X-exome sequencing in Finnish families with Intellectual Disability - four novel mutations and two novel syndromic phenotypes

Anju K Philips1, Auli Sirén2, Kristiina Avela3, Mirja Somer3, Maarit Peippo3, Minna Ahvenainen1, Fatma Doagu1, Maria Arvio5, Helena Kääriäinen4,6, Hilde Van Esch7, Guy Froyen8, Stefan A Haas9, Hao Hu10, Vera M Kalscheuer10 and Irma Järvelä1*

Abstract

Background: X-linked intellectual disability (XLID) is a group of genetically heterogeneous disorders characterized by substantial impairment in cognitive abilities, social and behavioral adaptive skills. Next generation sequencing technologies have become a powerful approach for identifying molecular gene mutations relevant for diagnosis.

Methods & objectives: Enrichment of X-chromosome specific exons and massively parallel sequencing was performed for identifying the causative mutations in 14 Finnish families, each of them having several males affected with intellectual disability of unknown cause.

Results: We found four novel mutations in known XLID genes. Two mutations; one previously reported missense mutation (c.1111C > T), and one novel frameshift mutation (c. 990_991insGCTGC) were identified in SLC16A2, a gene that has been linked to Allan-Herndon-Dudley syndrome (AHDS). One novel missense mutation (c.1888G > C) was found in GRIA3 and two novel splice donor site mutations (c.357 + 1G > C and c.985 + 1G > C) were identified in the DLG3 gene. One missense mutation (c.1321C > T) was identified in the candidate gene ZMYM3 in three affected males with a previously unrecognized syndrome characterized by unique facial features, aortic stenosis and hypospadia was detected. All of the identified mutations segregated in the corresponding families and were absent in > 100 Finnish controls and in the publicly available databases. In addition, a previously reported benign variant (c.877G > A) in SYP was identified in a large family with nine affected males in three generations, who have a syndromic phenotype.

Conclusions: All of the mutations found in this study are being reported for the first time in Finnish families with several affected male patients whose etiological diagnoses have remained unknown to us, in some families, for more than 30 years. This study illustrates the impact of X-exome sequencing to identify rare gene mutations and the challenges of interpreting the results. Further functional studies are required to confirm the cause of the syndromic phenotypes associated with ZMYM3 and SYP in this study.

Background

Intellectual disability (ID) can be defined as a significant impairment of cognitive and adaptive functions and has an estimated prevalence of 1.5-2% [1]. It is defined by an intelligence quotient (IQ) below 70 and an impairment in social and adaptive skills diagnosed before the age of 18 years [2]. ID can be caused by genetic as well as Anon-genetic factors. A consistent finding among individuals with ID has been the excess of males [1], indicating a role of defects on the X-chromosome. Mutations in X-linked genes contribute for 10%-15% of ID cases in males [3]. There is substantial evidence that X-linked ID (XLID) is genetically an extremely heterogeneous disorder [4].

The recent development of sequencing technologies has provided an effective tool to analyze the chromosomal distribution of mutations underlying ID [5-10]. Sequencing of the coding regions of the X-chromosome in families with multiple affected males has resulted in

* Correspondence: irma.jarvela@helsinki.fi
1Department of Medical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland
Full list of author information is available at the end of the article

© 2014 Philips et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
the identification of about 100 XLID genes to date [11]. Also in sporadic male patients, the possibility of a de novo mutation on the X-chromosome has to be taken into consideration [12].

The aim of this study was to identify the causative gene defects underlying XLID in Finnish families with two or more affected males.

Methods

Patients

Families with two or more affected males with ID were enrolled in this study (n = 14). Many of these patients have been under the care of experienced child neurologists and/or clinical geneticists since 1980. Despite detailed clinical investigations the diagnosis for these families has remained unknown. The Ethical Committee of the Helsinki University Central Hospital approved this study. Written informed consent was obtained from either the parents or the guardians of the patients. DNAs from Finnish blood donors were used as controls.

