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ARTICLE

Consequences of Chromsome18q Deletions

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Providing clinically relevant prognoses and treatment information for people with a chromsome18q deletion is particularly challenging because every unrelated person has a unique region of hemizygosity. The hemizygous region can involve almost any region of 18q including between 1 and 101 genes (30 Mb of DNA). Most individuals have terminal deletions, but in our cohort of over 350 individuals 23% have interstitial deletions. Because of this heterogeneity, we take a gene by gene approach to understanding the clinical consequences. There are 196 genes on 18q. We classified 133 of them as dosage insensitive, 15 (8%) as dosage sensitive leading to haploinsufficiency while another 10 (5%) have effects that are conditionally haploinsufficient and are dependent on another factor, genetic or environmental in order to cause an abnormal phenotype. Thirty-seven genes (19%) have insufficient information to classify their dosage effect. Phenotypes attributed to single genes include: congenital heart disease, minor bone morphology changes, central nervous system dysmyelination,

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expressive speech delay, vesicouretreral reflux, polyposis, Pitt-Hopkins syndrome, intellectual disability, executive function impairment, male infertility, aural atresia, and high frequency sensorineural hearing loss. Additionally, identified critical regions for other phenotypes include: adolescent idiopathic scoliosis and pectus excavatum, Virchow-Robin perivascular spaces, small corpus callosum, strabismus, atopic disorders, mood disorder, IgA deficiency, nystagmus, congenital heart disease, kidney malformation, vertical talus, CNS dysmyelination growth hormone deficiency and cleft palate. Together these findings make it increasingly feasible to compile an individualized syndrome description based on each person's individuated genotype. Future work will focus on understanding molecular mechanisms leading to treatment.

KEY WORDS: 18q deletion; hemizygosity; haploinsufficiency; chromosome 18; 18q-; gene dosage

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INTRODUCTION

Deletions of chromosome 18q were among the earliest of the chromosome deletions to be identified [de Grouchy et al., 1964]. Early on, it was appreciated even with basic cytogenetic techniques that the size of 18q deletions varied between different patients. Now, with the much higher resolution of contemporary molecular determinations, we know that no two unrelated individuals with simple deletions have identical hemizygous regions of 18q [Heard et al., 2009]. This fundamental finding presents an opportunity and a challenge. It also delineates the approach that will be required for understanding the full spectrum of 18q deletions.

Early on, it was appreciated even with basic cytogenetic techniques that the size of 18q deletions varied between different patients. Now, with the much higher resolution of contemporary molecular determinations, we know that no two unrelated individuals with simple deletions have identical hemizygous regions of 18q.

The opportunity this presents is that the variation in chromosome content can be used to explore genotype phenotype correlations. This, in turn, leads to identification of chromosome regions linked to specific phenotypes and ultimately to the discovery of specific dosage sensitive genes. The challenge is that, because there is no uniformity in the genotype, the phenotype is highly variable. Thus it is problematic to develop clinical anticipatory guidance using a "syndrome" approach, such as has been done for Down or Turner syndromes. In the past we have tried to impose this approach for the sake of conformity with other genetic references; however, this is only truly useful for the small number of individuals who have terminal deletions with breakpoints between the same two genes [Cody et al., 2014].

Most syndrome definitions originated based on phenotypic data, only later to be defined genotypically. 18q- originated as an abnormal genotype; someone with hemizygosity for a portion of the long arm chromosome 18, and then was described phenotypically. However, this genotype-based definition is extremely broad. Consequently, any two people with an 18q deletion may have very different phenotypes and prognoses.

Because 18q- is a condition defined by the genotype, the genotype guides our approach to understanding and treating each individual. The genotypic data will set the stage for both the research agenda and clinical care. We envision a future in which we precisely know all of the genes that are in hemizygosity and know which are dosage sensitive and therefore, have potential functional consequences for the affected individual. This then becomes the foundation for clinical care and anticipatory guidance.

This review is approached from this new perspective. We will first describe what is known about the dosage sensitive genes on 18q and their phenotypic consequences. Then we will discuss phenotypes for which a critical region has been defined, but the causative gene has not been identified. Next we will discuss phenotypes for which the genetic basis cannot yet be established and the approach for further investigations. Lastly, we will review treatment and outcomes data from the perspective of 25 years of experience with affected individuals and in light of contemporary genetic understanding. Together these data serve as the foundation for implementing a strategy whose ultimate goal is treatment.

MOLECULAR CHARACTERIZATION

Our cohort of more than 350 individuals is considerably larger than any other group reported in the literature, thus providing the opportunity to gain the broadest perspective on these conditions. Figure 1 illustrates the spectrum of chromosome 18 content detected in people with 18q-. This illustrates several features of 18q-. First, the location of the region of hemizygosity varies greatly and can include almost any region of 18q. Second, there are no recurrent deletions; each unrelated individual has a unique region of hemizygosity. Third, the size of the deletion varies greatly from 60 Kb (including a single gene) to 30 Mb. Fourth, 18q- hemizygosity can involve terminal or interstitial deletions.

