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Phenotypes and Variants in Cases Submitted for X-Linked Intellectual Disability (XLID) Gene Panel Testing

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Phenotypes and Variants in Cases Submitted for X-Linked Intellectual Disability (XLID) Gene Panel Testing

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Abstract

Intellectual disability (ID) refers to reduced cognitive function, apparent before the age of 18, that negatively affects a person's learning and adaptive capacity. Approximately 1-3% of the population is affected with ID, males more than females, and most in the mild-to-moderate range. ID creates financial, logistical and psychosocial challenges for affected persons and their families and caregivers. It is estimated that up to 50% of ID has a genetic cause. Molecular genetic diagnosis may help in obtaining services and has important implications for family members, but can be elusive. Genes causing ID are known to be over-represented on the X chromosome. Over 160 X-linked intellectual disability (XLID) syndromes and > 100 XLID genes have been identified to date. Greenwood Genetic Center (GGC) offers a next-generation sequencing panel of approximately 90 XLID genes. The diagnostic potential offered by large gene panels is offset by the challenges of interpreting variants of uncertain significance (VUS). In this study, molecular and clinical data from 592 cases submitted for XLID panel testing were evaluated for patterns of phenotype and genotype, in order to further the understanding of XLID. We found a low pathogenic hit rate, a high VUS-only rate and a general absence of statistically significant phenotypic patterns. These results highlight the need for appropriate patient selection, full and accurate phenotype reporting and open sharing of information in order to interpret and learn from the results of genetic testing.

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Chapter 1. Introduction

1.1 Intellectual Disability Overview

Intellectual Disability (ID) is defined as reduced intellectual capacity that is apparent before the age of 18 years, and which creates significant limitations on the individual's ability to learn, understand, communicate and adapt (American Association on Intellectual and Developmental Disabilities (AAIDD), 2014). Intellectual capacity is commonly reported in terms of an Intelligence Quotient, or IQ score. Average IQ is defined as a score of 100. ID is diagnosed when an individual's IQ falls $\geq$ 2 standard deviations below the age-appropriate mean (Tirosh & Jaffe, 2011). ID is considered to begin at IQ of 70-75 and below, and can be characterized as mild, moderate, severe or profound, with measurable decreases in the individual's functional capacity with increasing ID severity. Approximately 1-3% of the population is affected with ID, most in the mild-to-moderate range. Prevalence of ID is 30-40% higher in males, perhaps reflecting the influence of X-linked genes on brain development and function (Leonard & Wen, 2002; Nguyen & Disteche, 2006). Individuals with ID may have difficulty living independently, securing or maintaining employment or caring for themselves. This creates financial and logistical challenges for the affected individuals and for their families, caregivers, and communities.

ID may exist as part of a recognizable, consistent constellation of intellectual, physical and/or functional anomalies that are due to a single underlying etiology, in which case it is considered syndromic ID. Common examples include Down syndrome, Fragile X syndrome and untreated Phenylketonuria (PKU). On the other hand, in non-syndromic forms, ID may be the only finding, brought to attention by the child's failure to develop along a typical time course, or the loss of developmental milestones. There may be other anomalies that do not fall within a recognizable pattern. While some reports state that only 20% of all ID has an identifiable cause (Rauch et al., 2006), others estimate that genetic factors may contribute in up to 85% of all cases (Curry et al., 1997).

1.2 Living With and Caring For Intellectual Disability

By definition, individuals with ID face difficulties in conducting the tasks of daily living. They require assistance ranging from minor supervision and aid to round-the-clock, total care. In addition to their intellectual challenges, individuals with ID may have significant medical and behavioral problems that must be managed. Studies have demonstrated that the responsibility for care falls mainly on families, most particularly mothers, a majority of whom may not have additional, outside employment (Rowbotham, Cuskelly, & Carroll, 2011). Caregivers must personally provide care, arrange and attend medical and service appointments, negotiate with parties such as educators and third party payors, and pay for expenses related to care and services for their family member with ID. This, compounded with the additional expense of care for a disabled child or adult, places enormous strain on caregivers. The ability to provide care is directly related to socioeconomic status and age, with older and younger parents having fewer resources than those in the 45-to-54 age group (Parish, Rose, & Swaine, 2010). Moreover,

intellectual disability is more common in individuals of lower socioeconomic status, meaning that the population with the fewest resources for providing care and obtaining services for disabled individuals has the greatest need of those services (Emerson, 2012). Children with ID grow up to be adults with ID, who continue to require lifelong supervision and care. The burden and expense to their families/caregivers is therefore lifelong as well. As parents age, there can be considerable distress surrounding issues of care beyond the time the parents are able to provide it themselves (Dillenburger & McKerr, 2011). As many as 62% of caregivers have no alternative care plan in place for if or when they are no longer able to provide care personally (Anderson, Larson & Wuorio, 2011). This has become an issue of increasing significance as care and life expectancy for individuals with intellectual disability have improved markedly over the last several decades. For instance, life expectancy for individuals with Down syndrome in the 1980s was approximately 25 years, but in the late 1990s had increased to 49 and is currently estimated at the mid-50s (Coppus, 2013; Yang, Rasmussen, & Friedman, 2002). As with any aging persons, individuals with ID face increasing health concerns as they grow older, a factor that increases the complexity and cost of their care (Strydom et al., 2010), even as their parents experience the challenges of aging themselves (Bittles et al., 2002).

A major source of services for the intellectually disabled is state-funded assistance programs, the history of which is nicely reviewed by Harold Pollack (Pollack, 2011). The largest of these is Medicaid, which funds not only medical services, but other educational and disability resource services. The recent decline in the United States economy and associated tax revenue has necessitated cost-cutting measures among state

governmental agencies, including those programs providing services for the disabled. The early 2000s saw a dramatic drop in the average yearly increase in state funding for disability services, from 6.6% to less than 2%. Between 2008 and 2009, 47 states experienced percentage decreases and there was a drop in actual dollar expenditures for disability services in 23 states (Braddock et al., 2011). Perhaps most unfortunately, the states with the lowest pre-recession disability funding levels (that is, states in which the disabled were already at a disadvantage) were also the states that implemented the deepest cuts in disability services funding (Pollack, 2011).

In addition to the financial and logistical burdens borne by individuals with ID and their families, there are social burdens as well. Intellectual impairment remains one of the disabilities that is least understood and accepted in many modern cultures (Scior, 2011). The lack of awareness can lead to negative attitudes about people with ID, manifested in such ways as bullying, violent/hate crimes, discrimination, avoidance, lack of inclusion and stigmatization (Ali, Hassiotis, Strydom, & King, 2012; Scior, 2011; Werner, Corrigan, Ditchman, & Sokol, 2012). Stigmatization may be directed not just toward individuals with ID, but toward their families, friends and associates as well, known as "courtesy" or "affiliate" stigma (Ali et al., 2012). Tragically, affected individuals exposed to such negative attitudes often internalize them and adopt self-deprecating thoughts and attitudes ("self-stigma"), a phenomenon that has been documented in individuals with ID for many decades (Ali et al., 2012).

1.3 X-Linked Intellectual Disability (XLID)

By definition, X-linked ID follows an X-linked pattern of inheritance, whether or not a specific X-linked genetic cause has been identified. Alternatively, it is ID that is

caused by mutation of an X-linked gene, with or without a family history. Depending on the underlying etiology, an X-linked family history may present with affected males only; with both males and females affected, equally or differentially; or with only females affected, due to male lethality. XLID may be syndromic (ID plus additional features), non-syndromic (ID only), or neuromuscular (for instance, associated with Duchenne/Becker muscular dystrophy). Approximately 160 XLID syndromes have been described; however, the majority of XLID is non-syndromic (Stevenson, Schwartz & Rogers 2012; Lubs, Stevenson & Schwartz, 2012). It is estimated that ~10% of genes on the X chromosome, or at least 120 genes, may contribute to XLID; over 100 of these have been identified to date (Lubs, Stevenson & Schwartz et al., 2012).

1.4 Selected Features Often Associated with Intellectual Disability

1.4.1 Abnormal head size.

The biological function of the cranium (brain vault of the skull) is to enclose and protect the brain within it. Like a motorcycle helmet, this function is best served when the fit is neither too loose nor too tight. The dimensions of the cranium are therefore determined by the volume of the brain. Measurement of the occipito-frontal circumference (OFC) is a useful clinical tool for estimating whether an individual's brain volume falls within the expected range for their age and sex. Head size in ID may be smaller (microcephaly) or larger (macrocephaly) than expected. In general, a relative head size that changes over time (that is, an increasing or decreasing OFC percentile) is more worrisome than an OFC that remains stable in terms of percentile. It should be noted that a recent meta-analysis of worldwide human growth data has demonstrated that OFC means can vary significantly according to geographical, national or ethnic

backgrounds (Natale & Rajagopalan, 2014). The authors concluded that universal use of the World Health Organization (WHO) OFC standards may lead to inappropriate characterization of young children as having micro- or macrocephaly.

Microcephaly, its causes, evaluation and implications have been thoroughly discussed in a recent review (Woods & Parker, 2013). Primary microcephaly is often defined as an OFC at least two standard deviations smaller than the mean at birth (and often detected prenatally). Some would argue that three standard deviations is a more appropriate cutoff, and that head shape should be taken into consideration as well (Woods & Parker, 2013). In primary microcephaly, the fetal brain does not grow to typical size, therefore the cranium also remains small. Brain architecture may be essentially normal or may show underdevelopment and/or abnormal formation of specific structures. The causes of primary microcephaly are many (Woods & Parker, 2013) and include failure of neurogenesis due to insults such as single gene defects (e.g., autosomal recessive primary microcephaly); chromosomal imbalance (e.g., Trisomy 21 or 7q11.23 deletion a.k.a. Williams syndrome); or infection (e.g., cytomegalovirus (CMV) or Toxoplasmosis). It may also arise from physical damage such as hypoxia/ischemia or toxicity due to uncontrolled maternal PKU or other metabolic defects. Individuals with primary microcephaly will usually have some degree of intellectual disability and developmental delay, ranging from mild to severe.

Secondary, acquired or progressive microcephaly occurs when the brain (and head) fails to grow adequately during infancy and childhood. Head circumference may be small to normal at birth, but eventually becomes significantly smaller than average. The slowing or cessation of postnatal brain growth can be caused by any factor, intrinsic

or extrinsic, that interferes with proliferation and maintenance of neurons (Woods & Parker, 2013).

Macrocephaly, by contrast, refers to an OFC that is greater than two (or three; see above) standard deviations larger than average, suggesting a larger-than-average brain size. This is not necessarily due to an increased amount of brain tissue, but may be due to enlargement of the ventricles and accumulation of fluid within the brain (ventriculomegaly or hydrocephalus), which in itself can be medically problematic. Macrocephaly may or may not be seen with autism spectrum disorders, (Barnard-Brak, Sulak, & Hatz, 2011; Grandgeorge, Lemonnier, & Jallot, 2013), which are often but not always associated with intellectual deficits. Macrocephaly is a feature of many genetic conditions, with or without associated ID, but may also be a benign familial trait.

1.4.2 Seizures.

Seizures are involuntary, abnormal electrical events in the brain that alter the movement, senses and/or consciousness of the individual experiencing the seizure. Although the acute seizure activity is usually not continuous, individuals with seizure disorders demonstrate abnormal brain wave patterns by electroencephalogram (EEG) even when not undergoing a (recognizable) seizure. There is an extensive list of seizure disorder classifications according to such criteria as physical manifestations, area of the brain affected, precipitating events, etc. (Berg et al., 2010; Shorvon, 2011); however all seizures result from an alteration of the normal electrical discharge pattern(s) of the brain. Seizures are very common with ID disorders, an unsurprising finding given that both result from disruption of the normal neuronal network. Persons of normal intellect may

also have seizure disorders. Unfortunately, seizures themselves can result in damage to the brain and acquired ID.

1.4.3 Autism spectrum disorder.

Autism spectrum disorder (ASD) encompasses a range of conditions that share the features of impaired social relationships, impaired language and communication, and repetitive behaviors and/or narrow range of interests (Miles, McCathren, Stichter & Shinawi, 2013). Signs and symptoms are often apparent in early childhood or infancy. Features may develop gradually after an initially "normal" period of development, and in some cases (30%) may involve regression, or loss of developmental milestones. ASD may be syndromic ("complex") or non-syndromic ("essential"), but involves intellectual disability in 50-75% of cases. Seizure disorders are a common co-morbidity, occurring in about 25% of affected individuals. Up to 75% of people with ASD experience lifelong disability associated with the diagnosis. Even those with average intellectual ability often have trouble living independently as adults (Farley et al., 2009).

The prevalence of ASD in the United States has increased rapidly in recent years. The Autism and Developmental Disabilities Monitoring (ADDM) Network within the Centers for Disease Control and Prevention (CDC) periodically generates reports on ASD prevalence, based on data about 8-year-old children at selected surveillance sites around the country (Centers for Disease Control and Prevention, 2014). In 2000 and 2002, the ADDM Network-reported ASD prevalence was approximately 1 in 150 children. In 2006, the prevalence was 1 in 110, and in 2008, ASD was found in 1 in 88 children.

On March 28, 2014, the ADDM Network reported that, as of 2010, 1 in 68 children in the United States had been diagnosed with ASD (ADDM Network, 2014).

This figure varied considerably according to demographic characteristics. Consistent with prior data, males (1 in 42) were affected 4 to 5 times more commonly than females (1 in 189). Intellectual disability was reported in 31% of children, with another 23% falling in the borderline range (IQ 70-85). These rates are lower than previously reported, continuing the previous decade's trend of decreasing association of ID with ASD. It follows that the increased prevalence of ASD is composed substantially of individuals with average or higher intellectual ability. Females were more likely than males to have ASD with associated ID (36% of affected females vs. 30% of males). Caucasian (non-Hispanic) children were much more likely to be diagnosed with ASD than either Hispanic or African American children, and were less likely to have associated ID. ASD *with* ID was most frequent in African-American children. There was less demographic stratification among children with ASD *and* ID than in those with ASD alone. Regional prevalence varied also, from 1 in 175 in Alabama to 1 in 45 in New Jersey. The ADDM Network postulated that at least some of the demographic and temporal variation in prevalence is due to factors such as variable diagnostic practices and geographical migration driven by access to services.

1.5 Genetic Testing for Intellectual Disability

1.5.1 Genetic testing methods and intellectual disability.

The ability to perform genetic testing for ID was established in 1959 with the recognition that cells from individuals with Down syndrome contained 47 chromosomes (rather than 46) by cytogenetic or karyotype analysis (Lejeune, Gauthier, & Turpin, 1959). Cytogenetic analysis is able to detect large-scale alterations in genomic dosage or structure, as in the above-mentioned case of Down syndrome (the presence of an extra

entire chromosome 21 or significant portion thereof) or Fragile X syndrome where the causative trinucleotide expansion changes the appearance of the X chromosome under certain cell culture conditions, creating a "fragile" site (Lubs, 1969). Fragile X repeat expansions are now assessed using molecular methods: polymerase chain reaction (PCR) amplification of repeat regions and/or Southern blot protocols. Up to 15% of individuals with ID may have genomic aberrations that are cytogenetically detectable (Leonard & Wen, 2002). Although different preparations may affect the resolution of karyotyping, generally changes must encompass a minimum nucleotide size of approximately 5-10 megabases (Mb; one million nucleotide bases) to be visible by classical cytogenetic techniques. Interphase fluorescence in situ hybridization (FISH) may increase the resolution, with the ability to detect changes at the level of 100s of kilobases (kb; one thousand nucleotide bases) (Vorsanova, Yurov, & Iourov, 2010). This resolution is still at the whole-gene/several gene level in many cases. More recently, array-based techniques such as comparative genomic hybridization (or aCGH) have been employed to recognize chromosomal microdeletions and microduplications that are not visible by cytogenetic methods. While aCGH may identify putative pathogenic variants in an additional 15% of patients with ID over those whose underlying genetic etiologies are identified by the classical cytogenetic techniques, microarray studies have led to the discovery of an enormous degree of copy number variation between individual genomes, much of which is of unclear significance (Bui, Vetro, Zuffardi, & Shaffer, 2011). For instance, a recent study of aCGH in individuals with ID uncovered sixteen microduplications or microdeletions in twelve patients, only five of which were *de novo* and presumably pathogenic (Zrnova, Vranova, Slamova, Gaillyova, & Kuglik, 2011).

Resolution of microarray-based analysis may be as fine as several kilobases, dependent upon the specific microarray platform design. Some arrays are designed to assess the presence or absence of specific mutations only, including single-nucleotide changes. These are often carrier screening panels relying on foreknowledge of, and tiling of, probes for anticipated mutations; they do not assay any changes not specifically tiled on the array. All of these methods have clinical utility, but are incapable of detecting unanticipated changes at the single- or oligonucleotide level that may deleteriously alter gene function.

Thus, when hunting for causative mutations, full gene sequencing is often the technique most likely to yield results. There is a current expectation in the medical community that, due to the rapid advancement of technology and decreasing cost, whole-genome (or at least whole-exome) sequencing will soon become a routine, standard-of-care medical test (Brunham & Hayden, 2012; Drmanac, 2012). Such genome-wide sequencing will inevitably uncover thousands of variants, pathogenic or otherwise. The subsequent data analysis, data storage, genetic counseling and follow-up resource requirements, as well as ethical considerations regarding reporting of incidental or uncertain findings, are astronomical and currently impractical for most applications. A viable compromise is to perform full-gene or full-exome sequencing on a condition-specific gene set. This approach has the benefit of simultaneously reducing the data management burden and possible genetic counseling complexities while maintaining a relatively high mutation identification rate. Recently published studies have applied this strategy of gene panel sequencing to, for instance, childhood cancer (Plon et al., 2011);

epilepsy (Lemke et al., 2012); hereditary hearing loss (Baek et al., 2012); and mitochondrial disease (Calvo et al., 2012).