Exome sequencing

For enrichment of genomic DNA from 11 index patients we used the Agilent SureSelect Human X-Chromosome Exome kit (Agilent, California, USA) and for three index patients (D299, D301, D303) we performed droplet-based multiplex PCR (7,367 amplicons, 757 genes, 1.54 Mb) similarly to the previously described study [13]. DNAs were sequenced on the Illumina GAII and HiSeq2000 platforms, respectively. For all samples coverage of at least 10X was reached for >86% of the targeted regions. Average sequencing depth ranged between 149–583. The sequence files were then aligned against the hg19 reference genome by SOAP2.21 with the default settings. Variants in the form of SNVs, indels and CNVs were called by an in house software package (Medical Re-sequencing Analysis Pipeline (MERAP), developed at the Max Planck Institute for Molecular Genetics, Berlin. All sequence variants were screened against publicly available databases (dbSNP138, 1000 Genomes project, Exome Variant Server (ESP6500) and the in-house database of the Max Planck Institute, Berlin) for annotating likely non-pathogenic and previously reported neutral variants. In addition, the OMIM catalogue and the Human Gene Mutation Database (HGMD) were used as a filter to identify all previously described pathogenic changes.

Mutation analysis and Sanger sequencing

Validation of variants was performed using the standard Sanger sequencing protocol. First, primers (Oligomer Ltd, Helsinki, Finland) were designed to detect each individual mutation using their respective reference sequence. All PCR reactions were performed in 25 µl volume using Dynazyme polymerase (Finnzymes, Espoo, Finland) under standard conditions. PCR products were purified using Exo-SapIT (Affymetrix, Santa Clara, CA) according to the standard protocol. Sequencing reactions were performed using Applied Biosystems BigDye terminator v3.1 (Life Technologies, Carlsbad, CA) and products were analyzed on the ABI 3730 sequencer. Finally, the accurate genotype of each variant was confirmed by sequence analysis with the Sequencher software v4.8 (Life Technologies) in both the family members and the anonymous Finnish controls. Primer sequences are available on request.

X-inactivation analysis

X-inactivation study was performed in the families with symptomatic carrier females. 500 ng of DNA was divided into two fractions; one set was digested in a 20 µl volume of buffer solution with HpaII (New England Biolabs, UK) while the other set was digested with RsaI (Roche, Basel, Switzerland). Both aliquots were incubated at 37°C for 17 hours. Next, exon 1 of the androgen receptor gene (AR) was amplified from 2 µl of each of the HpaII and RsaI digested DNA samples using the forward 5’-labelled primer TCCAGAATCTGTTCCAGAGCGTGC and CTG GGAGCGAACCTCCTCTCTC reverse primer [14]. The amplification was performed for 35 cycles with an annealing temperature of 63.3°C. DNAs from healthy males were used as controls. Two µl of the PCR product was mixed with 10 µl of Hi-Di formamide (Life Technologies) and 0.025 µl of GeneScan 500 Liz size marker (Life Technologies). The samples were run on an ABI3730xl genetic analyzer. The peaks were analyzed using the GeneMarker software version1.4 (Life Technologies).

In silico analysis

Multiple programs, including SIFT, PolyPhen-2, PhD-SNP, PANTHER and SNPs&GO were used to predict the functional impact of the mutation on the protein function [15-19]. Multiple protein sequence alignment using orthologous species and human sequences was performed using ClustalX to verify the evolutionary conservation of the respective amino acid positions [20]. The HOPE server was used to predict the structural effects of the mutation [21]. In addition, we used the ConSeq server to analyze the evolutionary conservation of the structures of proteins [22].

Results

Computational analysis of the sequencing data revealed a total of 1,032 variants in coding and non-coding exons on chromosome X, or potentially affecting splice sites. Out of these, 405 protein changing mutations were either present in publicly available databases or were recurrent in our in-house dataset. The final set of 25 novel variants including those that were reported as heterozygous in
publicly available databases, the recurrent pathogenic SLC16A2 mutation present in HGMD and the recurrent variants investigated for segregation in this study are listed in the Additional file 1: Table S1. In four families, D299, D174, D172 and D301 a novel mutation in an X-chromosomal gene was found to co-segregate with the clinical phenotype. In one family L107, a recurrent pathogenic mutation in an X chromosomal gene was identified. In one family D222, a novel missense mutation in a candidate XLID gene was identified. The clinical phenotypes and the identified mutations are described below. In the remaining eight families no functionally relevant candidate genes were found.

Clinical description of the patients and X-exome sequencing results

**Family L107**

Family L107 has three affected males (II-1, II-2 and II-9, Figure 1a), who presented with moderate to severe ID. Patients II-1 born in 1950 and II-2 born in 1954 never walked. They have spastic diplegia in the lower limbs, athetosis in the upper limbs, alalia and dysarthria. In contrast, the index patient (II-9) born in 1966 who is the half-brother could walk but lost his motor skills during early childhood. Patients II-2 and II-9 could speak a few words. No data was available from patient II-1 concerning speech. Clinical features are summarized in Table 1.

