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The effects of congenital heart disease on brain development in individuals with 22q11.2 deletion syndrome

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Thesis

THE EFFECTS OF CONGENITAL HEART DISEASE ON BRAIN DEVELOPMENT IN INDIVIDUALS WITH 22Q11.2 DELETION SYNDROME

by

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DEDICATION

I would like to dedicate this work to my parents Andy and Sheryl Urtusuastegui, my grandmother Mary Brining, and Dr. Sarah Morton

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I would like to first and foremost thank Dr. Sarah Morton for allowing me to become a member of her lab, and her invaluable guidance and support throughout this project. I would also like to thank the amazing team of people who all contributed to this project: Cesar Alves, Stefanie Perrier, Ankush Kesri, Ellen Grant, Sheng He, and Yangming Ou. Lastly, I would like to thank Dr. Gwynneth Offner at BU school of medicine for her many contributions throughout my grad school journey.

THE EFFECTS OF CONGENITAL HEART DISEASE ON BRAIN DEVELOPMENT IN INDIVIDUALS WITH 22Q11.2 DELETION SYNDROME

MATTHEW URTUSUASTEGUI

ABSTRACT

Background:

22q11.2 deletion syndrome (22q11.2 DS) is a chromosomal microdeletion disorder, occurring in 1:4000 births, and often associated with neurologic and cardiologic manifestations. This autosomal dominant disorder is caused by a heterozygous deletion which occurs *de novo* in approximately 90% of cases. People with 22q11.2 DS can have variable penetrance and phenotypic heterogeneity, and most individuals with 22q11.2 DS require specialty care throughout their lives. Due to the high prevalence of neuropsychiatric disorders, many studies have assessed how brain development is altered by deletion of the 22q11.2 region. However, most studies agree that the haploinsufficiency of genes in the 22q11.2 region alone is insufficient to explain the broad spectrum of phenotypes and varying levels of severities observed. Despite this consensus however, there are limited studies of human development

that explore the correlations between non-neurological diagnoses and brain development in people with 22q11.2 DS. Specifically, the association between congenital heart disease (CHD) and structural brain development has been infrequently studied in 22q11.2 DS, despite CHD being present in ~75% of people with 22q11.2 DS and the main cause of mortality in this population.

Objective:

The objective of this study is to investigate correlations between clinical diagnoses and structural brain development in people with 22q11.2 DS. We aim to use analysis of brain magnetic resonance imaging (MRI) data to identify potential correlations between non neurological phenotypes such as CHD and brain development in people with 22q11.2 DS.

Material and Methods:

Individuals with 22q11.2 DS who had obtained a clinical brain MRI were identified via electronic medical record query. Confirmation of 22q11.2 DS was obtained via review of genetic testing, and MRI data availability was confirmed within radiology records. Retrospective chart review was conducted to extract clinical history of CHD and other diagnoses. Brain MRI data was quantitatively assessed with an age prediction deep learning algorithm. The difference between the true

chronological age of the individual and estimated brain age (by the algorithm) was calculated and labeled as "brain age gap" (BAG). The distributions of the BAG values for individuals with CHD and those without CHD were compared via Mann-Whitney U Test. A correlation matrix was then run to calculate Pearson coefficients between each of the individual phenotypic variables collected, as well as between the individual phenotypic variables and BAG.

Results, Discussion, and/or Key Learning:

We identified a significant difference in the median values and distribution sizes of brain age gap among people with 22q11.2 DS, depending on the presence or absence of CHD. This pilot experiment highlights a large potential role for CHD in brain health among people with 22q11.2 DS. We did not find any significant correlation between the specific subtype of CHD and age prediction values. We also did not find any strong correlations between the non-neurological phenotypes themselves.

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LIST OF ABBREVIATIONS

22q11.2 DS ..... 22q11.2 Deletion Syndrome

AI ..... Artificial Intelligence

BAG..... Brain Age Gap

BU..... Boston University

CHD ..... Congenital Heart Disease

DTI ..... Diffusion Tensor Imaging

EMR ..... Electronic Medical Record

IQ ..... Intelligence Quotient

IQR ..... Inter Quartile Range

LCR ..... Low Copy Repeats

MAPCA..... Major Aortopulmonary Collateral Arteries

MRI ..... Magnetic Resonance Imaging

SCA..... Subclavian Artery

CHAPTER ONE: Overview of 22q11.2 Deletion Syndrome

Background and Epidemiology

Before the Human Genome Project, many genetic conditions were identified and mapped due to their high prevalence among infants with congenital anomalies. One of these, 22q11.2 deletion syndrome (22q11.2 DS), is the second most common genetic cause of congenital heart disease (CHD) [1]. The 22q11.2 region of the human genome is a region of 7.4 million base pairs on the long arm of the 22nd chromosome, containing 46 known protein coding genes, 7 known miRNA encoding genes, and 10 non-coding RNAs (Figure 1) [1, 2, 3]. This region contains several low copy repeats (LCRs), which greatly increase its susceptibility to recombination error during meiosis [4]. In fact, ~90% of 22q11.2 DS cases are due to a *de novo* mutation [1, 2, 3].

The deletion occurs in approximately 1:4000 birth, and is the most common microdeletion order currently known [2, 4, 5]. The size and location of the deletion that occurs in 22q11.2 DS can be variable. The most common deletion, which occurs in ~85% of people, is a 2.54Mb deletion occurring from LCR A to LCR D. A smaller subset of approximately 5% of people have a 1.5Mb deletion which covers LCR A to LCR B. The remaining 10% of people with 22q11 DS have other

deletion types, including LCR B- LCR D nested deletions and distal deletions of LCR D-H [1, 2, 3, 6].

Manifestations of 22q11.2 DS are seen across several body systems as well, including immune, endocrine, cardiovascular, genitourinary, neurological, musculoskeletal, palatal and more. What's more, is that each of these phenotypes can occur to differencing levels of severity [1, 2, 3]. The question of why such heterogeneity exists in people with 22q11.2 DS is something that still remains under investigation.

While not a complete list, common phenotypes and lab findings that have been documented in people with 22q11.2 DS, as well as their approximate frequencies, are shown below in Tables 1 and 2.

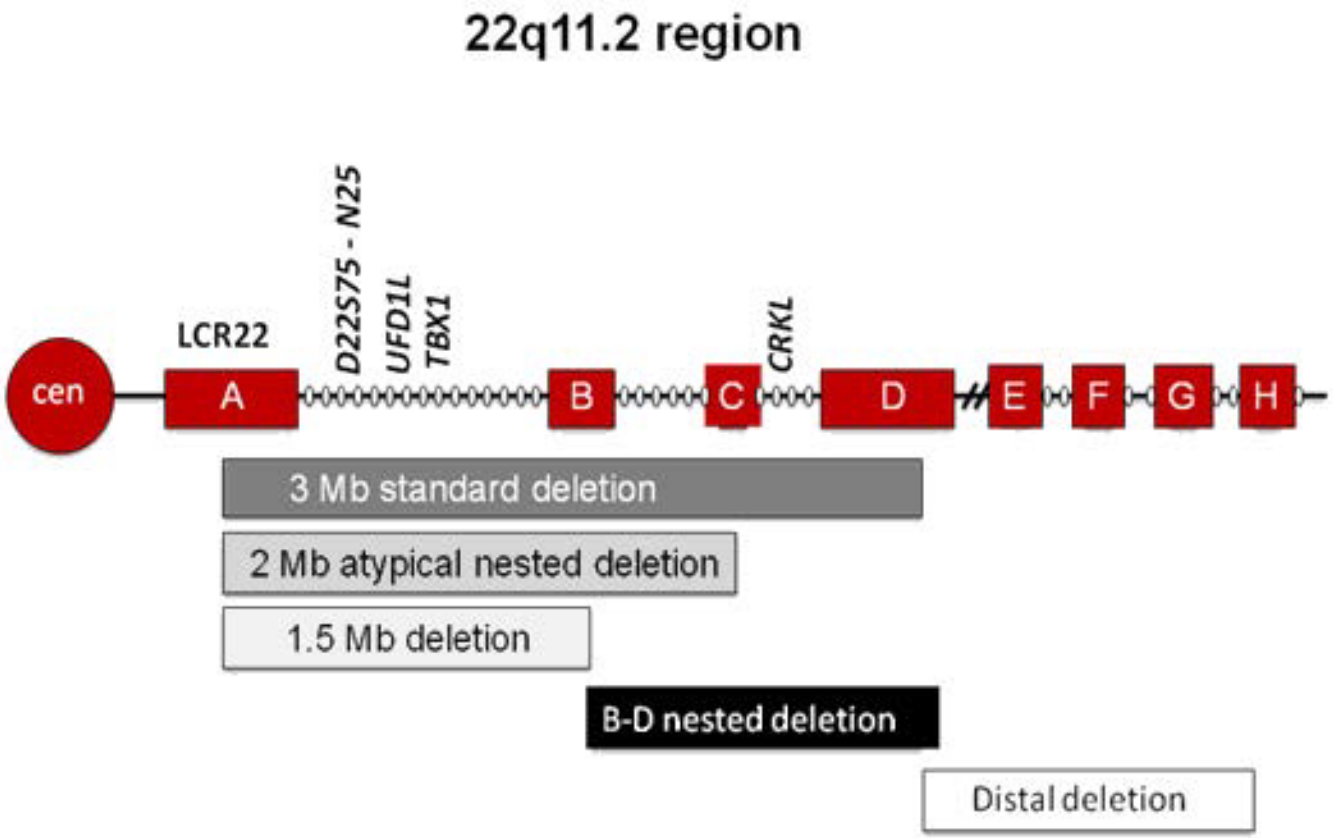


Figure 1: Visualization of 22q11.2 Region and Deletion Sizes. Figure adapted from McDonald-McGinn & Zackai [2008][(1)].