1.5.2 X-linked intellectual disability gene panel tests.

Current estimates suggest that > 10% of all ID affecting males may be due to X-linked genetic causes (Gecz, Shoubridge, & Corbett, 2009; Ropers, 2008). Genetic loci contributing to ID are significantly over-represented on the X chromosome compared with the autosomes (Lubs et al., 2012). In addition, X-linked genes are more likely to be highly expressed in brain than autosomal genes (Nguyen & Disteche, 2006). Therefore, XLID genes may be a reasonable place to look when searching for a genetic cause for ID, even when there is no clear family history with X-linked inheritance pattern. City of Hope Molecular Diagnostic Laboratory currently offers a Sanger sequencing panel of 8 XLID genes (City of Hope Molecular Diagnostic Laboratory, 2014). A handful of commercial laboratories are currently offering XLID NextGen sequencing panels: Ambry Genetics (81 genes, Ambry Genetics, 2014); the University of Chicago Genetic Services (75 genes, the University of Chicago Genetic Services, 2014); Emory Genetics Laboratory (92 genes, Emory Genetics Laboratory, 2014); and Greenwood Genetic Center (GGC) (90 genes, GGC, 2014).

1.5.3 Greenwood Genetic Center X-linked intellectual disability panel.

The Greenwood Genetic Center is a not-for-profit clinical and diagnostic genetics services provider based in Greenwood, SC. Genetic testing services are available for a wide variety of conditions, including intellectual disability. In 2010 GGC began offering a next-generation sequencing panel test of 92 genes associated with X-linked intellectual disability, developed in conjunction with Emory Genetics Laboratory. Since the panel's

inception, GGC has revised its reporting practices to conform with updated information regarding the involvement of specific genes in XLID. Current GGC literature states that the panel comprises 90 XLID genes; however, results from only 89 genes were being reported at the time of this study (M. Friez, personal communication, February 28, 2014). These genes are located throughout the length of the X chromosome and include genes associated with both well-defined syndromes and non-syndromic ID.

Coding exons and flanking intronic sequences of the genes within the XLID panel are amplified and sequenced using next-generation sequencing (NGS) methods. The results are then subjected to bioinformatic analysis (M. Friez, personal communication, May 16, 2013). Laboratory results are reviewed by at least two Directors, and identified variants are confirmed by Sanger sequencing. If necessary, sequence analysis of variants of unknown significance (VUS) is carried out on samples from appropriate family members (parents or siblings when available) to help distinguish pathogenic and benign variants. The testing process, including bioinformatics analysis, takes approximately three months to complete.

A checklist of clinical features is included as part of the test ordering documentation. Ordering physicians are asked to supply a pedigree and photographs of the affected individual. Cases submitted with complete clinical information, including photographs, are eligible for assessment by the GGC Intellectual Disability Evaluation and Advice System (IDEAS) team, an international panel of experts in clinical genetics. Of the first 100 cases submitted for XLID panel analysis, over 50% did not include clinical information. Only 22 of 100 cases were provided with sufficient clinical information, including photographs, to be eligible for clinical review by the IDEAS team.

To date, the proportion of samples provided with complete clinical information remains at about 20% (M. Friez, personal communication, April 4, 2013).

1.6 Selected X-linked Intellectual Disability Genes

1.6.1 FMR1: Fragile X syndrome and related disorders

Fragile X syndrome is the most common inherited cause of intellectual disability, with an incidence of about 1 in 3600 to 1 in 4000 males, and 1 in 4000 to 1 in 6000 females (National Fragile X Foundation, 2014). Males with Fragile X syndrome have recognizable physical characteristics, including a long, narrow face; large and prominent ears; high arched palate; connective tissue abnormalities such as hyperextensible joints and mitral valve prolapse; and macroorchidism (Gallagher & Hallahan, 2012; Saul & Tarleton, 2012). Intellectual function is usually in the moderate to severe disability range. Cognitively, individuals with Fragile X often fall within the autism spectrum and have a characteristic intellectual-psychological profile that includes executive and memory deficits; attention deficits; obsessive tendencies; anxiety, aggression and social avoidance. Females with Fragile X syndrome have a similar, but usually less severe, phenotype, with only ~25% of carrier females having IQ < 70 (Gallagher & Hallahan, 2012; Hagerman et al., 1992).

The gene responsible for Fragile X syndrome, FMR1, is located at Xq27.3. The gene product, Fragile X Mental Retardation Protein (FMRP), is an RNA-binding protein involved in translational regulation of many other proteins (Wang, Bray, & Warren, 2012). The Fragile X locus was originally located, and named, due to the appearance of a "fragile" site on affected chromosomes under certain cell culture conditions (Lubs, 1969). This locus was later found to contain the FMR1 gene (Verkerk et al., 1991). The

promoter region of the FMR1 gene contains a tract of CGG trinucleotide repeats, the length of which is highly variable. With an increasing number of repeats, the allele becomes more unstable and vulnerable to slippage during replication, leading to expansion (Fu et al., 1991). Interestingly, this instability is primarily manifested in oogenesis, therefore alleles may expand when passed from female carriers but are unlikely to expand when transmitted by a male carrier. Fully affected individuals have an allele size of more than 200, and sometimes many hundreds, of repeats. This significant expansion in the repeat tract triggers abnormal hypermethylation of the affected allele, shutting down gene expression and depriving the cell of FMRP. Loss of FMRP and the resultant misexpression of multiple proteins in neurons leads to improper synapse formation. This is thought to be directly responsible for the intellectual deficits seen with Fragile X syndrome.

Tracts of 55-200 CGG repeats are known as premutations. These unstable alleles carry a risk of expansion with each female generation that is correlated with the number of repeats; the larger the allele, the greater the chance of expansion to a full mutation. In unaffected individuals, the number of CGG repeats ranges up to about 54, however between 45-54 repeats is considered a "gray zone" that is at risk for slight expansion. This expansion may create a premutation allele, but not a full mutation. The chance of affected children for a "gray zone" allele carrier is therefore very low, but her grandchildren may be at risk if her daughter inherits a premutation allele.

In addition to the classic Fragile X syndrome, pre- and full-mutation alleles of FMR1 can lead to several other disorders. Up to 20% of female premutation carriers experience primary ovarian insufficiency or POI, a condition that causes early ovarian

failure/menopause and can lead to infertility (Hundscheid, Braat, Kiemeney, Smits, & Thomas, 2001; Allingham-Hawkins et al., 1999; Conway, Hettiarachchi, Murray, & Jacobs, 1995). POI is not associated with full mutation alleles. Female premutation carriers may also experience anxiety or major depressive disorders (Roberts et al., 2009) and other problems such as thyroid disease and fibromyalgia (Rodriguez-Revenga et al., 2009). Male or female premutation carriers are at risk for Fragile X-associated Tremor Ataxia syndrome (FXTAS), an adult-onset neurodegenerative condition involving both neuromuscular and cognitive decline (Coffey et al., 2008; Hagerman et al., 2001). Because of the association of autism spectrum features with Fragile X syndrome, Fragile X remains a first-tier genetic test for individuals with autistic features as well as those with ID.

1.6.2 ARX

A detailed review of ARX genotypes and phenotypes has recently been published (Shoubridge, Fullston, & Gecz, 2010). XLID due to ARX mutation ranges from mild to severe (Stromme, Mangelsdorf, Scheffer, & Gecz, 2002) and is most often syndromic, although ID may be isolated in some cases. When syndromic, ARX-related neurological features include seizures (including infantile spasms), autism, dysarthria (motor speech disorder) and dystonia (involuntary muscle contractions) (Shoubridge et al., 2010). When physical malformations are present, they are limited to the brain (lissencephaly, hydranencephaly and agenesis of the corpus callosum) and genitalia (Kato et al., 2004). Chronic diarrhea has also been reported in severely affected individuals (Kato et al., 2004). Named syndromes associated with ARX mutations include West syndrome (X-linked infantile spasms) (Kato, Das, Petras, Sawaishi, & Dobyns, 2003) and Proud

syndrome (X-linked intellectual disability, agenesis of the corpus callosum, and abnormal genitalia) also known as X-linked Lissencephaly Abnormal Genitalia syndrome or XLAG (Kitamura et al., 2002; Proud, Levine, & Carpenter, 1992).

Mutations in the ARX gene have been found in up to 7.5% of families demonstrating an apparent XLID (Shoubridge et al., 2010; de Brouwer et al., 2007). Almost every mutation class has been reported; however, by far the most common is a recurring, 24-base pair duplication that increases the gene's second polyalanine tract from 12 to 20 alanine residues. This duplication may cause syndromic or non-syndromic ID, tending toward the milder end of the spectrum, but demonstrates significant phenotypic variability both within and between families (Turner, Partington, Kerr, Mangelsdorf, & Gecz, 2002). In general, however, ARX genotype-phenotype correlation is fairly consistent (Olivetti & Noebels, 2012). Female carriers of ARX mutations range from completely asymptomatic (often associated with mutations causing milder phenotypes in males) to significantly affected (Bettella et al., 2013; Bonneau et al., 2002; Kato et al., 2004; Proud et al., 1992; Scheffer et al., 2002).

1.6.3 DMD

DMD is the largest gene in the human genome, spanning 2.4 megabases of genomic sequence on Xp21.2. DMD encodes the protein dystrophin, a component of a multiprotein complex responsible for linking the muscle cell cytoskeleton to the extracellular matrix, thereby stabilizing the cell against contraction-induced damage; hence, the dystrophinopathies are primarily muscular disorders. Incidence of dystrophinopathies is estimated at 1:3500 - 1:6000 male births (National Center for Biotechnology Information (NCBI), 2014; Flanigan, 2012). Affected males are normal at

birth, but begin to experience muscle degeneration and weakness in childhood, eventually losing the ability to walk or perform self-care. Cardiac and respiratory muscles degenerate as well, leading to death most commonly from cardiorespiratory failure. Disease severity is related to residual quantity and function of dystrophin; Becker type (BMD) displays later onset and slower progression associated with reduced dystrophin, while Duchenne type (DMD) has earlier onset, more rapid progression and (near) complete absence of dystrophin. Females may also express a DMD/BMD phenotype, due to various phenomena that expose DMD mutations, such as skewed X-inactivation; uniparental disomy (UPD) X; compound heterozygosity for DMD mutations; and monosomy X (Turner syndrome) (Darras, Miller & Urion, 2011). Approximately 8-18% of carrier females eventually develop cardiomyopathy (Hoogerwaard et al., 1999). Rare families display X-linked cardiomyopathy only in both males and females, associated with specific mutations in the DMD gene (Neri et al., 2007).

DMD gene mutations, including those responsible for BMD, are frequently deletions (60-70% of mutations) but also include point and other mutations distributed throughout the entire coding region (Dent et al., 2005; Flanigan et al., 2009). In general, deletions that maintain the reading frame allow production of a shortened, but partially functional, dystrophin molecule and milder phenotype (Monaco, Bertelson, Liechti-Gallati, Moser, & Kunkel, 1988). Only one DMD founder mutation has been reported (Flanigan, Dunn, von Niederhausern, Howard, et al., 2009). Consistent with its large gene size, DMD has a high forward mutation rate, and 30% of Duchenne and 10% of BMD mutations occur *de novo*. Because of the high frequency of pathogenic deletions (and duplications) in DMD, first-line testing when a dystrophinopathy is suspected is

multiplex ligation-dependent probe amplification or MLPA. This test gauges the presence or absence of specific exons according to whether (PCR-based) amplification is achieved. Second-tier testing for dystrophinopathies is sequence-based analysis for point mutations or small insertions/deletions not detectable by MLPA.

The DMD gene contains 79 exons and undergoes alternative splicing to generate many dystrophin isoforms, some of which are brain-specific. Although the primary feature of dystrophinopathies is muscle weakness and degeneration, up to one-third of boys with DMD mutations also display some degree of intellectual disability. Since the combination of intellectual and motor delays (i.e., global delay) is not uncommon in young children, it may not immediately be apparent that these signs in a toddler boy indicate the onset of a dystrophinopathy. A DMD mutation causing familial ID with no muscle weakness has recently been reported (de Brouwer et al., 2013). This in-frame deletion of a single amino acid affects the brain-specific dystrophin isoform Dp71. For these reasons, DMD is included in some XLID gene panels, with the understanding that only about 10-35% of pathogenic DMD mutations are point/small sequence changes (Darras et al., 2011). However, NextGen sequencing may still identify larger deletions because of failure to amplify or reduced read density compared to other exons or genes. Due to the large size of the gene, sequence variants that are benign or of uncertain significance are common (Flanigan, Dunn, von Niederhausern, Soltanzadeh, et al., 2009), creating challenges for interpretation of sequence-based results.

1.6.4 ATRX

ATRX is the causative gene in alpha-thalassemia mental retardation X-linked (ATR-X) syndrome (Gibbons, Picketts, & Higgs, 1995), which consists of severe

intellectual disability, facial, skeletal and urogenital abnormalities and variable, mild alpha-thalassamia (Gibbons, Brueton, et al., 1995; McPherson, Clemens, Gibbons, & Higgs, 1995). Incidence of ATRX spectrum disorders is not known. ATRX phenotypes occur along a continuum that includes mild, non-syndromic ID in the least-affected individuals (Guerrini et al., 2000; Yntema et al., 2002). Clinical features are rarely seen in female carriers, nearly all of whom (>95%) demonstrate highly skewed X-inactivation (XI) patterns (Stevenson, 2010). Reports of affected females demonstrating random XI (Wada, Sugie, Fukushima, & Saitoh, 2005) or highly skewed inactivation of the non-mutation-bearing X (Badens et al., 2006) suggest that the skewed XI in carrier females is protective. The ATRX protein is involved in chromatin remodeling processes, including DNA replication and gene expression, and has been shown to play a role in certain cancers. Abnormal chromatin regulation in the absence of ATRX function leads to dysregulation of multiple genes and pathways (Clynes & Gibbons, 2013; Clynes, Higgs, & Gibbons, 2013). This, then, provides a plausible selective disadvantage for cells with active, ATRX mutation-bearing X chromosomes and an explanation for the highly-skewed XI in unaffected female carriers (Migeon, 2007).

ATRX is a relatively large gene, at 350 kilobases of genomic sequence. Mutations are found in the zinc finger (exons 7-9) and helicase (exons 17-20) domains in about 90% of cases.

1.6.5 CASK

Unlike many forms of XLID, CASK-related ID is seen more often in females than in males. The CASK phenotype classically includes significant to severe ID accompanied by brain (including optic) malformations, marked microcephaly (onset

prenatally or within the first year of life) and distinctive facial features (Moog et al., 2011; Moog, Uyanik & Kutsche, 2013). Brain imaging consistently shows pontocerebellar hypoplasia with dilated fourth ventricle. There is reduced gyration in the cerebral cortex of some, but not all patients. When affected, the male phenotype is variable ID with or without congenital nystagmus, cerebellar hypoplasia and micro- or macrocephaly (Hackett et al., 2010; Piluso et al., 2009; Tarpey et al., 2009). CASK mutations may also be responsible for a subset of FG syndrome, comprising developmental delay, agenesis of the corpus callosum, macrocephaly, hypotonia, digestive disturbances and characteristic personality (Piluso et al., 2009).

Genotype-phenotype correlation studies have indicated that CASK mutations causing phenotypes in females tend to be null/inactivating and male lethal. Male viable mutations are generally missense and splicing mutations that are expected to be hypomorphic and may be asymptomatic or penetrant in carrier females (Hackett et al., 2010; Najm et al., 2008). Mutations causing nystagmus are clustered at the C-terminal of the protein (Hackett et al., 2010). The CASK protein is a ubiquitous serine protein kinase that is highly expressed in fetal brain, consistent with the phenotypes of ID and brain malformation.

1.6.6 PQBP1

PQBP1 encodes a polyglutamine-binding protein that is thought to play a role in transcriptional and splicing regulation. Mutations in PQBP1 have been identified in individuals/families with a variety of named syndromes, including Sutherland-Haan, Hamel, Renpenning, Porteous, and Golabi-Ito-Hall, as well as periventricular heterotopia; these can now be grouped under "Renpenning spectrum" disorders (Germanaud et al.,

2011; Gerrard & Renpenning, 1974; Kalscheuer et al., 2003; Kleefstra et al., 2004; Kunde et al., 2011; Lubs et al., 2006; Stevenson et al., 2005; Sutherland, Gedeon, Haan, Woodroffe, & Mulley, 1988). Incidence/prevalence figures are not known. Common features within the Renpenning spectrum include variable intellectual disability, severe microcephaly, craniofacial dysmorphism, velar dysfunction, sparse hair, short stature, lean body habitus, selective muscular atrophy and genital anomalies. However, there is variability in presentation within families, and between families with identical mutations (Kalscheuer et al., 2003). Some families demonstrate congenital heart defects, anal stenosis/atresia, spastic diplegia, hypermetropia, strabismus, hearing loss and/or other anomalies, while in others, ID is the only phenotype. Autism and neuropsychological/behavioral problems are common. Features may be stable or progressive. Female carriers are mostly of normal intelligence, with random XI (Kalscheuer et al., 2003) (one notable exception reported by Fichera et al., 2005), but may show a slightly reduced head circumference (Germanaud et al., 2011).

