophrenia has been hypothesized (Hata et al., 2003; Lohr et al., 1997; Green et al., 1989). Other authors believe that there is a constitutional predisposition for schizophrenia that is inherited and with the combination of environmental stressors, an individual break downs and become schizophrenic (Waddington et al., 1998a,b).

Gualtieri et al (1982) were among the first to study MPA in adult populations, specifically in schizophrenic and alcoholic adults. Similar to results obtained for adolescent and child behavior disorders, they found that schizophrenic adults, had an increased number of MPA compared to a control population. The alcoholic adults did not have a greater significance in anomalies, however the bimodal distribution was suggestive of some heterogeneity within this group. McGrath (1995) looked at several functional psychoses for MPA score and did not find any significant difference between the diagnostic groups, suggesting that MPA are not specific to schizophrenics.

Some studies have shown that males diagnosed with a psychosis, regardless of the disorder, have higher MPA compared to females (Alexander, 1994). Lal and Sharma (1987) looked at schizophrenic patients using first degree relatives as the control group to eliminate any genetic differences. They found a higher MPA score in female schizophrenics. They also found a relationship with a higher MPA score in the paranoid subgroup of schizophrenics when compared to the non-paranoid schizophrenics. That, as they concluded, might indicate the role of

an organic neurological impairment in the paranoid subgroup that leads to a separate entity for schizophrenia types.

A difference between early and late onset schizophrenia and the number of minor physical anomalies has been analyzed. Green et al. (1989) and Hata et al. (2003) found a correlation between the earlier age of onset for schizophrenia and the number of MPA present. As an explanation, he suggested that some patients may have a prenatal brain injury that in turn can be physically seen through MPA which then places them at a higher risk for schizophrenia. However, several investigators were unable to replicate these findings (Akabaliev and Sivkov, 2003b; Lohr and Flynn, 1993; O'Callaghan, 1991).

Attention has also focused on whether specific anomalies or body regions are more common in schizophrenic patients. It has been accepted that abnormal facial features can be reflective of brain abnormality, and that the underlying brain structure and overlying facial structure are intertwined (Smith, 1988). From the standpoint of embryogenesis, the face supports the brain during development, and a disturbance in normal brain growth may result in abnormal facial characteristics that become more pronounced later in life (Sperber, 1992). Hata et al. (2003) assessed the prevalence of MPA in schizophrenic adults and found significantly higher rates incidences of abnormalities associated with craniofacial anomalies including head circumference, epicanthus, high-steeped palate and furrowed tongue, which has been replicated in several other studies (Green et al., 1989; Lane et al., 1997). Green et al. (1989) specifically found an increase in abnormalities involving the mouth and head circumference, particular in females who were schizophrenic. Lane et al. (1997) found frequencies of MPA increased primarily in the craniofacial region. Sivkov (2003b) found that all three Waldrop mouth anomalies (high steeped palate, furrowed tongue, and tongue with spots) had higher frequencies in schizophrenics. Ismail et al. (1998) also looked at anomalies in addition to the Waldrop items (n=41) and found a higher levels of anomalies present in the eye, mouth and hand/foot regions in both schizophrenic patients and their normal siblings, supporting their genetic or shared environmental relationship with factors that increase the risk for schizophrenia. Gourion et al. (2004) used the same scale as Ismail (1998) to analyze patients with schizophrenia, their first degree relatives, and normal control. In the schizophrenic group, they found a high prevalence

of facial asymmetry, cleft palate, hair whorls, and abnormal palmer creases. However, facial asymmetry and craniofacial MPA were found to be increased in siblings of the schizophrenic patients suggesting that genetic or shared environmental factors may increase the risk for development of both minor physical anomalies and schizophrenia in these families.

### 3.4.1 Meta-analysis of MPA-schizophrenia research

With most of the literature focusing on the relationship between minor physical anomalies in the schizophrenic population, a meta-analysis is a way of combining and interpreting the abundant research publications. In 1976, Gene Glass created a method of incorporating the summarized results and findings from different groups performing similar research and coined the term, "meta-analysis" (Glass, 1976). A meta-analysis is a way of collecting results from individualized studies for the purpose of integration into valuable information that helps to make sense of to vast amount of literature.

This type of analysis can help to evaluate the relationships between a study design and outcome. In a meta-analysis, research studies are collected, evaluated, and interpreted using statistical methods. There are several benefits to performing a meta-analysis. This type of investigation can help to improve the power of small or inconclusive studies and help to identify sources of variation across various studies. Meta-analysis may analyze the heterogeneity among populations and determine whether heterogeneity plays a role among studies. However, a meta-analysis cannot improve or change the quality of a study. Other limitations come from the degree of interpretations for each study when often studies are not identical in their recruitment and analysis. Potential for underlying biases are present between researchers and cannot be incorporated into an analysis and so several assumptions are made in order to create homogeneity when comparing studies (Ioannidis and Lau, 1999).

#### 3.4.1.1 Study selection

A PubMed search was performed to obtain relevant articles published on the relationship between minor physical anomalies and schizophrenia. Search terms included "minor physical anomalies and schizophrenia", "MPA and schizophrenia" and "dysmorphic anomalies and schizophrenia." This search returned 71 possible hits in which the abstracts were evaluated, and

42 articles were found to be relevant to the study. Each of these articles was obtained from online sources or from the University of Pittsburgh Medical Center's Library. The references of these articles were reviewed and additional 5 articles that were missed from the original PubMed search were obtained.

Inclusion criteria were established and included the utilization of a case-control design, in which means were listed for the case and control group, the use of the Waldrop scale or the inclusion of items present in the Waldrop scale, and established criteria for the diagnosis of schizophrenia such as the Diagnostic and Statistical Manual (DSM). Several studies, which shared authors, sample populations, and methods of ascertainment, were assumed to have overlapping data. Out these overlapping studies, those with a larger sample size were chosen for inclusion.

A final total of ten studies fit the inclusion criteria for comparing minor physical anomalies in the schizophrenia population. The ten studies included in the analysis were Alexander et al., 1994, Gourion et al., 2004, Green et al., 1989, Gualtieri et al., 1982, Hata et al., 2003, Ismail et al., 1998, Lal et al., 1987, Lane et al., 1997, Lohr et al.., 1993, Sivkov and Akabaliev, 2003b. Descriptive characteristics regarding each of the studies are listed in Table 1.

**Table 1. Basic characteristics of the studies included in the meta-analysis**

| Study | Cases | | Controls | | Population | Schizophrenia | | | MPA Scale | | | Ascertainment Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Age | N | Age | | Criteria | Onset | Duration | Type | Items | R | |
| Gualtieri et al., 1982[1] | T: 64 | - | T:95 | - | USA[2] | DSM-III | - | - | Waldrop | 12[3] | Yes | Cases ascertained from Dorthea Dix hospital in Raleigh, NC had been hospitalized for at least 6 months and had no hx of alcohol/drug abuse. Controls from various location with no prior hx of alcoholism, drug abuse, psychiatric problems or childhood hyperactivity. |
| Lal and Sharma, 1987 | T:80 ♂:50 ♀:30 | - | T:80 ♂:50 ♀:30 | - | India | RDC[4] | - | - | Waldrop | 13 | No | Cases selected at random from the inpatient population of the Central Institute of Psychiatry in Ranchi, India. Controls comprised of one healthy first degree relative of each of the cases and were matched on sex. |
| Green et al., 1989 | T:67 ♂:53 ♀:14 | 31.6 | T:88 ♂:43 ♀:45 | 28.1 | USA | DSM-III | - | - | Modified Waldrop[5] | 18 | Yes | Cases recruited from Camarillo State Hospital in California and were excluded if had hx of drug or alcohol abuse, mental retardation or were over 55 years old. Controls obtained from Psychiatric Technician training program at the State hospital and undergraduate classes at local university. |
| Lohr and Flynn, 1993[1] | T:118 ♂:110 ♀:8 | 38.7 | T:31 ♂:25 ♀:6 | 43.7 | USA | DSM-III-R | 25.8 | - | Waldrop | 17[6] | Yes | Controls matched for SES were recruited from patients and volunteers at San Diego Veterans Affairs Medical Center. Controls excluded if they had any hx of psychiatric or neurological illness. No information on case recruitment. |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alexander et al., 1994[1] | T:41<br>♂:29<br>♀:11 | 32.8 | T:14<br>♂:7<br>♀:7 | 39.6 | USA | DSM-III-R | - | 14 | Waldrop | 14[7] | Yes | 34 cases current or former patients from Creedmoor Hospital in New York. The remaining 7 were inpatients at the Schizophrenia Research Unit of NY State Psychiatric Institute. Controls recruited from the MHCRC Normal Control Project at NY State Psychiatric Institute and screened for any psychosis or family hx of psychosis. |
| McGrath et al., 1995[1] | T: 79 | - | T: 63 | - | Australia | PSE[8] | - | - | Waldrop | 12[9] | No | Cases drawn from Camberwell Collaborative Psychosis Study, which enrolled psychotic patients consecutively admitted from three regional hospitals. Controls drawn from ER visitors and patients at Princess Alexandra Hospital and screened for hx of mental illness. Attempt was made to match cases and controls on sex. |
| Lane et al., 1997 | T:174<br>♂:127<br>♀:47 | 46.1 | T:80<br>♂:55<br>♀:25 | 46.2 | Ireland | DSM-III-R | - | - | Waldrop | 18 | Yes | Cases recruited from three psychiatric hospitals and included a combination of in-patients, rehabilitation cases and out-patients. Controls were from same region and ethnic background and consisted of retired individuals and hospital staff members. Controls were excluded if they had a personal or family hx of schizophrenia, major affective disorder or suicide. |
| Ismail et al., 1998[10] | T:60<br>♂:44<br>♀:16 | 38.2 | T:75<br>♂:59<br>♀:16 | 35.9 | Sweden | DSM-III-R | - | 14.8 | Expanded Waldrop | 41 | Yes | Cases recruited from psychiatric facilities in Malmo, Sweden and born in Scandinavia in 1941 or later. Both cases and controls with hx of psychoactive substance abuse, head trauma, neurological disorder, or somatic disorder with neurological components were excluded. Controls were comprised of various occupation groups and similar to cases in age and education. |
| Hata et al., 2003 | T:71<br>♂:39<br>♀:32 | 32.4 | T:65<br>♂:34<br>♀:31 | 30.5 | Japan | DSM-IV | 20.6 | 11.8 | Waldrop | 15[11] | Yes | Cases in- and out-patients recruited from the Department of Psychiatry at the Nara Medical University in Japan. Controls were comprised mostly of hospital staff and students. |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sivkov and Akabaliev, 2003 | T:76<br>♂:43<br>♀:33 | 31.5 | T:82<br>♂:42<br>♀:40 | 39.2 | Bulgaria | DSM-IV | - | 6.86 | Modified Waldrop[12] | 19 | Yes | Cases comprised of in-patients consecutively admitted to the Clinic of Psychiatry in Plovdiv. Both Cases and controls with hx of drug and alcohol abuse, neurological disorder, mental retardation or somatic disorder with neurological component were excluded. Controls were of Bulgarian decent and were matched for SES. Controls were excluded if they had a first-degree relative with hx of psychiatric disorder, major affective disorder or suicide. |
| Gourion et al., 2004[13] | T:40<br>♂:29<br>♀:11 | 29.0 | T:42<br>♂:15<br>♀:27 | 26.6 | France | DSM-IV | 20.3 | - | Expanded Waldrop | 41 | Yes | Cases diagnosed with schizophrenia or schizoaffective disorder were ascertained University Department of Sainte-Anne Hospital in Paris. Both cases and controls with hx of head injury or major somatic or neurological disorder were excluded. Controls recruited from hospital staff and screened for hx of Axis 1 disorder, substance abuse and no schizophrenia-related personality disorder. Further, controls had no family hx of schizophrenia, mood disorder or substance abuse up to the 2nd generation. Cases and controls matched on nationality, age and education level. |

Age = Mean age for total sample (years); Onset = Mean age of disease onset (years); Duration = Mean duration of illness (years); R = Assessment of scale reliability/rater-agreement

[1] Only schizophrenia subjects considered

[2] Both Caucasian and African-Americans included in sample

[3] Six items dropped because of poor reliability or assessment difficulties

[4] Research Diagnostic Criteria

[5] Weighting scheme for head circumference and intercanthal with altered from original Waldrop scale

[6] Unclear why only 17 items were included

[7] Four items dropped because of poor reliability

[8] Present State Examination (PSE) and case notes used to assign diagnosis according to Research Diagnostic Criteria

[9] Six items dropped because of concerns over reliability/validity in non-Caucasian subjects

[10] Sibs of cases not included here

[11] Three items (hair whorls, soft ears and spotted tongue) dropped; reasons unclear

[12] One item added by dividing adherent ear lobes into two separate items. Further, furrowed tongue was scored 1 for random and 2 for transverse.

[13] Parents of cases not included here

### 3.4.1.2 Statistical analysis

All statistical calculations were carried out using the software package Comprehensive Meta-Analysis version 2.0 (Biostat Inc., Englewood, NJ). For each of the ten studies used in the analysis, an effect size (*Hedge's g*) was calculated, which is a parametric effect size index used to compare the mean differences between two samples taking into account individual sample means, standard deviations, and population size (Hedges and Olkin 1985). *Hedges g* is calculated by:

$$g = dj$$

where d is the standardized mean difference between the schizophrenic and control group

$$d = \frac{M_1 - M_2}{S_p}$$

and $s_{p,}$ is the square root of the within-groups variance $s^2_{p,}$

$$\text{where } s^2_p = SS_w / df_w$$

$$SS_w = (n_1\text{-}1)\, s^2_1 + (n_2\text{-}1)\, s^2_2 \text{ and } df_w = n_1 + n_2 - 2$$

The correction factor j is calculated by:

$$j = 1 - \frac{3}{4}(df_w - 1)$$

An effect size between 0.2 and 0.5 is considered to be a weak, between 0.5 and 0.8 is a moderate effect, and greater than 0.8 is considered to be a large effect size (Cohen 1988). A 95% confidence interval was obtained to test whether the effect sizes of the studies significantly differed and whether there were differences between the schizophrenic and control groups (Klein 2004). A Cochran's heterogeneity statistic *Q* was obtained to test the null hypothesis that effect

sizes from each of the studies were similar enough that a common population effect size could be calculated (Cochran 1954). Cochran's *Q* was calculated using the following formula:

$$Q = \sum_{i=1}^{k} w_i (d_i - d_w)^2$$

where $d_i$ is the effect size index for the *i*th study, $w_i$ the weight for that particular effect size and $d_w$ is the weighted average effect size from all studies. The significance of *Q* is determined by a chi-squared distribution with k-1 degrees of freedom where k is the number of studies included in the analysis.

A sensitivity analysis was performed to determine whether a single study was contributing to the large effect size found in the initial meta-analysis and to test to robustness of the sample. In the sensitivity analysis, each of the ten studies is excluded one at a time and the remaining nine studies are used and a new pooled effect size is calculated. A meta-regression which is an extension to a meta-analysis is used to determine whether other study characteristics are contributing to the large variation among studies.

In order to perform this analysis, a list of study quality items was generated by assessing specific information described in each of the papers (Table 2). Each of the ten studies was carefully analyzed to determine whether each of the quality items was present (1) or absent (0). A total quality score was then given for each study. The quality scores ranged from 4 to 8 with the highest possible score of 10. Table 2 represents the quality scores given to each of the ten studies.

**Table 2. Assessment of methodological quality for each study included in the meta-analysis of total MPA scores**

| Study quality item | Included studies[1,2] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J |
| 1. Raters experienced or underwent formal training prior to assessment | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 |
| 2. Raters blind to subject's affection status | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 |
| 3. Cases and controls matched on demographic variables (e.g., SES and/or education level)[3] | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| 4. Cases and controls from same ethnic background | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| 5. Sex ratio approximately equal for cases and controls | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| 6. Sample size in case and control group at least 30 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| 7. Controls were a representative sample | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| 8. Controls screened for history of mental illness, neurological disorders, etc. | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| 9. Recognized assessment criteria (e.g., DSM) used in diagnosis | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 10. Reliability analysis was carried out | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Total quality score: | 4 | 4 | 4 | 5 | 6 | 7 | 7 | 7 | 8 | 8 |

A quality score of 0 = no or unclear; 1 = yes

[1] McGrath et al., 1995 not included here because only used in the regional MPA analysis

[2] Study A = Gualtieri et al., 1982; B = Lal and Sharma, 1987; C = Green et al., 1989; D = Lohr and Flynn, 1993; E = Alexander et al., 1994; F = Lane et al., 1997; G = Ismail et al., 1998; H = Hata et al., 2003; I = Sivkov and Akabaliev, 2003; J = Gourion et al., 2004

[3] Matched to cases or their families

In order to focus on whether or not specific regions of the body varied in anomaly scores compared to the control group, a separate meta-analysis using six studies that included raw data were used. McGrath et al. 1995 had raw data listed for each Waldrop score, however a mean score was not presented in the paper so it was not used for the mean analysis but was included in the region analysis. For each of the six studies, regional odds ratios (OR) were calculated using the equation:

$$OR = ad/bc,$$

where *a* and *c* are the total number of anomalies observed in each region for the cases (*a)* and controls (c), and *b* and *d* are the total anomalies present per region for cases (*b*) and controls (*d*). Quantities for *b* and *d* were calculated by taking the maximum number of anomalies possible for each of the regions subtracted by the actual number of anomalies observed, represented by *a* and *c*. For each region, the odds ratios for each study were combined using the Mantel-Haenszel method of continuity correction for empty cells (Sutten et al., 2000). A 95% confidence interval was obtained for each of the regions and Cochran's *Q* was also obtained to determine variation among study regions. The pooled odds ratios for each of the regions were compared across using a mixed-model ANOVA. This test was used to determine whether a particular region has a higher MPA score compared to other regions in schizophrenia.