Table 1: Common Clinical Phenotypes of 22q11.2 DS. When enough data was available, multiple reported frequencies were consolidated to give a single approximate frequency. If phenotype was reported without a numerical value, percentage was listed as "unspecified"

Phenotype Observed	**Approximate Frequency in 22q11.2 DS Population**
Congenital Heart Disease (CHD)	75% [1, 3, 7, 8, 9]
Velopharyngeal Abnormalities	71% [1, 3]
Immune Deficiencies (predominantly T-Cell)	77% [1, 3]
Developmental Delay or Learning Difficulties	77% [1, 3]
Schizophrenia	25% [1, 2, 3]
Genitourinary Tract Anomalies	24% [1, 3]
Hearing Loss	Unspecified [1]
Enamel Disturbances	46% [7, 10, 11]

Table 2. Potential Lab Findings in 22q11.2 DS. When enough data was available, multiple reported frequencies were consolidated to give a single approximate frequency. If phenotype was reported without numerical value, percentage was listed as "unspecified"

Laboratory Findings Observed	**Approximate Frequency in 22q11.2 DS Population**
Hypoparathyroidism and Hypocalcemia	57% [1, 3, 8]
Growth Hormone Deficiency	Unspecified [1]
Hypothyroidism	Unspecified [1]

Clinical Manifestations of 22q11.2 DS

Neurological Phenotypes of 22q11.2 DS

In the past 25 years, over 100 studies have focused on neurological and psychiatric manifestations among people with 22q11.2 DS, due to the pertinent nature of these characteristics in this population. It has now been well established that substantially higher occurrence of schizophrenia, learning disabilities, attention deficit hyperactivity disorder (ADHD), autism, and other psychiatric features are seen in individuals with 22q11.2 DS compared to controls [1, 2, 3, 5, 12].

In addition to a higher prevalence of psychiatric disorders, people with 22q11.2 DS often have the following structural differences on brain MRI when compared to healthy controls: whole brain volume reductions, decreased hippocampal volume, increased cortical thickness, increased presence of cavum septum pellucidum, and reduced cortical surface area [2, 13, 14, 15, 16, 17].

Future Neuroimaging with 22q11.2 DS

The next frontier in understanding the impact of 22q11.2 DS on brain development and health is to examine the microstructural differences in white matter connectivity via diffusion tensor imaging

(DTI) MRI. So far, a general trend of reduced axial, radial and mean diffusivity has been observed in people with 22q11.2 DS, indicating a distinct difference in white matter (WM) structure in people with 22q11.2 DS compared to controls [5, 12, 18]. However, what those phenotypical and biochemical differences are remains uncertain, as multiple factors can contribute to differences in neuronal diffusivity values (axonal diameter, demyelination, axonal damage, etc.) [5, 12].

Resting state functional MRI has also been explored in an attempt to provide better insight on the functional aspect of neurodevelopment in people with 22q11.2 DS. Results so far have indicated significant functional differences in neurological pathways between 22q11.2 DS individuals compared and controls. Most interestingly, as opposed to global increases or decreases in activity levels, increased or decreased activation is highly specific to the region or psychological function of the functional network in question [19, 20, 21].

Resting state functional MRI has a high promise for future applications and insights, due to a lack of complicated tasks for patients, and the ability to analyze connectivity of multiple specific functional tracts simultaneously. Similar DTI studies though, more

studies will be needed in order to properly understand the clinical implications of these findings.

CHD in 22q11.2 DS

22q11.2 DS is the second most common genetic cause of congenital heart disease behind trisomy 21 [1, 3]. As previously shown in table 1, CHD is present in approximately 75% of people with 22q11.2 DS, making it one of the most consistent phenotypes observed in these individuals. Furthermore, CHD is the primary cause of mortality among individuals with 22q11.2 DS, accounting for ~80% of all deaths, and a median age of death between 3-5 months [1, 22, 23, 24].

The presence of CHD also plays a crucial role in the diagnosis of 22q11.2 DS, as approximately 10% of newborns diagnosed with CHD who have genetic testing done will have a 22q11.2 deletion [25]. Due to this increased frequency of genetic testing in people with CHD, the median age of diagnosis of 22q11.2 DS is approximately 2.6 months in those with CHD, and 3.1 years in those without CHD [22].

Table 3 below shows the approximate frequencies of CHD subtypes seen in people with 22q11.2 DS. While these approximate overall frequences are fairly well documented, it is not conclusively known if these frequencies vary substantially between people with

22q11.2 DS who have different ethnic backgrounds or deletion sizes/regions.

Table 3: Prevalence of Specific CHD Phenotypes in the 22q11.2 DS Population. The table below shows the approximate prevalence of CHD subtypes within the 22q11.2 DS population.

*Note that Ventricular Septal Defect is one of the 4 components of Tetralogy of Fallot, so the approximate percentage of individuals with isolated VSD and without TOF is ~30%

CHD Phenotype	Approximate Frequency in 22q11.2 DS Population
Ventricular Septal Defect*	80% [9, 26]
Tetralogy of Fallot	50% [9, 25, 26, 27]
Pulmonary Atresia	15% [9, 26]
Major Aortopulmonary Collateral Artery	12% [26]
Right Aortic Arch	28% [26, 27]
Truncus Arteriosus	6% [9, 25, 26, 27]
Atrial Septal Defect	7% [9, 22, 26]
Patent Ductus Arteriosus	7% [22, 26]
Interrupted Aortic Arch	12% [9, 25, 26, 27]
Double Outlet Right Ventricle	5% [9, 25, 26]

CHAPTER 2: Factors in Brain Development

CHD and Brain Development

It has been well demonstrated that people with CHD face an increased risk of neurodevelopmental delay or disability [28].

The recent development of fetal MRI has proven crucial in understanding the specifics of CHD and neurodevelopment. It is now understood that fetal brain growth substantially drops off in the third trimester in fetuses with CHD compared to fetuses without CHD [28, 29, 30]. This drop off is hypothesized to be because the structural changes in the fetal heart cannot support the metabolic demand of the copious brain growth that occurs in the third trimester [28, 30].

During typical fetal development, approximately 25% of ventricular output is estimated to go to the brain, and the brain is estimated to be responsible for approximately 50% of total oxygen consumption in the developing fetus [31]. This is particularly meaningful in the context of CHD, since it has been established from fetal MRI that fetuses with CHD have lower cerebral perfusion and oxygen consumption than those without [30]. Expectedly, this lower oxygenation of developing neural tissues has in turn been correlated with increased risk of neurodevelopmental impairments [28, 30].

Structurally, approximately 25% of children with CHD are microcephalic at birth [29]. Other structural manifestations include: reductions in total brain volume, dysmature myelination, altered cortical folding, and ventricular asymmetry in CHD fetuses compared to controls [28, 29, 30, 31, 32]. Biochemically, decreased N-acetyl aspartate/choline ratio, and increased lactate levels are seen in fetuses with CHD [28, 29, 30, 31, 32].

Due to advances in surgical procedures and advanced care, many neonates born with CHD are now surviving until the teenage year and into adulthood. This wonderful increase in survival has also highlighted the potential long-term effects on the structural and psychological aspects to brain development. Compared to age matched controls, people with CHD are more likely to have motor developmental delays, reduced visuospatial integration, reduction in IQ, and reductions in executive performance [30, 33, 34].

Especially notable in the context of 22q11.2 DS, was that Tetralogy of Fallot was among the 3 subtypes of CHD noted to be associated with the largest reductions in measures of prenatal brain growth [32] and reduction in mean IQ scores for children/adolescents [31].