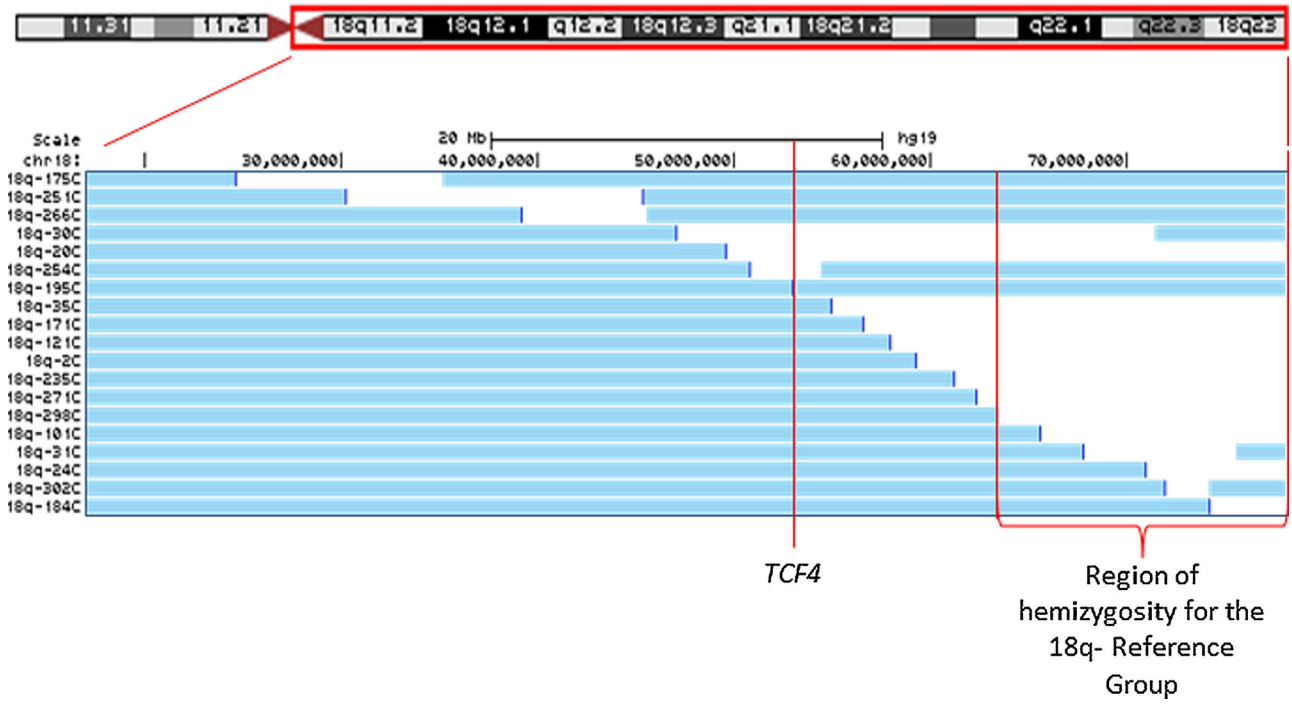

Figure 1. Chromosome 18 content for a selected group of individuals with 18q hemizygosity. The red box around a portion of the chromosome 18 ideogram at the top indicates the region highlighted below. Each individuals' intact chromosome is indicated by the light gray (blue) bar with the individuals study number written to the left. The dark gray (blue) line at the end of each bar depicts the breakpoint region. The gaps in the bar indicate the map location of the homozygous region. The individuals with interstitial hemizygosity have FISH confirmed interstitial deletions. The position of the *TCF4* gene; which has a significant phenotypic, effect is indicated as is the hemizygous region for the18q- Reference Group described previously [Cody et al., 2014].

In spite of the wide inter-individual genotypic variability, there are two regions of 18q that have not been found to be hemizygous in anyone; thus these two regions are potential hemizygous lethal regions. These regions are adjacent to the centromere (17,000,000–19,667,062) and at 18q21.1 (45,578,734–46,739,965). This essentially divides the chromosome into two regions with potential hemizygosity. We have termed the region between 19,667,062 and 45,578,734 as the Proximal 18q- Region and the region between 46,739,965 and the q telomere as the Distal 18q- Region. Photographs of individuals with deletions in these regions are shown in Figures 2 and 3, respectively.

Although each individual has a unique region of hemizygosity, there is a group of individuals whose breakpoints are between two genes that are 1.76 Mb apart. Because this is as as close to a recurrent genotype as we have detected and because these individual's deletions are about average in size compared to our entire cohort with terminal deletions we have referred to this as the Distal 18q- Reference Group [Cody et al., 2014]. Their region of hemizygosity is shown in Figure 1.

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The degree of variability seen with 18q deletions is unique among genome copy number changes. This can be illustrated by a comparison of chromosome 18 with chromosomes 17 and 19 (see Supplemental Figures in supporting information online). First, chromosome 18 is one of the most gene poor chromosomes per Mb of DNA. Thus, there is less of a chance that any particular deletion includes a dosage sensitive or haplolethal gene making a large deletion viable. A visual comparison of the DECIPHER database is shown in Supplemental Figure S1, showing that viable "pathogenic" deletions can be much larger for chromosome 18 than for 17 or 19. Secondly, except for people with whole p arm

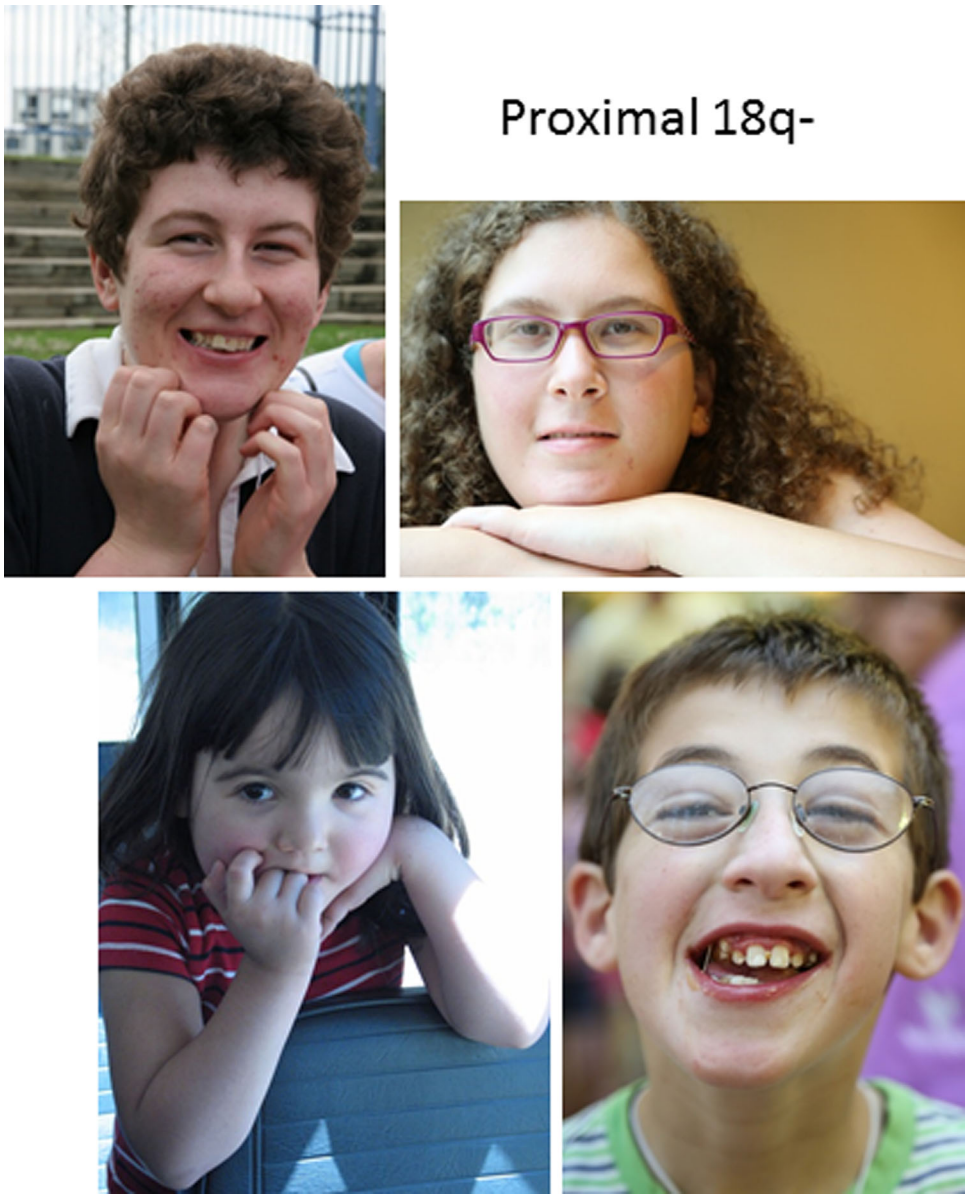

Figure 2. People with proximal 18q deletions.

duplications there are fewer recurrent duplications/deletions on chromosome 18 compared to 17 and 19. Recurrent deletions and duplications can result from segmental genomic duplications that are the substrates for nonallelic homologous recombination breakpoint clusters. Again, comparing chromosome 18 with 17 and 19, the sparse number and low homology of the segmental duplications on chromosome 18 can be appreciated (Supplemental Figure S2). Therefore, except for 18p whole arm deletions [see Hasi-Zogaj et al., this issue] chromosome 18 is not predisposed to recurrent interstitial deletions therefore breaks occur more randomly and are more likely to result in terminal deletions.