### 3.4.2 Results

The results of the meta-analysis along with descriptive statistics are presented in Table 3 and Figure 1. For each of the ten studies, a large effect size (represented by a score greater than 0) was present as calculated by Hedge's g (range of 0.38 to 2.06). Bolded studies in Table 3 represent a large effect (>0.8). Seven of the ten studies had large effect sizes. Only one study (Alexander et al. 1994) produced a weak effect (<0.5) where the mean different of MPA between schizophrenic patients and controls was not found to be significant (p=0.211). The pooled effect size among the ten studies was 1.228, representing a considerable difference in total MPA score between schizophrenic patient and controls. To determine if there was variation across the studies, a heterogeneity score (*Q*) was calculated and was found to be significant (*Q*= 53.530, df = 9; p<0.001). Due to significant heterogeneity present, a random-effects model was used to

estimate the cumulative effect size between studies (Sutton et al., 2000). A Rosenthal's fail-safe N statistic was then computed to evaluate how many additional studies with a zero effect size would be necessary to render the pooled effect size insignificant. The formula for calculation the fail-safe N statistics is:

$$N_{fs} = \frac{N(d_w - 0.20)}{0.20}$$

where $N$ is the total number of studies in the analysis and $d_w$ is the weighted average effect size. The value 0.20 is the value $d_w$ would equal if $N_{fs}$ number of studies with such a negligible effect size were added to the meta-analysis.

The Rosenthal's fail-safe N statistic ($N_{fs}$=1072) indicated that over 1,000 studies with a null effect size would be needed to disprove the significant difference between cases and controls, suggesting that publication bias plays a little or no role in producing the observed results.

**Table 3. Descriptive statistics and results for studies reporting overall mean MPA scores**

| Study | Case mean (sd) | Control mean (sd) | Effect size (g) | 95% LL | 95% UL | Z-Value | Sig | $N_{fs}$[1] |
|---|---|---|---|---|---|---|---|---|
| Gualtieri et al., 1982 | 4.00 (0.90) | 2.60 (0.90) | **1.548** | 1.190 | 1.907 | 8.466 | 0.000 | |
| Lal and Sharma, 1987 | 6.80 (2.00) | 2.90 (1.76) | **2.060** | 1.678 | 2.443 | 10.566 | 0.000 | |
| Green et al., 1989[2] | 2.19 (1.52) | 0.68 (0.84) | **1.271** | 0.924 | 1.617 | 7.190 | 0.000 | |
| Lohr and Flynn, 1993 | 1.53 (1.57) | 0.65 (0.84) | 0.603 | 0.204 | 1.003 | 2.960 | 0.003 | |
| Alexander et al., 1994 | 3.50 (1.40) | 2.90 (1.90) | 0.385 | -0.218 | 0.987 | 1.251 | 0.211 | |
| Lane et al., 1997 | 7.30 (2.30) | 4.20 (2.10) | **1.380** | 1.090 | 1.670 | 9.329 | 0.000 | |
| Ismail et al., 1998[3] | 6.37 (2.62) | 2.73 (1.68) | **1.685** | 1.292 | 2.078 | 8.405 | 0.000 | |
| Hata et al., 2003 | 3.32 (1.98) | 2.19 (1.18) | 0.682 | 0.338 | 1.026 | 3.884 | 0.000 | |
| Sivkov, and Akabaliev, 2003 | 4.95 (2.02) | 2.66 (1.57) | **1.266** | 0.925 | 1.606 | 7.286 | 0.000 | |
| Gourion et al., 2004[3] | 5.80 (4.00) | 2.20 (1.20) | **1.220** | 0.753 | 1.688 | 5.113 | 0.000 | |
| Pooled effect size (random effects model) | | | **1.228** | 0.936 | 1.521 | 8.232 | 0.000 | 1072 |
| Heterogeneity score | | | Q = 53.530; df = 9; p < 0.001 | | | | | |

[1] Rosenthal's fail-safe N statistic
[2] Male and female data combined
[3] Score for total 41 item scale

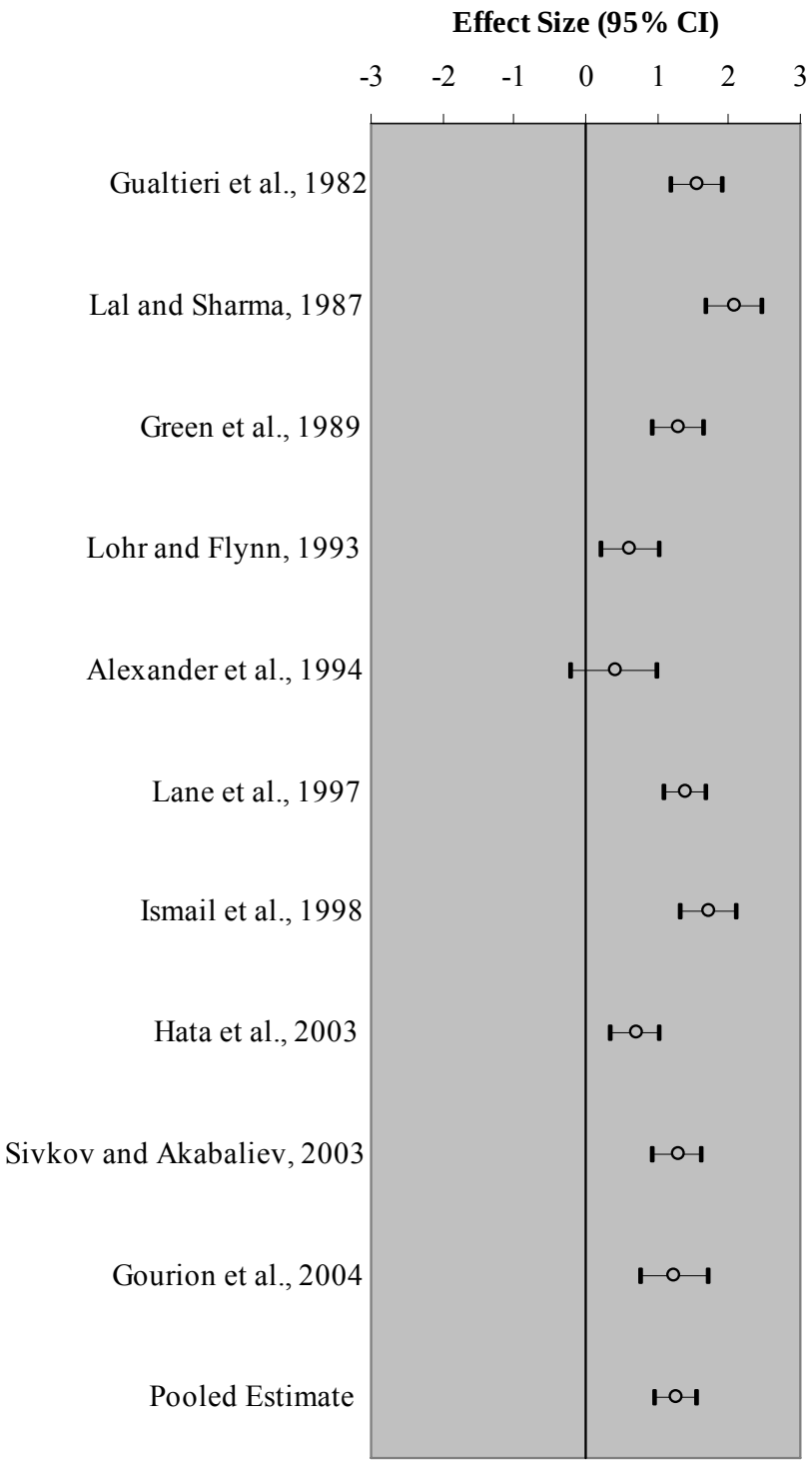



**Figure 1. Effect size for each study used in the meta-analysis**

To investigate the possible reasons for heterogeneity, year of publication, diagnostic criteria used, country of origin, effects of a single study, and the total number of items present on the scale was analyzed. The first variable that was assessed was the year of publication (Figure 1). As shown in Figure 1, which lists the studies in increasing order of publication date, the year of publication and effect size variation are not related. The change in the type of criteria used for schizophrenia diagnosis (DSMIII, DSMIIIR, DSMIV) also does not appear to influence effect size.

Results of the sensitivity analysis failed to reveal any specific study that was contributing to the high heterogeneity presented in Figure 2. The dashed borders represent the original pooled effect size including all ten studies. When each study is removed, the pooled effect size is still categorized as large (>0.8), suggesting that all ten studies were similarly influential on the overall pooled estimate. The removal of the study preformed by Lal and Sharma (1987) resulted in the greatest shift in effect size, although not large enough to be statistically significant.

The sensitivity analysis can also be useful in evaluating the effect of population bias for each study. Of the ten studies used in the meta-analysis, three originated from the United States and the other seven were each from a different country (India, Australia, Sweden, Japan, Bulgaria, and France). Because there are several countries represented in the meta-analysis, the sensitivity analysis can determine whether a particular country of origin contributes to the effect size. As previously noted, the sensitivity analysis did not reveal that any study was a major contributor to the magnitude of effect size. Therefore, the country of origin also does not appear to play a significant role in the large effect size observed.

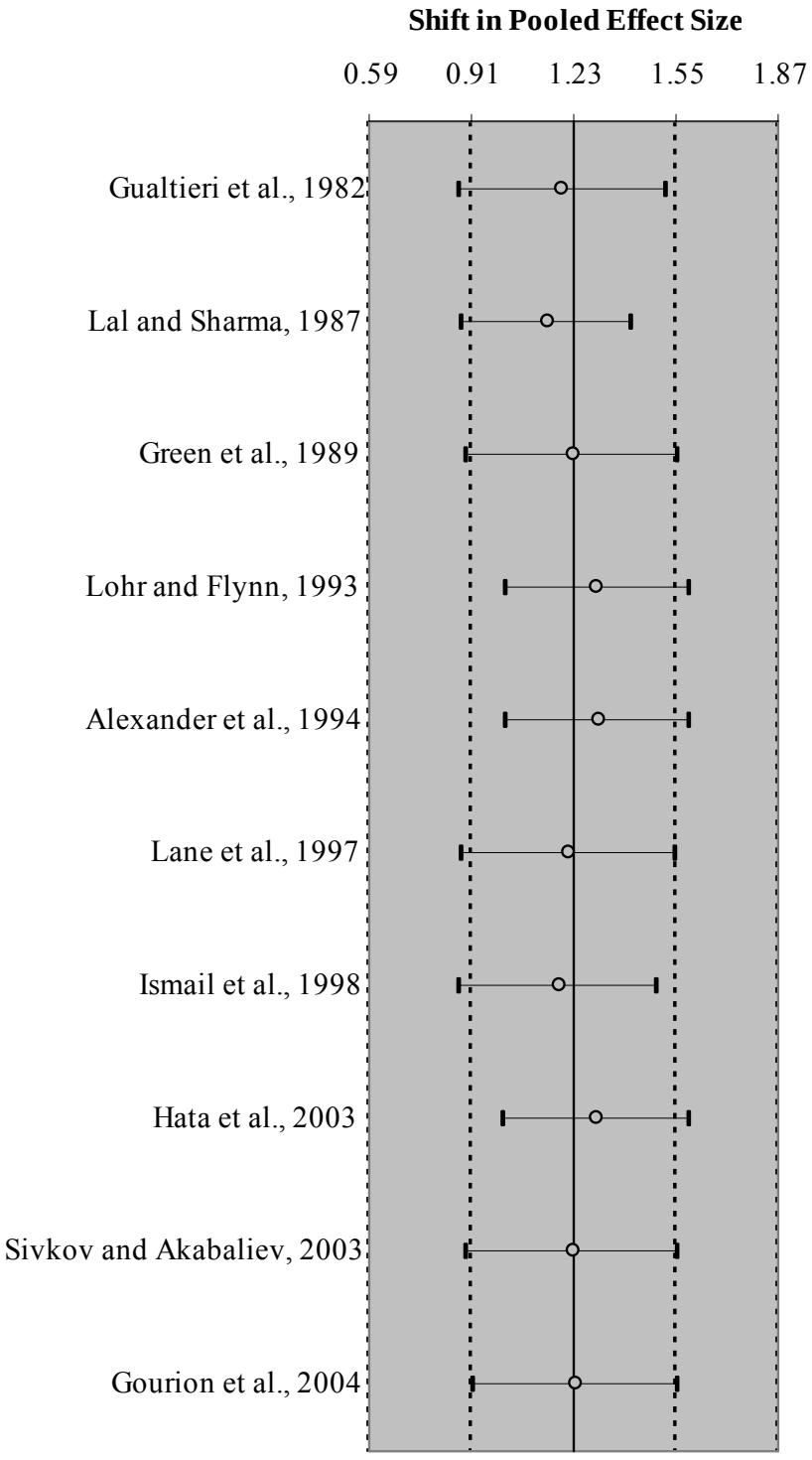



**Figure 2. Shifts in pooled effect size by removing each study from the analysis**

Evaluation of the heterogeneity detected among individual effect size differences was also carried out using a meta-regression for the number of items used in scale for each population. A tau-squared score was generated that can be used to determine how well or how poor the variable of interest is able to explain the variance in effect size across studies. Two studies (Ismail et al.,1998 and Gourion et al. 2004) used items (n=41) in addition to the original Waldrop scale (n=19 items). The other eight studies used variations of the Waldrop scale with total number of items ranging from 12 to 19. Both scores for the number of scale items used (tau-squared = 0.177; p=0.56) or the quality assessment of each study (tau-squared = 0.171; p=0.48) showed any significance.

Individual study and pooled results for the regional MPA analysis are represented in Table 4 and described graphically in Figure 3. The McGrath et al. (1995) study was not included in the evaluation of anomalies from the head region because it lacked the relevant data. Each of the six anatomical body regions (head, eyes, ears, mouth, hands, and feet) had a pooled effect size greater than 1, with the bolded value representing regions within each of the results that were significant. Two studies reported odds ratios less than one; Green et al. (1989) for the eye region and McGrath et al. (1995) for the ear and mouth region. Among the pooled effect sizes for each region, the mouth region was found have the largest difference (effect size of 3.19) and the greatest confidence interval. Five of six studies showed an effect sizes difference between the schizophrenic patients and controls in the head region. The ear region was found have the smallest effect size of 1.58 with the smallest confidence interval. One three of the six studies found significance in the ear region between schizophrenic patients and controls. A heterogeneity score determined by ANOVA did not a significant difference among each of the regions ($Q = 6.779$; $df = 5$; $p = 0.24$).

**Table 4. Results for studies reporting MPA frequencies by anatomical region**

| Study | OR (95%CI) by region[1,2] | | | | | |
|---|---|---|---|---|---|---|
| | Head | Eyes | Ears | Mouth | Hands | Feet |
| Green et al., 1989 | **2.35(1.05-5.23)** | 0.98 (0.48-2.00) | **22.87(1.32-397.58)** | **13.49(5.64-32.26)** | **5.02(1.61-15.61)** | 1.83(0.91-3.68) |
| McGrath et al., 1995 | - | 6.18(0.31-121.75) | 0.71(0.37-1.36) | 0.69(0.34-1.37) | **5.92(1.31-26.71)** | **5.38(1.21-23.94)** |
| Ismail et al., 1998 | **3.33(2.05-5.42)** | **4.41(2.32-8.39)** | **2.00(1.34-2.98)** | **3.64(2.20-6.02)** | **2.06(1.35-3.14)** | 1.44(0.88-2.37) |
| Hata et al., 2003 | 1.86(0.97-3.55) | 1.46(0.90-2.37) | 1.92(0.99-3.74) | **4.83(1.78-13.12)** | 1.60(0.81-3.13) | 1.41(0.66-3.00) |
| Sivkov and Akabeliev, 2003 | **2.23(1.42-3.49)** | **2.57(1.35-4.88)** | 1.36(0.98-1.88) | **2.50(1.61-3.89)** | 1.31(0.73-2.36) | **11.80(3.54-39.32)** |
| Gourion et al., 2004 | **3.09(1.92-4.97)** | **13.34(1.72-103.57)** | **1.80(1.08-2.99)** | **3.20(1.52-6.73)** | **4.84(2.45-9.53)** | 1.81(0.94-3.51) |
| Pooled Effect Size | **2.63(2.07-3.33)** | **2.38(1.29-4.38)**[3] | **1.58(1.30-1.94)** | **3.19(1.63-6.24)**[3] | **2.34(1.80-3.03)** | **2.10(1.57-2.79)** |
| Heterogeneity (Q) | 3.060 | 15.796** | 11.905 | 31.084*** | 12.906 | 13.014 |

[1] An OR > 1 indicates increased MPA frequency in the schizophrenic group
[2] Mantel-Haenszel method used for calculating OR
[3] Random effects model used to estimate pooled effect size
Bold figures indicate OR significant at least at the 0.05 level
** $p \leq 0.01$
*** $p \leq 0.001$

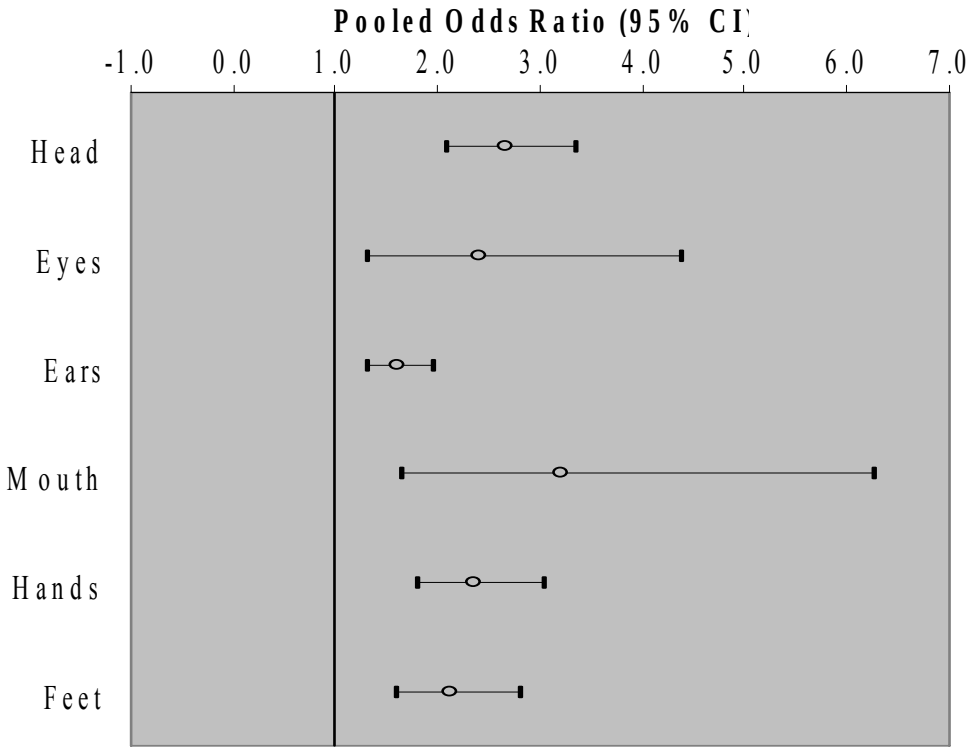



**Figure 3. Pooled odds ratios for studies for each anatomical body region**

### 3.4.3 Discussion

The meta-analysis shows that the number of MPA found in schizophrenic patients is increased despite significant heterogeneity among the studies. Several outcome variables were analyzed as possible explanations for a significant pooled effect size among the ten studies. Neither year of publication nor the criteria used for diagnosis were related to the effect size. The sensitivity analysis suggests that none of the ten studies had a significant impact on the results of the analysis. Each of the studies had a relatively small sample of schizophrenic cases ranging from a total of 41 to 174. The regression analysis showed no significant difference in studies with lower compared to higher methodological qualities that were present in their study. A regression analysis was also carried out for the number of items used in the total MPA score. Two authors (Ismail et al. 1998; Gourion et al. 2004) used additional items with the Waldrop scale to assess whether other anomalies should be examined in schizophrenic patients and to determine other biological etiologies that may have an underlying effect on the development of schizophrenia. With the regression model that was performed, it was found that there was not a significant difference that is contributing to the effect size by using a 41 item scale. This item regression analysis suggests that the original 19 item Waldrop scale may be sufficient when studying MPA differences between schizophrenic patients and normal control subjects.