The most common mutations seen in PQBP1 are deletions and duplications of AG dinucleotides within an $(AG)_6$ tract in exon 4 (Germanaud et al., 2011; Kalscheuer et al., 2003). These generate frameshifts, leading to premature termination of the protein. PQBP1 transcripts containing premature STOP codons likely undergo nonsense-mediated decay, resulting in reduction or absence of PQBP1 protein (Kalscheuer et al., 2003). Mutant PQBP1 protein has also been shown to be mislocalized throughout the cell rather than confined to the nucleus (Kalscheuer et al., 2003). Other mutations, including other deletions and nonsense changes, also lead to premature termination.

1.6.7 L1CAM

L1CAM at Xq28 is the gene underlying L1 syndrome spectrum disorders (Legius, Kaepernick, Higgins, & Glover, 1994; Schrander-Stumpel & Vos, 2010). Phenotypic features are variable between and within families and include X-linked hydrocephalus with or without stenosis of the aqueduct of Sylvius, seizures; ID, speech delay, hypotonia, spastic paraplegia, agenesis of the corpus callosum and adducted thumbs (Vos et al., 2010). Hirschsprung disease has been reported in individuals with L1 syndrome, but it is not clear whether this finding is coincidental or influenced by the L1CAM mutation. ID with L1CAM mutations can range from mild to severe. Brain MRI may be normal or may reveal subclinical hydrocephalus or other malformations. Approximately 5% of female carriers display mild features of L1 syndrome but are rarely reported to be severely affected (Kaepernick, Legius, Higgins, & Kapur, 1994; Vos et al., 2010). L1CAM mutations are almost always single- or oligonucleotide variants detectable by sequence analysis, and occur *de novo* about 7% of the time (Vos & Hofstra, 2010). Mutations that cause premature truncation of the L1CAM protein are associated with a greater likelihood of death in early childhood compared to missense mutations (Vos et al., 2010).

1.6.8 KDM5C

KDM5C (also known as JAR1D1C and SMCX) at Xp11.22 encodes lysine (K)-specific demethylase 5C, a ubiquitously expressed protein involved in chromatin remodeling through the removal of methyl groups from lysine 4 of histone H3 (Iwase et al., 2007; Tahiliani et al., 2007). KDM5C was identified as a cause of X-linked intellectual disability in 2005 and has since been shown to play a role in survival of neurons and dendritic development (Iwase et al., 2007; Jensen et al., 2005). Loss of

DNA methylation has been demonstrated at multiple loci in individuals with KDM5C mutations (Grafodatskaya et al., 2013), and its depletion via RNA interference results in de-repression of multiple neuronal target genes (Grafodatskaya et al., 2013; Tahiliani et al., 2007). Interestingly, downregulation of KDM5C has been proposed as a potential therapeutic approach in Huntington's disease, to reverse the overexpression of neuronal genes triggered by mutant huntingtin (Vashishtha et al., 2013).

Like many other XLID genes, KDM5C mutations may lead to syndromic or non-syndromic forms of ID. Estimates of the KDM5C mutation frequency in XLID families range from 0.6% to 2.8% (Goncalves et al., 2014; Jensen et al., 2005). When syndromic, associated features include variable ID, speech delay, short stature, dysmorphic facial features, genital anomalies, altered muscle tone, ataxia, spastic paraplegia and aggressive behavior (Goncalves et al., 2014; Jensen et al., 2005; Ounap et al., 2012). Females may be affected as well as males, at least in part because KDM5C is one of several X chromosome genes that escape inactivation and are more highly expressed in females than in males (Johnston et al., 2008; Ounap et al., 2012).

Chapter 2

Phenotypes and Variants in Cases Submitted for X-Linked Intellectual Disability (XLID) Gene Panel Testing[1]

2.1 Abstract

Intellectual disability (ID) refers to reduced cognitive function, apparent before the age of 18, that negatively affects a person's learning and adaptive capacity. Approximately 1-3% of the population is affected with ID, males more than females, and most in the mild-to-moderate range. ID creates financial, logistical and psychosocial challenges for affected persons and their families and caregivers. It is estimated that up to 50% of ID has a genetic cause. Molecular genetic diagnosis may help in obtaining services and has important implications for family members, but can be elusive. Genes causing ID are known to be over-represented on the X chromosome. Over 160 X-linked intellectual disability (XLID) syndromes and > 100 XLID genes have been identified to date. Greenwood Genetic Center (GGC) offers a next-generation sequencing panel of approximately 90 XLID genes. The diagnostic potential offered by large gene panels is offset by the challenges of interpreting variants of uncertain significance (VUS). In this study, molecular and clinical data from 592 cases submitted for XLID panel testing were evaluated for patterns of phenotype and genotype, in order to further the understanding of XLID. We found a low pathogenic hit rate, a high VUS-only rate and a general absence

[1] Hill-Harfe, K., Ramsey, T.B. Jr., Rogers, R.C., Walker, M. and M.J. Friez. To be submitted to *American Journal of Medical Genetics.*

of statistically significant phenotypic patterns. These results highlight the need for appropriate patient selection, full and accurate phenotype reporting and open sharing of information in order to interpret and learn from the results of genetic testing.

2.2 Introduction

Intellectual Disability (ID) is defined as reduced intellectual capacity that is apparent before the age of 18 years, and which creates significant limitations on the individual's ability to learn, understand, communicate and adapt (American Association on Intellectual and Developmental Disabilities, AAIDD, 2013). Intellectual capacity is commonly reported in terms of an Intelligence Quotient, or IQ score. Average IQ is defined as a score of 100. ID is diagnosed when an individual's IQ falls $\geq$ 2 standard deviations below the age-appropriate mean (Tirosh & Jaffe, 2011). ID is considered to begin at IQ of 70-75 and below, and can be characterized as mild, moderate, severe or profound, with measurable decreases in the individual's functional capacity with increasing ID severity. Approximately 1-3% of the population is affected with ID, most in the mild-to-moderate range. Prevalence of ID is 30-40% higher in males, perhaps reflecting the influence of X-linked genes on brain development and function (Leonard & Wen, 2002; Nguyen & Disteche, 2006).

ID may exist as part of a recognizable, consistent constellation of intellectual, physical and/or functional anomalies due to a single underlying etiology, in which case it is considered syndromic ID. Common examples include Down syndrome, Fragile X syndrome and untreated Phenylketonuria (PKU). Non-syndromic ID may be brought to attention by the child's failure to develop along a typical time course or the loss of developmental milestones in the absence of other significant findings.

By definition, individuals with ID face difficulties in conducting the tasks of daily living. In addition to their intellectual challenges, they may have significant medical and behavioral problems that must be managed. Financial and logistical responsibility for providing care and services falls primarily on families, which can place enormous lifelong strain on caregivers. In addition to the financial and logistical burdens borne by individuals with ID and their families, there are social burdens as well. Intellectual impairment remains one of the disabilities that is least understood and accepted in many modern cultures (Scior, 2011). The lack of awareness can lead to negative attitudes about people with ID, manifested in such ways as bullying, violent/hate crimes, discrimination, avoidance, lack of inclusion and stigmatization (Ali et al., 2012; Scior, 2011; Werner et al., 2012). Stigmatization may be directed not just toward individuals with ID, but toward their families, friends and associates as well, known as "courtesy" or "affiliate" stigma (Ali et al., 2012). Tragically, affected individuals exposed to such negative attitudes often internalize them and adopt self-deprecating thoughts and attitudes ("self-stigma"), a phenomenon that has been documented in individuals with ID for many decades (Ali et al., 2012).

The availability of state-funded services and assistance for individuals with ID can fluctuate considerably with changing economic climate. Having a specific molecular diagnosis may help families obtain needed services. Molecular diagnosis can also help guide medical management, for instance, by allowing monitoring for known complications, and provides a means for informed family planning decisions by family members. An explanation for the child's disability, however, can by itself provide benefit

to the family by reducing uncertainty and frustration and allowing future planning (Lewis, Skirton, & Jones, 2010; Rosenthal, Biesecker, & Biesecker, 2001).

While some reports state that only 20% of all ID has an identifiable cause (Rauch et al., 2006), others estimate that genetic factors may contribute in up to 85% of all cases (Curry et al., 1997). Over 100 genes implicated in ID are located on the X chromosome, accounting in part for the greater incidence of ID in males than in females. The advent of next-generation (NextGen) sequencing technologies has allowed simultaneous analysis of large groups of genes more quickly and at lower cost than more traditional Sanger (dideoxy-) sequencing. Within the last few years, several laboratories have begun offering NextGen X-linked intellectual disability panels, including Greenwood Genetic Center (GGC).

We hypothesized that analysis of the clinical and molecular data from cases submitted for GGC XLID panel testing might yield important insights into the genetic etiologies and phenotypic patterns of XLID, as well as the overall success of the XLID panel as a diagnostic tool.

2.3 Materials and Methods

2.3.1 Data collection and curation.

GGC maintains a MEDGIS (Medical Genetics Information System; PSA Computer Consultants, Winston Salem, NC) database to track and store information on all patients and samples submitted for analysis. MEDGIS can be queried to retrieve information based on user-specified search criteria. For this study, MEDGIS was queried for all samples submitted for clinical testing via the GGC XLID panel from its inception through June 30, 2013. Extracted data was transferred to an Excel spreadsheet for ease of

manipulation. Following generation of the Excel file, 10 samples were chosen at random and the information in the spreadsheet for those samples was verified against the MEDGIS record, in order to check for data transfer errors. Cases were then reviewed individually to make corrections and clarifications and to obtain data not retrieved in the initial inquiry. During this process, several samples or variants were found to be inappropriate for inclusion and were excluded from the final data set. These included 7 cases with a prior diagnosis of muscular dystrophy (MD; 6 Duchenne/Becker and one LAMA2-related) and one with clinically suspected MD; three cases for which the panel was ordered but never run; one of a pair of affected full brothers who did not represent independent cases; and seven variants in two genes (NXF5 and ZNF674) for which GGC is no longer reporting data. In 6/7 cases with NXF5 or ZNF674 variants, the removed variant was the only variant found, resulting in reclassification of the case from "VUS" to "No Variant."

2.3.2 Deidentification of data.

Following data curation, study identification numbers were assigned and personal identifiers were removed from the data set as follows. Patient data in the Excel spreadsheet was sorted alphabetically by patient's last/family name. In a new column, the RANDBETWEEN Excel function was used to generate a random number for each of the patients. The entire data set was then subjected to a 2-tier sort, first by the random number column and second, by the patients' GGC sample identifiers (Lab ID), to remove sequential alphabetical or chronological ordering from the list. The re-ordered samples were then assigned sequential Study Identification (Study ID) numbers. The columns containing the Study ID, Lab ID and patient name were copied into a new, password-

protected Excel workbook to generate a Study Sample Key. Finally, the columns containing identifying information were removed from the working dataset to create a deidentified data set for analysis.

2.3.3 Data preparation and statistical analysis.

The data set was prepared for statistical analysis using Microsoft Excel 2008 version 12.2.3 for Mac (Excel). Each patient/case was assigned to a single row. Data categories, including demographic, phenotypic, genotypic and family studies information, were assigned to columns. Phenotype information was parsed into the primary categories of: intellectual disability/developmental disability (ID/DD); autism; seizures; (craniofacial) dysmorphism; microcephaly; macrocephaly; integumentary (encompassing skin, hair, nails and teeth); skeletal; stature and/or habitus; heart; central nervous system (CNS); kidney; genital; hearing; and eyes (structural and/or functional). Additional columns were created to reflect combinations of phenotypes, for instance, "Any Urogenital" to reflect kidney, genital or both, or "Any Physical Anomaly" to include any physical phenotype reported. Each case was marked "Y" in a category if the phenotype was reported, and "N" if the phenotype was either specifically denied or was not reported. In one case, the phenotype was given as "multiple congenital anomalies" with no specific phenotypes reported; this case was marked "Y" for "Any Physical Anomaly" and "N" in all other categories.

XLID panel variants were classified according to the molecular nature of the variant and its predicted pathogenicity. Molecular classifications were: synonymous; missense; nonsense; frameshift (small insertions or deletions leading to a change in protein reading frame); deletion (larger deletions, including those in intronic regions);

insertion (larger insertions or duplications, regardless of whether reading frame was retained); and regulatory (mutations affecting one or more nucleotides in intronic or regulatory regions, potentially affecting splicing or translation initiation). Variants reported by GGC as "pathogenic" or "likely pathogenic" at the time of XLID panel analysis were considered pathogenic for the purposes of this analysis. All others were considered variants of uncertain significance (VUS).

Data analysis was performed using Excel and IBM's Statistical Package for Social Sciences version 22.0 (SPSS). Frequency statistics were generated using both Excel and SPSS. SPSS was used to test associations between data subsets with chi square analysis.

2.4 Results

2.4.1 Phenotype analysis.

Descriptive statistics of the entire XLID data set and the IDEAS and non-IDEAS subsets are presented in Table 2.1, Table 2.2 and Table 2.3, respectively. After curation, there were a total of 592 cases, comprised of 562 male (94.9%) and 30 female (5.1%). The most common phenotypes reported were ID/DD (80.9%); dysmorphism (35.5%); autism (29.4%); seizures (22.3%); microcephaly (12.8%); skeletal anomalies (12.2%); and macrocephaly (10.1%). All other phenotypic categories were reported in less than 10% of cases. In 15 cases (2.5%), one or more physical anomalies were reported, but ID/DD was not. In 7 cases (1.2%), autism was the only reported phenotype. There was no phenotypic information provided for 82 cases (13.9%).

The IDEAS set comprised 130 cases, 125 male (96.2%) and five female (3.8%). Over 99% (129/130) were reported to have ID/DD. The single male case not reported to have ID/DD was an infant with multiple physical anomalies. Other common phenotypes

were dysmorphism (64.6%); autism (41.5%); seizures (31.5%); microcephaly (20.8%); skeletal anomalies (20.0%); macrocephaly (17.7%); genital anomalies (17.7%) and heart defects (13.1%). All other phenotypic categories were reported in less than 10% of the IDEAS cases.

Within the non-IDEAS cases, there were 437 male (94.6%) and 25 female (5.4%) cases. Phenotype frequencies in this data subset were as follows: ID/DD 75.8%; dysmorphism 27.3%; autism 26.0%; seizures 19.7%; microcephaly 10.6%; skeletal 10.0% and all other categories < 10% each.

In order to identify phenotypic patterns, chi square analysis was employed to test associations between phenotypes and combinations of phenotypes within the full (Table 2.4) and IDEAS-only (Table 2.5) data sets. Within the full data set, the majority of associations examined were not statistically significant, and many failed the criteria for valid chi square analysis, because one or more cells had an Expected count of less than five. Among those that were both valid and statistically significant, 28 of 29 were positive associations. The single negative association was between autism and microcephaly ($p = 0.025$). Among the 28 positive associations, 22 involved ID/DD, including a greater likelihood of ID/DD reported in males than in females (82.2% vs. 56.7%, $p = 0.001$). Five positive associations involved seizures and physical anomaly categories. The final positive association was between autism and macrocephaly ($p < 0.001$).

In addition to associations between phenotypes, we looked at whether there was any association between the presence of a pathogenic variant and specific phenotypes, and whether the number of VUS (categorized as VUS Load: 0, low (1-2 VUS) or high (3

or more VUS) was associated with any phenotypes. No valid, statistically significant associations were found.

Within the IDEAS set, only two associations both met the criteria for analytic validity and returned a statistically significant result. These were a negative association between autism and microcephaly ($p = 0.002$) and a negative association between autism and any reported anomaly of head size or shape ($p = 0.035$). Autism and macrocephaly were not found to be significantly associated within the IDEAS data subset ($p = 0.50$).

2.4.2 Pathogenic variants.

Table 2.6 provides a summary of the pathogenic variants identified within our data set. There were 25 male cases (hit rate 4.4%) and three female cases (hit rate 10%) found to have a total of 28 pathogenic variants on XLID panel analysis. This yielded an overall hit rate of 4.7%. Within the IDEAS subset, pathogenic variants were identified in five males (4.0%) and one female (20.0%), giving a combined hit rate of 4.6%. The 28 pathogenic variants were found in 18 genes, or 20.2% of the 89 XLID panel genes for which GGC is currently reporting results. Multiple cases were found to have pathogenic variants in *ARX* (five cases) and *ATRX* (three cases). *ARHGEF9, L1CAM, OPHN1* and *PQBP1* each had two pathogenic variants, while the remaining 12 genes yielded one pathogenic variant each. In 15 of the 28 cases, the pathogenic variant was the only variant found. The remaining 13 cases had between one and four VUS in addition to the pathogenic variant.

2.4.3 Variants of uncertain significance.

In addition to the 13 cases with both pathogenic variants and VUS, 339 cases without pathogenic variants were found to have one or more VUS, giving a VUS-only rate of 57.3%. Sixty-two (18.3%) had synonymous variants only, while 277 (81.7%; or 46.7% of all cases) had one or more non-synonymous variants. The total number of VUS per case ranged from one to 10, however the majority of cases had only one (185 cases; 54.6%) or two (102 cases; 30.1%) VUS. VUS were found in 85 of 89 reportable genes on the panel. Sixty-seven genes (75.2%) yielded VUS only. All 18 genes for which pathogenic variants were found also yielded one or more VUS. As expected, the genes with the largest cDNA sizes, such as *HUWE1* (13.1 kb), *DMD* (11.1 kb), *FLNA* (7.9 kb), and *ATRX* (7.5 kb) gave the highest number of variants.