GENE DOSAGE MAP

Our approach for understanding the potential effects of an 18q deletion for any one individual is different from past approaches. Traditionally, clinicians have used a syndrome matching approach and looked for a patient or a group of patients with deletions just like their own patient in order to have some idea of the phenotypic outcome. This is the idea behind the DECIPHER and ISCA databases. This approach can be helpful for gathering data on numerous subjects with the same copy number change thereby identifying new syndromes. With this approach, the more patients in the dataset the more comprehensive the collated phenotype(s) can be. However, when there are many patients with unique copy number changes, this strategy may not be as helpful, and can

Figure 3. People with distal 18q deletions.

even be obfuscating. Scientifically, this approach correlates a set of phenotypes with a set of genes, not individual phenotypes with specific genes. Our approach seeks to link the individual phenotypes with specific genes thereby leading to insights necessary to develop specific treatments. We determine the potential for dosage effects for each individual gene by collating all available data on the gene's function. Then we apply that to what we know about

the phenotype of anyone with of copy number change for that gene.

Our approach seeks to link the individual phenotypes with specific genes thereby leading to insights necessary to develop specific treatments.

There are 196 confirmed genes on 18q. In order to determine which are likely to have dosage effects we monitor both the literature and information emerging from our own cohort [Cody et al., 2009b]. Reflecting the potential consequences of hemizygosity, the genes are tentatively assigned to one of five categories: haplosufficient, haploinsufficient, conditionally haploinsufficient, haplolethal, and unknown. The designations assigned to each gene may change as additional information emerges. The UCSC Genome browser custom tracks presents the most up-to-date data in a visual manner and allows links to the supporting materials and references (http://pediatrics.uthscsa.edu/centers/Chromosome18/dosage.asp). For ease of visualization, the genes are categorized by color according to their gene dosage category as are the phenotype-specific critical regions. This method should allow someone to look up a patient's region of hemizygosity, identify the dosage sensitive genes and learn about the potential phenotypes for their specific patient's deletion. Figure 4 shows an example of the 18q Gene Dosage Map with the haplosufficient genes hidden (current as of March 2015). The data supporting the classification of every gene on chromosome 18 can be viewed at the Gene Dosage Map link.

Currently we categorized 15 of the 196 genes on 18q as hemizygous leading to haploinsufficiency. This means hemizygosity of the gene can lead to an abnormal phenotype; although with variable penetrance. Those genes are:

***GATA6*(19,749,404-19,782,491)**
Complex congenital heart disease: Heterozygous mutations in this gene are associated with congenital heart defects and pancreatic agenesis [Allen et al., 2011; Yorifuji et al., 2012; Suzuki et al., 2014]. There are no participants in our cohort with deletions that include this gene. However, a patient with a deletion of this gene has been reported with complex congenital heart disease [Bui et al., 2013]. Deletions of this region have been identified in a control population indicating that this phenotype is not completely penetrant [MacDonald et al., 2013].

***ZNF521*(22,641,888-22,932,214)Minor bone morphology changes**: The heterozygous knock-out mouse has an increased hyoid bone size and abnormal clavicle morphology [Hesse et al., 2010]. Neither phenotype has not been noted in humans.

***SS18*(23,596,217-23,670,611)**
Growth failure: The heterozygous knock-out mouse has embryonic growth retardation and partial preweaning lethality [de Bruijn et al., 2006]. Structural variation data on control populations does not identify anyone with hemizygosity for this gene [MacDonald et al., 2013]. In our cohort of patients, we have a physically healthy individual with hemizygosity for this gene.

***DSG2*(29,078,027-29,128,814) Undetermined effect**:Heterozygous mutations in this gene in the mouse results in embryonic lethality before somite formation with partial penetrance [Eshkind et al., 2002]. Duplications but not deletions of this gene have been identified in control populations [MacDonald et al., 2013]. There are 6 individuals in our cohort with deletions including this gene therefore it probably has a less critical function in humans or a lower penetrance.

***ZNF24*(32,912,178-32,924,426) CNS dysmyelination**: The heterozygous knock-out mouse has tremors and CNS dysmyelination [Howng et al., 2010]. In our data, 4/5 individuals hemizygous for this gene have seizures and 4/5 have tremors. We have only evaluated one of these people using MRI and this person had normal myelination but had a history of seizures.

***SETBP1*(42,260,863-42,648,475)**
Expressive speech delay: Hemizygosity is associated with severe expressive speech delay with intact receptive language [Filges et al., 2011; Marseglia et al., 2012]. A frameshift indel was found to be a de novo mutation in autism [O'Roak et al., 2012]. A nonsense mutation was identified in a patient with IQ < 60 by exome sequencing [Rauch et al., 2012]. CNVs are also associated with ID [Coe et al., 2014]. In our own data set 7/7 individuals hemizygous for this gene have significant expressive speech delay.

***SLC14A2*(42,792,947-43,263,060)**
Vesicouretreral reflux/hydronephrosis: The heterozygous knock-out mice have 50% less urea concentrating capacity [Yang and Bankir, 2005]. Linkage studies of families with two or more children with vesicoureteral reflux identified significant linkage to a SNP in this gene [Briggs et al., 2010]. In our cohort, 30% (3/8) with hemizygosity for this gene have vesicouretreral reflux/hydronephrosis.

***SMAD4*(48,556,583-48,611,411)**
Polyposis: Heterozygotes for loss-of-function mutations develop polyposis of the glandular stomach and duodenum [Takaku et al., 1999; Chu et al., 2004]. A person with an interstitial 18q del was identified with juvenile polyposis syndrome (JPS) [Oliveira et al., 2014]. No members of our cohort have undergone a upper and lower endoscopy to evaluate JPS.

***TCF4*(52,889,562-53,303,188) Pitt-Hopkins syndrome**: Multiple individuals are identified who have hemizygous deletions of all or a part of this gene [Amiel et al., 2007; Zweier et al., 2007]. Hemizygosity of *TCF4* is one of the causes of Pitt-Hopkins syndrome [Rosenfeld et al., 2009]. Penetrance is very high since no individuals hemizygous for this