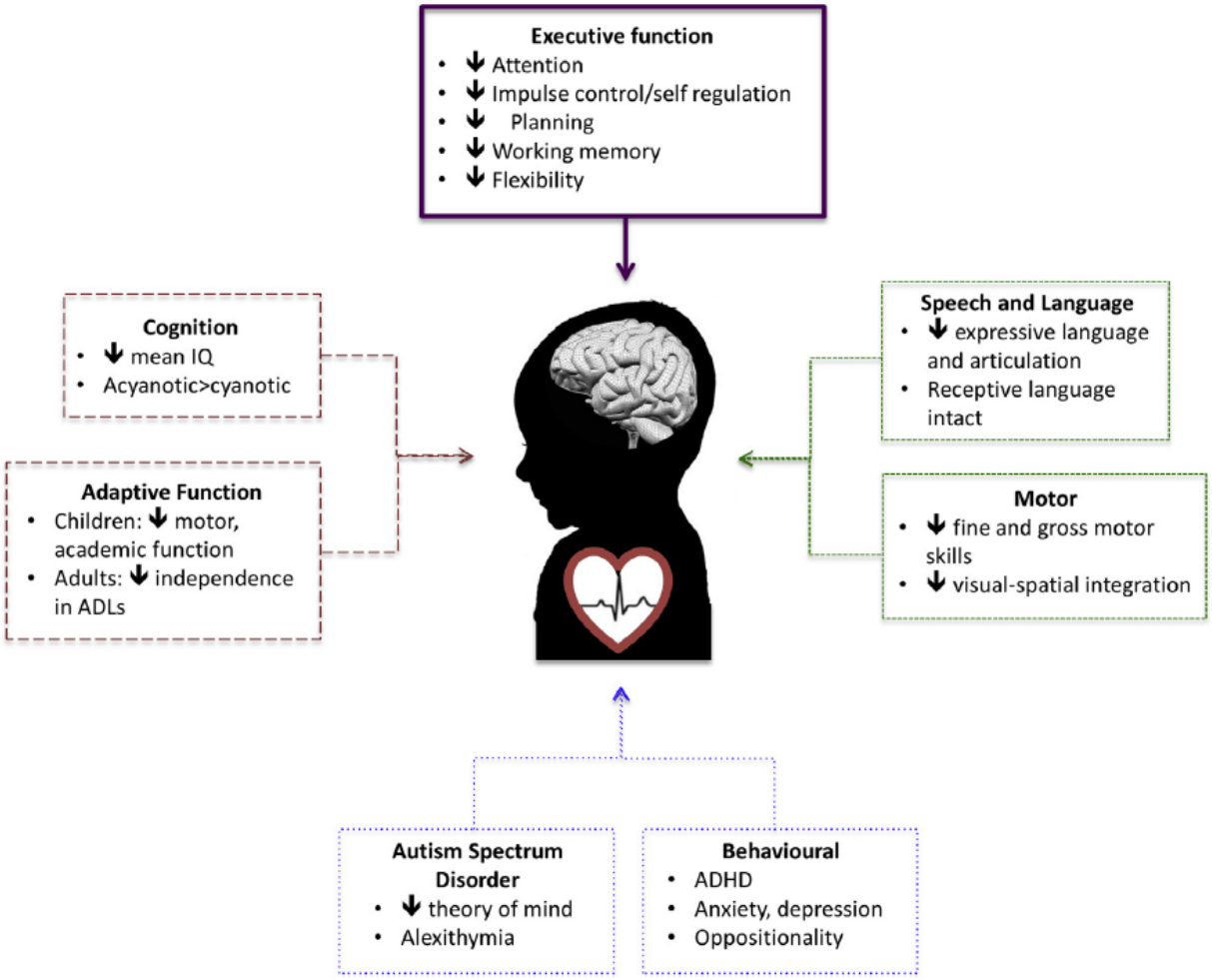


Figure 2: Summary of the Behavioral/Psychological Effects of CHD: Adapted from Nattel at. Al [2017] [34]

Genetics of 22q11.2 and Brain Development

Many of the cardiac, neurological, craniofacial and palatal tissues affected in 22q11.2 DS share a common embryologic origin (figure 3). Thus, much of the pathology in 22q11.2 deletion is attributed not just to disrupted gene expression in mature tissues, but disrupted gene expression crucial in the differentiation and interaction of embryologic cells [3].

Trying to determine how all of these genes interact to produce the phenotypic manifestations and severities of 22q11.2 DS remains largely unclear. Fortunately, the individual functions for most of these genes are well established, and there are many genes in the 22q11.2 region with known roles in neurological development and function.

One of these genes in question is *COMT*, which encodes catechol-O-methyltransferase. This enzyme is responsible for the degradation of catecholamines, including dopamine, epinephrine, and norepinephrine. *COMT* is shown to be highly expressed in the hippocampus and prefrontal cortex [3, 6], and *COMT* knockout mice have shown impaired cognition and increased aggressive behavior. Additionally, *COMT* polymorphisms have been linked to similar psychiatric phenotypes as those seen in 22q11.2 DS (such as ADHD)

[6], and *COMT* expression in 22q11.2 DS individuals has been found to be reduced by approximately 50% [2].

Another gene of substantial significance that is deleted in 22q11.2 DS is DiGeorge Syndrome Critical Region Gene 8 (*DGCR8*), named after its importance in DiGeorge Syndrome (now 22q11.2 DS). *DGCR8* encodes a subunit of a microprocessor complex that mediates the biogenesis of micro-RNA's (miRNAs), which are vital in post transcriptional gene regulation [2, 4, 6]. Animal models have shown extensive support for the role of *DGCR8* in neurological development and function. Mouse studies have shown haploinsufficiency in *Dgcr8* to cause excessive dopaminergic transmission [2], and *Dgcr8* knockout mice have shown altered synaptic transmission, memory deficits, and defective dendritic spines [3, 6].

Two factors make *DGCR8* of particular interest in the context of our study. The first, is that in addition to its well-defined role in neurological development, targeted loss of function studies in cardiac neural crest cells have shown loss of D*GCR8* to lead to cardiomyocyte related cardiomyopathy, interrupted aortic arch, truncus arteriosus, ventral septal defects [3, 6, 35]. This indicates that altered expression of *DGCR8* has both cardiac and neurological repercussions.

Secondly, *DGCR8* not only has substantial support from animal studies, but human studies as well to support its role in 22q11.2 DS. Forsyth et. al utilized neuroanatomical data from 386 people with 22q11.2 DS and 315 controls to obtain measures of cortical surface area and thickness. As previously confirmed, individuals with 22q11.2 DS had a reduction in cortical surface area and an increase in cortical thickness [36]. However, the investigators went one step further and found that increased expression of *DGCR8* was highly associated with severity of surface area reductions in people with 22q11.2 DS compared to controls [36].

Lastly, *HIRA* (aka *DGCR1)* is a histone cell cycle regulator encoding a histone chaperone that is conserved across eukaryotes, indicating its fundamental role in cell function [6]. Furthermore, HIRA levels are dramatically increased during embryonic neurogenesis [37], and neurulation defects are seen in *Hira* knockout mice [6]. Similar to *DGCR8*, *HIRA* is especially noteworthy in the context of our study, since *HIRA* expression is not only important in neurological development, but cardiopulmonary development as well [4, 6, 35].

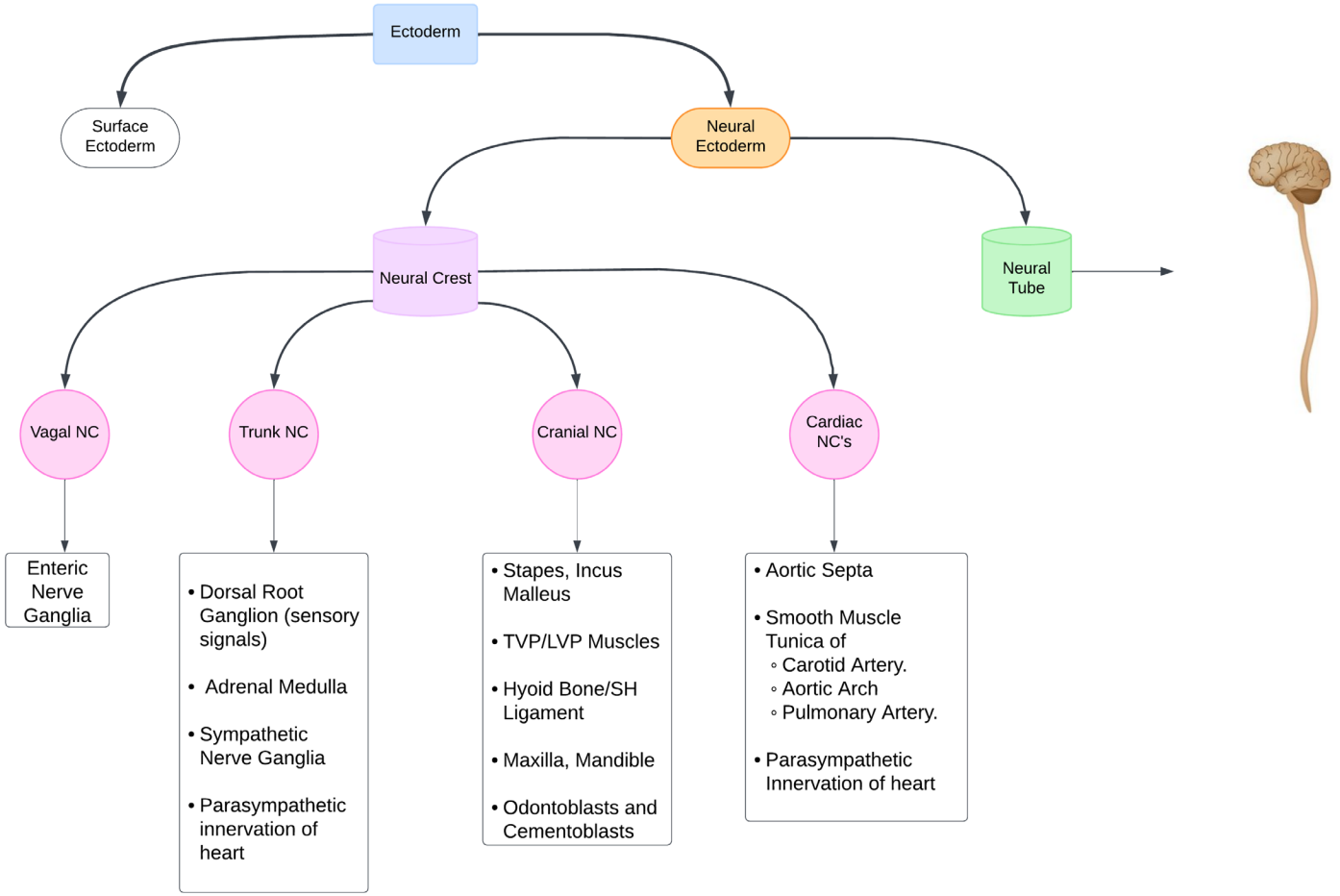


Figure 3: Embryologic Origins of 22q11.2 DS Pathologies. Many of the phenotypes seen in 22q11.2 DS either share a developmental origin as neural crest cells, or have crucial interactions with neural crest cells during development.

The Big Picture

In summary: CHD and altered neurological development are both highly prevalent phenotypes in the context of 22q11.2 Deletion Syndrome. Individually, CHD and hemizygosity of the 22q11.2 region have both been established as having substantial contributions to brain development.

Despite this however, only a handful of papers have attempted to investigate the relationship between these factors and neurological development. What's more, is that most of the available literature has examined the role between CHD and Psychological factors in 22q11.2 DS (e.g. IQ, Speech, Motor control) [22, 24, 34]. Thus, our study set out to determine if any substantial correlation existed between CHD status and structural brain development in people with a 22q11.2 Deletion Syndrome.