The regional analysis, examining whether certain regions of the body have higher MPA score compared to other regions, did not show any differences among regions. Although the mouth region was found to be the highest difference between cases and controls, the mouth region was not found to be significantly different compared to the body regions. This regional analysis also contradicts some studies that state that craniofacial anomalies are a more effective predictor for assessing schizophrenic patients compared to controls compared and other body regions do not show significant differences (Gourion et al., 2004).

There are some criticisms within each of the studies that were unable to be analyzed that may have affected the outcome of the analysis. Alexander et al. (1994) was the only study that mentioned in the methodology that raters were blind to the subject's diagnosis (schizophrenic versus control). Of interest, this study produced the lowest effect size compared to the other

studies and did not produce a significant difference between schizophrenic patients and controls. Future studies comparing MPA in schizophrenic patients may produce different results when raters are blind to the individual's diagnosis.

Another possible variable that could be contributing to variation among studies is that each paper uses different criteria for recruitment of their control subjects. Lal and Sharma (1987) use first degree relatives that are matched by sex as the control group. Their reasoning of using first degree relatives was to account for any MPA that may be heritable and would not produce differences between cases and controls. However, with using first degree relatives as a control group, there is the possibility that control subjects may have a familial risk for schizophrenia and at the time of this study, they may have been too young to develop schizophrenia. Other studies used controls that were easily assessable and recruited from the hospital staff or from local students located at the same site that the study was conducted (Green et al., 1989; Lohr and Flynn, 1993; McGrath et al., 1995; Hata et al., 2003; Gourion et al., 2004). Other studies used specific criteria to screen their subjects for inclusion. Inclusion criteria for the control group included no family history of mental illness, substance abuse, head trauma or a family history of schizophrenia or other psychotic disorders (Gualtieri et al., 1982; Alexander et al., 1994; Ismail et al., 1998; Sivkov and Akabaliev, 2003). While likely ascertainment methods for controls did not have a significant impact on the results found in each study, the fact that each study has different criteria set forth for controls can not be ignored.

In each of the studies, the male to female ratio used for cases and controls varied. Only one study by Hata et al. (2003) used equal numbers of males and females for cases and controls. The remainder of the studies use more males than females with most studies having a 2:1 ratio of male to female cases. In an epidemiological study by McGrath et al. (2004) 30 countries were studied for prevalence of schizophrenia. They found a male to female ratio median of 1.4. Several authors have suggested the interplay of sex hormones in neurodevelopment of psychosocial as an explanation for sex differences and age of onset (Seeman, 2002; Riecher and Hafner, 2000; Salem and Kring, 1998). Sex hormones may serve as a threshold effect that later in life would trigger the first episode of schizophrenia. Although sex hormones may play a role in the timing of onset between males and females, the effect of MPA development due to some prenatal disturbances in brain structure would have an equal effect on males and females. Lal and Sharma (1987) was the only study that found females to have a higher MPA score compared

to males, however only thirty females were used in the sample which may influenced the outcome. Green et al. (1989) found more mouth abnormalities and an increased head circumference in female schizophrenic patients compared to male schizophrenic patients and female controls. However, their study only included 14 female schizophrenics in which the small sample size may have skewed the results. In each of the ten studies a small sample size for the control group was used (range of 40 to 174). The small sample sizes of the studies may play a role in the heterogeneity found among studies however there are no large studies of MPA in schizophrenic patients in which to compare the results obtained from the meta-analysis.

From the results obtained from the meta-analysis, minor physical anomalies were not found to be higher in the craniofacial region compared to the other body regions. During embryogenesis, there is overlapping critical periods of sequential development that could result in multiple malformations in different areas of the body. The palatal morphology originates at 6-9 weeks gestation and has achieved its postnatal form at 16-17 weeks when the maxillary process begins to occur (Sivkov, 2003b). The development of the auricle of the ear begins during the 8$^{th}$ week of gestation where six hillocks begin to surround the first pharyngeal arch. Because several complications can arise in hillock formation, anomalies of the ear are not uncommon. Low set ears results as a sequence due to the failure of the mandible to form correctly and at the right time. The eyes also begin to form around the 8$^{th}$ week of gestation and continue to develop until late in pregnancy. Fingers and toes do not begin forming until after the 8$^{th}$ week when cell death occurs in the ectodermal ridge, which separates into five parts. Because of the overlapping developmental sequences in embryogenesis, an insult would need to occur early on in development, particularly before the 8$^{th}$ week, in order to find anomalies present in more than one region of the body.

Firestone et al (1978) suggests the role of genetic factors in the development of schizophrenia. They base their assumptions of the evidence that physical anomalies can result form chromosomal disorders as seen in Down syndrome and trisomy 13 (Smith, 1988). With schizophrenia, an increase in MPA may reflect a group of aberrant genes that are responsible for disoriented neurological development. Gross chromosomal anomalies have been documented in schizophrenia with particular interest in individuals with deletion of 22q (Bassett and Chow, 1999). O'Callaghan (1991) reported a high number of MPA in families with schizophrenia supporting a possible genetic explanation for schizophrenia.

It is overly simplistic to assume a single genetic or environmental cause solely responsible for schizophrenia. Another theory that has been suggested involves both genetic and environmental factors. Heterogeneity likely exists between the two factors, and there may be a multifactorial threshold that must be reached (McGuffin et al., 1994). Genetic factors may exist that predispose an individual to schizophrenia, and environmental stressors may be necessary to trigger the onset of schizophrenia later in life. Green et al (1994) found that MPA in schizophrenia are associated with dermatoglyphic fluctuating asymmetry (FA) suggesting that both MPA and FA may be related to developmental instability, which may account for the lack of specific MPA causal for schizophrenia. Several studies have also shown that schizophrenic patients have a greater FA compared to normal controls. Waddington (1988a,b) has proposed a lifetime trajectory model for schizophrenia that incorporates genetic and environmental effects that all likely have some impact on schizophrenia development (Figure 4).

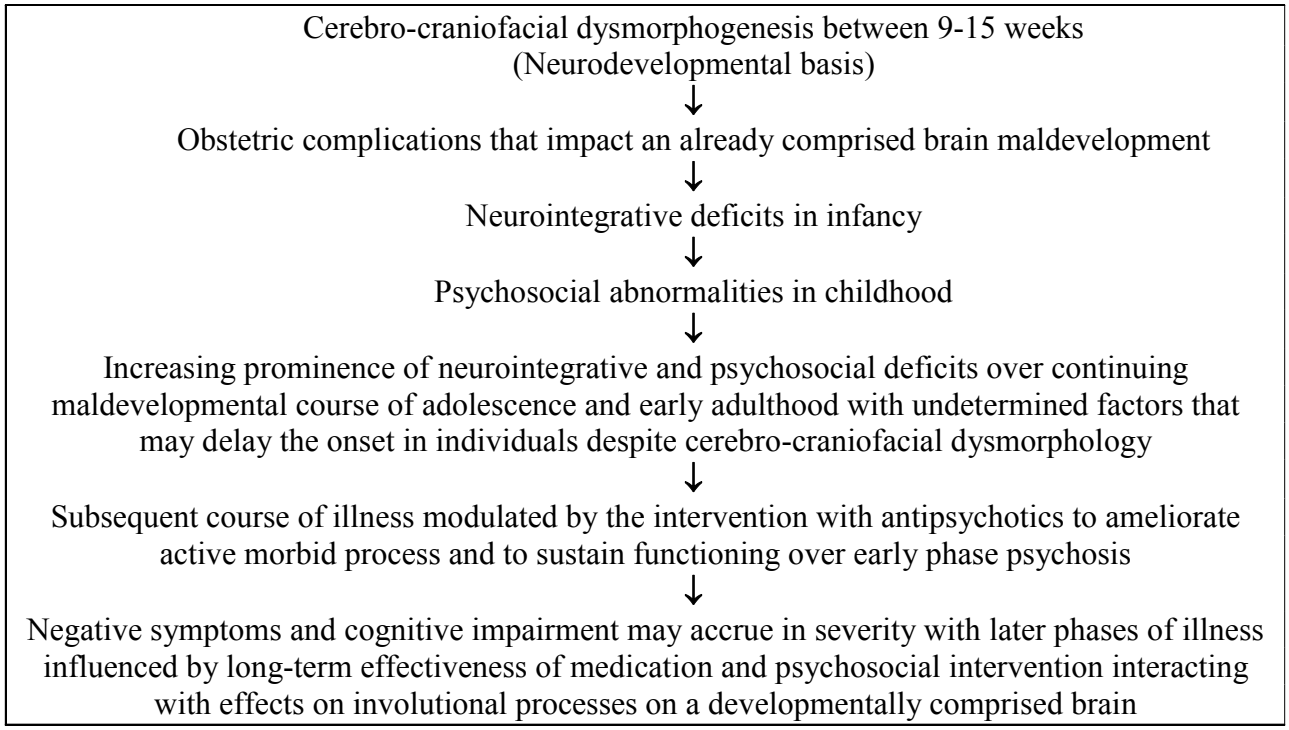



**Figure 4. Developmental model for schizophrenia (adapted from Waddington, 1988a,b)**

The increase in the prevalence of MPA found in schizophrenic patients is generally interpreted as a result of a neurodevelopmental abnormality or insult that occurs early during the first and second trimester of development. As shown in this meta-analysis, there is a strong correlation between the increase in MPA score and schizophrenia. However, an increase in the number of MPA is not only seen in the schizophrenic population, but as mentioned earlier, high MPA scores are also seen in hyperactive and learning disabled children, in adolescent delinquents, other psychoses, and has been associated with obstetric complications. Therefore, MPA cannot be indicated as a sole predictor of schizophrenia development, but instead as an additional predictor of behavioral outcomes.

### 3.4.4 Conclusions

This meta-analysis proves useful in several aspects. This research suggests that, although there are several limitations, results obtained demonstrate no single study characteristic is able to explain the variation seen among studies. The combination of several study characteristics may explain the variability seen among studies. This study provides evidence that minor physical anomalies are significantly increased in the schizophrenic population and that some underlying biological mechanism is likely responsible for producing these anomalies. The regional analysis was unable to confirm our theory that the craniofacial region would have a significantly larger impact compared to other body region. The regional analysis supports the theory that there may be some biological involvement that occurs early in development that alters brain structure. Early brain insults may result in altered physical development which can be seen in the form of minor physical anomalies. Although altered brain structure may not directly cause schizophrenia, environmental stressors may act on this predisposition for schizophrenia and triggering its onset.

# 4.0 TWIN METHODOLOGY

Today, approximately 3% of births are multiple births (Hoyert et al., 2006). Ninety-five percent of these multiple births are twin births while the other 5% are multiparity of a higher order (triplets, quadruplets, etc.). There has been a 67% increase in twin birth rates since 1980. Two related trends have been ashs: older age at childbearing (women in their thirties are more likely to have a multiple birth) and the more widespread use of fertility-enhancing drugs and procedures such as in vitro fertilization (Elliott, 2005). Other than age, several other factors can play a role in twinning, including a family history of twins, already having a set of twins, and the use of fertility treatment by in vitro fertilization or fertility drugs. With fertility assistance, the chance of having twins is as high as 20-25% whereas the chance of having twins naturally is 1 in 60 (near 2%). Race also is shown to have an influence. African-Americans have the highest probability of multiparity and Hispanics and Asians have been showed to have the lowest frequency of multiple births (Martin et al., 1997).

There are two types of twins, monozygotic (identical or MZ) and dizygotic (fraternal or DZ). Identical twins are not as common (1 in 250 births) and result when a single egg divides in half after fertilization that results in two separate individuals with the same genetic composition. DZ or fraternal twins result when two separate eggs become fertilized by two sperm. Fraternal twins do not share the exact same genetic composition but instead share 50% of their genetic material identical by decent, the same as other siblings would who were born at different times.

The concept of studying twins in research originated in the 19$^{th}$ century. Francis Galton was the first author to suggest using twins to study the different contributions of hereditary and environment by researching family resemblances and differences in twin pairs. Karl Pearson published a three-volume biography of Galton's work describing several studies of individuals differences and family resemblance and particularly the acknowledgment that differences between MZ and DZ twins could help to differentiate between genetic and cultural effects.

However, what Galton was lacking in his theories about inheritance was described by Gregor Mendel. Mendel's theory regarding inheritance of traits using plant hybridization stated that some traits are inherited together while other traits are independent of each other. It was not until 1918 that Ronald Fisher brought together the concepts of both Galton and Mendel. Fisher assumed what is now termed a *polygenic* model of inheritance, in which correlations between phenotypic traits of related individuals can be explained by the inheritance of segregating genes by an underlying Mendelian inheritance and variation occurs due to environment, assortative mating, and non-additive gene actions (Neale and Maes, 2004).

Today, family (twin) research is useful in helping to understand genetic relationships and the contribution of unknown genes that contribute to the variance of a trait. The extent to which MZ twins are dissimilar is attributed to non-shared environmental factors, which include measurement error. Given that MZ twins share the same genetic material and DZ twins share 50% of their segregating genes, the genetic correlation between MZ twins is 1 and between DZ twins is 0.5. The differences between DZ twins are due to both non-shared environmental and non-shared genetic factors (Posthuma et al., 2003).

Genetic variation among twins can be broken down into additive and dominant components. The total genetic variation of a trait is the sum of both the additive and dominant effects plus the variance due to the interaction of alleles at different loci (Bateson, 1909). If all alleles were to act additively, the correlation of genetic factors in DZ twins would be 0.5. However, when dominance and epistasis are considered, effects are reduced to 0.25, thus reducing the phenotypic resemblance of DZ compared to MZ twins. Assuming panmixia and the absence of gene and environmental action and epistatic effects, the phenotypic variance of a particular latent trait, $V_{P,}$ is composed of the additive genetic component, $V_A$, the dominant genetic component, $V_D$, and a shared, $V_C$, and non-shared or unique, $V_E$, environmental component represented by:

$$V_P = V_A + V_D + V_C + V_E$$

(Neale and Cardon, 1992). A shared environmental variance is due to environmental factors that increase family members' (twins) similarity. A non-shared environmental variance is due to factors that cause family members' (twins) dissimilarity, including measurement error. The

additive variance component represents the variance across a phenotype due to the additive effects of alleles across one loci. The dominant variance component represents the non-additive genetic variance due to the interaction between two alleles at one loci.

Path diagrams are visual representations of relationships between variables. In path diagrams, variables are connected by arrows depicting relationships between variables. A variance is indicated by a two-headed arrow with ends pointing to the same variable. Covariances or correlations depicted by the two-headed arrow can be used to quantify similarities between two related individuals. Partial regression, or path, coefficients are represented by a one-headed arrow that points to a hypothesized pathway or causal relationship between two different variables in which the variable at the tail of the arrow is transmitted to the variable at the head of the arrow. Observed phenotypic variables are indicated by rectangles and unobserved or latent variables are depicted by circles. Figure 5 represents the univariate twin model pathway.

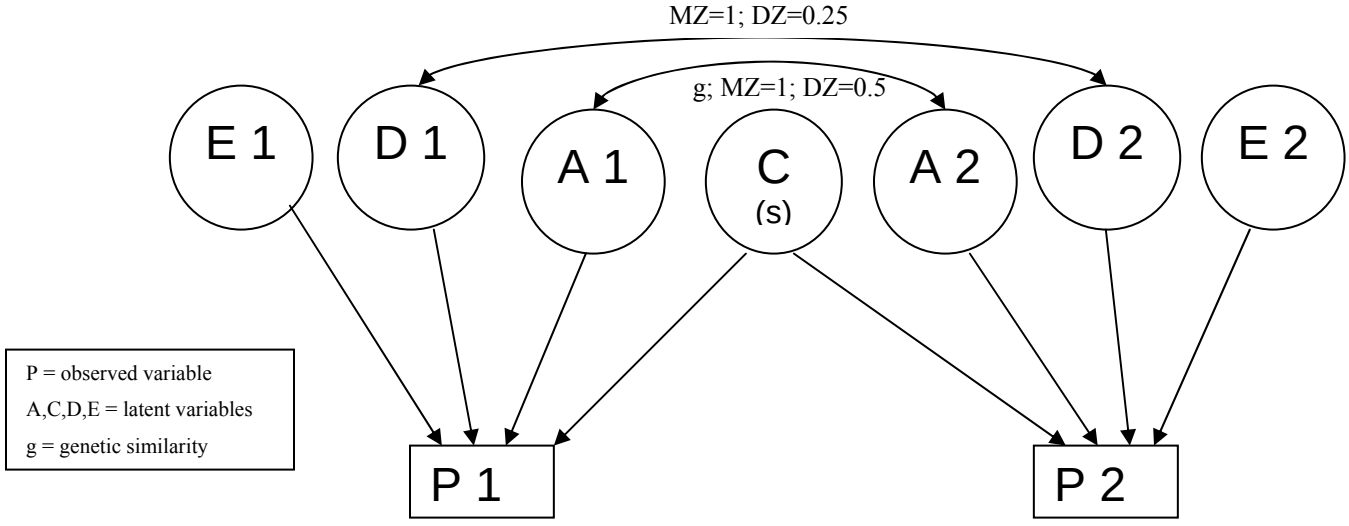



**Figure 5. Path diagram of the univariate twin model**

The relationships between twins (depicted by the numbers 1 and 2) can by calculated by tracing the pathways. Phenotypic variation from additive genetic influences is represented by two times the difference between MZ and DZ correlations ($V_A = 2[r_{MZ} - r_{DZ}]$). Contribution of a dominant influence can be calculated by subtracting four times the DZ correlation by twice the MZ correlation ($V_D = [2r_{MZ} - 4r_{DZ}]$). The contribution of shared environmental effects is

calculated by subtracting the MZ correlation from twice the DZ correlation ($Vc = 2r_{DZ} - r_{MZ}$) and the non-shared environmental influences is found by subtracting the MZ correlation from 1 ($V_E = 1 - r_{MZ}$).