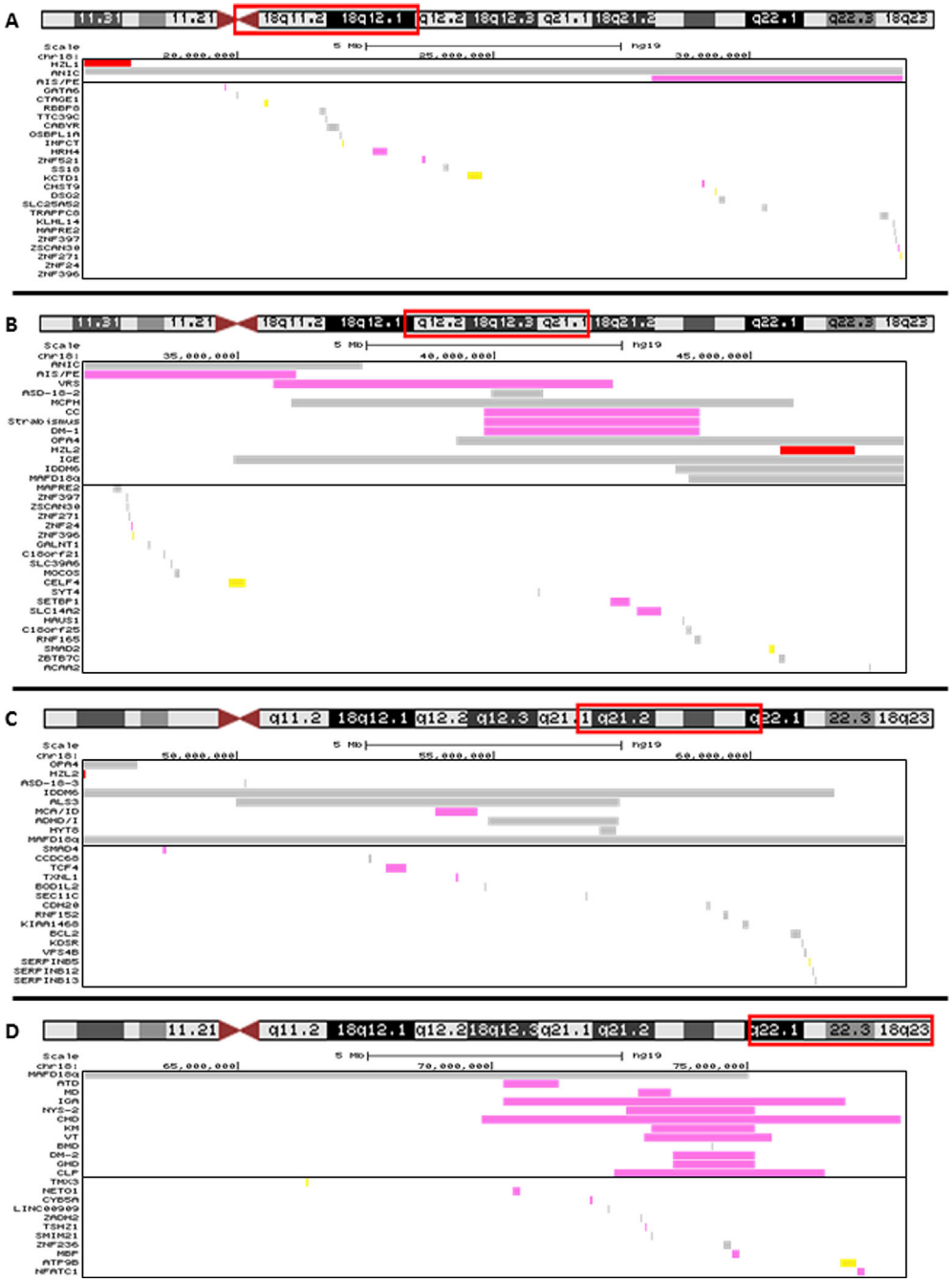

Figure 4. The Chromosome 18 Gene Dosage Map. Each of the four panels shows a section of 18q depicting the chromosome regions associated with phenotypes and the genes in the region. The abbreviations for the regions and genes are listed to the left. Both are color coded by the molecular mechanism of disease as it relates to gene dosage. Pink indicates that the mechanism of disease is haploinsufficiency, yellow indicates that the gene is a risk factor for disease, red indicates hemizygous lethality and gray means the mechanism of disease is unknown. Not included in this figure are those regions and genes not associated with gene dosage abnormalities. However, all the regions and genes in all dosage classifications can be viewed at (http://pediatrics.uthscsa.edu/centers/Chromosome18/dosage.asp) The data on this website are also continuously updated as new information on gene function is learned. ANIC, Anosmia, isolated congenital; T2D, Type 2 Diabetes; AIS/ PE, Adolescent idiopathic scoliosis & pectus excavatum; VRS, Virchow-Robin Perivascular spaces; ASD-18-2, Autism spectrum disorder/ play skills; MCPH, Microcephaly with simple gyration, epilepsy, and infantile diabetes; CC, Small Corpus callosum; SBM, Strabismus; DM-1, CNS Dysmyelination-1; OPA4, Optic atrophy-4; HZL2, Hemizygous lethal region 2; IGE, Idiopathic Generalized Epilepsy; HYT8, Essential Hypertension; ASD-18-3, Autism spectrum disorder/ play skills; IDDM6, Insulin-dependent diabetes mellitus-6; ALS3, Amyotrophic lateral sclerosis 3; MCA/ID, Multiple congenital anomalies, Speech delay, ID, sleep abnormalities; ADHD/I, Attention deficit hyperactivity disorder/ Inattention; MAFD18q, Major Affective Disorder, 18q; ATD, atopic disorders; MD, Mood Disorder; IGA, IgA deficiency; NYS-2, Nystagmus; CHD, Congenital heart disease; KM, Kidney malformation; VT, Vertical talus; BMD, Bone Mineral Density; DM-2, CNS Dysmyelination -2; GHD, Growth Hormone deficiency; CLP, Cleft palate.

gene have been identified without this phenotype [Hasi et al., 2011].

***TXNL1*(54,270,053-54,306,270) intellectual disability**: The product of this gene was found to be enriched in post synaptic densities [Bayés et al., 2011] implying a potential role in autism [Betancur et al., 2009]. One person with multiple congenital anomalies and a deletion of this gene was reported [van Diepen et al., 2011]. Since no deletions have been found in a control population, this may be a dosage sensitive gene [MacDonald et al., 2013]. There are three individuals in our cohort with interstitial deletions within which *TXNL1* is the only potentially dosage sensitive gene. These individuals have mild learning disabilities. One has a history of seizures.

***NETO1*(70,409,549-70,534,810) Executive function impairment**: The Neto1 heterozygous knock-out mouse has impaired spatial learning and memory compared to the wild type [Ng et al., 2009]. While this gene was found to be hemizygous in a control population [Kidd et al., 2008; Gusev et al., 2009; Park et al., 2010] the phenotype of impaired executive function might only be revealed by a specific type of evaluation that would not typically be done when recruiting control individuals. In our cohort of adults with hemizygosity for this gene, executive function difficulties are a universal challenge.

CYB5A (71,983,110-72,026,422) Male infertility/hypospadias: Homozygous inactivating mutations of this gene result in isolated 17,20 lyase deficiency thereby causing abnormal male sexual development [Giordano et al., 1994; Kok et al., 2010; Miller, 2012; Idkowiak et al., 2012]. However, these reports do not discuss the phenotypic presentation of male obligate carriers of the mutation, who would be analogous to males with 18q-. Abnormalities of male genitalia are found in 50–57% of males with 18q deletions. The common diagnoses include cryptorchidism, hypospadias, and/or micropenis [Cody et al., 1999]. Although *CYB5A* does not lie in the previously defined critical region for this phenoptye [Linnankivi et al., 2006] it does lie only 0.6 Mb distal to that critical region. One individual has been reported with an 18q deletion who had 17,20 lyase deficiency [Chaslow et al., 1986]. This individual also had micropenis, which was found in only two of the 57 males with 18q deletions that we have evaluated for this finding. We hypothesize that, due to the low incidence of this finding, this may be a case of a revealed recessive mutation.