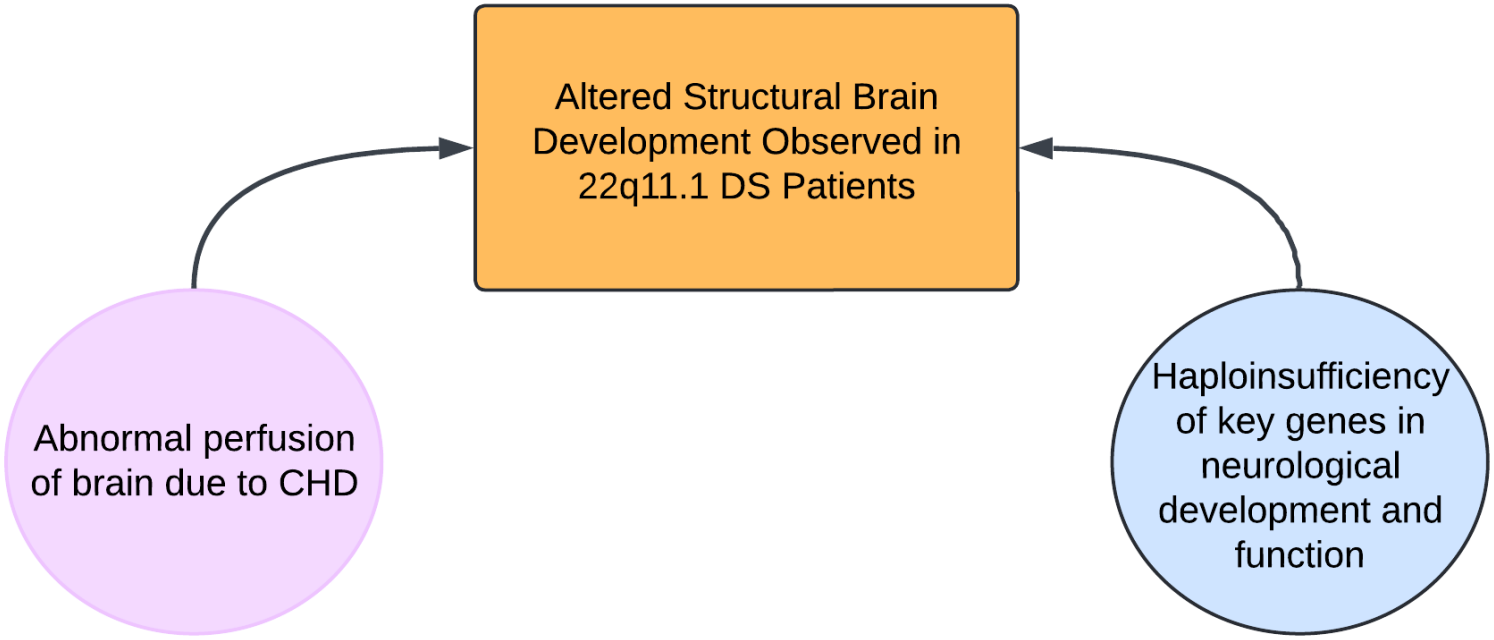


Figure 4: Visualization of Our Study: The above figure illustrates the fundamental question in our study: Does the presence or absence of CHD make a significant contribution in the brain development of 22q11.2 DS individuals?

CHAPTER 3: FiA-Net Deep Learning Algorithim

In addition to predominantly only looking at either CHD or presence of 22q11.2 DS in relation to brain development, past studies have often utilized similar methods in their investigations. The advancements of Artificial Intelligence (AI) and machine learning, especially in the past 3 years, provide us a new method to analyze brain MRI's. For this reason, we decided to analyze our study utilizing the FiA-Net deep learning algorithm developed by Shen He, et al [38].

The FiA-Net is a deep learning algorithm that is designed to predicted brain age. While most age predictor models utilize a single channel when analyzing MRI's, the FiA-Net algorithm splits the 3D image into 2 channels in order to recognize the differences due to the varying contributions of brain anatomy and feature layers across channels. Before FiA-Net, mutli-channel fusion had not been used in brain age prediction [38]. Furthermore, FiA-Net was the first to use attention-based fusion of the channels, instead of treating the different channels equally [38].

At initial development, the FiA-Net algorithm was trained on 16,705 samples 0-97 years of age. The algorithm achieved a mean absolute error of 3 years, and a Pearson correlation coefficient of r= 0.984 [38].

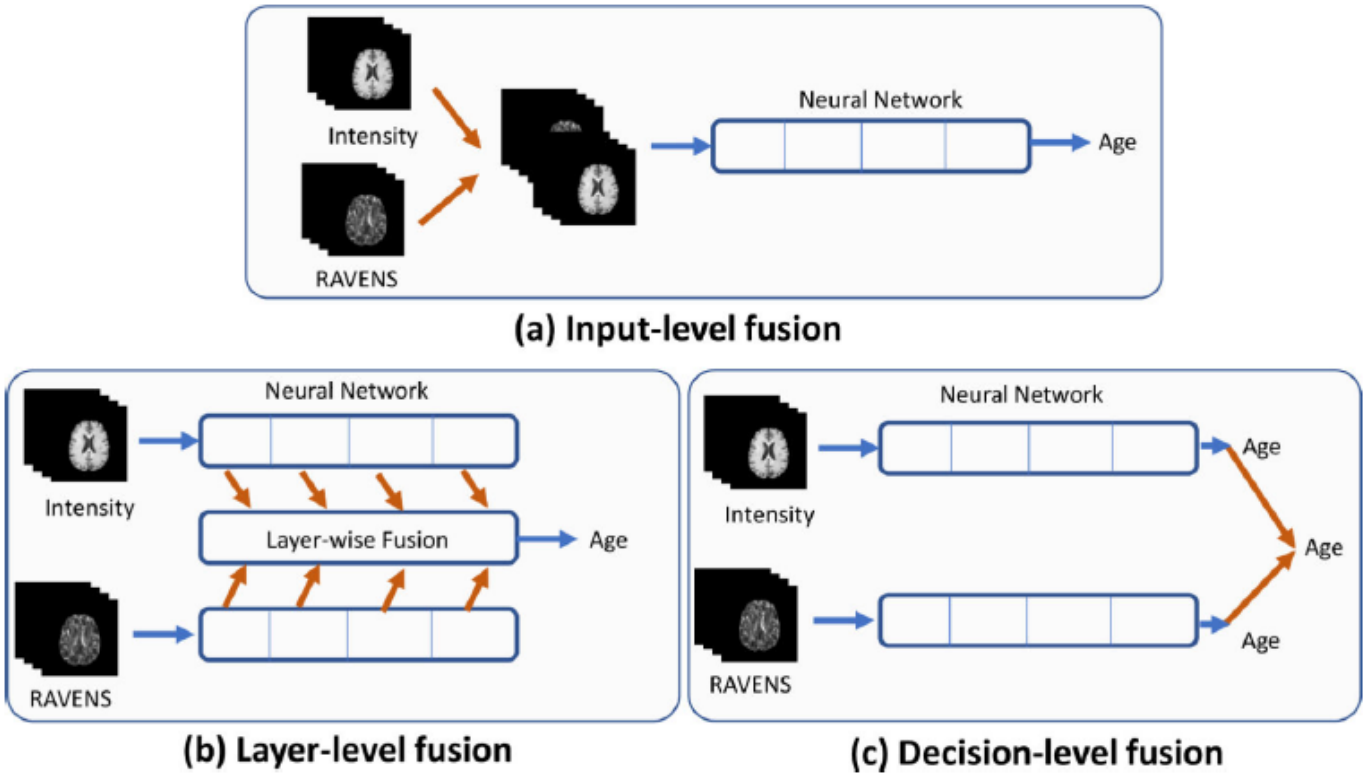


Figure 5: Layer Level Fusion Utilized in FiA-Net. The three main strategies to fuse information from multiple channels. Since FiA-Net split the MRI into two separate channels, the overall age predicted needed to fuse information from both of the channels. Figure adapted from He et al [2021] [38]