At a particular locus, there is often more than one allele present, contributing to phenotypic variation. Hardy-Weinberg equilibrium can be used to help illustrate this concept. When two alleles are present at a particular locus, for instance A1 and A2, their genotypic frequencies can be represented by the equation, $p^2 + 2pq + q^2 = 1$, which represents A1A1, A1A2, and A2A2 genotypes, respectively. The genotypic effect of A1A1 is termed, "*a*" while the genotypic effect of A2A2 is "-*a*" and A1A2 is termed "*d*". The midpoint between *a* and –*a* is 0. However, depending on how dominant the A1 allele is compared to the A2 allele, A1A2 may fall somewhere between 0 and the value of A1A1. If the A1 allele has no dominant affect, A1A2 genotype would fall at 0. However, most phenotypes are not due to a single locus and instead a threshold model must also be considered. A threshold model accounts for dichotomous traits or other ordinary phenotypes (e.g underweight, normal weight, overweight, obese). These traits measured on a continuous scale and are organized in contingency tables. In twin analysis, contingency tables contain the number of pairs for each zygosity group (MZ and DZ) with each combination of traits. Because a polygenic mode of inheritance is assumed in this model, an underlying quantitative liability exists with at least one threshold. Although liability cannot be measured, a normal distribution is assumed to account for liability. Thresholds are determined by calculating z-scores present within the standard normal distribution curve. Values that fall between the threshold limits or those values that fall between minus infinity to the one threshold value reflect the presence of the trait (category) of interest.

# 5.0 STUDY 2. TWIN STUDY OF MPA AND NEUROPSYCHOLOGICAL TRAITS

## 5.1 METHODS

### 5.1.1 Sample population

The original research site for twin assessment was a research tent at the annual Twins Day Festival held in Twinsburg, Ohio, August 6-7, 2005. The Twins Day Festival is an annual event that occurs every summer, in which twins and higher order multiples and their families are able to participate in contests and games for twins of all ages with carnival-type rides and stands. There is also a section of the park dedicated to twin research in which twins and their families can participate. For this study, twin pairs were recruited by two methods. PRIM parents whose twins fell into the age category of five to twelve years were contacted by phone and mail prior to the festival dates. Parents who responded to calls and letters and were interested in participation were scheduled an appointment time. Four families from PRIM were interested in participating in the research at the Twins Day Festival. Throughout the festival, active participants who met the age requirements were asked to participate. By this recruitment method, 35 more pairs were included in the analysis at the Twins Day Festival. Out of the total 39 families that participated in the research, 47 were twin pairs and 1 was a set of triplets. In the fall of 2005, the research was continued at the University of Pittsburgh Craniofacial and Dental Genetics Center in Pittsburgh, Pennsylvania. PRIM parents who were unable to attend the Twins Day Festival were asked if they would be willing to come to the university on a Saturday afternoon. Nine additional twin pairs were thus recruited. A total sample of 48 families was included.

The research conducted on the children was approved by the University of Pittsburgh's IRB (IRB#0506162). The study protocol was designed to learn more about developmental instability (DI) as a way to measure the response to stress during development. The main goal of

the study was to look at specific components of DI through physical and behavioral measurementsin twins. For this particular study, DI was not directly assessed but rather behavioral and physical measurements taken from the original study were used for analysis.

### 5.1.2 Zygosity determination

Parents completed consent forms for each twin participant. If multiples were 7 years of age or older, their assent was requested. To obtain zygosity information regarding same-sex twins, each parent was asked to complete the zygosity questionnaire used in the PRIM registry (Appendix B). This questionnaire and the zygosity determination algorithm were derived from Nichols and Bilbro (1966). The questionnaire consists of 15 questions regarding the similarities and differences between twin pairs. The questionnaire was found to predict zygosity with accuracy of 94% when compared with blood test results. For the triplet set, parents were asked to complete each questionnaire to compare each member of the triplet to one another.

### 5.1.3 Traits

#### 5.1.3.1 Minor physical anomalies and summary scales

Two separate investigators, myself and Dr. Kathy Neiswanger from the Center for Craniofacial and Dental Genetics assessed a list of 74 anomalies, 3 skin variations, and 7 neurological characteristics in each child. Items from the Waldrop scale were included into the list of physical variants assessed in this study as well as items from other sources (Appendix C). Although the original Waldrop scale items were weighted, items present in our assessment were categorized as present (1) or absent (0) due to the complexity of categorizing a trait as more severe (weight 2) compared to mild (1). Each variant was categorized under 8 different body regions: Head/Face, Ears, Nose, Mouth/Jaw, Hands/Arms, Feet/Legs, Skin, and Neuromuscular. A manual from the University of Pittsburgh's Craniofacial and Dental Genetics Center (Appendix C) describes and discusses the assessment of each of the minor physical anomalies. Two investigators performed the assessment, one for each member of the twin pair, to decrease the possibility of reporting bias that may occur when assessing one twin for MPA and then searching for similar MPA in the

other twin. The MPA assessment took an average of 15-20 minutes. The MPA assessment was set up apart from each other to reduce the possibility of reporting bias due to assessing twins next to each other.

After completion of the study and discussion regarding the reliability and ability to assess of each of the anomalies, discrepancies were found in scoring certain items, which were thus excluded from further analysis. Several items were not able to be assessed for every individual and were therefore removed (hair whorls, multiple buccal frenulae, missing and supernumerary teeth). Other items were removed due to the inability of the raters to agree upon a standard or due to the ability to assess the items in children (frontal bossing, sloping forehead, strabismus, nystagmus, arching one eyebrow, ear bump, angled columella, tongue with spots, hypoplastic fingernails, hyperconvex fingernails and toenails). Malformed ears included any abnormalities of the earlobe such as a simple ear and an overfolded helix. Abnormal palmar creases were combined and scored as present if an individual had either a single palmar, syndey, bridged, or deep creases. The "other" categories for each of the body regions were not included. The section including skin and neuromuscular analysis was not used in the analysis because of the inability to accurately assess these findings. After removal of these items, a total of 50 items were used for MPA analysis as represented in Table 5.

**Table 5. Minor physical anomalies used in the twin analysis**

| Regions | | | | | |
|---|---|---|---|---|---|
| **Head** | **Eyes** | **Ears** | **Nose/Mouth** | **Arms/Hands** | **Legs/Feet** |
| n=5 | n=4 | n=6 | n=10 | n=14 | n=11 |
| Fine hair | Epicanthal fold | Low set | Bulbous tip | Valgus elbow | Valgus/Varum knees |
| Widow's Peak | Ptosis | Malformed | Anteverted nares | Hypermobile joint | Pes planus |
| Frontal Cowlick | Heterochromia | Protruding | Smooth philtrum | Syndactyly | 2nd toe > 1st toe |
| Synophrys | Coloboma | Attached earlobe | Angled columella | Abnormal creases | 3rd toe > 2nd toe |
| Metopic ridge | | Preauricular tags | Thin upper lip | Short fingers | Syndactyly |
| | | Soft | Macroglassia | Broad fingers | Sandal gap |
| | | | Furrowed tongue | Tapering fingers | Overlapping toes |
| | | | Bifid uvula | Long fingers | Short toes |
| | | | Micrognathia | Clinodactyly | Deep creases on soles |
| | | | Grooved chin | Camptodactyly | Fetal pads |
| | | | | Overlapping fingers | Rotated little toe |
| | | | | Fetal pads | |
| | | | | Backwards thumb | |
| | | | | Bent index finger | |

### 5.1.3.2 Neurological indices

As part of the study, each child underwent two behavioral assessments. Conner's Continuous Performance Test II (CPT) is used to measure attention and learning disorders (Conners, 2000). The CPT II computer program is not recommended for use alone to reach a diagnosis, however the test provides information that helps in the assessment and diagnostic process when used with self and observer-reported data as well as behavioral and historical information (American Academy of Pediatrics, 2000). This test can be used for children beginning at age 4. Before taking the test, the administrator instructs the child to press the space bar of the computer keyboard when any letter appears on the screen except for the letter "X." The inter-stimulus intervals are 1, 2, and 4 seconds with a display time of 250 milliseconds. There are 6 blocks with 3 sub-blocks containing 20 letter presentations that vary between each block. The entire test

takes 14 minutes to complete. The CPT II is for children age 6 to 17. For children who were 5 years of age, a Conners' Kiddie CPT was used that is a shortened version of the original CPT II that uses different pictures instead of letters. Children are instructed to hit the space bar when each object appears on the screen except for when the soccer ball flashes. The Kiddie CPT is also a shortened version compared to the CPT II and only lasts for 6 minutes.

Two scores are given for the CPT assessment. The first is a commission error score that represents how often they did not see a target but pressed the space bar anyways. The second score is the omission score in which the child failed to not hit the space bar when the "X" or soccer ball appeared on the screen. Different response patterns can indicate various deficiencies such as inattentiveness as a result from a higher omission error score or impulsivity as represented by a high commission error score (Conner, 2000).

The other behavioral assessment, the Stroop Effect, was developed in 1935 to measure attention and cognitive inhibition (Archibald and Kerns, 1999). There are three parts to the task. The first part involves reading off the word colors listed in black ink that are presented in four columns of 25 words each. The color and word choices involve blue, green, and red. The child begins at the top left corner and reads down the column and then continues to the right column and so forth. The task is timed and after forty-five seconds, the child stops and the number of words read are counted. Results from the first section reflect basic reading rates that may be affected by learning or speech conditions (Golden et al., 2000).

In the second task, the child is to name the color of ink of a bar of X's (XXXXX in red, blue, or green ink). The same time limit and pattern of reading is used. This color task, like the previous word task, is useful in assessing learning disabilities and speech impairment in addition to colorblindness assessment.

In the final task the child is shown names of the three colors however the color name is not in the same color ink (e.g. if the word "green" is written in blue ink). In this task, an individual will say the word "green" more readily than they can name the color in which it is displayed, which in this case is "blue." The color-word task measurement helps to measure mental flexibility and the ability to inhibit a dominant response (ink color) and deal with interference (Wecker et al., 2000). A percent interference score from the Stroop Effect test used to assess cognitive inhibition is calculated by subtracting the "Color" score from the "ColorWord" score and then dividing the answer by the "Color" score.

### 5.1.4 Statistical methods

#### 5.1.4.1 Standard statistics

For each of the minor physical anomaly traits and behavioral assessment scores, the total number of pairs assessed, the means, standard deviation, and standard error of measurements were obtained. For all independent samples, a t-test was used to evaluate the differences in means between two groups. If variables were found to be normally distributed, variations of scores in the two groups (MZ and DZ; males and females) as well as age were calculated. In groups with equal variances, a pooled variance was calculated whereas groups found to have unequal variances, the Satterthwaite approximation assuming unequal variances was utilized. Equality of variances was calculated using an F-test obtained from SAS v.8.2 for Windows.

#### 5.1.4.2 Reliability estimation

To evaluate the reliability of the MPA scale, a reliability analysis was conducted. The assessment for the reliability scale is based on correlations between the individual traits relative to the variances of the items. The assessment for the reliability scale is based on correlations between the individual traits relative to the variances of the items. The most common index of reliability is Cronbach's alpha. This analysis can be used to assess a variety of items and accounts for how many items are present in a particular scale. Cronbach's alpha is equivalent to:

$$\alpha = \frac{N\mu}{1 + (N - 1)\mu},$$

where N represents the number of items and $\mu$ is the average inter-item correlation. When alpha is greater than 0.7, a scale is considered reliable. In this study, reliability was tested for several scales including: total MPA score, items only found on the Waldrop scale, and for each of the body regions assessed.

### 5.1.4.3 Heritability estimation

Intraclass correlations for the behavioral scores, total MPA score, total score using Waldrop items only, and total scores for each body region were obtained using SPSS v.13 for Windows. Intraclass correlations are used to measure the degree of similarity between two variables. In the case of twins, an ICC can be calculated to assess the relationship of a specific variable or trait between the twin pairs. Intraclass correlations are calculated based on the assumptions that the order of the twin pairs does not matter. The theoretical formula for the ICC is:

$$ICC = \frac{\sigma^2(b)}{\sigma^2(b) + \sigma^2(w)},$$

where $\sigma^2(w)$ is the pooled variance within twin pairs and $\sigma^2(b)$ is the variance of the trait between the twin pairs. A one-way model is used to measure the absolute agreement between the pairs. Heritability estimates can be obtained using ICC in the two categories of twins. Heritability ($H^2$) estimates the proportion of phenotypic variance $V_P$, due to the variance in a genotype, $V_G$ represented as:

$$H^2 = 2\,(r_{MZ} - r_{DZ}) = V_G / V_P,$$

where the genetic component ($V_G$) is composed of both additive and dominant effects ($V_A$ + 1.5*$V_D$). In the absence of a dominant effect, narrow sense heritability, $h^2$, can be calculated. Because it is not possible to estimate all components of phenotypic variance using twin data only, the following regularities are used to select a model (represented in Table 6):

**Table 6. Estimates of phenotypic variance based on twin correlations**

| Relationship | Interpretations |
|---|---|
| $r_{MZ} > 2\,r_{DZ}$ | Genetic dominance (or epistasis; shared environment is small) |
| $2r_{DZ} > r_{MZ} > r_{DZ}$ | Additive genes and shared environment (genetic dominance is small) |
| $r_{MZ} = 2\,r_{DZ}$ | Additive genetic effect either monogenic or polygenic |

The shared environmental component (CE) can be calculated by subtracting $H^2$ from the MZ intraclass correlation:

$$CE = r_{MZ} - H^2$$

and the non shared environmental component (SE) is computed by subtracting the MZ intraclass correlation from 1:

$$SE = 1 - r_{MZ}$$

The DeFries-Fulker regression-based model can be used for estimates of heritability based on twin analysis. The DeFries and Fulker regression equation is represented by:

$$S_{ij} = \beta_0 + \beta_3 S_{j-i} + \beta_4 R_{ji} + \beta_5 S_{j-i} R_{ji} + v_{ji},$$

where $\beta_5$ is twice the difference between MZ and DZ regression coefficients and heritability, $h^2$, is a direct estimate of $\beta_5$ under the assumption of an additive model, random mating, non-common environment of a DZ twin that is not correlated with her/her co-twins genes. $R_{ji}$ represents the coefficient of genetic relationship between MZ (given a 1) and DZ (given a 0.5). $\beta_3$ is an estimate of twin resemblance independent of genetic resemblance and is a direct estimate of common environmental influences, $c^2$, and $v_{ij}$ represents a stochastic disturbance term.

## 5.2 RESULTS

### 5.2.1 Sample statistics

Twin pairs ranged from 5 to 12 years of age with a mean age of 8.6 years. Out of the 100 total individuals, 46 were male and 54 were female. There were no age differences between sexes (female: mean = 8.91, sd = 2.47; SEM = 0.34; male: mean = 8.17, sd = 2.40, SEM = 0.34; p = 0.13). From this sample population, 44 zygosity questionnaires were completed: 17 twin pairs (38.6%) were found to be monozygotic, and 27 dizygotic (61.4%). In four families, zygosity questionnaires were not filled out by the parents. For the triplet set, each triplet was compared with the other two, making a total of 3 twin sets. No significant differences in zygosity were found between males and females (p=0.096). Table 7 is a cross tabulation of sex and zygosity for the data set.

**Table 7. Zygosity and sex cross-tabulation**

| | Female | Male | Female/Male | Total |
|---|---|---|---|---|
| MZ | 10 | 7 | n/a | 17 |
| DZ | 6 | 10 | 11 | 27 |
| Total | 16 | 17 | 11 | 44 |

The continuous performance task was administered to every individual with the Kiddie CPT version given to twin pairs who were 5 years of age. Individuals who were not able to read as determined by the "word" portion of the Stroop Effect test did not continue on. A total of 90 individuals completed the Stroop Effect assessment: 42 males and 28 females.

### 5.2.2 Trait statistics

One hundred individuals were assessed for each anomaly. Of the 36 items assessed, 12 have frequencies found to be present in greater than 10% represented in bold in table 8.

Bent index finger had the largest frequency (62%). Seventeen traits had a frequency of less than 4%, consistent with Waldrop's definition of a minor physical anomaly. Table 9 presents summary statistics for each body region (nmj represents the total score for the head, mouth, and jaw region) and mean scores for the behavioral assessments. Behavioral scores are represented by percent interference (%int), number of omission errors (omT) and commission errors (comT) and are corrected for age.