***TSHZ1*(72,997,498-73,000,596) Aural atresia**: Recently, *TSHZ1* (teashirt family zinc finger 1) was linked with the aural atresia phenotype [Feenstra et al., 2011]. Aural atresia without microtia is a very rare finding and because it is easily observable and highly penetrant (78%), it is the most characteristic clinical feature of distal 18q-. The heterozygous knock-out mouse shares this phenotype as well [Coré et al., 2007]. This gene also plays a role in olfaction in the heterozygous knock-out mouse and in humans with mutations in this gene [Ragancokova et al., 2014].

Recently, TSHZ1 (teashirt family zinc finger 1) was linked with the aural atresia phenotype. Aural atresia without microtia is a very rare finding and because it is easily observable and highly penetrant (78%), it is the most characteristic clinical feature of distal 18q-.

***MBP*(74,690,789-74,844,774) High frequency sensorineural hearing loss**: Vestibular dysfunction has seen described in the heterozygous shiverer mouse [Jones et al., 2005] as well as increased visual evoked potential [Martin et al., 2006]. Additionally, age-related high frequency sensorineural hearing loss in the aged wild type mouse is linked to myelin degeneration [Xing et al., 2012]. In the Distal 18q- Reference Group 43% had a sensorineural hearing loss, while in our larger cohort of participants with 18q deletions we found a 63% rate of high frequency sensorineural hearing loss. [Perry et al., 2014]. Therefore with regard to high frequency sensorineural hearing loss, *MBP* may be dosage sensitive and may explain this aspect of hearing loss.

***NFATC1*(77,160,326-77,289,323) Undetermined**: The heterozygous knock out mouse has abnormal lymphocyte cell number, immune system physiology, interferon-gamma secretion, interleukin-2 secretion and interleukin-4 secretion [Ranger et al., 1998; Aliprantis and Glimcher, 2010]. This gene was found to be hemizygous in a control population. Hemizygous deletions of *NFATC1* were found to be significantly correlated with degenerative lumbar scoliosis [Shin et al., 2011]. Additionally, *NFATC1* hemizygosity may actually be protective against autoimmune disease [Dietz et al., 2015]. It is our observation that this group has more autoimmune disease that might be expected, and is therefore a current area of investigation.

There are 10 genes that are categorized as conditionally dosage sensitive, meaning that when they are hemizygous they may contribute to, or be a risk factor for, an abnormal phenotype. Hemizygousity for most of these genes has been identified in control populations (see the Database of Genomic Variants) [MacDonald et al., 2013]. Hemizygosity for most of these genes are more common in individuals with multifactorial conditions such as autism [Pinto et al., 2010; Krumm et al., 2013]. The effects of some conditionally dosage sensitive genes may only be apparent in conjunction with a specific exposure, such

as a particular medication or environmental toxicant.

Not shown here are the conditions caused by recessive mechanisms. These conditions would not be anticipated to be present in someone with a hemizygous deletion of the gene. However, a recessive condition could be revealed if someone with a hemizygous gene deletion had a mutation in their remaining allele. Although these will be the rare phenotypes associated with hemizygosity, they are conditions that are partially contributed to by the hemizygosity. Space precludes us from listing each potential revealed recessive condition. However, when investigating a hemizygous region for a patient, it is important to note the small potential for revealed recessive conditions.

The Gene Dosage Map (Fig. 4) also displays the chromosome regions linked to a particular phenotype. Phenotypes associated with recessive conditions are not included in the figure. The figure only includes those phenotype caused by haploinsufficiency or by an unknown mechanism. However, the web-based version of this map includes all of the phenotypes, including ones caused by a recessive mechanism. These data primarily come from three sources, 18q-phenotype critical regions, linkage studies, or GWAS studies. The last two strategies are agnostic with regard to the molecular mechanism, so those phenotypes could be caused by haploinsufficiency. Since the mechanism is unknown, we want to remain aware that these phenotypes may be associated with hemizygosity of this region.

The phenotypes in Figure 4 associated with chromosome 18q hemizygosity for which a specific gene has not been identified as causative are:

Virchow-Robin perivascular spaces (VRS) (35,677,236-42,320,241.00): Three individuals in our cohort have Virchow-Robin perivascular spaces thereby defining a critical region. There is one person who is hemizygous for this region who does not have the phenotype and there are three additional people who do not have the phenotype but are only hemizygous for a portion of the critical region. Therefore until the exact causative gene is identified the penetrance is between 43% and 75%. There are five genes and three non-coding RNAs in this region.

Small corpus callosum (CC) (39,808,849-44,013,124): There are three individuals in our cohort with a small corpus callosum who define the smallest common region of hemizygosity. There are an additional four others who are also hemizygous for this region but do not have the phenotype making it 43% penetrant. There are 12 genes and two non-coding RNAs in this region. Three of the genes (*HAUS1, C18orf25* and *RNF165*) have unknown consequences when hemizygous and two of the genes (*SETBP1* and *SLC14A2*) have were shown to cause haploinsufficiency, however not for this phenotype.

Strabismus (SBM) (39,808,849-44,013,124): This region for strabismus is the same region as that for small corpus callosum and CNS dysmyelination. Although eight individuals in our cohort are hemizygous for this region, only four have strabismus making it 50% penetrant.

CNS dysmyelination-1 (DM-1) (39,808,849-44,013,124): Also linked to the same region is CNS dysmyelination. There are two individuals in our cohort with dysmyelination who have interstitial deletions and therefore are not hemizygous for the dysmyelination region at q23. These two individuals have a common region of hemizygosity that is much more proximal. However there are a total of eight individuals who are hemizygous for this region yet only two have dysmyelination therefore the gene in this region influencing myelination does so with only 25% penetrance.

Speech delay, intellectual disability, sleep abnormalities (MCA/ID) (53,867,461-54,680,656): This region is defined by a single patient reported by van Diepen et al. [2011].

Atopic disorders (ATD) (70,220,470-71,304,427): This critical region was defined by Linnankivi et al. [2006]. Eight out of 14 participants who were hemizygous for this region had atopic disorders making it 57% penetrant. In our own cohort the penetrance is 75%.

Mood disorder (MD) (72,854,624-73,497,405): We reported on mood disorders in people with 18q deletions [Daviss et al., 2013]. Data from five individuals in this cohort were reported in that manuscript, and three of five had a history of a mood disorder requiring ongoing treatment. A critical region for depression was identified and contains two genes: *TSHZ1 and ZADH2*. Although very little is known about the function of *ZADH2*, it is an alcohol dehydrogenase and was found to be under expressed in the brains of people with autism compared to controls [Voineagu et al., 2011]. Since numerous genes have recently been found to be involved in both autism and other psychiatric disorders, this is a reasonable link. There is currently no knock-out mouse available to help investigate the effects of this gene's dosage on behavior.