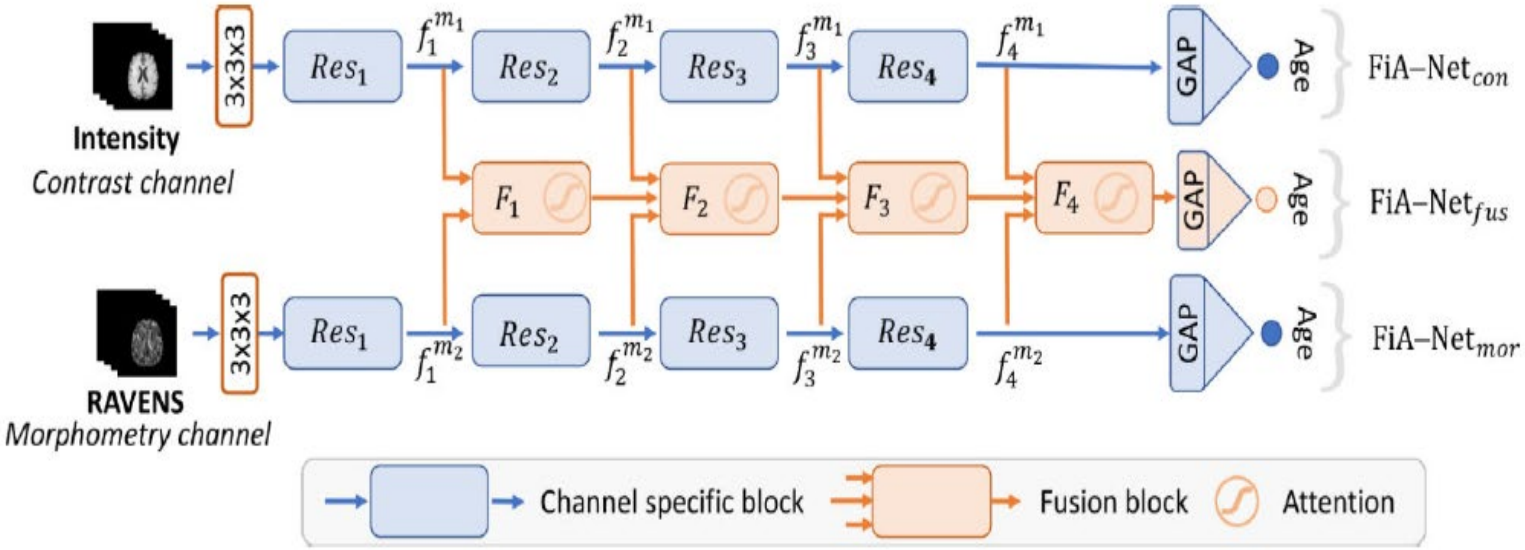


Figure 6: Multi-Channel Fusion with Attention Model. The age prediction deep learning algorithm not only utilized multi-level fusion, but was unique in its integration of attention to each fusion block. This allowed the algorithm to recognize and learn how different anatomic locations and feature levels could cause the channels to contribute differently to the overall computation. Figure adapted from He et al [2021] [38]

CHAPTER 4: Methods

Data Selection

Our study was a retrospective study, and data was obtained from the Boston Children's Hospital EMR. Our original cohort was obtained by doing a TrinetX search for individuals with a diagnosis of 22q11.2 deletion and at least one MRI present (263 people). We then excluded people who did not have genetic testing to confirm their 22q11.2 deletion, or those that did not have any MRIs of the brain (130 people excluded).

For the remaining 133 people, de-identified data collection was done. EMR extraction was performed through October 31, 2023 and data was recorded in RedCap software. Basic demographic data was collected such as sex, date of birth, and self-reported ethnicity. It was also recorded if the person was known to be deceased. The age at MRI was noted for every brain MRI for each person. If a person had multiple brain MRIs over the course of their care, each MRI was listed as a separate data point.

Select phenotypic data was also pulled for each person via chart search. Phenotypic categories of interest were selected based on both common findings in 22q11.2 DS (such as velopharyngeal or

craniofacial phenotypes) or based on phenotypes previously underreported in 22q11.2 DS, but thought to be likely based on the embryological origin as seen in figure 3 (such as dental or Otological phenotypes). Clinical notes were considered whether from Boston Children's Hospital or an outside institution to ensure the most thorough search possible. To ensure uniformity of data, each category of phenotypes (cardiac, craniofacial, dental, etc) contained checkboxes for certain commonly seen phenotypes, as well as a free text box to input any other relevant data.

A consort diagram showing individual person and MRI selection process is shown in figure 7. A summary of some of the demographic, phenotypic, and MRI data collected is shown in figures 8-10.

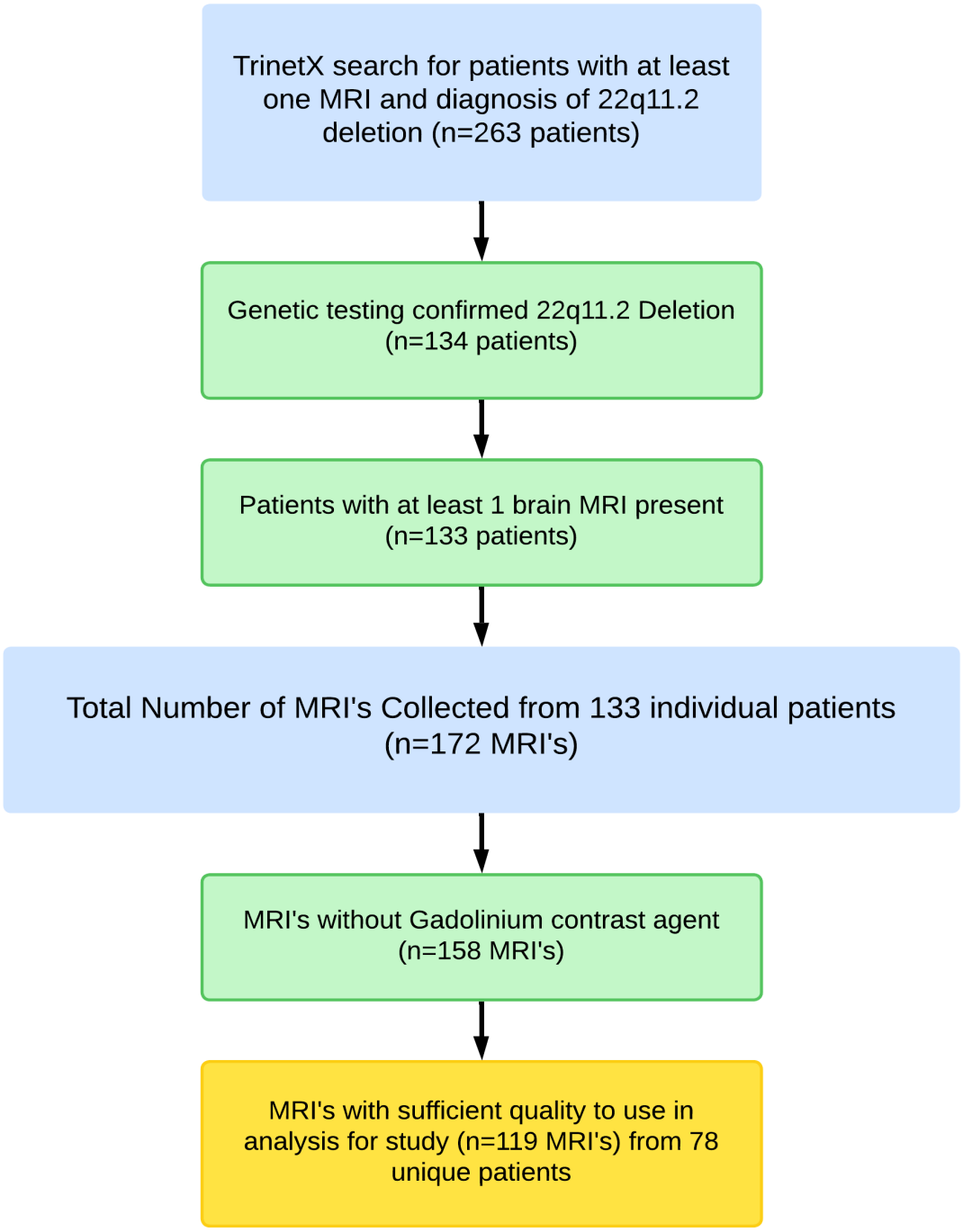
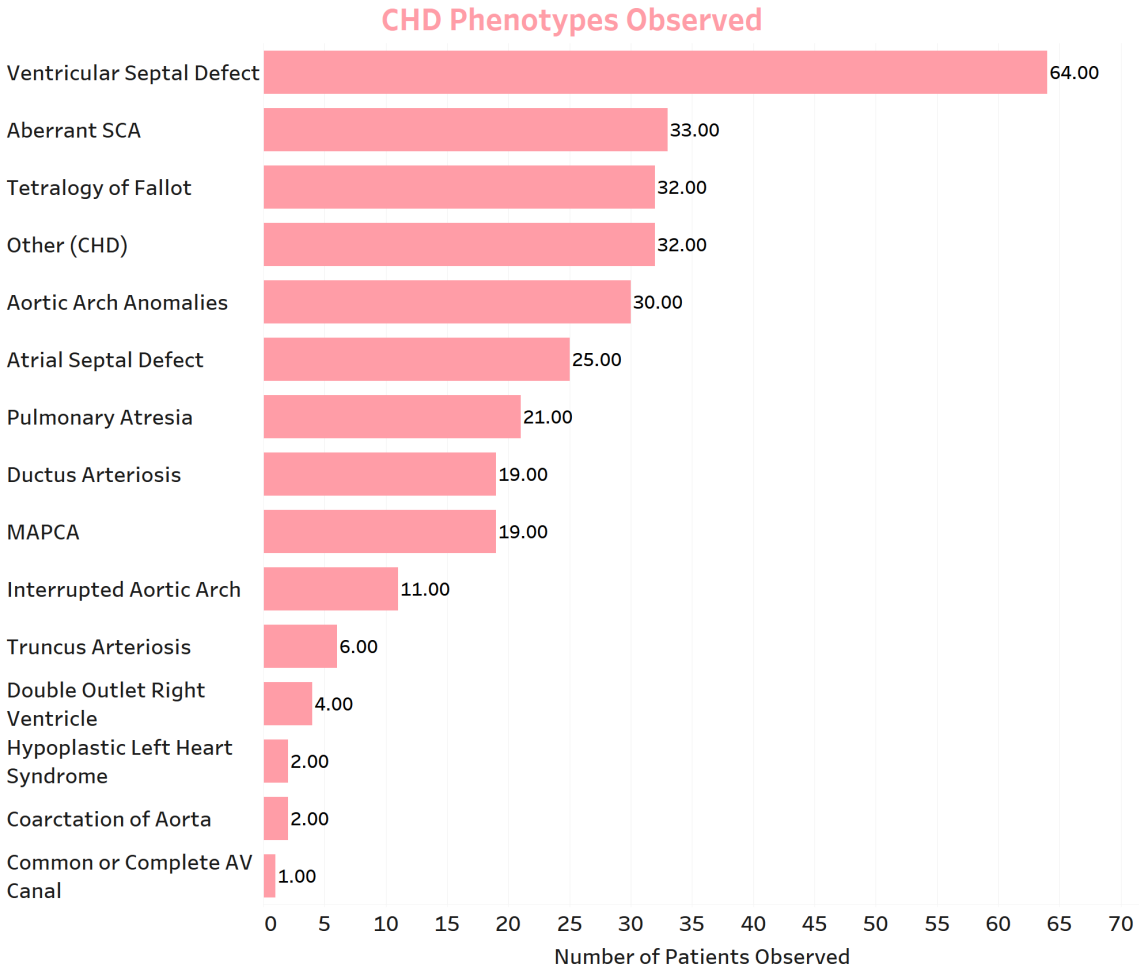


Figure 7: Cohort Diagram. Above is a cohort diagram illustrating our person and data selection process.