---

**Figure 1** Overview of the mutations reported in SLC16A2 gene in two Finnish families with Allan Herndon Dudley Syndrome.  

a) Family pedigree of L107 showing the inheritance of SLC16A2 mutation, open circles denote females; circles with a dot in the middle denote obligate carrier females, empty square denote males, the left half of the black squares denote affected males, the right half of the squares denote mutation positive males, crossed symbols denote deceased individuals, wt denote mutation negative subject,  
b) Sanger sequencing confirming the missense mutation c.1111C > T,  
c) Multiple species protein sequence alignment showing conservation of the mutated R371 residue,  
d) Family pedigree of D299 showing the inheritance of SLC16A2 mutation,  
e) Sanger sequencing confirming the frameshift insertion c.990_991insGCTGC.
Table 1 Overview of the clinical features of the previous and present families with Allan-Herndon-Dudley syndrome

| Clinical finding          | Clinical findings reported previously | D299                          | L107                          |
|--------------------------|--------------------------------------|--------------------------------|--------------------------------|
| Short stature            | +                                    | +                              | -                             |
| Scoliosis                | +                                    | +                              | +                             |
| Low weight               | +                                    | +                              | +                             |
| Microcephaly             | +                                    | +                              | -                             |
| Muscle hypoplasia        | +                                    | +                              | NA                            |
| Hypotonia                | +                                    | +                              | NA                            |
| Contractures             | +                                    | +                              | NA                            |
| Dystonic movements       | +                                    | +                              | +                             |
| Athetosis                | +                                    | +                              | +                             |
| Intellectual disability  | +                                    | +                              | /S                            |
| Absent speech            | +                                    | +                              | +                             |
| Dysarthria/Limited speech| +                                    | +                              | +                             |
| Seizures                 | +                                    | +                              | NA                            |
| Pectus excavatum         | +                                    | +                              | NA                            |
| Narrow long face         | +                                    | -                              | +                             |
| Round face               | +                                    | +                              | -                             |
| Valgus                   | +                                    | +                              | NA                            |
| Hyperreflexia            | +                                    | +                              | NA                            |
| Simple ears              | +                                    | +                              | -                             |
| Cupped ears              | +                                    | +                              | -                             |
| Spastic quadriplegia     | +                                    | +                              | -                             |

MO—Moderate, S—Severe, NA—No data available, +—clinical feature present, +/—clinical feature absent.

Metabolic analyses, EEG, EMG and imaging were normal. The thyroid hormone levels for TSH and T4 from the index patient II-9 were within the normal range. Data about the thyroid hormone level for T3 was unavailable. The thyroid hormone profiles of the index patient III-3 was T3-9 (Normal range: 2.6-5.7 pmol/L), T4-8.6 (Normal range: 9–19 pmol/L), TSH-2.16 (Normal range: 0.35-5 mU/L) and TGB-870 (Normal range: 233–490 nM/L). The patients have obtained thyroxin medication at the age of 2–3 years without any improvement.

Family D299

The index patient (III-3) is 16 years old and his two half-brothers (III-1, III-2) deceased at the ages of 21 years and 16 years respectively (Figure 1d). Clinical features are summarized in Table 1.

The three affected sons of family D299 had similar clinical features, characterized by severe psychomotor retardation, absent speech, seizures, hypotonia and contractures. The thyroid hormone profile of the index patient III-3 was T3-9 (Normal range: 2.6-5.7 pmol/L), T4-8.6 (Normal range: 9–19 pmol/L), TSH-2.16 (Normal range: 0.35-5 mU/L) and TGB-870 (Normal range: 233–490 nM/L). The patients have obtained thyroxin medication at the age of 2–3 years without any improvement.

X-exome sequencing of the index patient III-3 revealed two novel variants, one in SLC16A2 and the other in ODFZ1 (Additional file 1: Table S1). The 5 base-pair insertion identified in exon 3 of SLC16A2 leads to a frameshift and a premature stop codon [NM_006517.4:c.990_991insGCTGC; p.G334PfsX11] and segregated with the clinical phenotype. The mother (II-2) carries the mutation on one of her X-chromosomes. The mother’s sister (II-5) was also tested and she was found not to be a carrier. This novel mutation in the SLC16A2 gene was absent in 100 Finnish controls.

Family D174

Family D174 has three affected males (III-7, III-8 and II-5, Figure 2a). The patients aged 36, 35 and 57 years respectively, are characterized by severe ID with autistic features, epilepsy, short stature and behavioral problems such as self injury and aggressive outbursts (Table 2).