Four genes- *HSD17B10*, *NDP*, *PGK1* and *EBE2A*- showed no variants at all within this set of cases. Two hundred twenty-five cases, or 38.0%, were found to have no variants within the reportable genes on the XLID panel.

2.4.5 Family studies.

Family studies are often helpful when interpreting the pathogenicity of genetic variants. When considering X-linked variants, maternal X-inactivation status may provide additional clues, as unaffected female carriers of pathogenic mutations are sometimes found to have a protective skewing of X-inactivation. We sought to determine what proportion of the pathogenic variants and non-synonymous VUS in the full data set were inherited from a parent. Among the 28 individuals bearing pathogenic variants, parental inheritance studies were conducted for 15 (53.6%). The pathogenic variant was maternally inherited in 10 cases (66.7%) and was *de novo* in five cases (33.3%),

including both female cases. Maternal X-inactivation studies were performed for four of the 25 male cases with pathogenic variants (in *ARHGEF9* (two cases), *OPHN1* and *CASK*); all four mothers showed random XI. The patient's own XI pattern was studied for one female case with a *de novo* variant in *SLC16A2*, and her XI pattern was also found to be random.

There were 277 cases with one or more non-synonymous VUS but no pathogenic variants. Family studies were performed for 102 of these cases (36.8%), with a total of 166 non-synonymous VUS. Two cases (1.9%), were found to have a single *de novo* VUS each, amounting to a 1.2% *de novo* rate among non-synonymous VUS. For one female case, the VUS was found to be paternally inherited.

Maternal XI studies were performed for 36 cases with non-synonymous VUS but no pathogenic variants. In 26/36 cases, family studies had also been performed, showing maternal inheritance in 25/26. One VUS was *de novo*, with random maternal XI. Among the cases with maternally inherited variants, XI was random in 11 mothers, moderately skewed in eight mothers and highly skewed in six mothers. In the set of 10 cases for which maternal XI studies, but not maternal inheritance studies, were performed, two showed random XI, three had moderately skewed XI and five showed highly skewed XI. All combined, maternal XI was random in 14/36 cases (38.8%), moderately skewed in 11/36 cases (30.6%) and highly skewed in 11/36 cases (30.6%). A list of the genes with non-synonymous VUS found in cases with skewed maternal XI is presented in Table 2.8.

2.5 Discussion

The study presented here had two primary goals. The first was to look for phenotypic patterns within the GGC XLID cases that might be useful in constructing

genotype-phenotype correlations, to increase our understanding of X-linked intellectual disability. The second was to assess the utility of the current GGC XLID gene panel in identifying the molecular etiology of intellectual disability.

We found that the rate of pathogenic variants was virtually identical between the IDEAS group and the entire data set, at approximately 4.7%. This hit rate is substantially lower than those reported for next-generation sequencing panels in other conditions, such as familial hearing loss (62%) (Baek et al., 2012); hereditary retinal dystrophies (56%) (Chen et al., 2013); epileptic disorders (48%) (Lemke et al., 2012); and infantile mitochondrial disease (24%) (Calvo et al., 2012). Those studies differed from the current study in important ways. First, the studies investigated small numbers (8-42) of selected patients or families with well-defined clinical phenotypes, some including significant family history of the condition under investigation. Second, the epilepsy and retinal dystrophy studies examined panels of 265 and 189 genes, respectively, more than twice as many genes as the GGC XLID panel, while the mitochondrial disease study examined approximately 1000 genes.

We cannot exclude that there may be true pathogenic variants hiding among the numerous VUS in our data set. In this study, almost 50% of cases (without pathogenic variants) had one or more non-synonymous VUS with the potential to negatively impact protein function or gene regulation. While it was beyond the scope of this study to re-interpret VUS, it is likely that some of them are in fact pathogenic variants. Family studies are often the most useful tool in assessing the potential pathogenicity of VUS, however we found they were performed in less than 37% of cases with VUS. Nine XLID genes are consistently associated with skewed XI in carrier females (R.C. Rogers,

personal communication April 4, 2014). We found 21 cases with non-synonymous VUS and skewed maternal XI. Seven of these had variants in three genes (*ACSL4*, *ATRX* and *GRIA3*) associated with skewed XI in female carriers, potentially adding to the weight of evidence regarding their pathogenicity. Strikingly, six of the cases had VUS in *ATRX* and highly skewed maternal XI. The seventh case had variants in both *ACSL4* and *GRIA3* with moderately skewed maternal XI. Finally, while we did not find any statistically significant association between the "load" of VUS and phenotypes within our data set, the possibility remains that a multifactorial ID etiology may involve the additive effects of several slightly deleterious variants, both X-linked and autosomal, that current knowledge does not allow us to recognize.

With any gene panel test, the selection of which genes to assess is crucial to its success. Diagnostic laboratories design gene panels based on literature reporting causative associations between genes and phenotypes. However, the genetic landscape for many conditions, including XLID, is constantly shifting. As more data become available, it becomes clear that some variants and genes that were thought to be causative are instead benign. Recently, Piton, Redin & Mandel (2013) published a re-assessment of the roles of many X-linked genes in intellectual disability, based on large-scale exome sequencing data. Their study specifically questioned the roles of 10 genes in XLID, and called for additional data on another 15. Two of the genes considered highly questionable (*ZNF674 and NXF5*) are already omitted from GGC's panel reporting. One case in our study had a clearly deleterious (nonsense) mutation in *ZNF41*, also on the highly questionable list. We chose to consider that change a VUS in this analysis. Our

finding that only 18 of 89 genes yielded high-confidence pathogenic variants may have implications for future versions of the XLID panel.

The identification of phenotypic patterns is only possible if phenotypes are actually reported. One might expect that patients for whom an intellectual disability gene panel test is ordered would have ID/DD as a primary phenotype. Within the IDEAS set, theoretically submitted with full phenotypic information, ID/DD was reported in nearly 100% of cases. When looking at only the non-IDEAS cases, however, the frequency of reported ID/DD was reduced by almost 25%. The rank order of phenotype frequencies, however, was nearly identical between the IDEAS and non-IDEAS groups. This suggests that, *when* phenotypic information was provided, it tended to be equivalent, regardless of whether the referring provider requested IDEAS panel review. Indeed, despite specific guidelines for providing clinical information when requesting IDEAS review, a recent analysis of the first 55 IDEAS cases demonstrated that, even in this "best-case" scenario, phenotype reporting is consistently inconsistent (Hunter et al., 2014).

It is no surprise, then, that statistically significant associations between phenotypes were largely absent in this analysis. The strong positive correlation seen between macrocephaly and autism in these patients provided reassuring evidence that, when reported, phenotypes were reasonably accurate. The majority of significant associations, however, were between the *lack* of ID/DD and the *lack* of other reported phenotypes. In other words, an individual without reported ID/DD was highly likely ($p < 0.001$) to also have no other phenotypes reported, and this was true for nearly 14% of cases.

The apparently low success rate of the GGC XLID panel begs the question of whether a gene panel is the best approach for this condition, since its utility relies on certain assumptions (in this case, X-linked etiology and the causative roles of the included genes) that may not be valid. The higher hit rates seen when using larger gene panels for other genetically heterogeneous conditions suggest that, if choosing a panel approach, more genes may be better. A greater number of genes, however, inevitably means a greater burden of uncertain variants and incidental findings that must be interpreted and communicated. At some point the difference between analyzing a full exome vs. a large gene panel, for instance 1000 genes as in the mitochondrial disease study, becomes academic.

Full exome sequencing is increasingly common in clinical use, and will likely become routine in the near future as technology improves and costs decrease. Indeed, the current turnaround time and cost of clinical exome sequencing, for the proband alone or proband plus parents (trio sequencing) are equivalent to those of XLID panel testing (GGC, 2014; Iowa Institute of Human Genetics, 2014). Individuals with ID frequently have no other phenotypic findings or family history to aid in clinical diagnosis. For these patients, full exome sequencing may provide the most efficient method of molecular diagnosis. One way to manage the burden of exome interpretation may be to create virtual gene panels within the exome data. Analysis could proceed in a tiered fashion, looking at the most likely gene candidates first, and only moving to additional analysis if the previous group fails to provide an answer. This approach carries its own limitations. Sequencing coverage is less robust with a full exome vs. selected genes, making it more likely that a pathogenic variant may be missed. Full exome sequencing has the potential

to uncover medically actionable incidental findings, which may never come to light if exome data is only accessed for specific genes relevant to the indication at hand. The responsibilities and liabilities surrounding reporting of incidental findings are a current matter of intense discussion among genetics professionals. However, selective reporting of results as suggested for tiered exome reporting is in fact already in practice with the GGC XLID panel with the omission of some genes found on the Piton et al. (2013) highly questionable list.

It cannot be overstated that successful molecular diagnosis requires astute matching of patient to test. In the hearing loss study (Baek et al., 2012), for instance, their 8 chosen families had strong family histories showing autosomal dominant (AD) inheritance of a simple, well-defined phenotype; that is, a clear genetic cause, as opposed to a multifactorial, teratogenic, toxic or injurious etiology. In addition, their panel included *all* of the known causative genes for hearing loss, plus some additional candidate genes. Intellectual disability, by contrast, is enormously complex, heterogeneous in both presentation and etiology. Over 400 ID genes have been identified, roughly one-quarter of which lie on the X chromosome, but less than 10% of ID is estimated to be X-linked. When assessing the success of the XLID panel, a targeted parallel sequencing study of susceptibility genes in childhood cancer (Plon et al., 2011) may provide a more valid comparison. Like (X-linked) ID/DD, the condition is phenotypically and genetically heterogeneous, in many cases is multifactorial, and family history may be uninformative. Even with strict inclusion criteria, their hit rate (in 45 genes) was just 13%.

Unless a family history unmistakably demonstrates X-linked inheritance, the average case of non-syndromic ID is far more likely to have an autosomal etiology. Hunter et al. (2014) noted that information on family history and prior genetic evaluation(s) was often missing or incomplete in the early IDEAS cases, a circumstance that has not improved over time for the XLID panel submissions in general (M. Friez, personal communication, April 4, 2014). Further confounding the picture is the phenotypic heterogeneity displayed by many XLID genes. One gene may cause both syndromic and non-syndromic ID, which may or may not depend on the specific mutation. Carrier females may or may not display a recognizable phenotype. With non-syndromic ID, it is often simply not possible to match a patient to a specific genetic test, and it is likely that many of the submitted cases have been through several tiers of testing already. A large proportion of the XLID panel submissions may represent last-ditch diagnostic efforts rather than genuinely suspected XLID; in other words, poor XLID testing candidates. This returns us to the question of whether, if taking a shot in the dark anyway, it might be better to aim at a larger target (i.e., the exome).

The limitations of this study are in fact its most relevant findings. Plainly put, if progress is to be made in clarifying the genetics of intellectual disability, more information is needed, both from the referring physicians and the genetics community as a whole. Meaningful curation of variants requires complete, accurate and accessible information about the patient, the patient's genotype and the gene(s) in question. Ideally, the clinical and molecular evaluation of patients would proceed in a consistent, systematic way, and all reporting would be standardized and freely accessible. This concept is already firmly within the awareness of the genetics community, as the ability

to acquire genome-scale information has rapidly outpaced our ability to manage the information. The trend is moving toward more open sharing of variant information, but is not nearly universal, nor does the currently available molecular data necessarily include any phenotypic information (for instance, the Exome Sequencing Project of the National Heart, Lung and Blood Institute (NHLBI) in the National Institutes of Health (NIH), available at http://evs.gs.washington.edu/EVS/). In this example, allele frequencies in populations chosen for a specific condition (for instance, cardiovascular disease) are reported, and it is left to the investigator to assume that other genetic conditions are not present. The implementation of training and infrastructure to make comprehensive large-scale genetic data sharing possible will require a significant long-term commitment of funding, infrastructure and collaboration.

Table 2.1

Phenotype Frequencies, All Cases

	Male		Female		Total	
Feature	n	%	n	%	n	%
All cases	562	94.9	30	5.1	592	100.0
ID/DD	462	82.2	17	56.7	479	80.9
Dysmorphism	201	35.8	9	30.0	210	35.5
Autism	168	29.9	6	20.0	174	29.4
Seizures	123	21.9	9	30.0	132	22.3
Skeletal	70	12.5	2	6.7	72	12.2
Microcephaly	68	12.1	8	26.7	76	12.8
Macrocephaly	60	10.7	0	0.0	60	10.1
Integument	50	8.9	2	6.7	52	8.8
Genital	43	7.7	0	0.0	43	7.3
Heart	38	6.8	1	3.3	39	6.6
Eyes	37	6.6	0	0.0	37	6.3
Hearing	28	5.0	3	10.0	31	5.2
Kidney	20	3.6	2	6.7	22	3.7
Stature/Habitus	20	3.6	1	3.3	21	3.5
CNS	20	3.6	2	6.7	22	3.7
Autism only	7	1.2	0	0	7	1.2
No phenotype reported	74	13.2	8	26.7	82	13.9

Table 2.2

Phenotype Frequencies, IDEAS Cases

	Male		Female		Total	
Feature	n	%	n	%	n	%
IDEAS cases	125	96.2	5	3.8	130	100.0
ID/DD	124	99.2	5	100.0	129	99.2
Dysmorphism	82	65.6	2	40.0	84	64.6
Autism	52	41.6	2	40.0	54	41.5
Seizures	39	31.2	2	40.0	41	31.5
Skeletal	26	20.8	0	0.0	26	20.0
Microcephaly	26	20.8	1	20.0	27	20.8
Macrocephaly	23	18.4	0	0.0	23	17.7
Integument	12	9.6	0	0.0	12	9.2
Genital	23	18.4	0	0.0	23	17.7
Heart	17	13.6	0	0.0	17	13.1
Eyes	8	6.4	0	0.0	8	6.2
Hearing	6	4.8	0	0.0	6	4.6
Kidney	6	4.8	0	0.0	6	4.6
Stature/Habitus	4	3.2	0	0.0	4	3.1
CNS	2	1.6	0	0.0	2	1.5

Table 2.3

Phenotype Frequencies, Non-IDEAS Cases

	Male		Female		Total	
Feature	n	%	n	%	n	%
non-IDEAS cases	437	94.6	25	5.4	462	100.0
ID/DD	338	77.3	12	48.0	350	75.8
Dysmorphism	119	27.2	7	28.0	126	27.3
Autism	116	26.5	4	16.0	120	26.0
Seizures	84	19.2	7	28.0	91	19.7
Skeletal	44	10.1	2	8.0	46	10.0
Microcephaly	42	9.6	7	28.0	49	10.6
Macrocephaly	37	8.5	0	0.0	37	8.0
Integument	38	8.7	2	8.0	40	8.7
Genital	20	4.6	0	0.0	20	4.3
Heart	21	4.8	1	4.0	22	4.8
Eyes	29	6.6	0	0.0	29	6.3
Hearing	22	5.0	3	12.0	25	5.4
Kidney	14	3.2	2	8.0	16	3.5
Stature/Habitus	16	3.7	1	4.0	17	3.7
CNS	18	4.1	2	8.0	20	4.3
Autism only	7	1.6	0	0	7	1.5
No phenotype reported	74	16.9	8	32.0	82	17.7