**Table 8. Item statistics for individual MPA traits (n=100)**

| | Frequency |
|---|---|
| Fine hair | .04 |
| **Widows peak** | **.12** |
| **Cowlick** | **.19** |
| Synophrys | .02 |
| Epicanthal folds | .06 |
| Ptosis | .02 |
| Protruding ear | .03 |
| Malformed ear | .01 |
| **Attached Earlobes** | **.44** |
| Bifid uvula | .01 |
| Bulbous nasal tip | .06 |
| **Anteverted nares** | **.27** |
| Smooth philtrum | .08 |
| Thin upper lip | .04 |
| Furrowed tonge | .01 |
| Micrognathia | .04 |
| Grooved chin | .09 |
| **Valgus elbows** | **.28** |
| **Hypermobile joints** | **.20** |
| Abnormal palmar crease | .04 |
| Short fingers | .03 |
| **Tapered fingers** | **.11** |
| Long fingers | .08 |
| Clindodactyly | .06 |
| Fetal pads | .04 |
| **Backwards pointing thumb** | **.37** |
| **Bent index finger** | **.62** |
| Vagus knees | .01 |
| Pes planus | .03 |
| **$2^{nd}$ toe > $1^{st}$ toe** | **.20** |
| $3^{rd}$ toe > $2^{nd}$ toe | .02 |
| Syndactyly of toes | .02 |
| **Sandal gap** | **.55** |
| Overlapping toes | .01 |
| Short toes | .01 |
| **Rotated little toe** | **.22** |

**Table 9. Summary statistics for body regions and behavioral scores**

| | Region | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | head | ear | nmj | foot | hand | eye | total | waldrop | omt | comt | %int | Age |
| N | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 91 | 91 | 80 | 102 |
| Mean | 0.37 | 0.48 | 0.60 | 1.07 | 0.00 | 0.00 | 0.00 | 1.27 | 0.00 | 0.00 | 0.42 | 8.55 |
| Std. Error | 0.06 | 0.05 | 0.07 | 0.08 | 0.10 | 0.10 | 0.10 | 0.10 | 0.11 | 0.11 | 0.01 | 0.24 |
| Median | 0.00 | 0.00 | 0.50 | 1.00 | 0.12 | 0.26 | 0.04 | 1.00 | 0.22 | 0.25 | 0.43 | 8.00 |
| Mode | 0.00 | 0.00 | 0.00 | 1.00 | 0.14 | 0.08 | 0.29 | 1.00 | 0.20 | 0.90 | 0.40 | 5.00 |
| Std. Deviation | 0.56 | 0.54 | 0.67 | 0.78 | 1.01 | 1.00 | 1.01 | 0.96 | 1.01 | 1.01 | 0.11 | 2.46 |
| Skewness | 1.23 | 0.47 | 0.67 | 0.26 | 0.81 | 2.89 | 0.40 | 1.44 | 1.84 | -0.75 | -0.64 | -0.01 |
| Std. Error of Skewness | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.25 | 0.25 | 0.27 | 0.24 |
| Kurtosis | 0.56 | -0.99 | -0.60 | -0.46 | 1.59 | 7.11 | 0.51 | 3.61 | 5.39 | 0.13 | 1.01 | -1.38 |
| Std. Error of Kurtosis | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.50 | 0.50 | 0.53 | 0.47 |
| Minimum | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -1.53 | -2.88 | 0.06 | 5.00 |
| Maximum | 2.00 | 2.00 | 2.00 | 3.00 | 3.97 | 3.50 | 3.19 | 5.00 | 4.55 | 2.24 | 0.64 | 12.00 |

### 5.2.3 Sex differences

Significant differences between males and females were found in omission errors (omT) (p=0.0191) and the total score of the hand region (p=0.0068), as shown in table 8. Scores for the total number of MPA found in each body region are represented in table 10 as well as summary statistics for each of the three behavioral assessment scores (%int, omT, comT).

**Table 10. Sex differences for MPA regions and behavioral assessment scores**

| Variable | Gender | Mean | Std Dev | 95% CI | Sig. |
|---|---|---|---|---|---|
| Total | F | 4.6538 | 1.9492 | (4.1112 - 5.1965) | 0.212 |
| | M | 4.1875 | 1.7462 | (3.6805 - 4.6945) | |
| Head score | F | 0.3846 | 0.5655 | (0.2272 - 0.542) | 0.7883 |
| | M | 0.3542 | 0.5645 | (0.1902 - 0.5181) | |
| Eye score | F | 0.769 | 0.2691 | (0.002 - 0.1518) | 0.9072 |
| | M | 0.0833 | 0.2793 | (0.0022 - 0.1644) | |
| Ear score | F | 0.5385 | 0.576 | (0.3781 - 0.6988) | 0.2627 |
| | M | 0.4167 | 0.4982 | (0.272 - 0.5613) | |
| Nmj score | F | 0.5192 | 0.6414 | (0.3407 - 0.6978) | 0.2089 |
| | M | 0.6875 | 0.689 | (0.4874 - 0.8876) | |
| Hand score | F | 2.1154 | 1.2152 | (1.7771 - 2.4537) | **0.0068*** |
| | M | 1.5208 | 0.8989 | (1.2598 - 1.7819) | |
| Foot score | F | 1.10192 | 0.7273 | (0.8167 - 1.2217) | 0.5191 |
| | M | 1.125 | 0.8411 | (0.8808 - 1.369) | |
| % int | F | 0.4204 | 0.0987 | (0.3896 - 0.4512) | 0.8633 |
| | M | 0.4163 | 0.1154 | (0.3783 - 0.4542) | |
| omT | F | 44.887 | 4.8783 | (43.471 - 46.304) | **0.0191** |
| | M | 42.661 | 3.8978 | (41.461 - 43.861) | |
| comT | F | 51.092 | 12.059 | (47.591 - 54.594) | 0.814 |
| | M | 51.689 | 12.032 | (47.986 - 55.392) | |

*Satterhwaite unequal variances (all others pooled variances)
Bolded items represent statistical significance (p<0.05)

### 5.2.4 MZ-DZ differences

There was no significant difference found between MZ and DZ pairs (Table 11). Head score was the only variable that was significantly different (p=0.011) between MZ and DZ pairs (higher for DZ twins).

**Table 11. Zygosity differences for MPA regions and behavioral assessments**

| Variable | Zyg. | Mean | Std Dev | 95% CI | Sig. |
|---|---|---|---|---|---|
| Total | MZ | 4.5758 | 1.8205 | (3.9302 - 5.2213) | 0.4079 |
| | DZ | 4.2264 | 1.9379 | (3.6923 - 4.7606) | |
| Head score | MZ | 0.1818 | 0.3917 | (0.0429 - 0.3207) | **0.0053*** |
| | DZ | 0.4906 | 0.6084 | (0.3229 - 0.6583) | |
| Eye score | MZ | 0.0606 | 0.2423 | (-0.025 - 0.1465) | 0.7954 |
| | DZ | 0.0755 | 0.2667 | (0.002 - 0.149) | |
| Ear score | MZ | 0.4848 | 0.5075 | (0.3049 - 0.6648) | 0.4464 |
| | DZ | 0.3962 | 0.5313 | (0.2498 - 0.5427) | |
| Nmj score | MZ | 0.5152 | 0.6671 | (0.2786 - 0.7517) | 0.3338 |
| | DZ | 0.6604 | 0.6778 | (0.4736 - 0.8472) | |
| Hand score | MZ | 2.0606 | 1.2733 | (1.6091 - 2.5121) | 0.0994 |
| | DZ | 1.6415 | 1.0395 | (1.355 - 1.928) | |
| Foot score | MZ | 1.2727 | 0.7613 | (1.0028 - 1.5427) | 0.0801 |
| | DZ | 0.9623 | 0.8077 | (0.7396 - 1.1849) | |
| %int | MZ | 0.4118 | 0.0914 | (0.3749 - 0.4487) | 0.7521 |
| | DZ | 0.4205 | 0.1205 | (0.383 - 0.4581) | |
| omT | MZ | 43.614 | 3.8196 | (42.132 - 45.095) | 0.6442 |
| | DZ | 44.126 | 5.1155 | (42.688 - 45.565) | |
| comT | MZ | 49.819 | 14.558 | (44.173 - 55.464) | 0.5229 |
| | DZ | 51.656 | 10.659 | (48.658 - 54.654) | |

*Satterhwaite unequal variances (all others pooled variances)
Bolded items represent statistical significance ($p<0.05$)

### 5.2.5 Trait correlations

Pearson product-moment correlations of minor physical anomalies by body region, total score, behavioral scores for the CPTII and Stroop Effect test, and age are presented in Table 12. The main focus of Table 12 based on the hypothesis, is the correlations between the neuropsychological values and MPA scores.

**Table 12. Pearson correlations for MPA and behavioral assessments**

| | headscore | earscore | nmjscore | footscore | handscore | totalscore | eyescore | waldrop | omt | comt | %int |
|---|---|---|---|---|---|---|---|---|---|---|---|
| earscore | 0.141 | | | | | | | | | | |
| nmjscore | 0.022 | -0.106 | | | | | | | | | |
| footscore | -0.060 | -0.152 | 0.209* | | | | | | | | |
| handscore | -0.016 | 0.054 | 0.128 | 0.126 | | | | | | | |
| totalscore | 0.332*** | 0.272** | 0.499** | 0.506** | 0.667** | | | | | | |
| eyescore | 0.034 | -0.033 | -0.051 | -0.064 | 0.060 | 0.141 | | | | | |
| waldrop | 0.168 | 0.466*** | 0.060 | 0.283** | 0.246* | 0.568*** | 0.429*** | | | | |
| omt | -0.045 | -0.018 | 0.015 | 0.045 | -0.091 | -0.086 | -0.191 | -0.087 | | | |
| comt | 0.216* | 0.021 | 0.253* | -0.037 | -0.009 | 0.124 | -0.111 | 0.050 | 0.067 | | |
| _int | -0.155 | -0.224* | -0.018 | -0.205 | -0.131 | -0.288* | -0.067 | -0.168 | -0.046 | -0.176 | |
| Age | 0.135 | 0.115 | -0.029 | 0.052 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | -0.001 | 0.121 |

*** Correlation is significant at the 0.001 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

### 5.2.6 Heritability

Intraclass correlations were calculated for MZ and DZ twins for the traits studied and are presented in Table 13. Correlations found to be significant are bolded.

**Table 13. Intraclass correlations for MZ and DZ pairs**

| Variable | Zygosity | ICC | 95% CI | Sig. |
|---|---|---|---|---|
| Ear score | MZ | 0.280 | (-0.221- 0.669) | 0.132 |
| | DZ | 0.124 | (-0.264 - 0.480) | 0.266 |
| NMJ score | MZ | 0.101 | (-0.389 - 0.553) | 0.345 |
| | DZ | -0.305 | (-0.611 - 0.084) | 0.94 |
| Eye score | MZ | -0.004 | (-0.475 - 0.476) | 0.504 |
| | DZ | -0.168 | (-0.512 - 0.225) | 0.8 |
| Head score | MZ | 0.211 | (-0.29 - 0.626) | 0.203 |
| | DZ | 0.072 | (-0.312 - 0.439) | 0.358 |
| Foot score | MZ | 0.253 | (-0.249 - 0.652) | 0.158 |
| | DZ | 0.014 | (-0.364 - 0.391) | 0.47 |
| Hand score | MZ | 0.080 | (-0.407 - 0.538) | 0.376 |
| | DZ | -0.036 | (-0.407 - 0.347) | 0.571 |
| Total score | MZ | 0.046 | (-0.436 - 0.513) | 0.428 |
| | DZ | -0.012 | (-0.386 - 0.369) | 0.522 |
| Waldrop score | MZ | 0.160 | (-0.337 - 0.593) | 0.264 |
| | DZ | 0.214 | (-0.176 - 0.548) | 0.138 |
| % interference | MZ | 0.227 | (-0.330 - 0.674) | 0.21 |
| | DZ | 0.596 | (0.239 - 0.813) | **0.001** |
| Omission score | MZ | -0.023 | (-0.535 - 0.511) | 0.529 |
| | DZ | 0.002 | (-0.381 - 0.388) | 0.495 |
| Commission score | MZ | 0.638 | (0.179 - 0.872) | **0.006** |
| | DZ | 0.337 | (-0.053 - 0.640) | **0.044** |

Heritability was calculated for commission scores because intraclass correlations were found to be significant in both MZ and DZ pairs. (p=0.006 for MZ; p=0.044 for DZ). Fisher's z-test comparing MZ and DZ correlations showed the correlations to be significantly different (z=1.18; ns<1.96). Heritability, nonshared and shared environmental influences were calculated with results using both ICC-based equations and DeFries-Fulker regression (DeFries and Fulker 1985). Using intraclass correlations between MZ and DZ twins were calculated as follows:

$h^2 = 2\ (r_{MZ} - r_{DZ}\ ) = 0.602$

$c^2 = 2r_{DZ} - r_{MZ} = 0.036$

$e^2 = 1 - r_{MZ} = 0.362$

A DeFries-Fulker regression analysis was conducted with both twins' phenotypes interchangeably considered the dependent variables, followed by computing the average of the two regression coefficient corresponding to the heritability of the age-corrected omission rate (Table 14). The values obtained are consistent with the results of the ICC-based analysis. The heritability estimates (the average $h^2$=0.557) were not significantly different (Table 15).

**Table 14. DeFries-Fulker regression**

| | Model 1 | | | | Model 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | B | Std Error | Sig | 95% Confidence Interval | B | Std. Error | Sig | 95% Confidence Interval |
| (Constant) | 0.473 | 0.369 | 0.208 | (-0.277 – 1.222) | -0.376 | 0.507 | 0.463 | (-1.405 – 0.654) |
| R [a] | -0.564 | 0.518 | 0.283 | (-1.616 – 0.488) | 0.167 | 0.63 | 0.793 | (-1.114 – 1.448) |
| rcomt.2 | 0.04 | 0.343 | 0.907 | (-0.656 – 0.736) | 0.432 | 0.707 | 0.545 | (-1.004 – 1.868) |
| s2R,s1R [b] | 0.447 | 0.435 | 0.312 | (-0.438 – 1.332) | 0.667 | 0.789 | 0.404 | (-0.936 – 2.271) |

Model 1(2) represents twin 1(2) as the dependent variable
a. The coefficient of relationship (1 or 0.5)
b represents the twins' phenotypes

**Table 15. Heritability estimates from regression analysis**

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | .540(*) | .292 | .229 | .75589 |
| 2 | .517(*) | .267 | .203 | 1.01971 |

(*) Predictors: (Constant), sR, R, rcomt.

### 5.2.7 MPA scale properties (reliability)

Reliability assessment for the 12 items used on the original Waldrop scale was calculated and include fine hair, epicanthal folds, low set ears, attached earlobes, protruding ears, malformed ears, soft ears, clinodactyly, abnormal palmar creases, 3rd toe longer than 2nd, syndactyly of the 2nd and 3rd toes, and a sandal gap (Table 16). Cronbach's α for the 12 item Waldrop scale was 0.185.

**Table 16. Reliability estimates**

**Item-Total Statistics**

| | Scale Mean if Item Deleted | Scale Variance if Item Deleted | Corrected Item-Total Correlation | Cronbach's Alpha if Item Deleted |
|---|---|---|---|---|
| finehair | 1.2300 | .745 | .421 | .021 |
| epifolds | 1.2100 | .794 | .178 | .113 |
| lowset | 1.2700 | .926 | .000 | .186 |
| attached | .8300 | .749 | -.082 | .335 |
| protear | 1.2400 | .932 | -.105 | .226 |
| malear | 1.2600 | .901 | .079 | .173 |
| soft | 1.2700 | .926 | .000 | .186 |
| clinodac | 1.2100 | .753 | .280 | .060 |
| abnpalm | 1.2300 | .805 | .233 | .102 |
| big3toe | 1.2500 | .957 | -.183 | .238 |
| syndactoe | 1.2500 | .876 | .115 | .159 |
| sandal | .7200 | .668 | .010 | .245 |

## 5.3 DISCUSSION

The assessment of minor physical anomalies in the general population potentially as a predictor for behavioral indications has several clinical implications. If it were to be found that children who may be at risk for learning problems or other behavioral variations in the future were discovered early, additional services through the school setting as well as at the home could be implicated.

The goal of this study was to determine if there was a correlation between minor physical anomalies and behavioral assessments and also to determine the heritability of these traits by studying both monozygotic and dizygotic twin pairs. The sample obtained from recruitment at the Twins Day Festival as well as from our own PRIM families resulted in 47 twin pairs and 1 set of triplets. From the sample, there were no significant differences when corrected for sex, and age.

In this study, the frequencies for each of the anomalies that were assessed were obtained. A widow's peak, frontal cowlick, attached earlobes, anteverted nares, vagus elbow, hypermobile joints, tapered fingers, a backwards pointing thumb, a bent index finger, second toe longer than the first, a sandal gap and a rotated fifth toe were frequent in our sample population and found in greater than 10% of individuals. These 12 items, because of their high frequency in the general population, may represent normal phenotypic variants with no clinical signficiance and should not be indicated for use in a scale of items such as the Waldrop scale in predicting behavioral risks.

Among differences between males and females, females were found to have a higher total hand score compared to males. . Females also had a higher rate of omission errors compared to males, i.e., they more frequently failed to respond to the target stimulus. Omission errors are correlated with inattention, which suggests that females at the age range studied have a shorter attention span compared to males, who may be more competitive and try to obtain a higher score on the CPT test. However, no literature to support this hypothesis has been published.

In the differences between MZ and DZ pairs, the total head score was significantly higher in DZ compared to MZ pairs. One possible hypothesis is that DZ twins, who are genetically dissimilar, in contrast to genetically identical MZ twins, may compete in utero for nutrients, thus increasing the risk for developmental stress and MPA.

Several significant correlations were found between total MPA score, body region scores, and the three behavioral measurements. The total head score and total score for the mouth, nose, and jaw region were each correlated with commission errors. Although the sample size was small, these two regions of the head and midface which correlate with inattentiveness demonstrated by commission errors could be a predictor of learning problems, specifically ADHD and other learning problems associated with a short attention span. This finding could also lead to a hypothesis involving disruptions in development of the midline of the facial region as a response to brain growth and development. There may be a specific time period during later embryogenesis, in which some type of environmental influence or exposure may occur which could affect proper facial development (Waddington et al., 1999).

The heritability estimate for commission errors was not significant, due to the small sample size. This estimate, however, is based on significant the intraclass correlations for both MZ and DZ twins, and is comparable in magnitude with heritability estimates obtained for many

psychological traits. Other than the small sample size which is likely contributing to the insignificant findings, there are limitations present in the study. Interrater reliability was not assessed among the two raters of MPA scores. If an initial reliability estimate was established, this value could have provided information regarding the accuracy of the MPA assessment and to add to the validity of the correlations and heritability estimates that were obtained. Prior to the study, definitions were provided on how to recognize each of the anomalies. However, between raters there may have been a particular trait (or traits) on the scale that was assessed in a different fashion compared to the other rater. Without an interrater reliability score before the start of the MPA assessments, it is unknown whether certain traits were scored differently.