X-exome sequencing of the index patient III-8 revealed a novel missense mutation [NM_000828.4:c.1888G > C; p.Gly630Arg] in exon 12 of the GRIA3 gene that segregated in the family (Additional file 1: Table S1). The mutation was absent in 135 Finnish controls. Using PolyPhen-2, SIFT, PhD-SNP, SNPS&GO and PANTHER, the mutation was predicted to be probably damaging. The ConSeq server also revealed that this residue is highly conserved with a score of 9. Based on HOPE [21], the altered amino acid has a change in charge and can disturb the ionic interactions with the other transmembrane helices. The mutant cysteine residue is more hydrophobic than the wild type arginine residue, possibly affecting the hydrophobic interactions within the core of the protein or with the membrane lipids and, as a result, disturbing the correct folding of the protein. The mutation was absent in 100 Finnish controls.

Family D174

Family D174 has three affected males (III-7, III-8 and II-5, Figure 2a). The patients aged 36, 35 and 57 years respectively, are characterized by severe ID with autistic features, epilepsy, short stature and behavioral problems such as self injury and aggressive outbursts (Table 2).

X-exome sequencing of the index patient III-8 revealed a novel missense mutation [NM_000828.4:c.1888G > C; p.Gly630Arg] in exon 12 of the GRIA3 gene that segregated in the family (Additional file 1: Table S1). The mutation was absent in 135 Finnish controls. Using PolyPhen-2, SIFT, PhD-SNP, SNPS&GO and PANTHER, the mutation was predicted to be probably damaging. The ConSeq server also revealed that this residue is highly conserved with a score of 9. Based on HOPE [21], the altered amino acid has a change in charge and can disturb the ionic interactions with the other transmembrane helices. The mutant cysteine residue is more hydrophobic than the wild type arginine residue, possibly affecting the hydrophobic interactions within the core of the protein or with the membrane lipids and, as a result, disturbing the correct folding of the protein. The mutation was absent in 100 Finnish controls.

Family D174

Family D174 has three affected males (III-7, III-8 and II-5, Figure 2a). The patients aged 36, 35 and 57 years respectively, are characterized by severe ID with autistic features, epilepsy, short stature and behavioral problems such as self injury and aggressive outbursts (Table 2).

X-exome sequencing of the index patient III-8 revealed a novel missense mutation [NM_000828.4:c.1888G > C; p.Gly630Arg] in exon 12 of the GRIA3 gene that segregated in the family (Additional file 1: Table S1). The mutation was absent in 135 Finnish controls. Using PolyPhen-2, SIFT, PhD-SNP, SNPS&GO and PANTHER, the mutation was predicted to be probably damaging. The ConSeq server also revealed that this residue is highly conserved with a score of 9. Based on HOPE [21], the altered amino acid has a change in charge and can disturb the ionic interactions with the other transmembrane helices. The mutant cysteine residue is more hydrophobic than the wild type arginine residue, possibly affecting the hydrophobic interactions within the core of the protein or with the membrane lipids and, as a result, disturbing the correct folding of the protein. The mutation was absent in 100 Finnish controls.
common single nucleotide variations as they were present in 3/88 Finnish control samples.

The wild type residue glycine of the p.Gly630Arg is neutral and the mutant residue arginine is positively charged. HOPE [21] predicted that this can lead to disturbances in the ionic interactions with the other transmembrane helices. The mutant residue is larger than the wild type residue, which can disturb either the contacts with the other transmembrane domains or with the lipid membrane. This can also affect the hydrophobic interactions within the core of the protein or with the membrane lipids. The wild type residue, glycine, is considered to be the most flexible of all residues and this flexibility might be vital for this protein’s function. It is predicted that this glycine residue at this position is needed to make a special backbone conformation or to facilitate movement of the protein.