IgA deficiency (IGA) (62,548,985-76,923,991): Two individuals with IgA deficiency were identified by Linnankivi et al. [2006]. Although seven were assessed for this phenotype, only four were hemizygous for the entire critical region, making it 50% penetrant. In our cohort the penetrance was 33%.

Nystagmus (NYS) (72,632,502-75,158,616): Linnankivi et al. [2006] reported six individuals with nystagmus which defined a 6.7 Mb critical region. Of the seven people in their cohort hemizygous for this entire critical region, three had nystagmus making it 43% penetrant. In our cohort there are 24 individuals with nystagmus and who narrow the smallest common region of hemizygosity to 2.5 Mb. There are 44 individuals in our cohort who are also hemizygous for this entire critical region yet do not have

nyastagmus; making it 35% penetrant.

Congenital heart disease (CHD) (69,799,020-78,016,181): Recently, a critical region was reported for complex congenital heart disease [van Trier et al., 2013]. By comparing the regions of hemizygosity in two patients with those reported in the literature, they identified the terminal 8.17 Mb region of 18q as a critical region for cardiac abnormalities. In the Distal 18q- Reference Group 54% had a cardiac defect. However, the actual incidence of heart defects may be higher as ultrasound and ECG evaluations had not been performed on each individual. Within our entire cohort of individuals with 18q hemizygosity that have been evaluated at our center, 39/134 (29%) had a cardiac abnormality. Within this group with cardiac abnormalities, 43% has an ASD or VSD and 38% had pulmonic stenosis. A small critical region could not be identified implying that there is likely more than one gene on 18q whose hemizygosity can impact the development of the heart.

Kidney malformation (KM) (73107903-75158616): The critical region for kidney malformations proposed by our group [Cody et al., 2009a] has been narrowed at the proximal end by Margarit et al. [2012] who reported a mother and her two daughters with terminal deletion breakpoints at 71,236,981 (hg18). One of the daughters in this family had a hypoplastic left kidney, while the others had normal appearing kidneys. There is little evidence to support any particular candidate gene in this region. Genome-wide studies that have primarily focused on kidney function (as opposed to anatomy) have failed to identify candidate genes in this region. The penetrance in our cohort is 25%.

Vertical talus (VT) (72,980,819-75,485,284): The *TSHZ1* gene has recently been implicated in congenital vertical talus (CVT). This gene lies within the critical region for this phenotype identified by Feenstra et al. [2011]. More recently, Mark et al. [2013] identified the smallest region of overlap in three patients with 18q deletions and bilateral CVT. Although the authors report that the heterozygous *Tshz1* knock out mouse did not exhibit a similar phenotype, there were no data presented about the number of mice evaluated, which may have been insufficient if the phenotype is not completely penetrant [Coré et al., 2007]. In the mouse model of club foot; the Pitx1 mouse, the clubfoot phenotype was only present in 8.9% of the mice [Alvarado et al., 2011]. Thus, additional data are required to determine whether *TSHZ1* is indeed responsible for the CVT phenotype. If *TSHZ1* is responsible for CVT, then it may be responsible for the other distal 18q- foot malformations/deformations as well. While some of the participants in our cohort have vertical talus, they also have other abnormalities of the foot, including pes planus/pes cavus, abnormal toe placement, and metatarsus adductus. In general, the genes known to cause foot abnormalities have overlapping phenotypes including talipes equinovarus, valgus equinovarus, overlapping toes, and vertical talus. Conversely, multiple genes have been associated with foot abnormalities.

CNS Dysmyelination-2 (DM-2) (72,980,819-75,485,284): It has long been hypothesized that hemizygosity of the *MBP* gene is the cause of dysmyelination of the brain in people with distal 18q- [Miller et al., 1990, Gay et al., 1997]. Although this phenotype is 100% penetrant in individuals hemizygous for the dysmyelination critical region that includes *MBP* [Cody et al., 2009a], additional data is required to confirm this genotype-phenotype correlation. No individuals with dysmyelination have been identified with a mutation in or deletion of only the *MBP* gene. The shiverer mouse, which has a deletion of most of the *Mbp* gene, has not provided clues because the dysmyelination phenotype is largely discordant between the mouse and humans [Popko et al., 1987; Readhead et al., 1987]. In the homozygous state, the shiverer mouse has thin myelin sheaths and exhibits tremors but the heterozygote has no tremors and has histologically normal myelin [Shine et al., 1992].

Growth hormone deficiency (GHD) (73,540,560-75,158,616): Growth hormone deficiency has been a key feature of 18q- with significant treatment implications [Ghidoni et al., 1997; Cody et al., 2005]. We have reported previously on the spectrum of the phenotype [Hale et al., 2000] and the associated critical region of the chromosome as well as the high penetrance of 90% [Cody et al., 2009a].

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Cleft palate (CLP) (72,379,769-76,526,497): A critical region for cleft palate was identified by Eudy et al. [2010] who reported six individuals in one family with an interstitial deletion and clefts of the soft palate. This region includes nine known genes. This entire critical region is hemizygous in the Distal 18q- Reference Group, 57% of whom had a palatal abnormality. Within our larger cohort of 112 people with terminal deletions of 18q who were evaluated by our team, all of whom are hemizygous for the cleft palate critical region, five had a cleft lip and palate, nine had a cleft palate alone, and five had a cleft of the soft palate or a bifid uvula.

Therefore even in this larger population, while the overall prevalence of palate abnormalities is lower, there are still no cases of cleft lip in the absence of a cleft palate. There are two candidate genes that have been identified as potential causes of clefts on distal 18q, within the critical region. *TSHZ1*, in addition to playing a role in aural atresia and potentially congenital vertical talus, has also been associated with soft palate formation in the heterozygous knock out mouse [Coré et al., 2007]. An additional gene in the critical region, *ZNF236*, was implicated in cleft palate in a genome wide association study (GWAS), but only in the presence of maternal smoking [Beaty et al., 2011]. Additionally, *SALL3*, a gene lying 200 kb distal to this critical region is involved in craniofacial development in mice [Parrish et al., 2004]. While the heterozygous knock out animals, analogous to people with 18q deletions, were indistinguishable from the wild type, the homozygous animals had a palate and epiglottis that were reduced in size and the tongue was widened anteriorly. These defects caused a failure of the oral pharynx to close appropriately during feeding thereby preventing necessary suction and allowing aspiration resulting in perinatal death. It is therefore not clear if this gene in the hemizygous state could play a role in the palate abnormalities found in people with 18q deletions; either alone or in combination with other tightly linked genes.

As mentioned previously, there are two potential hemizygous lethal regions. These regions are adjacent to the centromere (17,000,000-19,667,062) and at 18q21.1 (45,578,734-46,739,965). While identifying these regions does not impact clinical care since no one has hemizygosity for those regions, it does highlight the potential for identifying key genes in development as well as potential key genes influencing phenotype in trisomy 18.