In terms of minor physical anomaly assessment, the internal consistency of the scale and items present from the original Waldrop scale were not found to be reliable. This study may suggest that items originally on the Waldrop scale may not be an accurate assessment when attempting to correlate a high MPA scores with behavioral variations. Several studies do not support the validity of the Waldrop scale. Trixler et al. (1997) point out that although research has found positive correlations among minor physical anomalies and cognitive and behavioral deviations, the scale fails to show a distinction between minor malformations that arise during organogenesis from phenotypic variants that appear after organogenesis. Opitz (1985) makes a clear distinction between the use of minor malformations and phenotypic variants. Minor malformations begin and will always be abnormal due to interferences in organogenesis, however phenotypic variants are variations that have no clinical or medical implications. Trixler's group focused on 56 informative variants in schizophrenic and alcohol-dependent patients and made a distinction between minor malformations and phenotypic variants. They found that schizophrenic patients had higher rates of both some minor malformations (furrowed tongue, multiple buccal frenula and hemangioma) as well as phenotypic variants (protruding auricle and large tongue). They recommended that finer diagnostic criteria need to be established for defining differences in trait variations in schizophrenics which may lead to future research in neurodevelopmental reasoning for schizophrenia development.

Sivkov and Akabaliev (2003a) published an article regarding the internal consistency of the Waldrop scale. As in our calculations, they took a sample of 82 healthy subjects to examine correlations among items as well as to determine the reliability of the Waldrop scale. They found that among the healthy subjects, the 19 anomalies of the original Waldrop scale was low

(Cronbach's $\alpha = 0.085$). The highest value obtained for the scale constructed of 10 best items was still very low (0.309). These data are consistent with the results of our study of the 12-item scale (respectively, 0.185, and 0.335 in single-item deletion analysis). The authors suggest that "low correlation among variables to be due to variables such as location, character, period of prenatal origin, developmental heterochronia, and induction process."

From the meta-analysis, it may be reasonable to also assume that MPA are not useful as a diagnostic tool in the general population for behavior variation but instead may be more significant in understanding the etiology of the populations in which MPA are strongly associated with a particular behavioral abnormality, specifically schizophrenia. As the meta-analysis shows, the schizophrenia population has a significantly higher number of anomalies compared to the control populations. A physiological explanation for the development of schizophrenia could be provided by research on specific anomalies that have a higher correlation within the schizophrenic population.

It is important to note that when assessing the general population, specifically children for predictors of behavioral variation, other explanations such as dysmorphology assessment should be considered. At the time that Waldrop and colleagues developed the minor physical anomaly scale, their population of interest was Down syndrome patients. At that time, an extra copy of chromosome 21 as the cause of Down syndrome was not known. Today, there are hundreds of chromosomal and genetic conditions that have been identified linking physical anomalies with behavior patterns. In children with a high number of anomalies who present with learning problems and behavioral variations, the cause may not be prenatal brain injury but instead a single gene mutation or a rearrangement within and among the chromosomes.

Future studies into the biological origin of the MPA in relating and creating a more reliable scale may prove beneficial. This study demonstrated that our scale as well as the scale items from the Waldrop scale did not hold up as a reliable measurement. Closer examination of each individual trait, specifically those that we did not find in more than 4% of the sample or those that are thought to be a true minor malformation compared to a phenotypic variant may be more reliable and have future implications. By capturing children with poor scores on both of our behavioral assessments and re-examining them for minor physical anomalies may prove to be useful in determining which MPA are found in higher numbers in these children.

Future research in predictive behavioral assessment may benefit from investigating other types of physical data. Investigators have studied asymmetrical facial measurements and lateralization which may serve as physical evidence for underlying brain development (Blinkerhorn 1997; Shackelford and Larsen 1997; Leask and Crow 2005). Children who are found to have greater asymmetry may benefit from the use of behavioral assessments as a possible early predictor for behavioral variation.

## 5.4 CONCLUSIONS

In this study, a meta-analysis comparing schizophrenic patients and controls found a significant effect size difference between physical anomaly scores that could not be explained by any study characteristic. The regional analysis also showed no significant differences between the body regions proving that the craniofacial region, as hypothesized, did not contribute a higher total score compared to other body regions.

A neuropsychological characteristic, commission error rate, an indicator of impulsivity, was estimated to be moderately heritable ($h^2$=0.6)

The internal consistency analysis of the MPA scale that was used in the study found that the scale was not statistically reliable.

Researchers believe that when a high number of minor physical anomalies as determined by the Waldrop scale are present, there are implications for behavioral variations. Recently, the literature is focused on the schizophrenic population. Although our study did not confirm the usefulness of screening the general population for minor physical anomalies as a predictor for behavior, the results from the meta-analysis demonstrate that the schizophrenic population has significantly more minor physical anomalies compared to healthy controls.

In future studies, it may be useful to start with the schizophrenic population and examine which Waldrop anomalies are more often found in this population and to further research the biological origin of these traits to formulate a biological explanation for schizophrenic development. By understanding the biological origin of MPA development, this might help in future research with predicting schizophrenia early, which may in turn lead to early treatment and support for this population.

# APPENDIX A

## LIST OF ANOMALIES AND SCORING WEIGHTS

| ANOMALY | WEIGHT |
|---|---|
| **HEAD** | |
| Two or more hair whorls | 0 |
| Circumference out of normal range: | |
| For each age level, > 1.5 σ | 2 |
| > 1.0 σ ≤ 1.5 σ | 1 |
| Fine, electric hair: | |
| Completely awry | 2 |
| Becomes awry after combing | 1 |
| **EYES** | |
| Epicanthus: | |
| Where the upper and lower lids join the nose, point of union is: | |
| Deeply covered | 2 |
| Partly covered | 1 |
| Hypertelorism: | |
| Approximate distance between tear ducts: | |
| For 6- and 7-year-olds, = 3.2 cm | 1 |
| ≥ 3.3 cm | 2 |
| For 8- and 9-year-olds, = 3.3 cm | 1 |
| ≥ 3.4 cm | 2 |
| For 10- and 11-year-old,= 3.4 cm | 1 |
| ≥ 3.5 cm | 2 |
| **EARS** | |
| Low-set ears: | |
| Where top juncture of ear is below line extended from nose bridge through outer corner of eye by: | |
| ≤ 0.5cm | 1 |
| <0.5 cm | 2 |
| Adherent lobes: | |
| Lower edge of ears extended: | |
| Upward and back toward crown of neck | 2 |
| Straight back towards rear of head | 1 |
| Malformed ears | 1 |
| Asymmetrical ears | 1 |
| Soft and pliable ears | 1 |
| **MOUTH** | |
| High palate where roof of mouth: | |
| Definitely steeped | 2 |
| Flat and narrow at top | 1 |
| Furrowed tongue (with steep ridges) | 1 |
| Smooth, rough spots on tongue | 0 |
| **HANDS** | |
| Fifth finger: | |
| Markedly curved inward toward other fingers | 2 |
| Slightly curved inward toward other fingers | 1 |
| Single transverse palmar crease | 1 |
| **FEET** | |
| Third toe: | |
| Definitely longer than second toe | 2 |
| Appears equal in length with second toe | 1 |
| Partial syndactylia of the two middle toes | 1 |
| Gap between first and second toes | 1 |

**APPENDIX B**

**ABOUT YOUR TWINS QUESTIONNAIRE**

## For each question, please fill in completely <u>one</u> of the squares

Are your twins of opposite sex?

☐Yes ☐No

If YES, do not continue

1. Are there differences in the *shade* of your twins' hair?
   ☐ None ☐Only slight difference ☐ Clear difference

2. Are there differences in the *texture* of your twins' hair (fine or coarse, straight or curly, etc?)
   ☐ None ☐Only slight difference ☐ Clear difference

3. Are there differences in the color of your twins' eyes?
   ☐ None ☐Only slight difference ☐ Clear difference

4. Are there differences in the shape your twins' ear lobes?
   ☐ None ☐Only slight difference ☐ Clear difference

5. Did the twins' teeth begin to come through at about the same time?

☐ The twins had matching teeth on the same side come through within a few days of each other

☐ The twins had matching teeth on opposite sides come through within a few days of each other

☐ The twins had different teeth come through within a few days of each other

☐ The twins' first teeth did not come through within a few days of each other

☐ The twins' teeth have not come through yet

6. Do you know of any physical differences between your twins that are not clear from looking at them (e.g. differences in internal organs?)

☐ YES ☐ NO

7. Do you know your twins' ABO blood group and Rhesus (Rh) factors?

☐ YES ☐ NO

If YES, are they:

1st born: ☐ A ☐ B ☐ AB ☐ O ☐ Rh+ ☐ Rh-

2nd born: ☐ A ☐ B ☐ AB ☐ O ☐ Rh+ ☐ Rh-

8. If there are differences between your twins, are they because of anything like problems at birth, an accident or illness?

☐ YES ☐ NO ☐ Don't know ☐ There are no differences

9. As your twins have gotten older, has the likeness between them:

☐ Remained the same ☐ Become less ☐ Become more

10. When looking at a new photograph of your twins, can you tell them apart (without looking at their clothes or using any other clues)?

☐ Yes, easily ☐ Yes, but it is hard sometimes ☐ No, I often confuse them in photographs

11. Do any of the following people ever mistake your twins for each other?

| | | | | |
|---|---|---|---|---|
| Other parent of the twins | ☐ YES, often | ☐ Yes, sometimes | ☐ Rarely or never | ☐ No other parent |
| Older brothers or sisters | ☐ YES, often | ☐ Yes, sometimes | ☐ Rarely or never | ☐ No older sibling |
| Other relatives | ☐ YES, often | ☐ Yes, sometimes | ☐ Rarely or never | |
| Babysitter/daycare | ☐ YES, often | ☐ Yes, sometimes | ☐ Rarely or never | ☐ No babysitter |
| Parents' close friends | ☐ YES, often | ☐ Yes, sometimes | ☐ Rarely or never | |
| Parents' casual friends | ☐ YES, often | ☐ Yes, sometimes | ☐ Rarely or never | |
| People meeting the twins for the first time | ☐ YES, often | ☐ Yes, sometimes | ☐ Rarely or never | |

12. If the twins are ever mistaken for one another, does this ever occur when they are together?

☐ Yes, often ☐ Yes, sometimes ☐ No, almost never ☐ They are not mistaken

13. Would you say your twins:

☐ Are as physically alike as "two peas in a pod" (virtually the same)

☐ Are as physically alike as brothers and sisters are

☐ Do not look very much alike

14. Have you ever been told by a *health professional* (for example doctor, nurse, consulatant) that your twins are identical (monozygotic) or non-identical (fraternal, dizygotic)?

☐ Yes, identical ☐ Yes, non-identical ☐ NO

15. Do *you* think that your twins are identical or non-identical?

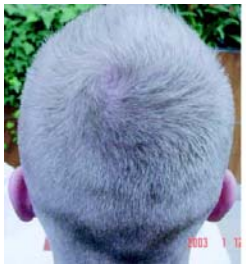
☐ Identical ☐ Non-identical

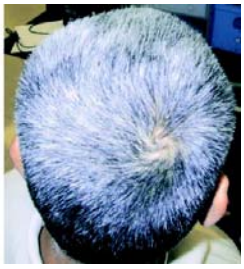
# APPENDIX C

## MINOR PHSYICAL ANOMALY VARIANTS MANUEL FROM THE UNIVERSITY OF PITTSBURGH CENTER FOR CRANIOFACIAL AND DENTAL GENETICS

**1. Fine Hair:*** Hair shafts that are unusually thin; hair may break easily or grow slowly. Feel the hair if necessary to score. Hair may or may not be sparse as well. Please score balding men as "not rated".

**2. Hair Whorl:*** Most people of non-African ethnicity have a single hair whorl located on the upper posterior portion of their scalp. Some people have two whorls (i.e., a double whorl), or, rarely, none. Africans often have a diffuse hair pattern with no obvious hair whorl. African-Americans can show either a hair whorl or a diffuse pattern.





(Klar, 2003)

Counterclockwise Center

Clockwise Right

Score the number of hair whorls (0, 1, 2). For each whorl, score the horizontal position (left, center, right) and the direction that the hair whorls (clockwise or counterclockwise). Males are generally easy to score; for females or males with long hair, it is necessary to search through the hair for a point at which the hair appears to circle. If a female is not willing or able to let down her hair or a person is bald, be sure to note "Not Rated."

**Widows' Peak:**** A slight to large V-shaped hairline at the midline of the forehead.

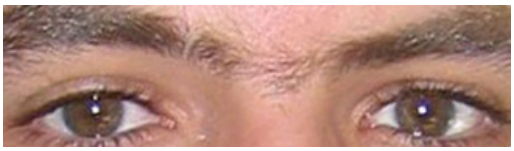
Widow's Peak

Frontal Cowlick

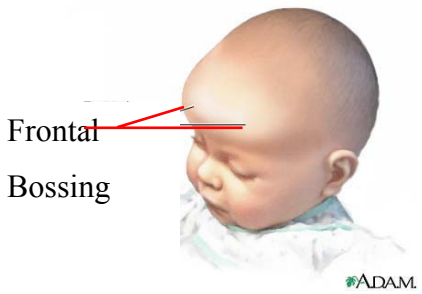
**4. Frontal Cowlick:**** A projecting tuft of hair along the hairline of the forehead that grows in a different direction from the rest of the hair and will not lie flat. If a frontal cowlick is present, score its position laterally (left, center or right). Please note, some people

may wear their hairstyle a certain way to conceal the frontal cowlick, so self-report may be necessary for adults.

**5. Synophrys:*** An excess of hair in the midline between the eyebrows (in extreme cases, a unibrow). People will remove their excess hair; if you suspect some degree of synophrys, you may have to elicit this from the subject.

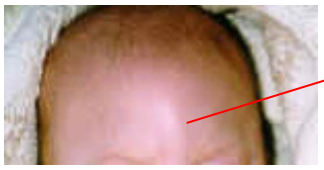


**6. Frontal Bossing:*** Localized hyperostosis in which there is a bony prominence or mound on each side of the forehead, vertically above each eye. Distinguish this from a simple prominence of the forehead, in which the entire forehead bulges through the midline, with no distinct prominences. If frontal bossing is present asymmetrically, examine the head shape from a bird's-eye view and note if there is plagicephaly (asymmetrical flattening of the head) in the "Other" field for the Head and Face.



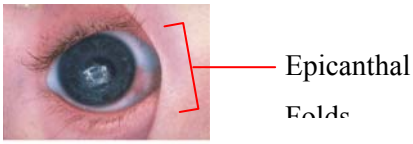

**7. Sloping Forehead:**** Marked backward slanting of the forehead. May be a normal variant, or may be associated with hypoplasia of the frontal lobes and microcephaly.

**8. Metopic Ridge:**** A crest running down the midline of the forehead. A metopic ridge occurs if the metopic suture fuses prematurely. It can range in severity from a minor feature of no clinical consequence to a cranial growth problem needing surgical repair.

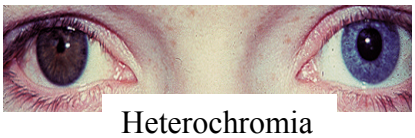



**9.** Other Variants of the Head and Face: Noticeable variations in the head and face include a number of unusual head shapes: plagiocephaly (asymmetric head), brachycephaly (short, broad head), dolichocephaly (elongated head), turricephaly (tower or cone-shaped head), trigonocephaly (triangular shaped head). An unusually large (macrocephaly) or small (microcephaly) head can be scored. Minor variations in hair include unusually sparse or patchy hair and premature greying.

**10. Epicanthal Folds:*** A crescent-shaped fold of excess skin that can cover the inner canthus of the eye. It may originate below the eye or at the margin of the lower lid, and may have a variety of shapes. Epicanthal folds are the norm in Asiatic populations, but can be a minor variant or anomaly in other racial groups.

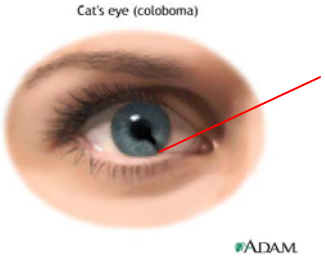



**11. Ptosis:*** Drooping of the upper eyelid, either unilateral or bilateral. Individuals with bilateral ptosis tend to tilt their heads back for better vision. If ptosis is present, note its laterality.

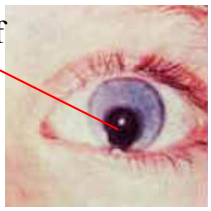


Heterochromia

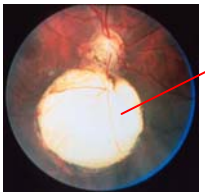
**12. Heterochromia:*** The irises of the eyes are two different colors. Each iris may be completely one color, or one iris may have noticeable segments of two different colors.

**13. Coloboma:*** An apparent absence or defect in some ocular tissue, which may be a notch in an upper or lower eyelid (coloboma of the eyelids), a black intrusion of the pupil into the iris like a keyhole (coloboma of the iris), or an elliptical pupil (coloboma of the retina). If coloboma is present, note its laterality.



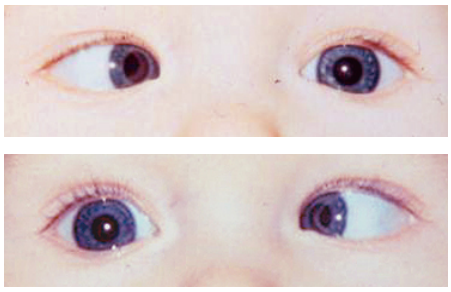

Colobomas of the Iris





Coloboma of the retina (might see an elliptical iris)

**14. Strabismus:****A fine motor disturbance that results in a deviation of the optic axis of the eyes from parallel in any direction of gaze. In convergent strabismus (cross-eyes or esotropia) and divergent strabismus (wall-eyes or exotropia), the angle between the optic axes remains constant no matter where the subject looks. If one eye is used for fixation and the other eye deviates, then the deviant eye is labeled eso- or exotropic. Alternating strabismus occurs when either eye can be used for fixation. If strabismus is observed, note the laterality and the type (eso- or exotropia). With adults, strabismus may have been corrected during childhood, and thus a self-report should be elicited.