Table 2.4

Chi Square Analysis of Phenotypic Associations, All Cases

Feature(s)	ID/DD	Autism	Seizures	Sex	VUS Load	PathVar
Sex	**0.001**[a]	0.246	0.298	-	(0.200)	(0.163)
Pathogenic Variant (PathVar)	0.865	0.343	0.414	(0.163)	(0.329)	-
VUS Load	0.729	0.907	0.976	(0.200)	-	(0.328)
ID/DD	-	**< 0.001**	**< 0.001**	**0.001**[a]	0.729	0.762
Autism	**< 0.001**	-	0.633	0.246	0.907	0.343
Seizures	**< 0.001**	0.633	-	0.298	0.976	0.411
ID/DD + Autism, no Seizures	-	-	-	0.540	0.651	0.665
ID/DD + Seizures, no Autism	-	-	-	(0.181)	0.682	(0.122)
ID/DD + Autism + Seizures	-	-	-	(0.426)	(0.200)	(0.474)
Any Physical Anomaly or Dysmorphic	**<0.001**	0.351	< 0.001	0.277	0.917	0.380
Physical Anomaly, not Dysmorphic	**<0.001**	0.313	0.043	0.554	0.429	0.136
Dysmorphic, no Physical Anomalies	**<0.001**	0.748	0.233	(0.345)	0.892	(0.810)
Physical Anomaly other than Dysmorphic	**<0.001**	0.490	0.011	0.615	0.359	0.531
Dysmorphic	**<0.001**	0.810	0.058	0.520	0.312	0.706
ID/DD + any Physical Anomaly or Dysmorphic	-	0.123	**< 0.001**	0.055	0.288	0.447
ID/DD + Dysmorphic	-	0.617	**0.038**	0.094	0.327	0.821
ID/DD + Physical Anomaly, not Dysmorphic	-	0.134	**0.014**	0.662	0.726	0.230
ID/DD + Physical Anomaly other than Dysmorphic	-	0.141	**0.002**	0.095	0.260	0.568
ID/DD + Physical Anomaly + Dysmorphic	-	0.773	**0.026**	0.105	0.242	0.563
Microcephaly	**0.001**	0.025	**0.037**	(0.020)	0.897	(0.164)
Macrocephaly	**< 0.001**	**< 0.001**	0.131	(0.059)	0.563	(0.069)
Any Head	**< 0.001**	0.146	**0.006**	0.690	0.829	0.863
Integument	**0.003**	0.387	0.578	(0.674)	0.543	(0.712)
Skeletal	**0.005**	0.748	0.356	(0.345)	0.405	(0.345)
Stature and/or Habitus	(0.996)	0.686	0.482	(0.948)	(0.882)	(0.292)
Heart	0.146	0.105	0.904	(0.461)	(0.786)	(0.510)
CNS	(0.658)	0.239	(0.106)	(0.381)	(0.026)	(0.326)
Kidney	(0.507)	0.484	(0.961)	(0.381)	(0.094)	(0.326)
Genital	**0.004**	0.569	0.823	(0.116)	(0.350)	(0.980)
Kidney and Genital	(0.745)	(0.086)	(0.688)	(0.539)	(0.220)	(0.553)
Any Urogenital	**0.005**	0.751	0.757	(0.554)	0.112	(0.413)
Hearing	0.066	0.653	0.355	(0.229)	(0.626)	(0.203)
Eyes	**0.029**	0.963	0.760	(0.147)	(0.933)	(0.549)
Hearing or Eyes	**0.011**	0.843	0.781	(0.995)	0.798	(0.247)
Hearing and Eyes	(0.142)	0.225	(0.108)	(0.485)	(0.049)	(0.501)
Skeletal and Integument	(0.077)	(0.912)	(0.545)	(0.400)	(0.731)	(0.067)
Skeletal and Kidney	(0.879)	(0.112)	(0.187)	(0.569)	(0.373)	(0.583)
Skeletal and Genital	(0.119)	0.765	(0.210)	(0.306)	(0.483)	(0.226)
Skeletal and any Urogenital	(0.149)	0.545	0.079	(0.804)	(0.713)	(0.431)
Skeletal and Heart	(0.938)	(0.031)	(0.741)	(0.539)	(0.940)	(0.456)
Skeletal and Hearing	(0.745)	(0.431)	(0.688)	(0.263)	(0.407)	(0.553)
Skeletal and Eyes	(0.275)	(0.131)	(0.340)	(0.604)	(0.178)	(0.617)
Skeletal and Hearing or Eyes	(0.539)	(0.083)	(0.996)	(0.405)	(0.502)	(0.501)
Skeletal and Microcephaly	(0.250)	(0.946)	(0.937)	(0.382)	(0.267)	(0.003)
Skeletal and Macrocephaly	(0.461)	(0.032)	(0.860)	(0.461)	(0.498)	(0.477)
Skeletal and any Head	(0.089)	0.168	(0.865)	(0.281)	(0.207)	(0.292)
Integument and Kidney	(0.745)	(0.962)	(0.688)	(0.004)	(0.521)	(0.553)
Integument and Genital	(0.232)	(0.265)	(0.187)	(0.569)	(0.878)	(0.583)
Integument and any Urogenital	(0.338)	(0.346)	(0.636)	(0.064)	(0.957)	(0.436)
Integument and Heart	(0.958)	(0.601)	(0.229)	(0.126)	(0.799)	(0.617)
Integument and Microcephaly	(0.394)	(0.410)	(0.741)	(0.539)	(0.009)	(0.456)
Integument and Macrocephaly	(0.167)	**(**0.004)	(0.503)	(0.511)	(0.980)	(0.526)
Integument and any Head	(0.119)	(0.216)	(0.488)	(0.968)	(0.047)	(0.324)
Heart and Kidney	(0.668)	(0.784)	(0.853)	(0.335)	(0.585)	(0.297)
Heart and Genital	(0.338)	(0.328)	(0.353)	(0.419)	(0.451)	(0.436)
Heart and any Urogenital	(0.436)	(0.590)	(0.901)	(0.876)	(0.315)	(0.820)
Heart and Microcephaly	(0.232)	(0.147)	(0.340)	(0.604)	(0.577)	(0.617)
Heart and Macrocephaly	(0.275)	(0.147)	(0.042)	(0.604)	(0.799)	(0.617)
Heart and any Head	(0.941)	(0.040)	(0.034)	(0.461)	(0.825)	(0.477)

Note: p values < 0.05 were considered significant. Valid positive associations are indicated in boldface. Valid negative associations are underlined. Associations that did not meet the criteria for valid chi square analysis are indicated in parentheses. VUS Load was 0, Low (1-2 VUS) or High (≥ 3 VUS). No cases were reported with both heart and CNS anomalies.

[a]Associated with males.

Table 2.5

Chi Square Analysis of Phenotypic Associations, IDEAS Cases

Feature(s)	ID/DD	Autism	Seizures	VUS Load
Sex	ND	ND	ND	ND
Pathogenic Variant	(0.825)	(0.676)	(0.923)	(0.092)
VUS Load	(0.481)	0.304	(0.489)	-
ID/DD	-	(0.397)	(0.496)	(0.481)
Autism	(0.397)	-	0.437	0.304
Seizures	(0.496)	0.437	-	(0.489)
ID/DD + Autism, no Seizures	-	-	-	(0.665
ID/DD + Seizures, no Autism	-	-	-	(0.771)
ID/DD + Autism + Seizures	-	-	-	(0.258)
Any Physical Anomaly or Dysmorphic	(0.659)	0.893	0.749	(0.481)
Physical Anomaly, not Dysmorphic	(0.633)	0.064	0.834	(0.314)
Dysmorphic, no Physical Anomalies	(0.642)	0.796	0.900	(0.944)
Physical Anomaly other than Dysmorphic	(0.480)	0.958	0.822	(0.856)
Dysmorphic	(0.458)	0.147	0.841	(0.223)
ID/DD + any Physical Anomaly or Dysmorphic	-	0.948	0.637	(0.342)
ID/DD + Dysmorphic	-	0.198	0.746	(0.169)
ID/DD + Physical Anomaly, not Dysmorphic	-	0.064	0.834	(0.314)
ID/DD + Physical Anomaly other than Dysmorphic	-	0.917	0.726	(0.739)
ID/DD + Physical Anomaly + Dysmorphic	-	0.134	0.656	(0.123)
Microcephaly	(0.050)	0.002	0.810	(0.608)
Macrocephaly	(0.642)	0.500	0.174	(0.191)
Any Head	(0.204)	0.035	0.387	0.478
Integument	(0.749)	(0.014)	(0.888)	(0.193)
Skeletal	(0.045)	0.722	0.131	(0.282)
Stature and/or Habitus	(0.858)	(0.727)	(0.775)	(0.554)
Heart	(0.010)	0.276	0.359	(0.961)
CNS	(0.900)	(0.807)	(0.571)	(0.130)
Kidney	(< 0.001)	(0.206)	(0.319)	(0.683)
Genital	(0.030)	0.234	0.535	(0.749)
Kidney and Genital	(< 0.001)	(0.140)	(0.185)	(0.616)
Any Urogenital	(0.045)	0.213	(0.571)	(0.792)
Hearing	(0.825)	(0.206)	(0.923)	(0.576)
Eyes	(0.797)	(0.327)	(0.232)	(0.336)
Hearing or Eyes	(0.749)	(0.222)	(0.245)	(0.689)
Hearing and Eyes	(0.900)	(0.230)	(0.571)	(0.351)
Skeletal and Integument	(0.900)	(0.091)	(0.571)	(0.351)
Skeletal and Kidney	(< 0.001)	(0.230)	(0.333)	(0.884)
Skeletal and Genital	(< 0.001)	(0.327)	(0.232)	(0.791)
Skeletal and any Urogenital	(0.001)	(0.441)	(0.127)	(0.529)
Skeletal and Heart	(< 0.001)	(0.055)	(0.678)	(0.725)
Skeletal and Hearing	(0.930)	(0.397)	(0.496)	(0.481)
Skeletal and Eyes	(0.930	(0.234)	(0.496)	(0.011)
Skeletal and Hearing or Eyes	(0.900)	(0.807)	(0.333)	(0.117)
Skeletal and Microcephaly	(0.001)	(0.150)	(0.127)	(0.324)
Skeletal and Macrocephaly	(0.858)	(0.727)	(0.775)	(0.543)
Skeletal and any Head	(0.760)	(0.316)	(0.319)	(0.513)
Integument and Kidney	(0.930)	(0.397)	(0.139)	(0.595)
Integument and Genital	(0.930)	(0.234)	(0.496)	(0.011)
Integument and any Urogenital	(0.900)	(0.807)	(0.571)	(0.130)
Integument and Heart	(0.930)	(0.234)	(0.496)	(0.011)
Integument and Microcephaly	(0.900)	(0.807)	(0.571)	(0.351)
Integument and Macrocephaly	(0.877)	(0.038)	(0.946)	(0.211)
Integument and any Head	(0.841)	(0.075)	(0.678)	(0.162)
Heart and Kidney	(< 0.001)	(0.087)	(0.420)	(0.779)
Heart and Genital	(< 0.001)	(0.327)	(0.246)	(0.597)
Heart and any Urogenital	(< 0.001)	(0.223)	(0.388)	(0.583)
Heart and Microcephaly	(< 0.001)	(0.140)	(0.946)	(0.616)
Heart and Macrocephaly	(0.877)	(0.140)	(0.010)	(0.392)
Heart and any Head	(< 0.001)	(0.035)	(0.058)	(0.693)

Note: p values < 0.05 were considered significant. Valid negative associations are underlined. Associations that did not meet the criteria for valid chi square analysis are indicated in parentheses. VUS Load was 0, Low (1-2 VUS) or High (≥ 3 VUS). No cases were reported with both heart and CNS anomalies.

Table 2.6

Pathogenic Variants

Study ID	Sex	Gene	Molecular	Protein	Class
534	M	AP1S2	c.138C>A	p.C46X	N
227	M	ARHGEF9	c.865C>T	p.R289X	N
354	M	ARHGEF9	c.691_709del19	NS	D
164	M	ARX	c.441_464dup24	NS	I
508	M	ARX	c.315_335dup21	NS	I
040	M	ARX	c.441_464dup24	NS	I
024	M	ARX	c.441_464dup24	NS	I
068	M	ARX	c.441_464dup24	NS	I
080	M	ATRX	c.536A>G	p.N179S	M
082	M	ATRX	c.109C>T	p.R37X	N
372	M	ATRX	c.4654G>T	p.V1552F	M
472	M	CASK	c.1811T>A	p.L604X	N
324	M	CUL4B	c.857delT	FS	FS
244	M	IDS	c.1180+2T>C	NS	R
252	M	KDM5C	c.3125delG	FS	FS
135	F	KIAA2022	c.964C>T	p.R322X	N
066	M	L1CAM	c.3531-12G>A	NS	R
598	M	L1CAM	c.1261G>A	p.V421I	M
527	M	MAOA	deleted	NS	D
006	M	OPHN1	deletion includes exon 20	NS	D
336	M	OPHN1	c.1489C>T	p.R497X	N
170	F	PDHA1	c.947dupC	FS	FS
197	M	PQBP1	c.586C>T	p.R196X	N
239	M	PQBP1	c.691G>A	p.A231T	M
355	M	RPS6KA3	c.1492G>T	p.G498X	N
029	F	SLC16A2	c.1A>T	p.M1?	R
328	M	SLC9A6	c.1236+2T>A	NS	R
057	M	UPF3B	c.674_677delGAAA	NS	D

Note: M = male. F = female. NS = not specified. Class = mutation classification. N = nonsense. D = deletion. I = insertion. M = missense. FS = frameshift. R = regulatory.

Table 2.7

Genes with Variants of Uncertain Significance

Gene	Total Variants	Total Cases	M	F	Pathogenic Variants	Total VUS	Synonymous	Missense	Regulatory	Nonsense	Insertions/ Deletions
FLNA	49	44	41	3	0	49	21	18	9	0	1
DMD	39	36	33	3	0	39	7	25	7	0	0
ATRX	35	35	33	2	3	32	8	20	6	1	0
HUWE1	27	25	23	2	0	27	6	7	14	0	0
NHS	18	17	15	2	0	18	5	9	1	0	3
SHROOM4	16	16	16	0	0	16	3	11	0	0	2
BCOR	15	14	13	1	0	15	7	8	0	0	0
L1CAM	16	14	13	1	2	14	4	5	7	0	0
GRIA3	14	13	12	1	0	14	1	4	9	0	0
PCDH19	14	14	10	4	0	14	8	6	0	0	0
BRWD3	13	13	12	1	0	13	8	3	2	0	0
FANCB	13	13	12	1	0	13	2	7	4	0	0
MED12	12	11	11	0	0	12	4	4	3	0	1
SYN1	12	11	11	0	0	12	3	5	4	0	0
PDHA1	11	11	9	2	1	10	3	0	6	2	0
ACSL4	10	10	8	2	0	10	1	4	5	0	0
ATP7A	10	10	10	0	0	10	1	7	2	0	0
FGD1	10	10	8	2	0	10	3	4	3	0	0
NSDHL	10	9	9	0	0	10	4	3	3	0	0
CASK	10	9	9	0	1	9	0	2	7	1	0
CUL4B	10	10	10	0	1	9	1	5	4	0	0
IDS	10	9	8	1	1	9	2	5	3	0	0
NLGN4X	9	9	9	0	0	9	3	6	0	0	0
OFD1	9	9	9	0	0	9	2	3	4	0	0
MAOA	9	8	8	0	1	8	2	2	3	0	2
AFF2	8	8	6	2	0	8	3	4	1	0	0
MTM1	8	8	7	1	0	8	3	2	3	0	0
SMC1A	8	7	7	0	0	8	1	2	5	0	0
ARX	14	14	14	0	5	7	4	3	0	0	7
OPHN1	9	9	9	0	2	7	2	3	2	1	1
AGTR2	7	7	7	0	0	7	3	4	0	0	0
ARHGEF6	7	6	6	0	0	7	1	4	2	0	0

Table 2.7 Continued

Gene	Total Variants	Total Cases	M	F	Pathogenic Variants	Total VUS	Synonymous	Missense	Regulatory	Nonsense	Insertions/ Deletions
LAMP2	7	7	7	0	0	7	1	2	4	0	0
PAK3	7	7	7	0	0	7	4	2	1	0	0
PHF8	7	7	7	0	0	7	3	1	3	0	0
ZNF81	7	7	7	0	0	7	1	6	0	0	0
CDKL5	6	6	6	0	0	6	1	2	2	0	1
KLF8	6	6	3	3	0	6	3	2	1	0	0
MECP2	6	6	6	0	0	6	1	5	0	0	0
OTC	6	6	5	1	0	6	1	1	4	0	0
KDM5C	6	6	6	0	1	5	1	4	0	0	1
KIAA2022	6	6	3	3	1	5	5	0	0	1	0
UPF3B	6	6	6	0	1	5	0	1	2	0	3
ABCD1	5	5	5	0	0	5	1	1	3	0	0
DLG3	5	5	5	0	0	5	4	0	1	0	0
FTSJ1	5	4	4	0	0	5	2	1	2	0	0
GPC3	5	5	5	0	0	5	1	2	2	0	0
IL1RAPL1	5	5	5	0	0	5	1	3	1	0	0
SLC16A2	5	5	4	1	1	4	2	2	1	0	0
DKC1	4	4	4	0	0	4	1	0	3	0	0
FMR1	4	4	4	0	0	4	4	0	0	0	0
GDI1	4	4	4	0	0	4	0	0	4	0	0
GK	4	3	3	0	0	4	3	0	1	0	0
MBTPS2	4	4	4	0	0	4	1	2	0	0	1
MID1	4	4	3	1	0	4	2	2	0	0	0
RPL10	4	4	4	0	0	4	2	1	1	0	0
SOX3	4	4	4	0	0	4	1	2	0	0	1
SRPX2	4	4	4	0	0	4	1	3	0	0	0
SYP	4	4	4	0	0	4	2	1	1	0	0
RPS6KA3	5	5	5	0	1	3	0	2	2	1	0
ATP6AP2	3	3	2	1	0	3	0	1	2	0	0
HCCS	3	3	3	0	0	3	1	0	2	0	0
MAGT1	3	3	1	2	0	3	2	0	1	0	0
OCRL	3	3	3	0	0	3	0	2	1	0	0
PHF6	3	3	3	0	0	3	0	0	3	0	0
PORCN	3	3	2	1	0	3	2	1	0	0	0