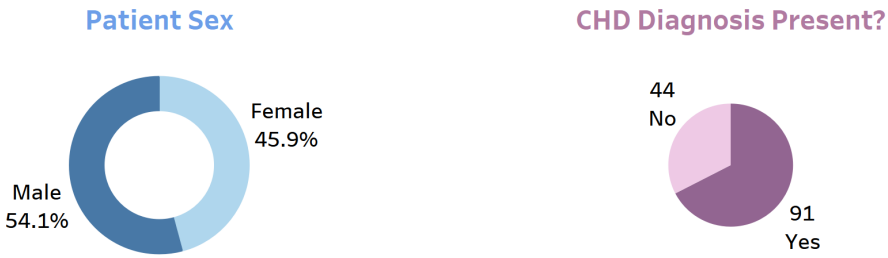


Figure 8: Cohort Data, Part 1. (top) The frequency of specific CHD phenotypes. (bottom) CHD prevalence and sex breakdown in our cohort.

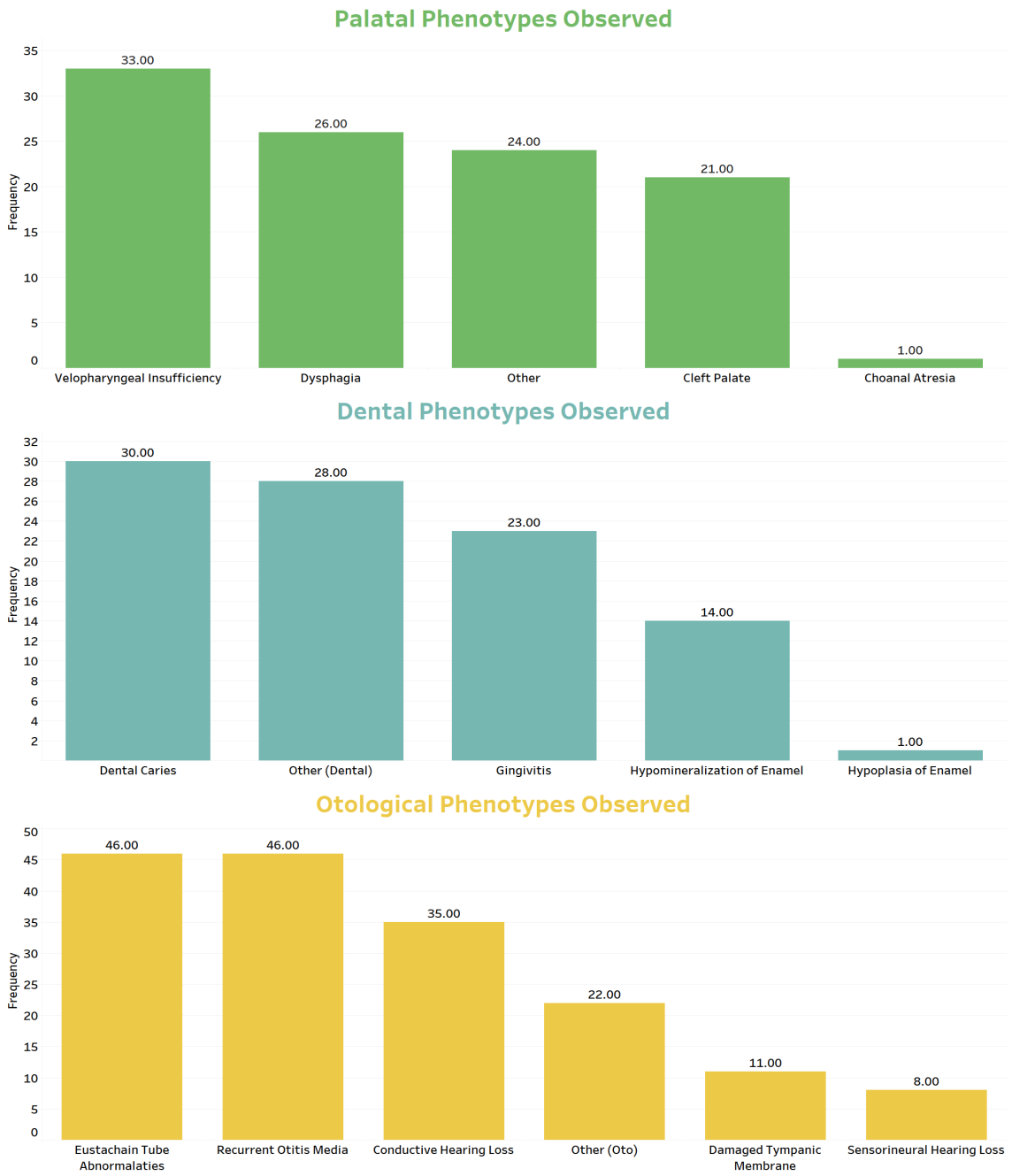


Figure 9: Cohort Data, Part 2. More frequencies of the phenotypic data obtained from our chart review. (top) palatal phenotypes, (middle) dental phenotypes, (bottom) otological phenotypes. Above the bar is the frequency with which the given phenotype was observed in individuals

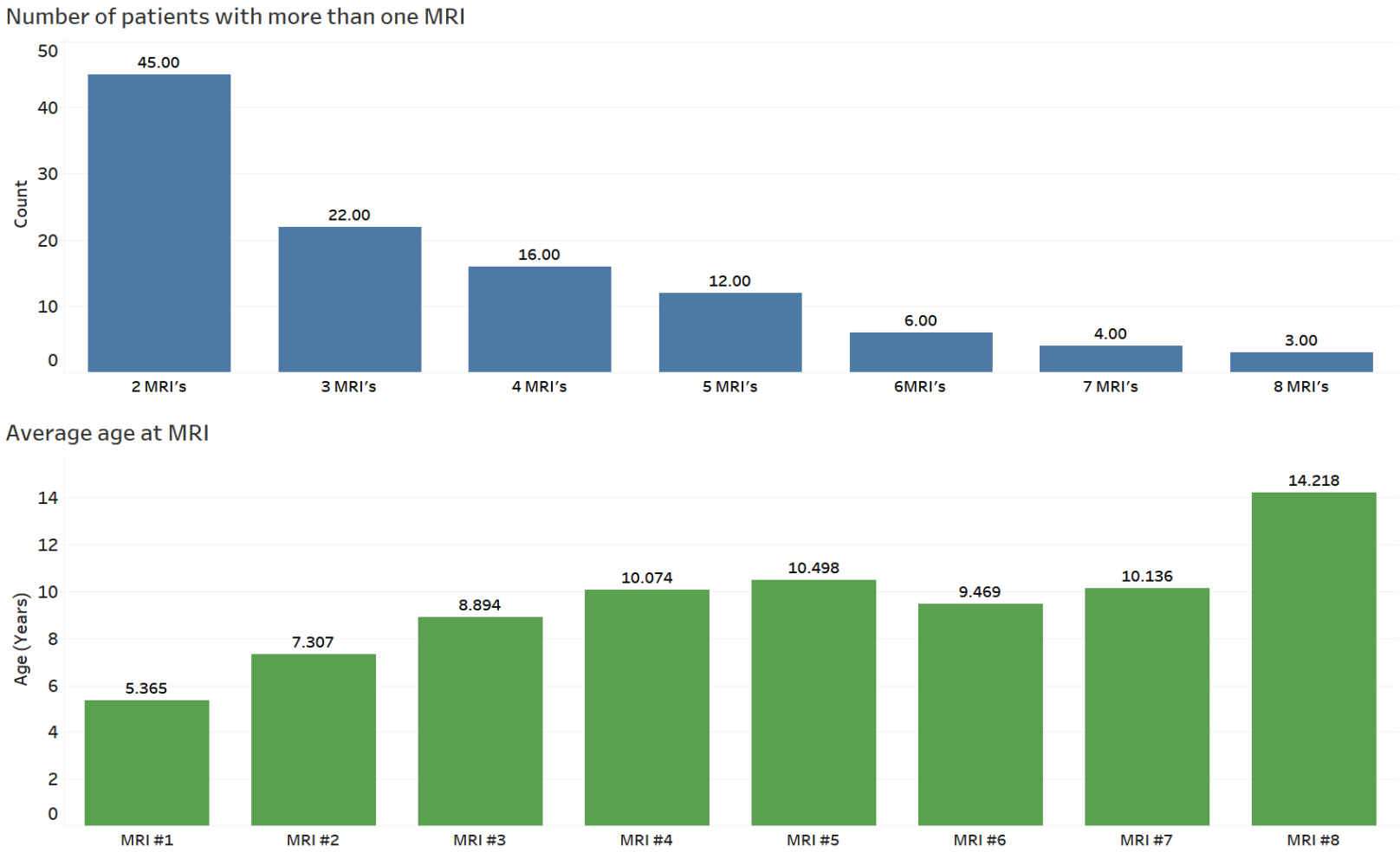


Figure 10: Individual MRI Data. The figure above shows the number of people with more than 1 MRI, and the average age of the person at each MRI.