There are also several phenotypes linked to chromosome 18q for which a causative mechanism has not been established. Therefore haploinsufficiency could be the mechanism and these phenotypes may be a component of or a low penetrance phenotype for someone with hemizygosity of this region. Regions with an as yet unknown molecular mechanism are: anosmia, isolated congenital (ANIC), type 2 diabetes (T2D), adolescent idiopathic scoliosis and pectus excavatum (AIS/PE), bone mineral density (BMD), autism spectrum disorder/play skills (ASD-18-2), microcephaly with simple gyration, epilepsy, and infantile diabetes (MCPH), optic atrophy-4 (OPA-4), idiopathic generalized epilepsy (IGE), essential hypertension (HYT8), autism spectrum disorder/ play skills (ASD-18-3), insulin-dependent diabetes mellitus-6 (IDDM6), amyotrophic lateral sclerosis 3 (ALS3), inattention (ADHD/I), essential hypertension (HYT8) and major affective disorder (MAD18q).

These data serve to illustrate the huge advances made in recent years to uncover the molecular underpinnings of 18q hemizygosity. Of the 196 known genes on chromosome18q we have data to support the hypotheses that 133 (68%) of them are not dosage sensitive and only 15 (8%) of them are clearly associated with an abnormal phenotype. These data guide future work by identifying target genes to be investigated regarding their role in development and homeostasis and potentially their up-regulation.

The ultimate goal is to make 18q- a completely treatable condition. To this end, we pursued two parallel strategies. The first is the long-term strategy to identify and understand the dosage sensitive genes; just outlined above. Concurrently, we sought to identify all potential conventional therapies that might be applicable to this population. Investigating the cause of growth failure led to the finding that many individuals with 18q- were growth hormone deficient [Ghidoni et al., 1997; Hale et al., 2000]. More importantly, growth hormone replacement therapy in this population had beneficial effects on height, performance IQ (pIQ) (Fig. 5A) and myelination [Cody et al., 2005]. In this treatment group there were three individuals whose pIQ did not demonstrate a change because their scores were below the lower limit of measurement for the instruments. We now know that three of these individuals had terminal deletions of 18q that included the *TCF4* gene. Since a deletion that includes *TCF4* has a dramatic effect on cognitive development [Hasi et al., 2011] we reanalyzed the data from these three individuals. Instead of analyzing their cumulative pIQ scores we determined their mental age by determining the mental age of their most advanced skills. These analyses also showed that the changes in the most advanced skills over the course of treatment did not improve in individuals with deletions that included the *TCF4* gene (Fig. 5B). However, we also reanalyzed our previously published MRI data by separating the two groups, those with two copies of *TCF4* (*TCF4*+/+) and those with a deletion that includes *TCF4* (*TCF4*+/-) (Fig. 6). In this analysis there was a significant effect of growth hormone treatment in group with deletions that included *TCF4*. Though *TCF4*+/− and *TFC4*+/+ groups both had a reduction in T1 relaxation times post GH the change was significantly larger in the *TFC4*+/+ group, with relaxation times tending toward normal values. In the *TFC4*+/+ group the reduction in relaxation times was larger in the Caudate and Insula than in frontal white matter. While reduced relaxation times could be related to increases in myelin, this likely would have had more effect in the frontal white matter. The reduction in relaxation times for all tissues indicates micromolecular changes in these tissues that could be a result of reduced tissue water fraction.

While we appreciate that the usual review of a condition should serve to clarify and simplify the topic, in this case it may at first not appear to be simplified. This is in part due to the nature of a text document that covers all possibilities for a continuous gene condition. It should also be apparent why a table with the frequency of each phenotype in a population of people with 18q deletions has little clinical utility.

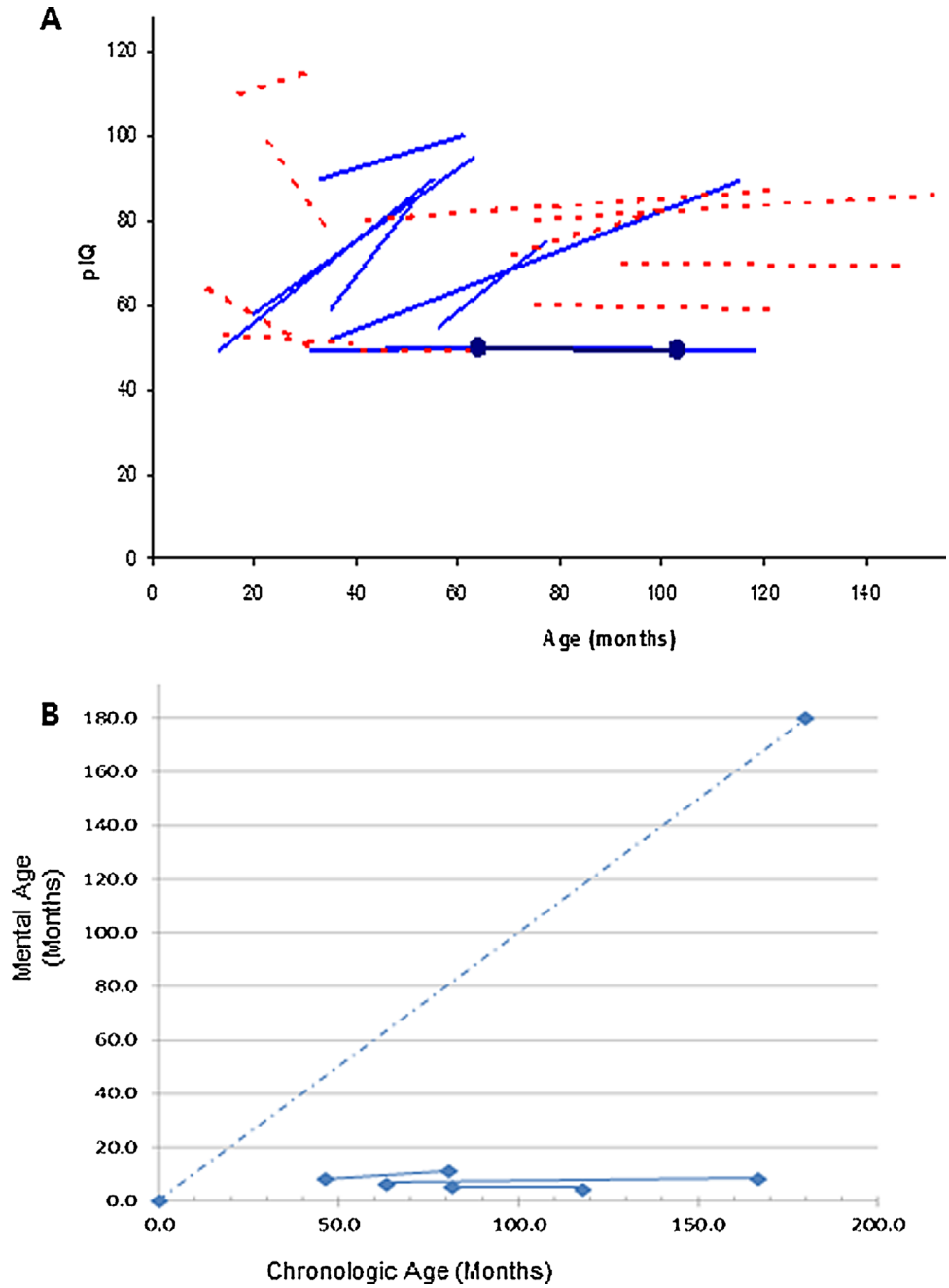

Figure 5. Effect of growth hormone treatment on performance IQ. Panel A was originally published in 2005 (Cody et al.) showing the changes in performance IQ over time for children with 18q- who were treated (solid blue lines) compared to those who were untreated. Panel B shows a reanalysis of the same data used in Panel A for those individuals whose pIQ did not show a change in the original analysis. The dashed lined indicates the expected change in mental age correlated with chronologic age. The solid lines show the changes in mental age over time in the 3 participants in panel A whose pIQ did not change.