Alternating

Exotropia

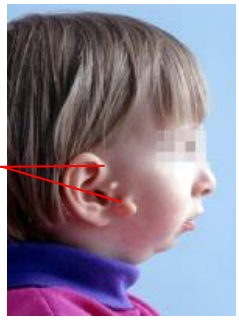
**15. Nystagmus:**** Fine motor disturbance involving "a fine rhythmic oscillation of the eyes" (Bates, 173). Horizontal nystagmus is the most common, and can occur at rest or only when the eyes are moving. It can be likened to eyes watching a moving train. Nystagmus can be so mild as to have no impact on vision or it can severely impede normal vision. To assess for

nystagmus, stand directly in front of the person about 16 inches away and ask them to follow your finger in a continuous line in these directions:

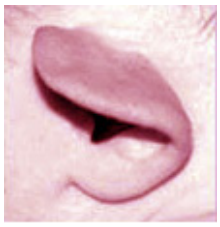
**16. Other Variants of the Eyes:** A rule of thumb for normal spacing between the eyes is one "eye-width" between the two eyes. Significant deviations from this are **hypertelorism** (eyes far apart) and **hypotelorism** (eyes close together). The palpebral fissures should be horizontal. If a line through the inner canthi runs above the outer canthi, then the palpebral fissures are **down-slanting**; if the line runs below the outer canthi, then the palpebral fissures are **up-slanting**.

**17. Preauricular pits or skin tags:*** Small pits or skin tags that appear just in front of the auricle, near the tragus or helical root. The pit will resemble a pin-sized dimple. They can be isolated, or part of a syndrome. If ear tags or pits are present, note their laterality.

Preauricular Skin Tags



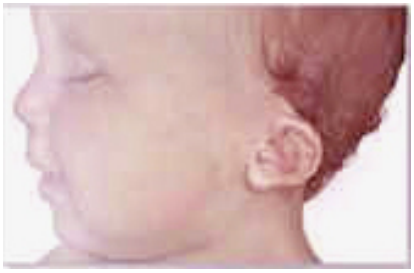
Soft or Lop Ear



**18. Soft ears:*** Ears in which the cartilaginous structure is underdeveloped or partially or completely absent; floppy or lop ears.

**19. Low Set:*** During development, ears migrate laterally and rotate upward from their initial position near the mandible. If this process is disrupted or delayed, the ears will be abnormally positioned, usually both low-set and posteriorly rotated (See #20). To ascertain this, make a visual inspection of the ears with the facial plane vertical, and note if helical root is substantially below the outer canthus.

**20. Posteriorly Rotated:**** If the auricles are rotated more than 20$^{o}$ from vertical, they are posteriorly rotated. They will often be low-set as well (See #19).



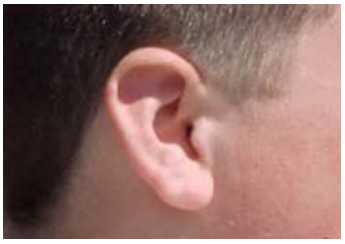
Low-Set, Posteriorly Rotated Ears

Protruding Ears

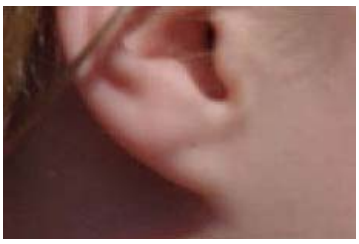
**21. Protruding/Prominent:**** The ears should project out from the head at an angle of 15$^{o}$ or less. Note if they project out at a larger angle, or if they appear unusually large or prominent.

**22. Ear Bump:*****An extra cartilaginous bump can sometimes be present on the back side of the auricle, on the upper posterior portion of the concha. When palpated, it may feel like the head of a pin embedded in the cartilage, or it may be substantially raised. If ear bumps are found, note their laterality.

**23. Attached Earlobes:*** Earlobes may or may not be attached to the side of the head. If the earlobes are attached, note "Yes" for this trait.

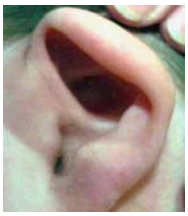


Unattached Earlobe



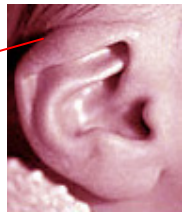
Attached Earlobe

**24. Malformed ear:**** An ear missing some of the normal structural features may be termed a "simple" ear. Simple ears are often present in individuals with Down syndrome, or they can be a minor variant. A relatively common variation of the helix, in which the upper helix is folded over on itself. In the above figure on the right, the overfolded helix results in a cup ear.



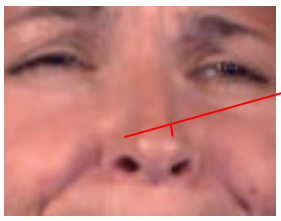
Simple Ear of an Individual with Down syndrome

Overfolded Helix



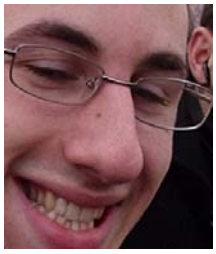
**25. Other Ear Malformations:** Other variant ear shapes include **lop** or **cup ears**, when the muscles holding the auricle to the head are absent or under-developed. **Microtia** is an unusually small ear; this can be essentially normal in shape or show structural problems. Forms of microtia include the **absence of the upper helix**, or a portion of the **upper antihelix**,. If an abnormal ear is observed, describe it in the note field, including its laterality. If ***any*** ear abnormality is noted, ask about hearing loss, since external ear problems can increase the risk of hearing loss.

26. **Bifid Nasal Tip:**** In it's mildest form, a midline vertical indentation or groove through the nasal tip. In severe cases, the nasal tip is completely bifurcated.



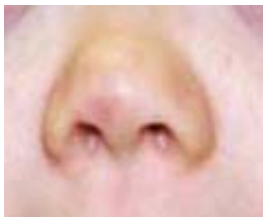
Bifid Nasal Tip

Bulbous Nasal Tip



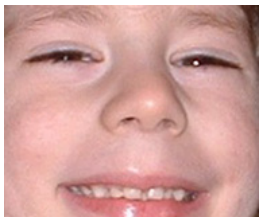
**27. Bulbous Nasal Tip:**** A large, rounded, overhanging nasal tip. This can be a minor variant or part of several genetic syndromes.

**28. Anteverted Nares:*** Nares that are easily visible with the head in a vertical position, because the nasal tip is pulled up. Anteverted nares are common in babies. They can be a minor variant or part of several genetic syndromes.



Anteverted Nares

Smooth Philtrum

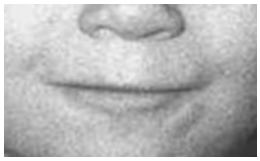


**29. Smooth Philtrum:**** A philtrum lacking the typical groove down the midline. This can be a minor variant or part of several syndromes, e.g., fetal alcohol syndrome.

**30. Angled Columella:†** The base of the nose between the nares, covering the outer end of the nasal septum. The columella usually forms a right angle with the top of the philtrum. An angled columella is connected to the philtrum at an angle noticeably larger than $90^{o}$.

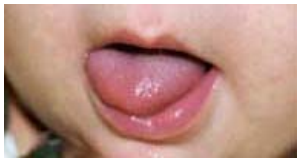
**31. Other Nose Variants:** The nose and philtrum together comprise the mid-face. An overly **long nose** will lead to a **short philtrum**, and vice-versa. The nasal bridge is often flat or low in young children; a **low nasal bridge** in older children and adults is a minor variant. A **broad nasal bridge** resembles a low nasal bridge; examination of the nose in profile distinguishes the two. The nasal tip has several variant forms, including a **beaked nose**, with the nasal tip extending below the alae. **Hypoplastic alae** lead to a pinched appearance of the nasal tip.

**32. Thin Upper Lip:**The upper and lower lips are usually about the same width. When the upper lip is markedly smaller than the lower lip, this is a minor variant.



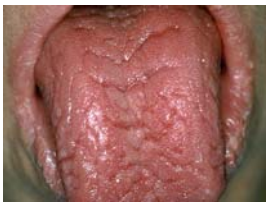
Thin Upper Lip

Macroglossia



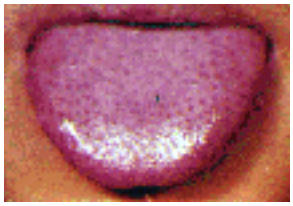
**33. Macroglossia:**** An enlarged tongue. Macroglossia will lead a child to keep their mouth open continuously. It can be part of Down syndrome or other general overgrowth syndromes, e.g., Beckwith-Wiedeman syndrome, but it can also be a minor variant. If you think that the person may have macroglossia but are unsure, ask him if he bites his tongue often.

**34. Furrowed Tongue:*** A tongue with deep grooves or furrows throughout the upper surface.



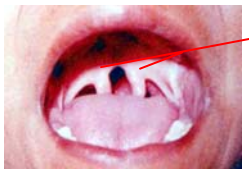
Furrowed Tongue

Tongue With Spots

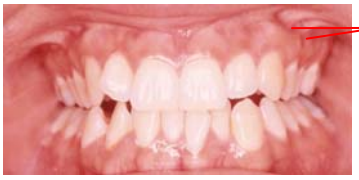


**35. Tongue with Spots:*** A tongue in which the fungiform papilli are unsually large, forming pink spots against the white background of the smaller fusilliform papilli.

**36. Bifid Uvula:*** The uvula represents the posterior midline termination of the soft palate. It may range from a minor midline swelling to a large, fleshy mass, and can occasionally be split, or bifid. A bifid uvula may be an isolated variant, may indicate the presence of a more extensive cleft of the soft palate, or may be part of a genetic syndrome. To determine if the uvula is bifid, visualize the back of the throat. This is usually possible if the subject says "Ah." If necessary, use a glove and depress the tongue with your finger.



Bifid Uvula



Multiple Buccal Frenula

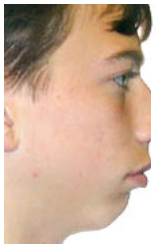
**37. Multiple Buccal Frenula:**** Frenula are small skin or mucous folds that check or anchor movement. Normal frenula in the mouth include the midline frenulum that anchors the underside of the tongue (lingual frenulum) and the two midline frenula between the central incisors and the lips (superior and inferior labial frenula). In addition, there is often a buccal frenulum that connects the gums in each quadrant of the mouth to the cheeks. More than one buccal frenulum in a single quadrant is considered a minor variant, and should be noted. To check for multiple buccal frenula, insert your finger between the cheek and gums, and pull

outward gently, until there is enough tension to bring the frenula into focus. All four quadrants of the mouth should be checked.

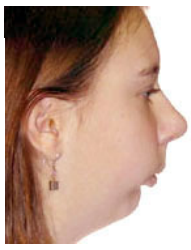
**38. Missing Teeth:**** People occasionally have congenitally missing or extra (i.e., supernumerary, see #43.) permanent teeth. Most often the wisdom teeth (3rd molars) will be missing, but other teeth, such as the 2nd upper premolar, can be commonly missing as well. Extra permanent teeth are also not that uncommon; they are usually extracted. Ask adult subjects if they have extra or missing teeth (not due to an accident). In children who do not yet have their complete permanent dentition, it is very difficult to assess missing or extra teeth. If a congenitally absent or extra tooth is detected, note the quadrant and the tooth (or teeth). [This may have to be assessed by dentally trained research staff.]

**39. Supernumerary Teeth:**** See #38. above.

**40. Micrognathia:*** An unusually small mandible. Infants often appear to have relatively small (micrognathia) or recessed (retrognathia) mandibles, which usually catch up during childhood. If a small jaw persists, this should be noted. It will usually be accompanied by crowding of the lower teeth and an overjet (misalignment of teeth, with upper teeth significantly in front of lower teeth).



Moderate and Severe Micrognathia

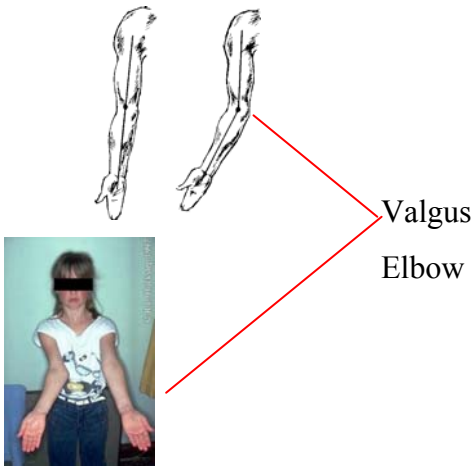


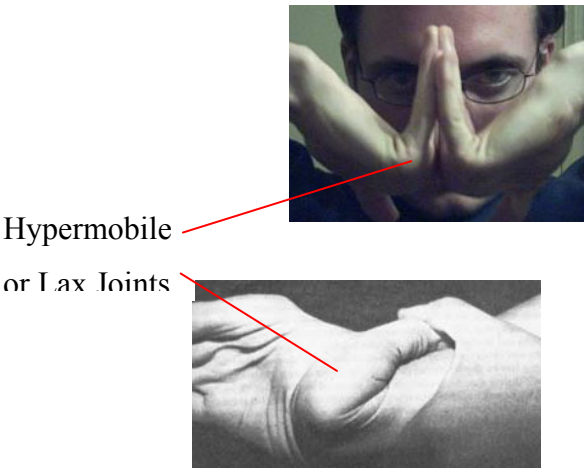
Grooved Chin

**41. Grooved Chin:**** A impression in the midline of the chin, from a dimple to a deep vertical groove.

**42. Other Variants of the Mouth and Jaw:** The mouth can be unusually large (***macrostomia***) or small (***microstomia***), and the mandible can protrude (***prognathia***) or be unusually large (***macrognathia***). ***Lower lip pits*** are occasionally observed. There can be an unusually large gap between the upper central incisors (***diastema***).

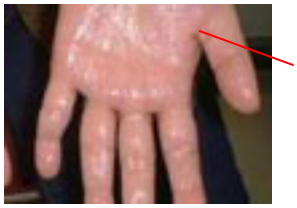
**43. Valgus Elbows:**** With the arms extended at the side and the palms facing forward, the forearm and hands are held slightly away from the body due to the normal carrying angle of the elbow, which is 0 to 15 degrees. A valgus elbow occurs when the carrying angle of the arm is noticeably greater than 15 degrees. It can be a minor variant, part of a larger genetic problem, or due to a poorly healed fracture. Also called cubitus valgus or excessive carrying angle.



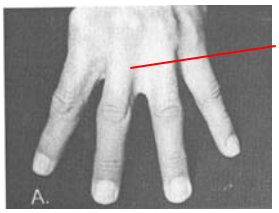




**44. Hypermobile (Loose) Joints:**** Joints that can be moved beyond their normal range, due to ligamentous laxity. If this is widespread and severe, it can be part of several disorders, e.g., Ehlers-Danlos syndrome. It is also a minor variant. Specifically examine the fingers, wrists and elbows. If the fingers can be extended more than $90^{o}$ backwards, score them as hypermobile joints, and note "distal, fingers." If a subject has hypermobile fingers, wrists, or elbows, ask about other joints.

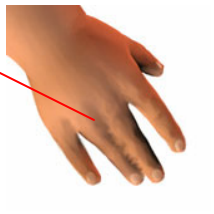
**45. Palmar Hyperhidrosis:†** Excessive sweating, specific to the palms, not due to nervousness.



Palmar Hyperhidrosis



Mild and Complete Cutaneous Syndactyly

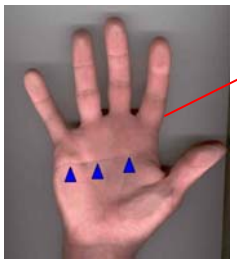


(Aase, 1990)

**46. Syndactyly:**** Fusion of one or more digits into a single mass. The most minor form of this is mild, cutaneous syndactyly, in which the webbed skin between two fingers is unusually extensive. Cutaneous syndactyly can extend the entire length of two fingers. The digit bones can also be fused, although this form of syndactyly is a rarer, major malformation. If present, note which hand and the involved fingers, as well as the extent.

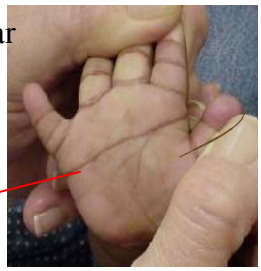
**47. Variant Palmar Creases:** The major palmar flexion creases are noted on the figure of the hand above. Flexion creases can vary greatly. They reflect both the relative sizes of the underlying bones and the degree to which the hands were flexed during prenatal development. The three common variants described in #51 - 53 should be checked and scored if present on either or both hands.

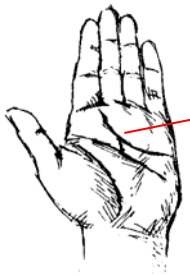
**47a. Single:*** Fusion of the distal and proximal palmar creases into one crease.



Single Palmar Crease

Sydney Crease





Bridged Crease

(Aase, 1990)

**47b. Sydney:*** Complete extension of the proximal palmar crease to the ulnar edge of the palm.

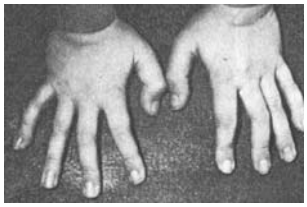
**47c. Bridged:*** A noticeable connecting crease that looks like a bridge, usually between the distal and proximal palmar creases. Often the distal and proximal palmar creases are unusually close together as well.

**47d. Deep:*** Unusually deep palmar creases, not secondary to fat hands.

**48. Short:*** Fingers that are noticeably shorter than the proportions described above. This is a general term that will usually apply to all fingers on both hands. If one finger (either unilateral or bilateral) is disproportionately short, note which one and describe the variation.

**49. Broad:**** Broad fingers may or may not be an unusual length, but they are noticeably wider than normal. If the fingers appear out of proportion, it is important to ascertain the lengths of the fingers relative to the palm, before determining if the fingers are short/long, broad, or both.

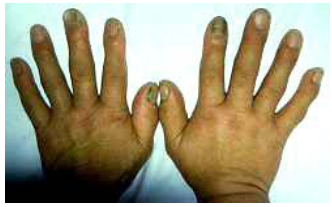
**50. Long/Arachnodactyly:**** If the 3rd finger is almost as long as the palm, score this variant and note "Long." Arachnodactyly is an even more extreme form of long fingers, in which the fingers are longer than the palm, as well as being slender and spider-like.