MRI Processing

After MRI and phenotypic data was collected, T1 weighted brain MRI's underwent pre-processing, which included bias correction, skill stripping, and image registration.

Bias correction was done to change the brightness or darkness of the image to remove any difference that is caused by inhomogeneity of magnetic fields in the scanner. Multi-Atlas Skull Stripping as outlined by Doshi et al [39] was then performed to remove non-brain structures from the MRI. Lastly, registration was done to ensure that the same (x, y, z) coordinate location represented the same anatomy across all the subjects. Registration also allowed for the splitting of each image into contrast and morphometry channels seen in figures 5 and 6.

Processed images were then run through the FiA-Net deep learning algorithm previously described. The algorithm computed 40 predicted ages for each MRI, one for each of the neural networks that compose the algorithm. The median and mean value for predicted age was then obtained from the distribution of predicted ages for each MRI. The difference between these respective values and the true age of the person in the MRI, the value we denoted as "brain age gap" was

also calculated. An example dataset is shown below in Table 4 to better understand this process.

Table 4: Sample Dataset and Calculations. The table below utilizes mock numbers to illustrate how our data was collected and calculated.

*Note that the dataset below only utilizes age predictions from 3 individual neural networks, whereas our actual dataset utilized 40 individual neural networks.

Individual Medical ID Number	MRI number	Age Prediction from Neural Network 1	Age Prediction from Neural Network 2	Age Prediction from Neural Network 3	Mean age predicted	**Median age predicted**	**True Age**	**Brain Age Gap (using median predicted age)**
12345	1st	5	4	12	7	**5**	**5**	**0**
24680	1st	3	10	14	9	**10**	**12**	**-2**
24680	2nd	12	18	30	30	**18**	**16**	**2**
35791	1st	3	14	7	8	**7**	**2**	**5**
35791	2nd	2	2	26	10	**2**	**1**	**1**

Final Exclusion Criteria

After the age prediction algorithm was run, MRI data were then excluded from the study due to either: 1) the image being a low-resolution MRI, causing unreliable data or 2) radioactive Gadolinium contrast being present in the MRI, which the deep learning model was not trained for. Quality of MRI and/or presence of contrast was confirmed by a board-certified Radiologist.

Statisical Analysis

Statistical analysis was then performed on the remaining 119 MRIs available (constituting 78 unique individuals). All statical tests were done in IMB SPSS Statical Software, version 29.0.2.0

Since the distribution of ages for almost every MRI precited by the 40 neural networks followed a non-normal distribution, the brain age gap value we used for statistical analysis in our study was the based on the median predicted age for an MRI, instead of the mean value. Also because of the non-normal distribution of the data, a Mann-Whitney U test was chosen to determine any statistical significance. A correlation matrix was also run between the phenotypic data collected and BAG values. The correlation matrix was also run between cardiac and non-cardiac phenotypes.

CHAPTER 5: Results

Age Prediction Results

Box and Whisker plots were generated to see the difference in distributions and mean values for brain age gap between the CHD and non-CHD populations (figure 11). Whiskers were set to incorporate data from Q1-1.5*IQR, up to Q3+1.5*IQR, to avoid incorporating any outliers. Box plots were color coded based on CHD status (orange for individuals with CHD, blue for individuals without CHD). For the non-CHD group, median brain age gap=3.51, with Q1=2.18 and Q3=4.83. For the CHD group, median brain age gap= 4.96, with Q1=3.08 and Q3= 8.99.

The Mann Whitney test calculated that there was a statically significant difference (p=0.005) between the median BAG values and distribution of BAG values between the CHD and non-CHD groups.

To visually see where the median age prediction values landed in comparison to the true age for each MRI, a scatterplot was generated using the median predicted age value vs true age value (figure 12). Data points were color coordinated based on CHD status of the person, and a reference line was inserted at Y=X.

Correlation Matrix Results

The correlation matrix showed no correlations with an absolute value greater than 0.5 between the subtype of CHD and brain age gap value (r values all between -0.066 and 0.429, with 9 p-values greater than 0.05, and 5 p-values below 0.05). Additionally, it did not show any correlations with an absolute value greater than 0.3 between the non-CHD phenotypes collected and brain age gap value (r values all between -0.176 and 0.269, with p-values greater than 0.05).

It did however, show correlations with an absolute value above 0.5 between certain subtypes of CHD and some of the non-CHD phenotypes collected. Aortic arch anomalies, Hypoplastic left heart syndrome and Pulmonary atresia all were correlated with Dysphagia (Pearson correlation values between r=0.51 and r=0.57; p values all less than 2.9×10^{-9}). Truncus arteriosus had a correlation with Gingivitis (Pearson correlation value of r=0.53; $p < 6.5 \times 10^{-10}$).

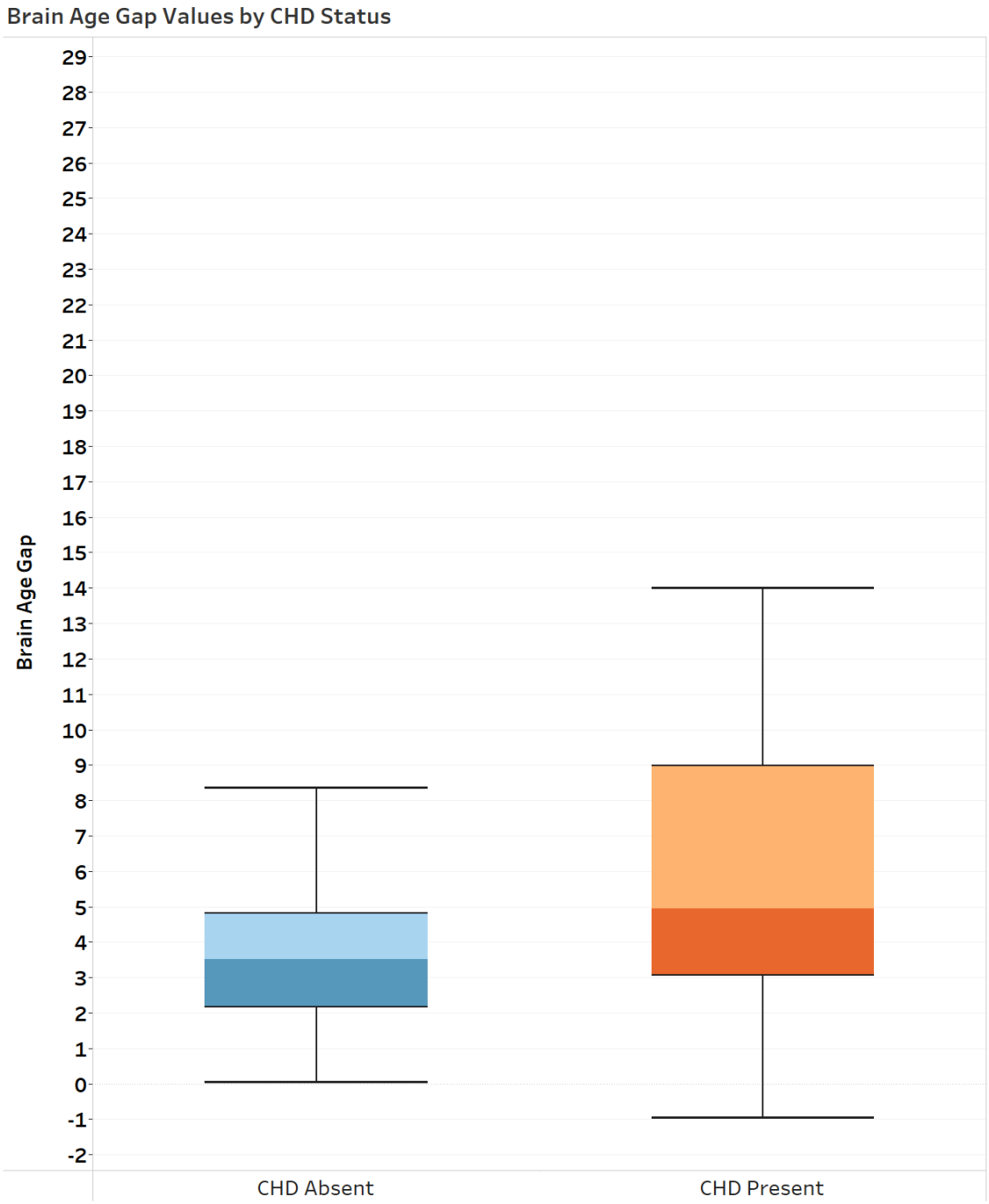


Figure 11: Box Plots of Brain Age Gap Values, separated by CHD and non-CHD groups. Outlier values not shown. Light colors indicate upper hinge and dark colors indicate lower hinge.