For example, if everyone with an 18q deletion is included, then the chance of having CNS dysmyelination is 80%. But to counsel a patient that the chance of CNS dysmyelination is 80% is not at all accurate. This is in part because there are two critical regions for dysmyelination. If the patient's deletion includes the distal dysmyelination region then 100% of these individuals have dysmyelination. If their deletion includes the proximal dysmyelination region their likelihood of having dysmyelination is 25%. If their deletion does not include either of these regions, which is the case for 18% of the people with 18q deletions, then their risk is equal to the background population risk of having CNS dysmyelination. All these frequencies are knowable based on the clinical molecular cytogenomics report.

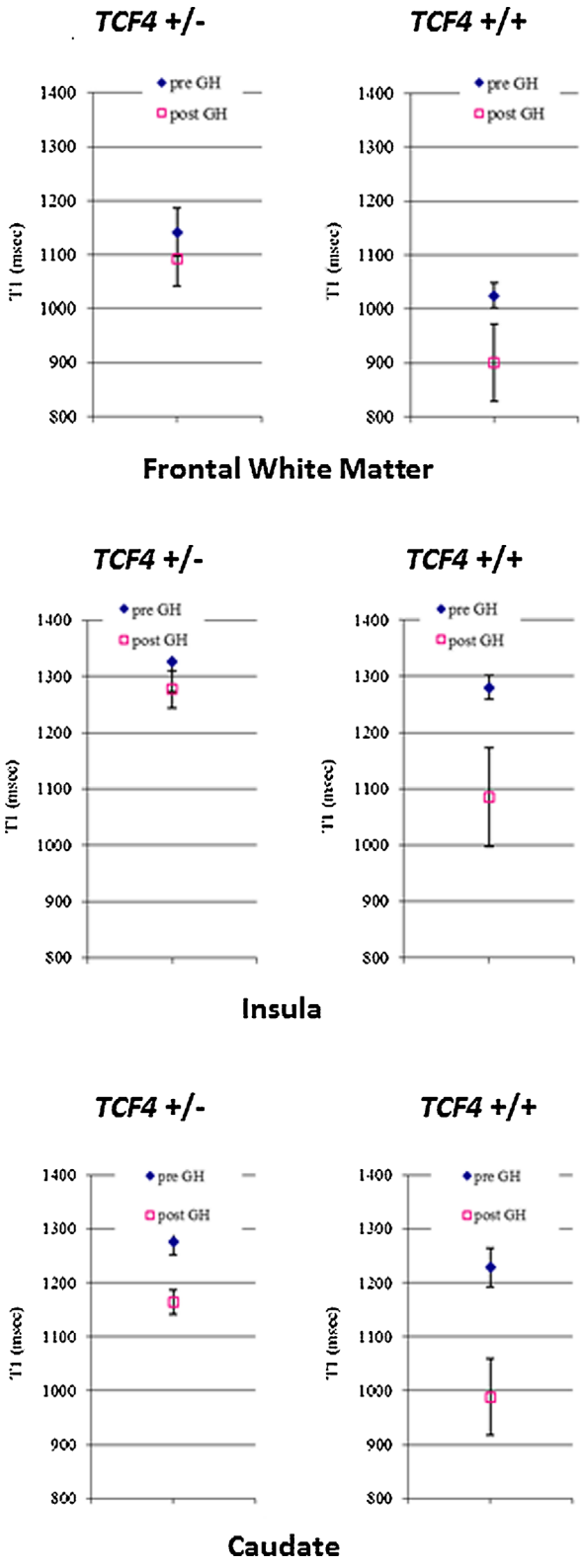

Figure 6. Quantitative MRI changes in response to growth hormone treatment. T1 values for three different brain regions, pre and post growth hormone (GH) treatment. These data compare the data from children with terminal deletions of 18q- whose deletion does not include *TCF4* to those whose deletion does include *TCF4*. Al data are age corrected to 48 months for comparison purposes.

Therefore counseling a patient with an 18q deletion requires the assembly of an individual management plan. This can be done by going to the Chromosome 18 Gene Dosage Annotation on our website (http://pediatrics.uthscsa.edu/centers/Chromosome18/dosage.asp). Clicking on the link to the "Gene Dosage Map or the "Phenotype Map" which will connect to the UCSC Genome Browser. Then, in the "search terms" box, type the coordinates to the patient's deletion preceded by "chr18:". The color coded genes in the region will be in the display window. By clicking on each of the pink dosage sensitive genes a new window will open, then by clicking on the gene name again a details page will open and then by clicking again on the gene name a new page will open with the dosage relevant information for that gene. By repeating the process with the "Phenotype Map" a list of potential phenotypes can be assembled for the specific patient.

Advantages of this approach are that all body systems and ages of the phenotypic presentation are considered. Most "syndrome descriptions" are focused on congenital morphometric differences and profound cognitive or behavioral differences. Phenotypes that are apparent later in life—be they developmental such as executive function or adult onset such as a cancer predisposition are often absent from syndrome descriptions.

Ultimately the Phenotype Map will be whittled down as phenotypes are transitioned to their respective genes. Then the information for each gene will include information about penetrance, expressivity and treatment. In order to get to this place much more work needs to be done.

The genotype/phenotype mapping strategy has worked well for congenital physical malformations such as ear canal atresia cause by hemizygosity of the *TSHZ1* gene. However, this approach is more challenging for the behavioral and cognitive phenotypes which slowly evolve and are unmasked over time and are often states as opposed to traits. Ideally, one would like to have sensitive biomarkers that could be used to track early changes in these phenotypes in the

same way that cholesterol is a biomarker of atherosclerosis and an EEG is for seizure activity. Our current focus is to identify biomarker sentinels of the abnormal biochemistry or physiology underlying the behavioral or cognitive deficits. Since clinically relevant biomarkers for behavioral and cognitive deficient are not currently known, we need to develop them beginning with mouse models. The gene dosage map helps to identify a small number of key genes and consequently the corresponding heterozygous knock-out mice to investigate. In addition, the heterozygous knockout mice are a necessary step in the development of pharmaceuticals to correct the abnormal phenotypes.

Lastly, natural history studies are a critical component for both clinical guidance and treatment development. Very little is known about the aging process in people with 18q deletions. Are there new adult onset conditions? Do some of the congenital problems resolve or get worse over the years? Were there early signs of adult onset conditions that were not appreciated in children that could be remediated or ameliorated if the early first signs and symptoms were appreciated? Are there new as yet unappreciated aspects of the functions of known genes?

The science of chromosome abnormalities and 18q- have made dramatic advances in the last 50 years. In the next 50 years we will see the medicine of 18q- make equal strides making it a completely treatable condition.

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