Table 2.7 Continued

Gene	Total Variants	Total Cases	M	F	Pathogenic Variants	Total VUS	Synonymous	Missense	Regulatory	Nonsense	Insertions/ Deletions
TSPAN7	3	3	3	0	0	3	2	1	0	0	0
ZNF41	3	2	2	0	0	3	0	1	1	1	0
ARHGEF9	4	4	4	0	2	2	1	1	0	1	1
PQBP1	4	4	4	0	2	2	1	1	1	1	0
SLC9A6	3	3	3	0	1	2	0	1	2	0	0
IGBP1	2	2	2	0	0	2	0	0	2	0	0
PLP1	2	2	2	0	0	2	1	0	1	0	0
ZNF711	2	2	0	2	0	2	2	0	0	0	0
AP1S2	2	2	2	0	1	1	0	0	1	1	0
DCX	1	1	1	0	0	1	1	0	0	0	0
HPRT1	1	1	1	0	0	1	0	0	1	0	0
NDUFA1	1	1	1	0	0	1	0	1	0	0	0
NLGN3	1	1	1	0	0	1	0	1	0	0	0
PRPS1	1	1	1	0	0	1	1	0	0	0	0
RAB39B	1	1	1	0	0	1	1	0	0	0	0
SMS	1	1	1	0	0	1	0	1	0	0	0
TIMM8A	1	1	1	0	0	1	0	0	1	0	0
ZDHHC15	1	1	1	0	0	1	0	1	0	0	0
ZDHHC9	1	1	1	0	0	1	0	0	1	0	0

Table 2.8

Non-synonymous Variants Associated with Skewed Maternal X-inactivation

Study ID	Gene	Molecular	Protein	Maternal	Maternal XI
104	CDKL5	c.555-19C>G	NS	-	HS
105	ATRX[a]	c.5048 A>G	p.Y1683C	Y	HS
125	DMD	c.2630T>G	p.V877G	-	HS
143	ATRX[a]	c.5038A>T	p.I1680F	Y	HS
196	ATRX[a]	c.2923G>A	p.D975N	Y	HS
196	FANCB	c.127T>A	p.L43I	Y	HS
196	ZNF81	c.8C>T	p.A3V	Y	HS
291	ATP7A	c.1009G>A	p.A337T	Y	HS
291	HCCS	c.1-2C>T	NS	Y	HS
357	OFD1	c.1543-19 C>G	NS	-	HS
545	ATRX[a]	c.5786A>G	p.K1929R	-	HS
545	HUWE1	c.4824+8_4824+9insA	NS	-	HS
573	ATRX[a]	c.4981C>T	p.R1661C	Y	HS
573	SHROOM4	c.3411_3413delGGA	NS	Y	HS
592	MAGT1	c.769-16T>C	NS	Y	HS
592	NSDHL	c.268-15_268-14insT	NS	Y	HS
596	ATRX[a]	c.5786A>G	p.K1929R	-	HS
596	HUWE1	c.4824+8_4824+9insA	NS	-	HS
062	GDI1	c.991+7 C>T	NS	Y	MS
062	SYN1	c.1056-14_1056-8del CTTGTC	NS	Y	MS
092	FANCB	c.676A>G	p.I226V	Y	MS
156	FGD1	c.1202C>T	p.A401V	Y	MS
308	FANCB	c.362G>A	p.R121H	-	MS
308	TIMM8A	IVS1-6C>T	NS	-	MS
356	PHF8	c.1-1 G>A	NS	Y	MS
360	DMD	c.5182C>T	p.R1728C	-	MS
410	AFF2	c.2569-21G>A	NS	Y	MS
410	IDS	c.641C>T	p.T214M	Y	MS
434	SRPX2	c.980 A>G	p.N327S	Y	MS
473	ATP6AP2	c.38-5T>C	NS	Y	MS
473	BCOR	c.2423T>A	p.L808H	Y	MS
473	BCOR	c.2424T>C	p.L808H	Y	MS
473	FLNA	c.3035C>T	p.S1012L	Y	MS
473	FLNA	c.5290G>A	p.A1764T	Y	MS
473	OFD1	c.2387+11C>T	NS	Y	MS
473	PDHA1	c.832-24_832-21delAACT	NS	Y	MS
473	SYN1	c.528-19C>T	NS	Y	MS
521	ACSL4[a]	c.1384A>G	p.I462V	-	MS
521	DMD	c.668C>T	p.P223L	-	MS
521	GRIA3[a]	c.2647G>A	p.G883S	-	MS
600	DMD	c.5355G>C	p.Q1785H	Y	MS

Note: all cases with non-synonymous VUS and skewed maternal XI were male. NS = not specified. Y = maternally inherited. - = no family studies performed. HS = highly skewed. MS = moderately skewed.

[a] Gene associated with skewed XI in female carriers

Chapter 3: Conclusions

The limitations of this study are in fact its most relevant findings. Plainly put, if progress is to be made in clarifying the genetics of intellectual disability, more information is needed, both from the referring physicians and the genetics community as a whole. Meaningful curation of variants requires complete, accurate and accessible information about the patient, the patient's genotype and the gene(s) in question. Ideally, the clinical and molecular evaluation of patients would proceed in a consistent, systematic way, and all reporting would be standardized and freely accessible. This concept is already firmly within the awareness of the genetics community, as the ability to acquire genome-scale information has rapidly outpaced our ability to manage the information. The trend is moving toward more open sharing of variant information, but is not nearly universal, nor does the currently available molecular data necessarily include any phenotypic information (for instance, the Exome Sequencing Project of the National Heart, Lung and Blood Institute (NHLBI) in the National Institutes of Health (NIH), available at http://evs.gs.washington.edu/EVS/). In this example, allele frequencies in populations chosen for a specific condition (for instance, cardiovascular disease) are reported, and it is left to the investigator to assume that other genetic conditions are not present. The implementation of training and infrastructure to make comprehensive large-scale genetic data sharing possible will require a significant long-term commitment of funding, infrastructure and collaboration.

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Appendix A: X-linked Intellectual Disability Panel Variants

Table A.1

X-linked Intellectual Disability Panel Variants

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
070	M	ABCD1	c.1634+12 C>T	NS	R	VUS
446	M	ABCD1	c.1489-6delC	NS	R	VUS
450	M	ABCD1	c.108G>A	p.V36V	S	VUS
456	M	ABCD1	c.1489-6delC	NS	R	VUS
225	M	ABCD1	c.1429G>A	p.E477K	M	VUS
070	M	ACSL4	c.308G>C	p.S103T	M	VUS
109	M	ACSL4	c.929+3 A>G	NS	R	VUS
122	M	ACSL4	c.1978+11 A>G	NS	R	VUS
152	M	ACSL4	c.1821-10 G>A	NS	R	VUS
193	F	ACSL4	c.1448A>G	p.Y483C	M	VUS
257	M	ACSL4	c.82A>G	p.I28V	M	VUS
298	M	ACSL4	c.1054-9T>A	NS	R	VUS
521	M	ACSL4	c.1384A>G	p.I462V	M	VUS
529	M	ACSL4	c.72C>T	p.A24A	S	VUS
553	F	ACSL4	c.1514-7T>C	NS	R	VUS
133	M	AFF2	c.3088A>C	p.I1030L	M	VUS
222	M	AFF2	c.3268T>A	p.F1090I	M	VUS
250	F	AFF2	c.3837 A>G het	p.T1279T	S	VUS
362	F	AFF2	c.3701C>G	p.T1234S	M	VUS
365	M	AFF2	c.180T>C	p.Y60Y	S	VUS
410	M	AFF2	c.2569-21G>A	NS	R	VUS
575	M	AFF2	c.496 C>T	p.P166S	M	VUS
490	M	AFF2	c.294A>G	p.P98P	S	VUS
233	M	AGTR2	c.402delT	p.F134F	S	VUS
304	M	AGTR2	c.127C>T	p.H43Y	M	VUS
220	M	AP1S2	c.289-10delT	NS	R	VUS
534	M	AP1S2	c.138C>A	p.C46X	N	P
144	M	ARHGEF6	c.2331+9G>C	NS	R	VUS
200	M	ARHGEF6	c.2051C>T	p.S684F	M	VUS
200	M	ARHGEF6	c.334+7C>T	NS	R	VUS
500	M	ARHGEF6	c.540A>G	p.S180S	S	VUS
509	M	ARHGEF6	c.2093T>C	p.I698T	M	VUS
224	M	ARHGEF6	c.1483 A>G	p.K495E	M	VUS
283	M	ARHGEF6	c.2309G>A	p.S770N	M	VUS
119	M	ARHGEF9	c.1094G>A	p.R365H	M	VUS
227	M	ARHGEF9	c.865C>T	p.R289X	N	P
354	M	ARHGEF9	c.691_709del19	NS	D	P
570	M	ARHGEF9	c.30C>T	p.I10I	S	VUS
018	M	ARX	c.1318_1320 dup GGC	NS	I	VUS
053	M	ARX	c.665_670dupGCACCG	NS	I	VUS
088	M	ARX	c.303A>G	p.A101A	S	VUS
164	M	ARX	c.441_464dup24	NS	I	P
196	M	ARX	c.1671G>A	p.T557T	S	VUS
267	M	ARX	c.904G>A	p.A302T	M	VUS
508	M	ARX	c.315_335dup21	NS	I	P