Family D172

This family has three affected males in two generations (III-5, III-8 and IV-3, Figure 3a). X-exome sequencing of the index patient (III-8) revealed two novel variants, one in *DLG3* and the other in *ZBTB33* (Additional file 1: Table S1). The *ZBTB33* variant was excluded because it did not co-segregate with the phenotype. A donor splice site mutation [NM_021120.3:c.357 + 1G > C] in intron 1 of the *DLG3* gene segregates in the family. The affected males who presented with mild to moderate ID, normal growth, narrow thorax, molar hypoplasia, short up-sloping palpebral fissures, a high vaulted palate, and hypotonia. Daytime wetting has been a problem until adult age (Table 3). In addition to the aforementioned features the index patient (III-8) had an external strabismus in his right eye. Chromosomes (300 bands), FRAXA, vacuolated lymphocytes, urinary metabolic screen, ophthalmology and hearing examinations showed normal
| Type of change | Gece et al. [1999] [31] Female patient | Wu et al. [2007] [34] Male patients | Bonnet et al. [2009] [36] Male/male patient | Chiyonobu et al. [2007] [35] Male patient | D174 Male patient (III-7) | D174 Male patient (III-8) | D174 Male patient (II-5) |
|---------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|----------------|----------------|----------------|
| n = 1         | n = 3                                 | n = 4                                 | n = 2                                 | n = 2                                 |                |                |                |
| Feature       |                                      |                                      |                                      |                                      |                |                |                |
| Intellectual disability | +                                   | +                                    | +                                    | +                                    | +              | +              | +              |
| Mild          | -                                    | -                                    | -                                    | -                                    | -              | -              | -              |
| Moderate      | +                                    | +                                    | +                                    | +                                    | -              | -              | -              |
| Severe        | -                                    | -                                    | -                                    | -                                    | -              | -              | -              |
| Autistic features | ND                                  | ND                                   | ND                                   | ND                                   | +/ND           | +              | -              |
| Behaviour troubles | +                                   | ND                                   | +                                    | ND                                   | +              | +              | +              |
| Self injury   | ND                                   | ND                                   | ND                                   | ND                                   | +              | +              | +              |
| Aggressive outbursts | ND                                  | ND                                   | ND                                   | ND                                   | -              | -              | -              |
| Dysmorphic features | ND                                  | ND                                   | ND                                   | ND                                   | ND             | +              | +              |
| Brachycephaly | ND                                   | ND                                   | ND                                   | ND                                   | ND             | +              | +              |
| Macrocephaly  | ND                                   | +                                    | ND                                   | ND                                   | ND             | -              | -              |
| Deep set eyes | ND                                   | ND                                   | ND                                   | ND                                   | ND             | +              | +              |
| Aesthetic habitus | ND                                  | ND                                   | ND                                   | ND                                   | ND             | -              | -              |
| Myclonic jerks | ND                                   | ND                                   | ND                                   | ND                                   | ND             | ND             | ND             |
| Hyporeflexia  | ND                                   | +                                    | ND                                   | ND                                   | ND             | ND             | ND             |
| Prominent supraorbital ridges | ND                                  | ND                                   | ND                                   | ND                                   | +              | +              | +              |
| Short stature | ND                                   | ND                                   | ND                                   | ND                                   | ND             | +              | +              |
| Epilepsy      | +                                    | ND                                   | ND                                   | ND                                   | ND             | ND             | ND             |
| Inguinal hernia | -                                   | ND                                   | ND                                   | ND                                   | ND             | +              | +              |
| Brain MRI abnormalities | ND                                  | ND                                   | ND                                   | ND                                   | ND             | ND             | ND             |
| Bowel occlusions | ND                                  | ND                                   | ND                                   | ND                                   | ND             | ND             | ND             |
| Malposition of feet | ND                                | ND                                   | ND                                   | ND                                   | ND             | ND             | ND             |
| Hydronephrosis | ND                                   | ND                                   | ND                                   | ND                                   | ND             | ND             | ND             |
| Ren arcuatus  | ND                                   | ND                                   | ND                                   | ND                                   | ND             | ND             | ND             |

ND—No data, +—clinical trait present, -—clinical trait absent.
Figure 3 Overview of the two splice donor mutations identified in DLG3 in two Finnish families. a) Pedigree of family D172 showing the inheritance of the DLG3 splice donor mutation c.357 + 1G > C, open circles denote females; circles with a dot in the middle denote obligate carrier females, empty square show males, the left half of the black squares denote affected males, the right half of the squares denote mutation positive males, crossed symbols denote deceased individuals, wt denote mutation negative subject, b) Sanger sequencing result showing the mutation in one affected male, c) Pedigree of family D301 showing the inheritance of the DLG3 splice donor mutation c.985 + 1G > C, d) Sanger sequencing result showing the mutation in one affected male, e) Schematic representation of the DLG3 gene, along with overview of previously reported mutations and mutations reported in the current study.