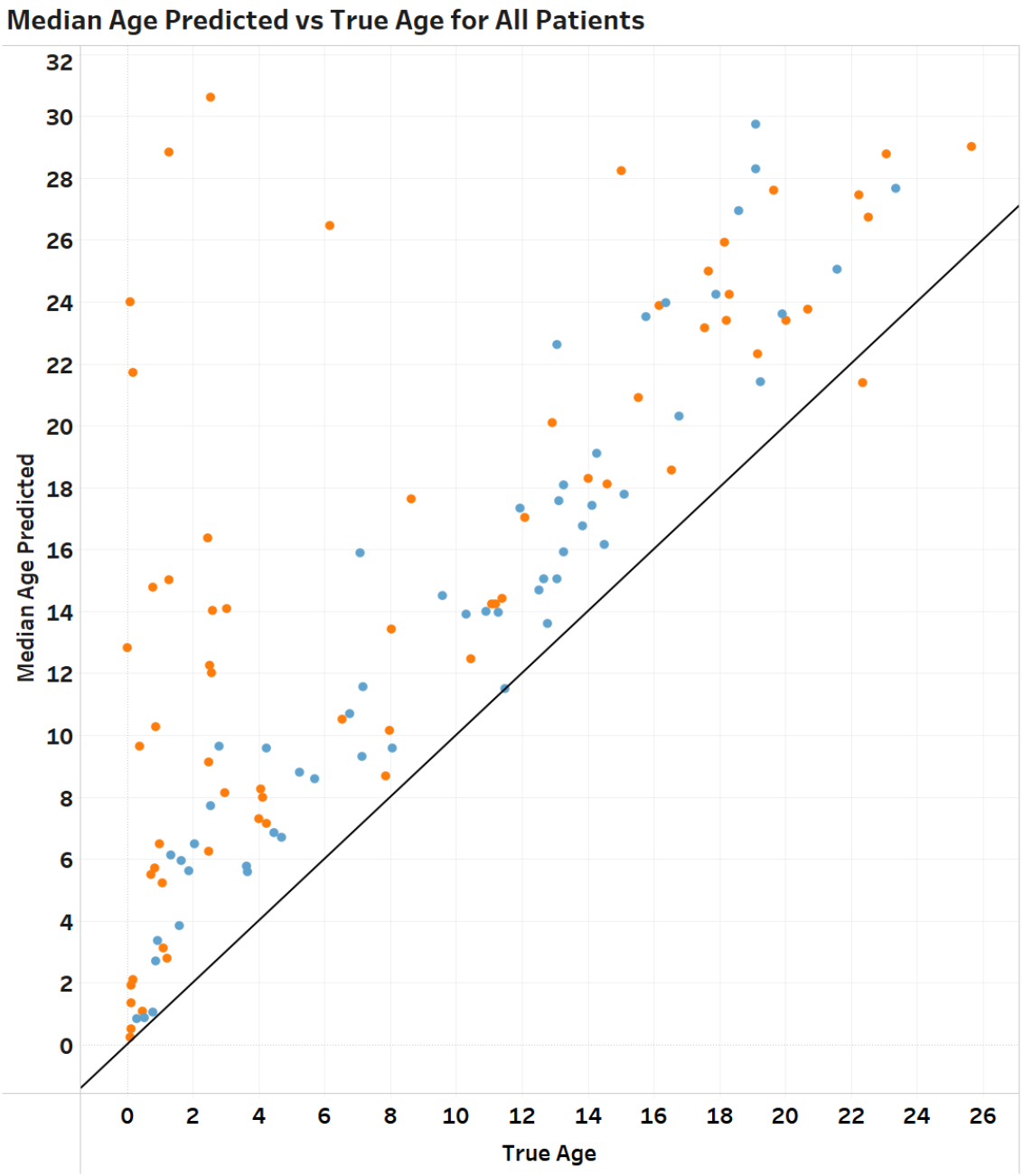


Figure 12: Mean Predicted Age vs True Age. A scatterplot containing all 120 values for mean predicted age vs true age. A 45-degree reference line is present at Y=X to visualize if median predicted age was above/below true age. Blue dots represent people without CHD and orange dots represent people with CHD.

CHAPTER 6: Discussion

CHD Status vs Brain Development

Overall, our statistical test revealed that there was a significant difference in the median brain age gap values, and distribution of brain age gap values, for people with 22q11.2 DSS with CHD vs those without CHD. This pilot analysis supports the hypothesis that the presence or absence of CHD has a significant impact the structural brain development of people with 22q11.2 DS.

From the distribution of the values, it can be concluded that the presence of CHD in 22q11.2 DS individuals leads to a much wider variety in structural brain development, as seen on MRI. Furthermore, it can be said that people with 22q11.2 DS and CHD generally tend to present as appearing "older" based on structural brain MRI presentation, compared to people with 22q11.2 DS and no CHD.

While there has not been much research in this area, the small amount that has been done further supports the conclusion that CHD does have a significant impact on structural brain development in 22q11.2 DS individuals. One study by Schaer et al. found that people with 22q11.2 DS with CHD had notably reduced gyrification compared to those without CHD (40). Another study, also by Schaer et al., found

that 22q11.2 DS individuals with CHD had distinctly lower total brain volumes than 22q11.2 DS individuals without CHD [21].

Perhaps one of the most intriguing aspects about our finding, is the fact that despite the clear evidence for structural brain alteration in people with 22q11.2 DS based on the presence or absence of CHD, the available literature does not support any psychological difference in people with 22q11.2 DS based on presence or absence of CHD [22, 24, 41]. It should be noted though, that some of these conclusions were made with p values of 0.14 and above, so their statistical significance is in question [22].

Apparent Brain Age in 22q11.2 DS

From figure 12, it can also be visually inferred that people with 22q11.2 DS were almost always predicted to be older than their true age, regardless of CHD status. This is particularly intriguing, as another study by Licht et al. did an analysis on total maturation score between 22q11.2 DS individuals with and without CHD, and concluded that brain maturation in those with CHD appeared to be approximately one month younger than controls [42].

Phenotypic Correlation Analysis

Overall, the results of the correlation matrix between phenotypes reflected the vast heterogeneity of phenotypic presentation seen in the 22q11.2 DS population. Most notably, the results showed that no specific CHD subtype or non-CHD phenotype showed a strong correlation to the brain age gap value. This was surprising, considering the shared embryological origin of many of these structures. It did show moderate correlations between certain CHD subtypes and non-CHD phenotypes, such as truncus arteriosus and gingivitis.

Due to the number of people lacking any data in our EMR that specified the presence or absence of non-cardiac phenotypes however, the conclusion there is no correlation between these factors and the BAG values, as well any conclusions about the correlations between cardiac and non-cardiac phenotypes, cannot be made with great certainty.

Impact in Clinical Setting

It has been reported previously that positive brain age gaps are associated with increased risk of neurological pathologies such as: neurodegeneration, Alzheimer's disease, cognitive impairment, psychosis, mortality, and more [43].

The positive BAG seen for almost every person in our 22q11.2 DS cohort, combined with the fact that 22q11.2 DS individuals are already known to have a notable increased risk/prevalence of psychiatric disorders (see chapters 1&2 and table 1), further supports the importance of close monitoring and early intervention in this population as a whole.

Furthermore, the known effects of isolated CHD in relation to brain development, combined with the preliminary data that CHD significantly alters brain development within the 22q11.2 DS population, indicates that clinicians should be especially cognizant of CHD status when caring for people with 22q11.2 DS.

It is obvious from the limited number of studies however, that more research needs to be done on the interplay of CHD and both structural and psychological brain development within the 22q11.2 DS population. Only then can we have a truly comprehensive understanding and deliver the most accurate care possible.

CHAPTER 7: Limitations and Future Considerations

Limitations of Study

While this was a relatively large cohort compared to many other studies on 22q11.2 DS, it must be acknowledged that one of the limitations of the study is the small sample size, especially when dividing the overall sample size into smaller subsets based on CHD status. A larger sample size would increase statistical power, and maybe illuminate potential phenotypic correlations that were not found in this study.

A second limitation of this study was the time constraint. Time permitting, a qualitative assessment by a pediatric neuro-radiologist and a FreeSurfer mapping analysis of the MRI's would have greatly contributed to the findings and conclusions of this study.

Lastly, a limitation of this study was incomplete chart data for phenotypes outside of CHD. Many people did not have otological or dental phenotypes available in their charts, as these would most likely be noted in an outpatient setting. This severely limited the ability to draw any meaningful conclusions about the correlations between the non-CHD phenotypes observed, as well as correlation of these other phenotypes with brain development or CHD.

Future Considerations

The double-edged sword to using a deep learning algorithm is that the model utilizes millions of data points for maximum predictive accuracy, and thus it is impossible to know which specific parameters affected the age prediction (and to what degree). In an attempt to obtain a rough idea on which factors affected our model's prediction, one of the next steps would be to have the MRI's undergo a qualitative analysis by a pediatric neuro-radiologist.

After this, statistical analysis can be done to see if any traits (i.e. enlarged ventricles, presence/intensity of lesions, decreases in gray matter, etc…) play a significant role in the age prediction of our deep learning model. Furthermore, this could allow us to run a correlation analysis on whether certain neurological pathologies or structural findings are correlated with any of the phenotypic data obtained for each person.

Another step in our attempt to delineate the neurological structural differences between 22q11.2 DS individuals with CHD and without CHD, will be to run an analysis utilizing the open-source software FreeSurfer (https://surfer.nmr.mgh.harvard.edu/). FreeSurfer was developed by the Laboratory for Computational Neuroimaging at the Athinoula A. Martinos Center for Biomedical

Imaging, and is described on the website as "an open-source package for the analysis and visualization of structural, functional, and diffusion neuroimaging data from cross-sectional and longitudinal studies".

This analysis with FreeSurfer can also help us determine the presence of any structural differences between 22q11.2 DS individuals with and without CHD, in parameters such as cortical thickness, gyrification index, whole brain volume, and more.

Lastly, an interesting aspect would be to see how deletion size correlated with phenotypes and brain development. While there is not an abundant amount of literature on the subject, there is some evidence to suggest that people with the LCR B-D deletion or distal deletion present with less severe pathologies [22]. Unfortunately, not all of the genetic data on our cohort was specific for deletion type, but rather just the presence of a 22q11.2 deletion. Gleaming any significant results for this also requires a much larger sample size since such a small subset of people with 22q11.2 DS have these less common deletions.

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