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
519	M	ARX	c.611G>A	p.R204H	M	VUS
559	M	ARX	c.590G>A	p.G197D	M	VUS
562	M	ARX	c.336A>G	p.A112A	S	VUS
568	M	ARX	c.1671G>A	p.T557T	S	VUS
040	M	ARX	c.441_464dup24	NS	I	P
024	M	ARX	c.441_464dup24	NS	I	P
068	M	ARX	c.441_464dup24	NS	I	P
338	F	ATP6AP2	c.268C>G	p.P90A	M	VUS
403	M	ATP6AP2	c.397-13A>G	NS	R	VUS
473	M	ATP6AP2	c.38-5T>C	NS	R	VUS
046	M	ATP7A	c. 3801+6T>C	NS	R	VUS
186	M	ATP7A	c.3589A>G	p.N1197D	M	VUS
219	M	ATP7A	c.4312G>A	p.V1438I	M	VUS
291	M	ATP7A	c.1009G>A	p.A337T	M	VUS
311	M	ATP7A	c.3801+6T>C	NS	R	VUS
429	M	ATP7A	c.1400C>G	p.T467S	M	VUS
456	M	ATP7A	c.1302A>C	p.A434A	S	VUS
587	M	ATP7A	c.3790A>G	p.I1264V	M	VUS
603	M	ATP7A	c.1009G>A	p.A337T	M	VUS
547	M	ATP7A	c.4223 A>G	p.K1408R	M	VUS
005	M	ATRX	c.4120+4A>C	NS	R	VUS
047	M	ATRX	c.2127T>C	p.D709D	S	VUS
053	M	ATRX	c.2692G>C	p.D898H	M	VUS
080	M	ATRX	c.536A>G	p.N179S	M	LP
082	M	ATRX	c.109C>T	p.R37X	N	P
106	M	ATRX	c.1257G>A	p.A419A	S	VUS
116	M	ATRX	c.4317G>A	p.K1439K	S	VUS
142	M	ATRX	c.366 A>G	p.P122P	S	VUS
143	M	ATRX	c.5038A>T	p.I1680F	M	VUS
148	M	ATRX	c.1467C>T	p.T489T	S	VUS
178	M	ATRX	c.2595C>G	p.H865Q	M	VUS
180	M	ATRX	c.6110+15A>G	NS	R	VUS
190	M	ATRX	c.5395G>A	p. V1799V	S	VUS
196	M	ATRX	c.2923G>A	p.D975N	M	VUS
206	F	ATRX	c.2785C>G	p.Q929E	M	VUS
208	M	ATRX	c.5811 G>A	p.G1937G	S	VUS
217	M	ATRX	c.2806 G>C	p.V936L	M	VUS
235	M	ATRX	c.5579A>G	p.N1860S	M	VUS
238	M	ATRX	c.4120+4 A>C	NS	R	VUS
359	M	ATRX	c.2521A>G	p.I841V	M	VUS
362	F	ATRX	c.662+11T>C	NS	R	VUS
372	M	ATRX	c.4654G>T	p.V1552F	M	P
430	M	ATRX	c.5579A>G	p.N1860S	M	VUS
442	M	ATRX	c.6327-18C>T	NS	R	VUS
458	M	ATRX	c.763A>C	p.A255C	M	VUS
473	M	ATRX	c.5349A>G	p.P1783P	S	VUS
486	M	ATRX	c.2000C>T	p.P667L	M	VUS
491	M	ATRX	c.242+9A>G	NS	R	VUS
512	M	ATRX	c.5579A>G	p.N1860S	M	VUS
524	M	ATRX	c.3065G>A	p.R1022Q	M	VUS
545	M	ATRX	c.5786A>G	p.K1929R	M	VUS
573	M	ATRX	c.4981C>T	p.R1661C	M	VUS
591	M	ATRX	c.563G>C	p.R188T	M	VUS
596	M	ATRX	c.5786A>G	p.K1929R	M	VUS
105	M	ATRX	c.5048 A>G	p.Y1683C	M	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
022	M	BCOR	c.1448 C>T	p.P483L	M	VUS
072	M	BCOR	c.909C>T	p.A303A	S	VUS
126	M	BCOR	c.4899 G>A	p.S1633S	S	VUS
135	F	BCOR	c.64A>G	p.M22L	M	VUS
164	M	BCOR	c.1257A>G	p.K419K	S	VUS
176	M	BCOR	c.1241C>G	p.A414G	M	VUS
218	M	BCOR	c.4113G>A	p.S1371S	S	VUS
258	M	BCOR	c.277T>G	p.L93V	M	VUS
308	M	BCOR	c.1791C>T	p.H597H	S	VUS
354	M	BCOR	c.1084G>A	p.A362T	M	VUS
418	M	BCOR	c.3036C>T	p.P1012P	S	VUS
471	M	BCOR	c.3378C>T	p.H1126H	S	VUS
473	M	BCOR	c.2423T>A	p.L808H	M	VUS
473	M	BCOR	c.2424T>C	p.L808H	M	VUS
541	M	BCOR	c.4829C>T	p.T1610I	M	VUS
046	M	BRWD3	c.3875G>A	p.R1292Q	M	VUS
050	M	BRWD3	c.492 A>G	p.S164S	S	VUS
082	M	BRWD3	c.1644C>T	p.Y548Y	S	VUS
233	M	BRWD3	c.3194G>A	p.G1065E	M	VUS
280	M	BRWD3	c.1386+11T>C	NS	R	VUS
345	M	BRWD3	c.33G>A	p.E11E	S	VUS
407	M	BRWD3	c.4210T>C	p.Y1404H	M	VUS
427	M	BRWD3	c.3540T>C	p.T1180T	S	VUS
428	F	BRWD3	c.5100T>C	p.G1700G	S	VUS
438	M	BRWD3	c.1206 G>A	p.V402V	S	VUS
542	M	BRWD3	c.4008 T>G	p.G1336G	S	VUS
555	M	BRWD3	c.1521+13T>C	NS	R	VUS
604	M	BRWD3	c.858T>C	p.T286T	S	VUS
115	M	CASK	c.1314+18 T>C	NS	R	VUS
173	M	CASK	c.1503+3 A>G	NS	R	VUS
201	M	CASK	c.1-11C>T	N/A	R	VUS
218	M	CASK	c.1234-20A>G	NS	R	VUS
218	M	CASK	c.1668+10T>C	NS	R	VUS
361	M	CASK	c.2506-11T>C	NS	R	VUS
424	M	CASK	c.1718C>T	p.T573I	M	VUS
472	M	CASK	c.1811T>A	p.L604X	N	P
513	M	CASK	c.1289G>A	p.R430H	M	VUS
529	M	CASK	c.1034-6C>T	NS	R	VUS
101	M	CDKL5	c.413C>T	p.P138L	M	VUS
104	M	CDKL5	c.555-19C>G	NS	R	VUS
272	M	CDKL5	c.1431T>C	p.S477S	S	VUS
436	M	CDKL5	c.103A>C	p.T35P	M	VUS
499	M	CDKL5	c.1455_1460delGGCCAA	p.A486_K487del	D	VUS
289	M	CDKL5	c.145+17 A>G	NS	R	VUS
009	M	CUL4B	c.831-12C>T	NS	R	VUS
057	M	CUL4B	c.65G>A	p.G22D	M	VUS
087	M	CUL4B	c.1183C>T	p.R395W	M	VUS
167	M	CUL4B	c.2298 G>T	p.E766D	M	VUS
248	M	CUL4B	c.1311-19delT	NS	R	VUS
273	M	CUL4B	c.831-12C>T	NS	R	VUS
324	M	CUL4B	c.857delT	NS	R	LP
462	M	CUL4B	c.1911C>T	p.C637C	S	VUS
474	M	CUL4B	c.1796 G>T	p.G599V	M	VUS
130	M	CUL4B	c.373 C>G	p.L125V	M	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
107	M	DCX	c.339C>A	p.I113I	S	VUS
186	M	DKC1	c.771+13G>A	NS	R	VUS
292	M	DKC1	c.1167 G>A	p.K389K	S	VUS
326	M	DKC1	c.171+14delA	NS	R	VUS
407	M	DKC1	c.171+14delA	NS	R	VUS
141	M	DLG3	c.1405+5_1405+7 del GAG	NS	R	VUS
204	M	DLG3	c.1782 G>A	p.P594P	S	VUS
253	M	DLG3	c.1800T>C	p.Y600Y	S	VUS
345	M	DLG3	c.1938C>T	p.S646S	S	VUS
332	M	DLG3	c.2280 T>C	p.Y760Y	S	VUS
070	M	DMD	c.1537 A>G	p.M513V	M	VUS
070	M	DMD	c.10836 C>T	p.S3612S	S	VUS
071	M	DMD	c.7542+13 A>G	NS	R	VUS
125	M	DMD	c.2630T>G	p.V877G	M	VUS
136	M	DMD	c.6409G>C	p.E2137Q	M	VUS
141	M	DMD	c.2352 T>C	p.A784A	S	VUS
160	M	DMD	c.8110 T>C	p.W2704R	M	VUS
160	M	DMD	c.10565 C>T	p.A3522V	M	VUS
162	M	DMD	c.6732G>C	p.Q2244H	M	VUS
178	M	DMD	c.4798G>C	p.V1600L	M	VUS
182	M	DMD	c.6614+8G>A	NS	R	VUS
198	M	DMD	c.5146G>A	p.V1716M	M	VUS
218	M	DMD	c.483C>T	p.T161T	S	VUS
219	M	DMD	c.3951G>A	p.E1317E	S	VUS
222	M	DMD	c.7472A>G	p.Q2491R	M	VUS
256	M	DMD	c.2380+11 G>A	NS	R	VUS
287	M	DMD	c.1993-2_1993-1insCACA	NS	R	VUS
301	M	DMD	c.7183 G>A	p.A2395T	M	VUS
336	M	DMD	c.10262+1G>A	NS	R	VUS
338	F	DMD	c.4529A>G	p.K1510R	M	VUS
360	M	DMD	c.5182C>T	p.R1728C	M	VUS
370	M	DMD	c.6202 C>T	p.P2068S	M	VUS
379	M	DMD	c.1095 A>C	p.Q365H	M	VUS
393	F	DMD	c.8543A>G	p.H2848R	M	VUS
399	M	DMD	c.4233+2C>T	NS	R	VUS
427	M	DMD	c.538C>T	p.L180L	S	VUS
448	M	DMD	c.7602C>T	p.A2534A	S	VUS
469	M	DMD	c.1337A>G	p.H446R	M	VUS
488	M	DMD	c.3674T>C	p.I1225T	M	VUS
492	F	DMD	c.10442A>G	p.Q3481R	M	VUS
496	M	DMD	c.6571C>T	p.R2191W	M	VUS
501	M	DMD	c.8308 G>A	p.D2770N	M	VUS
511	M	DMD	c.1252A>T	p.T418S	M	VUS
511	M	DMD	c.5154+14T>A	NS	R	VUS
521	M	DMD	c.668C>T	p.P223L	M	VUS
538	M	DMD	c.7321A>C	p.T2441P	M	VUS
551	M	DMD	c.9033 G>A	p.P3011P	S	VUS
600	M	DMD	c.5355G>C	p.Q1785H	M	VUS
404	M	DMD	c.9352G>A	p.A3118T	M	VUS
557	M	DMD	c.2900T>G	p.L967R	M	VUS
018	M	FANCB	c.1105-3_1105-2insTATT	NS	R	VUS
067	M	FANCB	c.1769T>C	p.F590S	M	VUS
092	M	FANCB	c.676A>G	p.I226V	M	VUS
135	F	FANCB	c.493G>C	p.G165R	M	VUS
196	M	FANCB	c.127T>A	p.L43I	M	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
217	M	FANCB	c.513 G>A	p.Q171Q	S	VUS
219	M	FANCB	c.1197+19_1197+21delCTT	NS	R	VUS
308	M	FANCB	c.362G>A	p.R121H	M	VUS
518	M	FANCB	c.1105-3_1105-2insTATT	NS	R	VUS
598	M	FANCB	c.507T>C	p.S169S	S	VUS
601	M	FANCB	c.1327-3delT	NS	R	VUS
603	M	FANCB	c.2090C>T	p.P697L	M	VUS
544	M	FANCB	c.1177C>T	p.P393S	M	VUS
034	M	FGD1	c.2043 C>T	p.V681V	S	VUS
119	M	FGD1	c.110C>T	p.A37V	M	VUS
156	M	FGD1	c.1202C>T	p.A401V	M	VUS
219	M	FGD1	c.676G>A	p.A226T	M	VUS
225	M	FGD1	c.2082G>A	p.T694T	S	VUS
414	F	FGD1	c.2581-6C>T	NS	R	VUS
456	M	FGD1	c.942C>A	p.P314P	S	VUS
492	F	FGD1	c.1936-11A>C	NS	R	VUS
540	M	FGD1	c.676G>A	p.A226T	M	VUS
152	M	FGD1	c.1101+20	NS	R	VUS
005	M	FLNA	c.63C>T	p.V21V	S	VUS
025	M	FLNA	c.1029C>T	p.S343S	S	VUS
029	F	FLNA	c.5239_5250del12	NS	D	VUS
044	M	FLNA	c.2410G>A	p.V804I	M	VUS
044	M	FLNA	c.6769+17C>G	NS	R	VUS
064	M	FLNA	c.5972C>T	p.S1991L	M	VUS
066	M	FLNA	c.6303G>A	p.T2101T	S	VUS
123	M	FLNA	c.2331C>T	p.P777P	S	VUS
131	M	FLNA	c.5217+13 G>T	NS	R	VUS
139	M	FLNA	c.6651 G>A	p.K2214K	S	VUS
156	M	FLNA	c.5193G>A	p.V1731V	S	VUS
166	M	FLNA	c.4866 C>T	p.Y1622Y	S	VUS
176	M	FLNA	c.4451A>G	p.Q1484R	M	VUS
184	M	FLNA	c.1286C>T	p.T429M	M	VUS
207	M	FLNA	c.220G>C	p.G74R	M	VUS
214	F	FLNA	c.3429C>G	p.T1143T	S	VUS
223	M	FLNA	c.1900 C>G	p.R364G	M	VUS
271	M	FLNA	c.3650A>G	p.H1217R	M	VUS
272	M	FLNA	c.2845G>A	p.V949I	M	VUS
284	M	FLNA	c.1693C>T	p.P565S	M	VUS
284	M	FLNA	c.4499T>C	p.V1500A	M	VUS
305	M	FLNA	c.1812 C>T	p.D604D	S	VUS
316	M	FLNA	c.5313+18A>G	NS	R	VUS
316	M	FLNA	c.720+8C>T	NS	R	VUS
321	M	FLNA	c.1829-13T>C	NS	R	VUS
321	M	FLNA	c.6516G>A	p.Q2172Q	S	VUS
345	M	FLNA	c.3915G>A	p.T1305T	S	VUS
354	M	FLNA	c.1429+8C>T	NS	R	VUS
371	M	FLNA	c.4517C>T	p.T1506I	M	VUS
414	F	FLNA	c.1875C>T	p.D625D	S	VUS
427	M	FLNA	c.7221C>T	p.N2407N	S	VUS
436	M	FLNA	c.6612C>T	p.P2204P	S	VUS
455	M	FLNA	c.5911A>T	p.I1971F	M	VUS
462	M	FLNA	c.861C>T	p.Y287Y	S	VUS
473	M	FLNA	c.3035C>T	p.S1012L	M	VUS
473	M	FLNA	c.5290G>A	p.A1764T	M	VUS
481	M	FLNA	c.1875C>T	p.D625D	S	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
485	M	FLNA	c.4598+8G>C	NS	R	VUS
532	M	FLNA	c.6412 G>T	p.G2138C	M	VUS
546	M	FLNA	c.622+16G>A	NS	R	VUS
550	M	FLNA	c.6408C>T	p.G2136G	S	VUS
560	M	FLNA	c.3876 C>T	p.H1292H	S	VUS
563	M	FLNA	c.6412G>T	p.G2138C	M	VUS
579	M	FLNA	c.1608C>T	p.G536G	S	VUS
585	M	FLNA	c.7351 G>A	p.V2451I	M	VUS
224	M	FLNA	c.5392 A>C	p.T1798P	M	VUS
351	M	FLNA	c.1716 C>T	p.T572T	S	VUS
353	M	FLNA	c.5687-12 G>A	NS	R	VUS
431	M	FLNA	c.6651G>A	p.K2217K	S	VUS
345	M	FMR1	c.1857C>T	p.D619D	S	VUS
350	M	FMR1	c.1813C>T	p.L605L	S	VUS
354	M	FMR1	c.1695T>C	p.N565N	S	VUS
442	M	FMR1	c.309C>T	p.Y103Y	S	VUS
003	M	FTSJ1	c.984+8C>T	NS	R	VUS
003	M	FTSJ1	c.362-20C>T	NS	R	VUS
043	M	FTSJ1	c.219T>C	p.A73A	S	VUS
117	M	FTSJ1	c.831G>A	p.T277T	S	VUS
552	M	FTSJ1	c.349G>A	p.G117R	M	VUS
062	M	GDI1	c.991+7 C>T	NS	R	VUS
104	M	GDI1	c.587+15G>T	NS	R	VUS
331	M	GDI1	c.154-20T>A	NS	R	VUS
381	M	GDI1	c.1191+12 G>A	NS	R	VUS
372	M	GK	c.877-12delT	NS	R	VUS
459	M	GK	c.1479 G>A	p.A493A	S	VUS
592	M	GK	c.1575T>C	p.S525S	S	VUS
592	M	GK	c.501C>T	p.A167A	S	VUS
053	M	GPC3	c.1426A>T	p.M476L	M	VUS
065	M	GPC3	c.1574-7_1574-4delTTGA	NS	R	VUS
363	M	GPC3	c.1680C>T	p.S560S	S	VUS
494	M	GPC3	c.1285G>A	p.V429M	M	VUS
533	M	GPC3	c.1167-8T>C	NS	R	VUS
038	M	GRIA3	c.1501-8T>A	NS	R	VUS
170	F	GRIA3	c.1501-13dupT	NS	R	VUS
195	M	GRIA3	c.1-17T>C	NS	R	VUS
195	M	GRIA3	c.-2_-1insG	NS	R	VUS
221	M	GRIA3	c.-16T>C	NS	R	VUS
222	M	GRIA3	1878-3T>C	NS	R	VUS
314	M	GRIA3	c.1501-13_1501-12insT	NS	R	VUS
317	M	GRIA3	c.1852C>A	p.Q618K	M	VUS
426	M	GRIA3	c.1501-8T>A	NS	R	VUS
442	M	GRIA3	c.1181G>A	p.R394Q	M	VUS
521	M	GRIA3	c.2647G>A	p.G883S	M	VUS
535	M	GRIA3	c.2097C>T	p.Y699Y	S	VUS
552	M	GRIA3	c.1878-3T>C	NS	R	VUS
555	M	GRIA3	c.419A>G	p.Q140R	M	VUS
018	M	HCCS	c.608+13 G>A	NS	R	VUS
291	M	HCCS	c.1-2C>T	NS	R	VUS
364	M	HCCS	c.654 C>T	p.C218C	S	VUS
336	M	HPRT1	c.384+19A>G	NS	R	VUS
001	F	HUWE1	c.7338+15C>T	NS	R	VUS
005	M	HUWE1	c.6914A>G	p.D2305G	M	VUS
059	M	HUWE1	c.10621A>G	p.T3541A	M	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
122	M	HUWE1	c.5885-8T>C	NS	R	VUS
140	M	HUWE1	c.5716+5G>A	NS	R	VUS
140	M	HUWE1	c.6031-4T>A	NS	R	VUS
144	M	HUWE1	c.694-8dupT	NS	R	VUS
161	M	HUWE1	c.9817 C>T	p.H3273Y	M	VUS
162	M	HUWE1	c.3966G>A	p.L1322L	S	VUS
186	M	HUWE1	c.5091A>G	p.G1697G	S	VUS
194	M	HUWE1	c.12860C>T	p.S4287F	M	VUS
234	M	HUWE1	c.144+18C>T	NS	R	VUS
245	M	HUWE1	c.5716+15C>T	NS	R	VUS
270	M	HUWE1	c.8298A>G	p.Q2766Q	S	VUS
278	M	HUWE1	c.6031-4T>A	NS	R	VUS
278	M	HUWE1	c.5716+5G>A	NS	R	VUS
304	M	HUWE1	c.5885-8T>C	NS	R	VUS
370	M	HUWE1	c.3624 G>C	p.K1208N	M	VUS
421	M	HUWE1	c.3966G>A	p.L1322L	S	VUS
431	M	HUWE1	c.7662G>A	p.T2554T	S	VUS
445	M	HUWE1	c.646-18 T>C	NS	R	VUS
492	F	HUWE1	c.12364C>T	p.R4122C	M	VUS
523	M	HUWE1	c.1673-12 A>C	NS	R	VUS
545	M	HUWE1	c.4824+8_4824+9insA	NS	R	VUS
592	M	HUWE1	c.3912A>G	p.T1304T	S	VUS
596	M	HUWE1	c.4824+8_4824+9insA	NS	R	VUS
604	M	HUWE1	c.3433G>A	p.E1145K	M	VUS
193	F	IDS	c.1159G>A	p.A387T	M	VUS
244	M	IDS	c.1180+2T>C	NS	R	LP
292	M	IDS	c.23G>T	p.R8L	M	VUS
410	M	IDS	c.641C>T	p.T214M	M	VUS
433	M	IDS	c.1222C>T	p.P408S	M	VUS
458	M	IDS	c.123C>G	p.L41L	S	VUS
458	M	IDS	c.126C>T	p.I42I	S	VUS
530	M	IDS	c.1222C>T	p.P408S	M	VUS
535	M	IDS	c.1181-13C>T	NS	R	VUS