Table 3 Comparison of the clinical features of the patients of two families with DLG3 mutation

| Clinical features | Family D172 | Family D301 |
|------------------|-------------|-------------|
|                  | III-5       | III-8       | IV-3       | III-5 | III-9 | IV-3 | V-1 | V-2 |
| Male             | +/MO        | +/M         | +/MO       | +/S   | +/S   | +/MO | +/MO| +/MO|
| Male             | +           | ND          | +          | ND    | +     | +    | +   | +   |
| Male             | +           | ND          | +          | +     | +     | +    | +   | +   |
| Male             | +           | -           | +          | -     | +     | -    | -   | -   |
| Male             | -           | -           | +          | -     | +     | +    | -   | -   |
| Male             | -           | -           | ND         | ND    | +     | +    | +   | +   |
| Male             | -           | -           | +          | -     | -     | -    | -   | -   |
| Male             | -           | +           | +          | -     | +     | +    | -   | -   |
| Male             | -           | +           | +          | -     | +     | +    | -   | -   |

ND—No data, M—Mild, MO—Moderate, S—Severe, +—clinical feature present, -—clinical feature absent.
results. Females II-2, III-2 and III-12 are heterozygous carriers of this mutation. Female III-2 has passed regular basic education and completed vocational schooling. Her younger son (IV-5) has epilepsy but is of normal intelligence and does not have the DLG3 mutation. Female II-2 is 77 years old, who is also a mutation carrier and has no cognitive or memory problems. All females showed a normal X-inactivation pattern in their blood lymphocytes (data not shown).

Family D301
Family D301 has five affected males in three generations (III-5, III-9, IV-3, V-1 and V-2, Figure 3c). In the index patient, V-1, etiological investigations such as urine metabolic screening, karyotype analysis, Fragile-X testing, FISH on 22q11.2 locus, creatinine kinase, lactate, carnitine, very long fatty acids, EEG and brain MRI were normal.

The phenotype consists of delayed motor and language development. Three males had strabismus and attention deficit hyperactivity disorder (ADHD). The males do not have other dysmorphic features except a bifid uvula in patients V-1 and V-2. Only one patient had experienced seizures in childhood. The cognitive performance in the affected males varied from severe to moderate ID, with ID being less severe in the younger generations (Table 3). Female III-6 participated in the cognitive evaluation at the age of 60 years at the level of moderate ID; she had not been evaluated earlier. She had received her basic and vocational education in a special school. She has not been able to work but has lived independently and needs assistance in all paperwork and contacts with authorities. The mother (IV-2) of patients V-1 and V-2 and their grandmother (III-2) graduated from regular schools and completed vocational schooling.

X-exome sequencing of the index patient (V-1) identified a novel donor splice site mutation [NM_021120.3:c.985+1G > C] in intron 6 of the DLG3 gene (Additional file 1: Table S1). One affected carrier female (III-6) had a skewed X-inactivation pattern of 80:20 (Additional file 2: Figure S1).

Family D222
Family D222 has three affected males who are half-brothers (III-1, III-2 and III-3, Figure 4a). The phenotype consists of small gestational age (SGA), hypospadias, mild aortic stenosis and leakage of the aortic valve, arcus aortae dexter, horseshoe kidney, slenderness and cup formed ear lobes. Their motor and language development were slightly delayed and they continue to have nocturnal enuresis up to school age. Two of them have ADHD. The cognitive performance is at the level of mild ID. The development of the youngest brother (III-4) is normal (Table 4).

X-exome sequencing of the index patient (III-1) identified novel variants in PTCHD1, RAB40A, ABCD1, COL4A5 and ZMYM3, respectively (Additional file 1: Table S1). PTCHD1 and RAB40A were excluded as a candidate gene because their variants did not segregate in the family (Additional file 1: Table S1). We suggest that the variant in COL4A5 is unlikely pathogenic because mutations in this gene have been previously associated with Alport syndrome [OMIM#301050].

The variant in ABCD1 was excluded as a causative mutation as mutations in ABCD1 previously have been linked to adrenoleukodystrophy [OMIM#300100]. The novel missense mutation [NM_201599.2:c.1321C > T; p.Arg441Trp] in exon 7 of the ZMYM3 gene was present in all three affected males and absent in their healthy brother. The mother (II-2) was found to be a carrier of this mutation. This mutation leads to substitution of the amino acid arginine with tryptophan (p.Arg441Trp) and was absent in 100 Finnish anonymous blood donors.

PolyPhen-2, SIFT, PhD-SNP, SNPS&GO and PANTHER predicted this mutation to be damaging. The ConSeq server also revealed that this residue is highly conserved with a score of 9. HOPE [21] predicted that the mutant residue is larger and more hydrophobic than the wild type residue. The amino acid substitution will lead to loss of hydrogen bonds in the core of the protein, thus preventing correct folding. The difference in the amino acid properties is likely to disturb a Zinc-finger domain known to bind DNA.