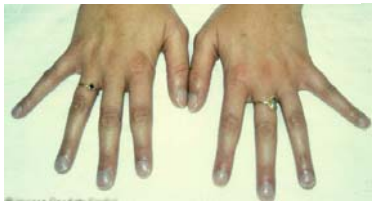
(Aase, 1990)

**Arachnodactyly:**
(Note "Arachnodactyly in #57.)

**Long, Broad Fingers** (Due to acromegaly): Score as "long" in #57 (Note "long;" and score #56 Broad "Yes.")

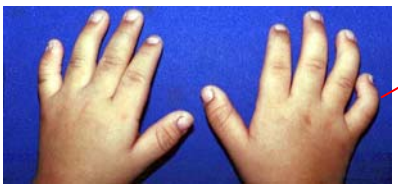


**Long, Tapering Fingers:** (Note "Long" or "Arachnodactyly" for #57, and Score # 58 Tapering "Yes.")



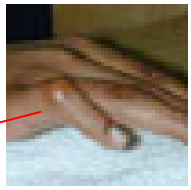
**51. Tapering:*** If the fingers taper from the proximal to distal phalanges, so that they look more cone-like than cylindrical, score this trait "Yes."

**52. Clinodactyly:*** This term applies to fingers that are bent in the plane of the palm, usually toward the midline. It is most commonly seen as a deviation of the 5th finger towards the 4th.



Clinodactyly

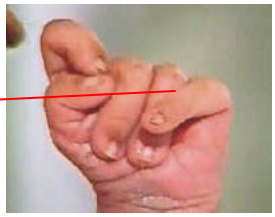
Camptodactyly



**53. Camptodactyly:**** When fully extended, the fingers should be straight, at 180°. If they cannot fully extend, they are exhibiting camptodactyly. This can occur for a single finger, or apply to them all.

**54. Overlapping:*** Clinodactyly can be so extreme that the fingers overlap. If this occurs, note which fingers are involved. This is present in some trisomies, as well as being an isolated variant. If a subject has unusual hands or some degree of clinodactly, have them make a fist, and see if the fingertips overlap.

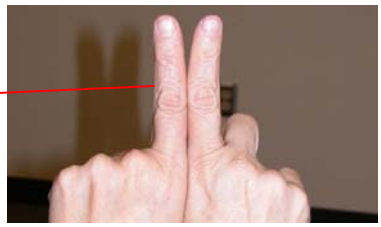
5th Finger overlaps 4th



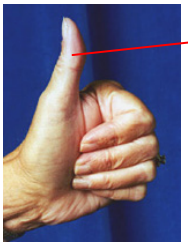
**55. Fetal Pads:**** By the eighth week of embryologic development, large pads of soft tissue appear on the palms and soles, and on the ventral surfaces of the distal fingers and toes. The shape of the distal pads will determine, in part, the dermatoglyphic patterns that will be laid down by the 19th week of gestation. The fetal pads slowly shrink, and are usually gone before birth. Occasionally they persist on the fingertips and toes, and should be scored.

**56. Bent Index Fingers:***** Have the subject bring their 2nd fingers together while keeping them straight. Make sure that the fingers touch at the knuckle and at the proximal interphalangeal joint. If the fingers bend away from each other distally, then the fingers are bent, and this trait should be scored "Yes." (NOTE: This is the common situation; straight fingers are unusual.)

Bent Index Fingers

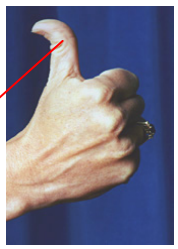


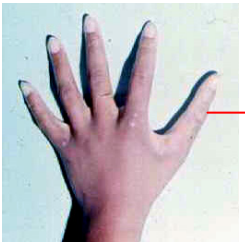
**57. Hitch-Hiker's Thumb:**** Have the subject give a thumbs-up, fully extending their thumb. Note whether there is a backwards projection of the finger tip, like a hitch-hiker.



Thumbs Regular

Hitchhiker

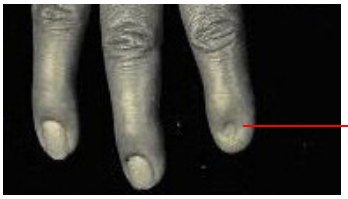




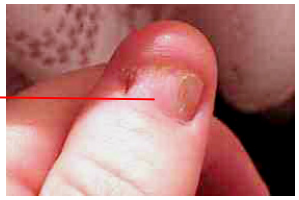
Triphalangeal Thumb

**58. Abnormal Thumb:**** Thumbs can have many minor variations in shape and position on the hand. Some of the more common include an extra phalange (***triphalangeal thumb***, which looks more like a finger, see figure above right), an underdeveloped thumb (***hypoplastic thumb***), and a **proximally placed thumb**. If you note an unusual thumb, describe it in the note box.

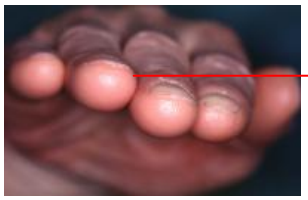
**59. Hypoplastic:*** Small, underdeveloped fingernails, which can be diminished in both length and width, or can be unusually short.

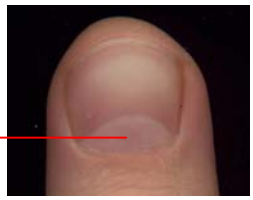


Hypoplastic Fingernails



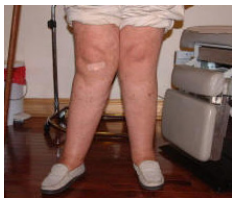
**60. Hyperconvex:*** When looking end-on at a finger, the arc of the fingernail is broad in children, and often flat in adults. If the arc is pronounced, as often happens with narrow nails, then score the nail as "hyperconvex."

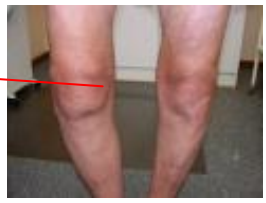



**61. Other Variants of the Hands and Arms:** Note if the hands are unusually **large** or **small**. One of the more common minor malformations of the hand is the presence of an extra finger (**polydactyly)**, which can occur either on the side of the thumb (**preaxial polydactyly**) or the little finger (**postaxial polydactyly**). Extra digits will likely have been removed, and there may not be any sign of them. However, subjects will likely volunteer such information during the course of this exam. Incomplete to absent mobility of the interphalangeal joints (**symphalangism**) may be detected by the **absence of interphalangeal flexion creases**. Other variants of the fingernails include spoon nails (**koilonychias**—common in infants), in which the main part of the nail is depressed and the distal, free nail turns upward, unusual **nail grooves**, **potter's thumb**—a very short and broad distal phalanx with an overly large thumb nail, and unusually **thin** or **thick nails**. Many of these conditions can be caused by specific illnesses or trauma, which must be excluded before they are scored.

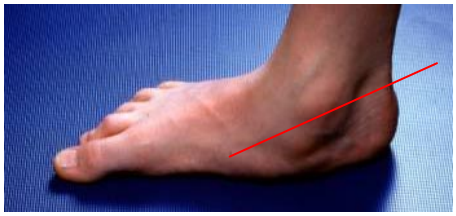
**62a. Knock-Kneed (Genu Valgum):**** When the subject is standing with their legs together, they should be able to have their feet close together with their knees touching. If their lower legs show a noticeable outward deviation so that they can't bring their feet together, score "knock-knee" as present.



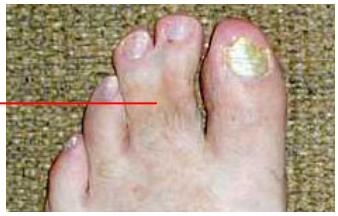
**62b. Bowlegged (Genu Varum):**** The opposite deviation of the legs from knock-knee. The patient can bring their feet together, but their knees will not touch. Instead, their legs will bow outward noticeably. This can be due to diseases or nutritional deficiencies during childhood, which should be ruled out before scoring.

**63. Pes planus (Flat Feet):**** A benign laxity of the supporting ligaments of the foot, so that the longitudinal (plantar) arch of the foot is very low or completely absent. This is usually bilateral; if it is present on only one foot, ask if there was an injury.



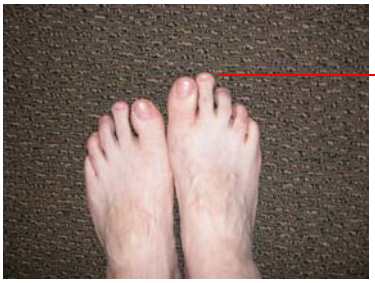
Pes Planus

Complete Cutaneous Syndactyly, 2 – 3 Toes, Left Foot



**64. Syndactyly:*** Fusion of one ***Variants of the Toes:*** Normal feet have toes of varying shapes and sizes. The toes are numbered from 1 (big toe) to 5 (pinkie). In general, the big toe (**hallux**) is much enlarged, compared to the other toes. It is also usually the longest toe, with the second toe equal to, or shorter than the first, and the rest of the toes progressively smaller.

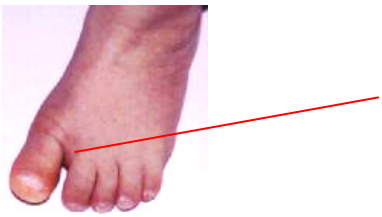
**65. 2nd Toe > 1st Toe**:** A very common variant occurs when the 2nd toe is longer than the first. This should be noted for each foot separately, only when the 2nd toe is noticeably longer.



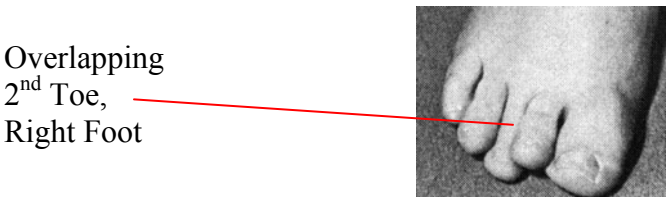
2nd Toe Longer than 1st Toe, Right Foot Only

**66. 3rd Toe > 2nd Toe:**** Another, less common variant occurs when the 3rd toe is longer than the 2nd. Again, score this separately for each foot, and only if it is noticieable.

**67. Sandal Gap:*** A large gap between the 1st and 2nd toes, almost as if the subject is wearing flip-flops.




**68. Overlapping:*** If one of the metatarsal bones is a bit short, it can result in toes that are overlapping or overriding, on an otherwise normal foot.

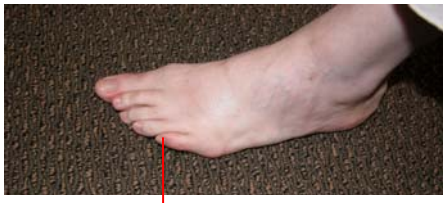



(Aase, 1990)

**69. Short:*** Toes that are noticeably shorter than usual. This is a general term that will usually apply to all toes on both feet. If one toe (either unilateral or bilateral) is disproportionately short, note which one.

**70. Fetal Pads:**** By the eighth week of embryologic development, large pads of soft tissue appear on the palms and soles, and on the ventral surfaces of the distal fingers and toes. The fetal pads slowly shrink, and are usually gone before birth. Occasionally they persist on the fingertips and toes, and should be scored, if there is a noticeable extra pad of tissue on the bottom of the toes.

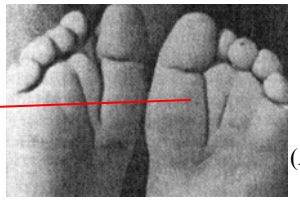
**71. Rotated Little Toe:***** Occasionally the 5th toe is rotated along its axis so that the toenail points away from the foot in a direction parallel to the floor. This can occur on one or both feet, and may be an autosomal dominant trait.



Rotated Little (5th) Toe

**72. Deep Creases on Soles:*** Newborns will sometimes have a **deep longitudinal plantar crease** if the foot has been compressed in utero. This should fade with time. Deep creases in older individuals, either the plantar crease or others, should be scored. They can be due to several genetic syndromes, or be a minor variant.

Deep Plantar Crease



(Aase, 1990)

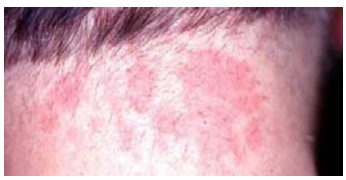
**73. Hyperconvex Toenails:*** As with the fingernails, the arc of the toenail is basically flat or only slightly curved. If there is a pronounced arc, score the toenail as "hyperconvex."

**74. Other Variants of the Feet and Legs:** Infants can be born with **congenital dislocation of the hips**, which requires correction and should be known by the parents. Infants can also be born with a number of isolated positional feet abnormalities, i.e., **clubfoot** or **talipes**, which can be deformed in different directions, and which may have been corrected. Ask to see if the parents know the type of clubfoot. Toes can often show **clinodactyly** or **polydactyly**. Sometimes the first toe is overly broad, known as a **broad hallux**. **Hammertoes** are toes which are dorsiflexed (flexed upward).

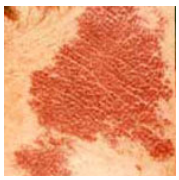
**Variation in the Skin**

**75. Dry Skin:**** Ask subjects if they have a problem with dry skin, that is more severe than the problems many people have during the winter. Is their skin unusually cracked? Do they have a problem even with extensive use of lotions? Have they been to a dermatologist. They may have a problem limited to a specific area of the body, such as one hand, which should be noted.

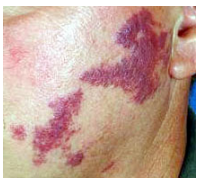
**76. Hemangiomas:**** A hemangioma is a benign tumor made up of new-formed blood vessels, i.e., a birthmark. There are many types, including 1) mild flat pink-red zones of superficial dilated capillaries (nevi flammeus) that can affect up to half of newborns and usually fade in a few years (**Stork bites** on the back of the neck); 2) regional dilation/engorgement of mature capillaries (nevi flammeus, or **port-wine** stains); 3) localized persistence of angioblastic cells that create raised vascular nevi (capillary hemangiomas or strawberry nevi). These may increase in early childhood and then spontaneously regress over several years; 4) deep cavernous hemangiomas that can persist. Ask if the subject has, or did have, a reddish birthmark, and note the location and approximate size.

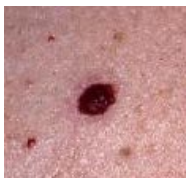


Stork Bite

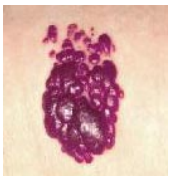


Port-Wine Stains



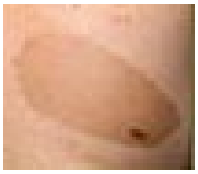


Cherry Spot

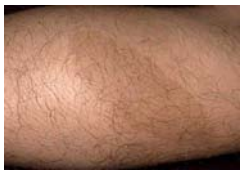


Strawberry

**77. CALS (café-au-lait spots):**** Flat, pigmented regions that range in size from a few millimeters to several centimeters. They are usually only slightly darker than the surrounding skin, and may or may not have well defined borders. They are common in different populations (5% in Caucasians; 15% in U.S. Blacks). If an adult subject has 6 or more CALS over 15 mm, they should be referred to a geneticist for a discussion of possible neurofibromatosis I.



Large CALS

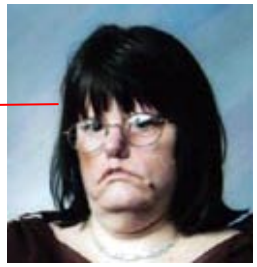


**Neuromuscular or Other General Variation**

**78. Expressionless/Dull Face:**** An expressionless face with virtually no muscle movement may be due to a general or localized neurological problem, e.g., Moebius syndrome, or to muscle weakness. An asymmetry in facial expression is also possible, due to a variety of neurological impairments. If a subject has a noticeably expressionless, droopy, or asymmetric face, discuss this, to see if they have had medical care.

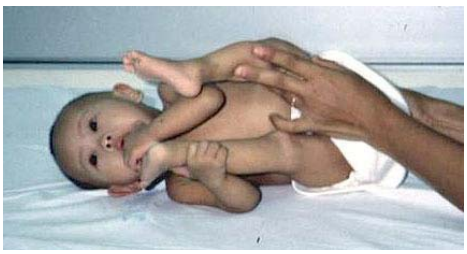
Expressionless Faces
Moebius Syndrome

Myotonic Dystrophy



**79. Hypotonia:**** Low muscle tone. In infants this is manifest as a "floppy baby," who will hang limply when supported, and not show any resistance to being held. As a hypotonic child grows, they may sit or walk at a later age, and may eventually exhibit a slumped sitting posture or an abnormal gait, or have generalized joint laxity. They may have trouble with gastroesophageal reflux.

Hypotonic
Infant



**80. Tremor:**** This should be a relatively slow moving, continuous (or intermittent, but not a single twitch or tic) involuntary motion, apparent to an observer. If present, note the subject's description/name for it.

**81. Developmental Delay:**** This is a complex trait marked by slowness in making developmental milestones, along with learning problems and possible mental retardation. Delay can be in motor development, cognitive areas, language acquisition, or any combination of these areas. For the purposes of this questionnaire, this trait should only be scored if the subject or their parents are aware of specific delays or learning problems that were worrisome or required intervention. Thus use subject/parental report to determine if a noticeable delay has occurred or is ongoing.

**82. Sneezes from Dark to Light:***** Some people have an autosomal dominant condition known as the ACHOO syndrome, which causes them to sneeze when they move from a dark room or tunnel into a bright light. Usually they sneeze only once or twice, but some people sneeze so many times that they have problems driving through tunnels.

**83. Motion Sickness:***** Ask subjects if they have motion sickness, including sea sickness or trouble driving or on airplanes. Do NOT score this for amusement park rides, which can make many people sick. For adults, this is usually easy to ascertain; children can be more difficult, since many children have some motion sickness that they will grow out of. If a child says "Yes," ask how severe it is: Have they thrown up? Does their family have to plan driving to help them? What do their parents think about their motion sickness?

**84. Other Variations:** This is a catchall category for anything else that comes up during the evaluation, but does not fit one of the other traits. If you find something to score, note that it is present, and name the variation or describe it in detail, including laterality, if relevant.

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