568	M	IDS	c.1181-13C>T	NS	R	VUS
311	M	IGBP1	c.872-3T>C	NS	R	VUS
329	M	IGBP1	c.-18 G>C	NS	R	VUS
241	M	IL1RAPL1	c.1927A>G	p.I643V	M	VUS
282	M	IL1RAPL1	c.1490G>A	p.R497Q	M	VUS
436	M	IL1RAPL1	c.1136A>G	p.K379R	M	VUS
483	M	IL1RAPL1	c.1202-6_1202-5insT	NS	R	VUS
555	M	IL1RAPL1	c.1605G>T	p.T535T	S	VUS
013	M	JARID1C	c.1607G>T	p.G536V	M	VUS
384	M	JARID1C	c.3778G>T	p.A1260S	M	VUS
190	M	KDM5C	c.1203C>T	p. A401A	S	VUS
252	M	KDM5C	c.3125delG	NS	FS	P
588	M	KDM5C	c.2726G>A	p.R909Q	M	VUS
605	M	KDM5C	c.3755G>A	p.R1252H	M	VUS
135	F	KIAA2022	c.964C>T	p.R322X	N	P
183	F	KIAA2022	c.1983 C>T	p.H661H	S	VUS
360	M	KIAA2022	c.813A>G	p.E271E	S	VUS
362	F	KIAA2022	c.637A>C	p.R213R	S	VUS
565	M	KIAA2022	c.2088C>T	p.G696G	S	VUS
418	M	KIAA2022	c.3756C>T	p.S1252S	S	VUS
135	F	KLF8	c.322A>G	p.I108V	M	VUS
225	M	KLF8	c.411T>G	p.T137T	S	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
360	M	KLF8	c.93T>C	p.S31S	S	VUS
396	F	KLF8	c.97C>T	p.R33W	M	VUS
553	F	KLF8	c.1-14C>T	NS	R	VUS
559	M	KLF8	c.795G>A	p.S265S	S	VUS
066	M	L1CAM	c.3531-12G>A	NS	R	LP
085	M	L1CAM	c.3057T>G	p.D1019E	M	VUS
100	M	L1CAM	c.649A>C	p.R217R	S	VUS
152	M	L1CAM	c.1268-10 C>T	NS	R	VUS
207	M	L1CAM	c.2243T>C	p.V748A	M	VUS
304	M	L1CAM	c.3457+18C>T	NS	R	VUS
340	M	L1CAM	c.1704-18_1704-15delGACA	NS	R	VUS
345	M	L1CAM	c.695-21C>T	NS	R	VUS
404	M	L1CAM	c.870C>T	p.Y290Y	S	VUS
444	M	L1CAM	c.1704-18_1704-15delGACA	NS	R	VUS
531	M	L1CAM	c.984 C>T	p.T328T	S	VUS
531	M	L1CAM	c.2432-12 G>A	NS	R	VUS
552	M	L1CAM	c.1880C>T	p.T627M	M	VUS
552	M	L1CAM	c.964C>T	p.R322W	M	VUS
553	F	L1CAM	c.2274G>A	p.G758G	S	VUS
598	M	L1CAM	c.1261G>A	p.V421I	M	LP
038	M	LAMP2	c.661G>A	p.G221R	M	VUS
078	M	LAMP2	c.929-9 T>C	NS	R	VUS
106	M	LAMP2	c.741+11C>T	NS	R	VUS
462	M	LAMP2	c.591G>A	p.V197V	S	VUS
522	M	LAMP2	c.-4G>C	NS	R	VUS
598	M	LAMP2	IVS1093+9C>T	NS	R	VUS
602	M	LAMP2	c.907A>T	p.M303L	M	VUS
026	F	MAGT1	c.798A>G	p.Q266Q	S	VUS
592	M	MAGT1	c.769-16T>C	NS	R	VUS
396	F	MAGT1	c.438A>T	p.I146I	S	VUS
004	M	MAOA	c.412-6C>G	NS	R	VUS
038	M	MAOA	c.1389C>T	p.L463L	S	VUS
074	M	MAOA	c.504G>T	p.K168N	M	VUS
137	M	MAOA	c.923T>C	p.M308T	M	VUS
215	M	MAOA	c.702C>T	p.L234L	S	VUS
222	M	MAOA	306+16C>A	N/A	R	VUS
527	M	MAOA	----	NS	D	P
588	M	MAOA	c.1262+19G>A	NS	R	VUS
011	M	MBTPS2	c.485 C>T	p.T162M	M	VUS
241	M	MBTPS2	c.1237C>T	p.H413Y	M	VUS
247	M	MBTPS2	c.366_383del18	NS	D	VUS
424	M	MBTPS2	c.846A>G	p.L282L	S	VUS
017	M	MECP2	c.527 C>A	p.P176H	M	VUS
050	M	MECP2	c.646 A>G	p.S216G	M	VUS
142	M	MECP2	c.1135 C>T	p.P379S	M	VUS
303	M	MECP2	c.1233C>T	p.S411S	S	VUS
318	M	MECP2	c.206G>T	p.G69V	M	VUS
359	M	MECP2	c.925C>T	p.R309W	M	VUS
054	M	MED12	c.4428G>A	p.L1476L	S	VUS
142	M	MED12	c.2981+13 G>A	NS	R	VUS
158	M	MED12	c.2325C>G	p.T775T	S	VUS
158	M	MED12	c.106C>A	p.L36M	M	VUS
231	M	MED12	c.1248+15T>C	NS	R	VUS
294	M	MED12	c.2784T>G	p.A928A	S	VUS
371	M	MED12	c.4984G>A	p.G1662S	M	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
407	M	MED12	c.3355-8_3355-7 ins T	NS	R	VUS
483	M	MED12	c.2351G>A	p.R784H	M	VUS
491	M	MED12	c.82G>A	p.D28N	M	VUS
528	M	MED12	c.6315_6320delACAGCA	p.Q2106_Q2107del	D	VUS
567	M	MED12	c.4449 G>A	p.S1483S	S	VUS
220	M	MID1	c.1443A>C	p.T481T	S	VUS
250	F	MID1	c.704C>T	p.T235I	M	VUS
516	M	MID1	c.2000C>T	p.P667L	M	VUS
591	M	MID1	c.1242C>T	p.Y414Y	S	VUS
026	F	MTM1	c.582C>T	p.L194L	S	VUS
053	M	MTM1	c.422C>T	p.A141V	M	VUS
132	M	MTM1	c.742G>A	p.G248S	M	VUS
350	M	MTM1	c.528+7T>C	NS	R	VUS
441	M	MTM1	c.1701C>T	p.Y567Y	S	VUS
456	M	MTM1	c.546T>C	p.H182H	S	VUS
527	M	MTM1	c.1260+17A>G	NS	R	VUS
348	M	MTM1	c.64-14 T>C	NS	R	VUS
567	M	NDUFA1	c.94 G>C	p.G32R	M	VUS
014	M	NHS	c.204A>G	p.P68P	S	VUS
021	F	NHS	c.176G>A	p.R59Q	M	VUS
021	F	NHS	c.177C>A	p.R59Q	M	VUS
063	M	NHS	c.1499T>C	p.V500A	M	VUS
078	M	NHS	c.3323 C>T	p.P1108L	M	VUS
114	M	NHS	c.310_345 del 36	NS	D	VUS
126	M	NHS	c.4416 C>A	p.T1472T	S	VUS
162	M	NHS	c.310_345 del 36	NS	D	VUS
221	M	NHS	c.1533G>A	p.E511E	S	VUS
222	M	NHS	c.211C>T	p.P71S	M	VUS
250	F	NHS	c.513 C>T het	p.L171L	S	VUS
304	M	NHS	c.2056G>T	p.A686S	M	VUS
421	M	NHS	c.211C>T	p.P71S	M	VUS
529	M	NHS	c.566-11dupT	NS	R	VUS
542	M	NHS	c.618 G>A	p.P206P	S	VUS
583	M	NHS	c.211C>T	p.P71S	M	VUS
229	M	NHS	c.1690 T>C	p.S564P	M	VUS
498	M	NHS	c.302_337dup36	NS	I	VUS
221	M	NLGN3	c.238G>C	p.G80R	M	VUS
025	M	NLGN4X	c.695G>A	p.R232Q	M	VUS
044	M	NLGN4X	c.2405A>G	p.Q802R	M	VUS
053	M	NLGN4X	c.1194C>T	p.S398S	S	VUS
061	M	NLGN4X	c.2295C>G	p.R765R	S	VUS
104	M	NLGN4X	c.1785T>A	p.P595P	S	VUS
378	M	NLGN4X	c.2020G>A	p.E674K	M	VUS
543	M	NLGN4X	c.1249 C>T	p.R417W	M	VUS
222	M	NLGN4X	c.1262A>G	p.K421R	M	VUS
468	M	NLGN4X	c.1381 G>A	p.A461T	M	VUS
130	M	NSDHL	c.585 C>G	p.T195T	S	VUS
175	M	NSDHL	c.267+10 C>G	NS	R	VUS
217	M	NSDHL	c.987 C>T	p.V329V	S	VUS
427	M	NSDHL	c.83A>G	p.D28G	M	VUS
427	M	NSDHL	c.987C>T	p.V329V	S	VUS
435	M	NSDHL	c.773C>T	p.S258L	M	VUS
457	M	NSDHL	IVS789+16C>T	NS	R	VUS
466	M	NSDHL	c.1084A>G	p.T362A	M	VUS
548	M	NSDHL	c.351T>C	p.F117F	S	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
592	M	NSDHL	c.268-15_268-14insT	NS	R	VUS
506	M	OCRL	c.897G>A	p.M299I	M	VUS
561	M	OCRL	c.2585C>T	p.T862I	M	VUS
588	M	OCRL	c.39+10G>A	NS	R	VUS
042	M	OFD1	c.1416 A>G	p.L472L	S	VUS
144	M	OFD1	c.216C>T	p.G72G	S	VUS
186	M	OFD1	c.2927A>C	p.K976T	M	VUS
238	M	OFD1	c.1742 G>A	p.C581Y	M	VUS
357	M	OFD1	c.1543-19 C>G	NS	R	VUS
363	M	OFD1	c.2929-18C>T	NS	R	VUS
473	M	OFD1	c.2387+11C>T	NS	R	VUS
507	M	OFD1	c.2177G>A	p.R726H	M	VUS
086	M	OFD1	c.*+2C>T	NS	R	VUS
006	M	OPHN1	del incl. exon 20	NS	D	LP
164	M	OPHN1	c.1722G>T	p.P574P	S	VUS
180	M	OPHN1	c.133G>A	p.A45T	M	VUS
222	M	OPHN1	c.902C>T	p.T301M	M	VUS
288	M	OPHN1	c.702+11A>C	NS	R	VUS
326	M	OPHN1	c.832+16G>A	NS	R	VUS
336	M	OPHN1	c.1489C>T	p.R497X	N	P
442	M	OPHN1	c.1890C>G	p.P630P	S	VUS
482	M	OPHN1	c.2144C>T	p.A715V	M	VUS
008	M	OTC	c.216+23G>A	NS	R	VUS
250	F	OTC	c.429 C>T het	p.Y143Y	S	VUS
421	M	OTC	c.718-14T>C	NS	R	VUS
431	M	OTC	c.299-7A>T	NS	R	VUS
493	M	OTC	c.83 G>A	p.G28E	M	VUS
469	M	OTC	c.216+9C>T	NS	R	VUS
094	M	PAK3	c.1518 G>A	p.R506R	S	VUS
204	M	PAK3	c.101 C>T	p.P34L	M	VUS
273	M	PAK3	c.483A>G	p.A161A	S	VUS
280	M	PAK3	c.993-20C>A	NS	R	VUS
345	M	PAK3	c.208A>G	p.I70V	M	VUS
560	M	PAK3	c.531 G>A	p.E177E	S	VUS
589	M	PAK3	c.45 A>G	p.P15P	S	VUS
055	M	PCDH19	c.2502 C>G	p.N834K	M	VUS
061	M	PCDH19	c.2328G>A	p.L776L	S	VUS
135	F	PCDH19	c.540G>A	p.T180T	S	VUS
209	M	PCDH19	c.1321G>C	p.V441L	M	VUS
262	F	PCDH19	c.591C>T	p.D197D	S	VUS
301	M	PCDH19	c.1877 C>T	p.T626I	M	VUS
361	M	PCDH19	c.2646T>C	p.A882A	S	VUS
362	F	PCDH19	c.1137C>T	p.G379G	S	VUS
378	M	PCDH19	c.3175C>G	p.R1059G	M	VUS
400	M	PCDH19	c.1745G>C	p.G582A	M	VUS
506	M	PCDH19	c.6G>A	p.E2E	S	VUS
509	M	PCDH19	c.540G>A	p.T180T	S	VUS
524	M	PCDH19	c.2355C>T	p.F785F	S	VUS
580	F	PCDH19	c.2821A>C	p.N941H	M	VUS
170	F	PDHA1	c.947dupC	NS	FS	P
173	M	PDHA1	c.831+15 C>T	NS	R	VUS
191	M	PDHA1	c.831+15 C>T	NS	R	VUS
200	M	PDHA1	c.*15G>C	NS	R	VUS
281	M	PDHA1	c.604-14 G>A	NS	R	VUS
381	M	PDHA1	c.1170 T>C	p.S390S	S	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
427	M	PDHA1	c.1008+7T>G	NS	R	VUS
473	M	PDHA1	c.832-24_832-21delAACT	NS	R	VUS
520	M	PDHA1	c.1009-24_1009-17delTTTACACT	NS	D	VUS
537	M	PDHA1	c.342C>T	p.L114L	S	VUS
580	F	PDHA1	c.984T>C	p.N328N	S	VUS
213	M	PHF6	c.729+4A>G	NS	R	VUS
312	M	PHF6	c.241-17 T>C	NS	R	VUS
601	M	PHF6	c.730-11T>G	NS	R	VUS
048	M	PHF8	c.175C>T	p.P59S	M	VUS
102	M	PHF8	c.2376G>A	p.E792E	S	VUS
140	M	PHF8	c.2129+8C>G	NS	R	VUS
249	M	PHF8	c.2547G>A	p.V849V	S	VUS
278	M	PHF8	c.2129+8C>G	NS	R	VUS
371	M	PHF8	c.222A>G	p.T74T	S	VUS
356	M	PHF8	c.1-1 G>A	NS	R	VUS
072	M	PLP1	c.666C>T	p.S222S	S	VUS
112	M	PLP1	c.763-3C>T	NS	R	VUS
053	M	PORCN	c.474C>T	p.Y158Y	S	VUS
206	F	PORCN	Heterozygous c.642C>T	p.L214L	S	VUS
474	M	PORCN	c.808 G>A	p.E270K	M	VUS
100	M	PQBP1	c.*+6C>T	NS	R	VUS
197	M	PQBP1	c.586C>T	p.R196X	N	P
239	M	PQBP1	c.691G>A	p.A231T	M	P
370	M	PQBP1	c.264 G>A	p.S88S	S	VUS
303	M	PRPS1	c.942C>T	p.S314S	S	VUS
595	M	RAB39B	c.330C>T	p.H110H	S	VUS
107	M	RPL10	c.633C>T	p.A211A	S	VUS
167	M	RPL10	c.218 A>G	p.N73S	M	VUS
182	M	RPL10	c.330-7A>G	NS	R	VUS
486	M	RPL10	c.630G>A	p.R210R	S	VUS
355	M	RPS6KA3	c.1492G>T	p.G498X	N	P
484	M	RPS6KA3	c.631+18G>A	N/A	R	VUS
497	M	RPS6KA3	c.1000-11 T>C	NS	R	VUS
552	M	RPS6KA3	c.1884A>T	p.E628D	M	VUS
581	M	RPS6KA3	c.1362T>G	p.D454E	M	VUS
050	M	SHROOM4	c.4066 G>A	p.V1356I	M	VUS
106	M	SHROOM4	c.2165G>A	p. R722H	M	VUS
152	M	SHROOM4	c.995 A>G	p.D332G	M	VUS
171	M	SHROOM4	c.1320G>A	p.Q440Q	S	VUS
179	M	SHROOM4	c.3408_3413 del GGAGGA	NS	D	VUS
182	M	SHROOM4	c.731A>G	p.N244S	M	VUS
234	M	SHROOM4	c.437G>A	p.R146Q	M	VUS
288	M	SHROOM4	c.2646C>T	p.Y882Y	S	VUS
304	M	SHROOM4	c.436C>T	p.R146W	M	VUS
347	M	SHROOM4	c. 2896G>A	p.E966K	M	VUS
430	M	SHROOM4	c.4066 G>A	p.V1356I	M	VUS
444	M	SHROOM4	c.2481C>T	p.D827D	S	VUS
447	M	SHROOM4	c.4195G>C	p.E1399Q	M	VUS
504	M	SHROOM4	c.1913C>G	p.S638C	M	VUS
533	M	SHROOM4	c.2629T>C	p.C877R	M	VUS
573	M	SHROOM4	c.3411_3413delGGA	NS	D	VUS
029	F	SLC16A2	c.1A>T	p.M1?	R	LP
458	M	SLC16A2	c.345C>T	p.P115P	S	VUS
473	M	SLC16A2	c.345C>T	p.P115P	S	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
506	M	SLC16A2	c.487G>A	p.A163T	M	VUS
523	M	SLC16A2	c.17G>A	p.G6E	M	VUS
161	M	SLC9A6	c.794-5 A>G	NS	R	VUS
227	M	SLC9A6	c.1703G>A	p.R568Q	M	VUS
328	M	SLC9A6	c.1236+2T>A	NS	R	P
076	M	SMC1A	c.1913C>T	P.T638I	M	VUS
138	M	SMC1A	c.1698 G>A	p.E566E	S	VUS
138	M	SMC1A	c.2600 T>C	p.M867T	M	VUS
256	M	SMC1A	c.412-10 C>T	NS	R	VUS
384	M	SMC1A	c.2421-20 C>T	NS	R	VUS
390	M	SMC1A	c.299-11T>C	NS	R	VUS
575	M	SMC1A	c.3702+18	NS	R	VUS
557	M	SMC1A	c.298+19A>G	NS	R	VUS
469	M	SMS	c.44C>A	p.A15D	M	VUS
198	M	SOX3	c.733_735dupGCC	NS	I	VUS
286	M	SOX3	c.732A>C	p.A244A	S	VUS
304	M	SOX3	c.946G>A	p.G316S	M	VUS
529	M	SOX3	c.307C>A	p.P103T	M	VUS
103	M	SRPX2	c.481C>A	p.R161R	S	VUS
271	M	SRPX2	c.605G>A	p.R202Q	M	VUS
434	M	SRPX2	c.980 A>G	p.N327S	M	VUS
443	M	SRPX2	c.605G>A	p.R202Q	M	VUS
052	M	SYN1	c.1369G>A	p.A457T	M	VUS
053	M	SYN1	c.1063C>T	p.L355L	S	VUS
062	M	SYN1	c.1056-14_1056-8 del CTTGTC	NS	R	VUS
070	M	SYN1	c.377+5 G>A	NS	R	VUS
222	M	SYN1	c.152C>G	p.A51G	M	VUS
222	M	SYN1	c.1699A>G	p.T567A	M	VUS
410	M	SYN1	c.1869C>T	p.P623P	S	VUS
458	M	SYN1	c.1699A>G	p.T567A	M	VUS
473	M	SYN1	c.528-19C>T	NS	R	VUS
501	M	SYN1	c.1056-14_1056-8 del CTTGTC	NS	R	VUS
546	M	SYN1	c.292_293delinsTT	p.Q98L	M	VUS
552	M	SYN1	c.1063C>T	p.L355L	S	VUS
138	M	SYP	c.687 C>T	p.A229A	S	VUS
273	M	SYP	c.705G>C	p.A235A	S	VUS
331	M	SYP	c.782G>A	p.G261E	M	VUS
400	M	SYP	c.615+12G>A	N/A	R	VUS
308	M	TIMM8A	IVS1-6C>T	NS	R	VUS
330	M	TSPAN7	c.78 C>T	p.F26F	S	VUS
381	M	TSPAN7	c.78 C>T	p.F26F	S	VUS
533	M	TSPAN7	c.515C>A	p.P172H	M	VUS
044	M	UPF3B	c.-13 G>C	NS	R	VUS
047	M	UPF3B	c.263+19dup12	NS	I	VUS
057	M	UPF3B	c.674_677delGAAA	NS	D	LP
208	M	UPF3B	c.-13 G>C	NS	R	VUS
271	M	UPF3B	c.263+19dup12	NS	I	VUS
526	M	UPF3B	c.388A>G	p.I130V	M	VUS
164	M	ZDHHC15	c.178C>T	p.L60F	M	VUS
447	M	ZDHHC9	c.881+3G>A	NS	R	VUS
246	M	ZNF41	c.2012C>T	p. A671V	M	VUS
246	M	ZNF41	c.2023C>T	p.R675X	N	VUS
510	M	ZNF41	c.296-19T>C	NS	R	VUS
001	F	ZNF711	c.1968A>G	p.T656T	S	VUS
021	F	ZNF711	c.363C>A	p.T121T	S	VUS

Table A.1 Continued

Study ID	Sex	Gene	Molecular	Protein	Class	Interpretation
056	M	ZNF81	c.828T>G	p.C276W	M	VUS
171	M	ZNF81	c.18C>T	p.D6D	S	VUS
172	M	ZNF81	c.554C>T	p.S185L	M	VUS
196	M	ZNF81	c.8C>T	p.A3V	M	VUS
219	M	ZNF81	c.1495A>G	p.I499V	M	VUS
499	M	ZNF81	c.290G>C	p.G97A	M	VUS
572	M	ZNF81	c.554C>T	p.S185L	M	VUS