Family D175
This family has 9 affected males in three generations (II-1, II-13, III-2, III-5, III-11, III-13, III-14, IV-1 and IV-2, Figure 5a). The affected males are characterized by mild to moderate ID, normal growth, and dysmorphic facial features with prominent supraorbital ridges, deep set eyes, short philtrum, and prominent chin suggesting a novel syndrome (Figure 5b). One patient has epilepsy (III-5). Patients III-5, IV-1 and IV-2 have mild brain abnormalities in MRI (corpus callosum hypoplasia, mild cortical atrophy). Only limited clinical data is available for patients III-11, III-13 and III-14.

X-exome sequencing of the index patient (IV-1) identified a recurrent missense variant in exon 6 of the SYP gene [NM_003179.2:c.877G > A; p.Gly293Ser]. This missense variant was found in all the affected males. Variants in DMD, FAM47A, USP9X variants were not found in all of the affected males (Additional file 1: Table S1). Variant in FAM47B was not analyzed.

Discussion
In six families we identified five pathogenic mutations in three known XLID genes (SLC16A2, GRIA3 and DLG3).
and one in the candidate XLID gene ZMYM3. In addition, two novel syndromes were identified, one with a novel missense mutation in a candidate XLID gene, ZMYM3 [23] and the other with a previously reported benign variant in SYP in a large family (D175).

Table 4 Summary of clinical findings of patients with the ZMYM3 mutation

| Clinical trait                  | Patient III-1 | Patient III-2 | Patient III-3 |
|--------------------------------|---------------|---------------|---------------|
| Age (y)                        | 15.5          | 8.8           | 7.3           |
| Weight at birth (g)            | 2530          | 2460          | 3590          |
| Head circumference (cm)        | 55.5, −1 SD   | 49.6, −3 SD   | 49.5, −2.7 SD |
| Hypospadia                     | +             | +             | -             |
| Horseshoe kidney               | -             | +             | -             |
| Enuresis nocturna              | +             | +             | rarely        |
| Large cupped formed ear lobes  | +             | +             | +             |
| ADHD                           | +             | +             | ND            |
| Arcus aortae dexter            | -             | +             | -             |
| Bicuspid aortic valve          | +             | +             | +             |
| Sleep disorder                 | +             | +             | +             |
| Intellectual disability        | +/M           | +/M           | +/M           |

M—Moderate, +—clinical trait present, −—clinical trait absent, SD—standard deviation.

Figure 4 Clinical presentation of a novel syndrome in a Finnish family with XLID and segregation of the ZMYM3 missense mutation.

a) Pedigree showing the inheritance of the ZMYM3 mutation in family D222, open circles show females, circles with a dot in the middle show obligate carrier females, empty square show males, the left half of the black squares show affected males, the right half of the squares show mutation positive males, crossed symbols denote deceased individuals, wt denote mutation negative subject. b) Photographs of the three affected males, c) Sanger sequencing confirming the mutation, d) Multiple species protein alignment showing conservation of the mutated R441 residue in ZMYM3.

We identified two pathogenic mutations in SLC16A2. Mutations in this gene are known to cause Allan-Herndon-Dudley syndrome (AHDS; OMIM #300523). AHDS is typically characterized by developmental delay, poor head control, poor or no speech and muscle hypoplasia [24]. The clinical features of the patients investigated in this study are characteristic of AHDS. Previously, mutations in the SLC16A2 gene have been reported in more than 45 families across the world and one de novo translocation t(X;9) (q13.2;p24) has been reported in a female patient [24-29]. SLC16A2 belongs to the SLC16 gene family [30] and encodes the monocarboxylate transporter 8 (MCT8) protein, which is expressed in brain, liver, heart, intestine, placenta, kidney and thyroid. In the central nervous system MCT8 is present in neurons and astrocytes and is an essential thyroid hormone transporter involved in the transport of thyroid hormone (TH) across the blood–brain barrier. The SLC16A2 gene consists of six exons and encodes a protein of 539 amino acids [24]. MCT8 contains twelve transmembrane domains (TMD) with intracellular amino- and carboxy-terminal domains [24]. In vitro studies showed that mutations in this gene lead to a reduced or absent supply of triiodothyronine (T3) to neurons [30]. The novel frameshift mutation p.G334PfsX11 identified in family D299 is located in the seventh TMD and the recurrent p.Arg371Cys
amino acid substitution present in family L107 lies in the eighth TMD. The same missense mutation has previously been identified in a sporadic case (annotated as c.1333C > T, p.Arg445Cys using a different SLC16A2 isoform [29]. It has been previously shown that in a moderately affected individual with c.1333C > T missense mutation resulted in a more severe decrease in T3 uptake [29]. In the article by Capri et al. [29], the affected patient, aged 13 years, is able to walk with aid and can speak a few words. In family L107 the phenotype is variable as the two oldest patients (II-1 and II-2) never walked whereas the youngest II-9 could walk until early childhood. The patients in family L107 could speak some words. Based on the available data p.Arg445Cys mutation seems to be associated with variable phenotypes. To our knowledge, these are the first families with AHDS in the Finnish population.

We identified a missense mutation c.1888G > C (p.Gly630Arg) (RefSeq NM_000828.4) in the glutamate receptor, ionotropic, AMPA 3 (GRIA3) gene. GRIA3 belongs to a class of an alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-sensitive glutamate receptor that operates as a ligand-gated ion channel in the central nervous system and has an essential role in excitatory synaptic transmission [31]. These receptors contain three functional domains – transmembrane, ligand binding, and receptor channel core [32]. Ionotropic glutamate receptors (iGluRs) are related to learning and memory [33]. In vitro functional studies on GRIA3 missense variants have shown that mutations in functional domains of GRIA3 are associated with kinetic changes in AMPA receptor function leading to significant reduction in iGluR3 channel function which is found to be linked with moderate ID [34,35]. Previously, four missense mutations, one whole gene deletion and three duplications have been reported in GRIA3 [34-38]. One of the missense mutations (p.Arg631Ser) [34] affected the amino acid residue adjacent to the Gly630 mutated in the family described here. The patients of these families have facial dysmorphic features in common and these are different from the other reported patients with a mutation in GRIA3. The affected individuals of family D174 exhibit a severe phenotype. They are all severely intellectually disabled with behavioral disturbances. Three of the four previously reported patients with a GRIA3 mutation were moderately intellectually disabled, whereas one patient with a missense

Figure 5 A clinical presentation of a novel syndrome in a Finnish family (D175) with XLID. a) Family pedigree showing the inheritance of the benign variant in SYP, open circles denote females, circles with a dot in the middle denote obligate carrier females, empty square denote males, the left half of the black squares denote affected males, the right half of the squares denote mutation positive males, crossed symbols denote deceased individuals, wt denote mutation negative subject. b) Photographs of the six affected males. c) Sanger sequencing confirming the benign variant. d) Multiple species protein alignment showing conservation of the mutated Gly293 residue in SYP. e) Schematic presentation of the SYP protein domains, location of the published mutations and the polymorphism identified in this study.
change (p.Met706Thr) had mild ID [34]. We conclude that there is wide variation in the severity of ID among patients with mutations in the GRIA3 gene. Further phenotype studies are needed to confirm the syndromic features underlying GRIA3 mutations.

The DLG3 gene, located at Xq13.1, encodes the synapse-associated protein SAP102 [39]. SAP102 is a member of the membrane-associated guanylate kinase (MAGUK) protein family [39,40]. SAP102 is highly expressed in both young and mature neurons and localizes to the postsynaptic density of excitatory synapses [41]. It is the first XLID gene to be associated to glutamate receptor-mediated postsynaptic signaling, a process which is crucial for the regulation of synaptic formation and plasticity in brain development [40]. Mutated DLG3 has been identified as a rare cause of XLID with, so far, only five families diagnosed, with a total of 17 affected males presenting with moderate to severe ID [41,42]. Of eleven mutation carrier women, one has mild ID and in another family a female carrier has mild ID and a history of seizures. These expressing females, however, do not have a skewed X-inactivation pattern [41,42]. Our families expand the phenotype spectrum of DLG3 mutations to mild ID.

The missense mutation c.1321C>T (p.Arg441Trp) (RefSeq NM_201599.2) is the first variant in the coding region of ZMYM3 reported to date. Previously, a chromosomal breakpoint in the 5’ UTR of ZMYM3 has been reported in an intellectually disabled female. In addition to ID, the patient had scoliosis and spotty hyperpigmentation of the skin. She also had slight facial asymmetry and clinodactyly [23]. However, the patients described in this study do not have any of the reported clinical features except for mild ID. Recently, a missense variant of unknown significance (c.356A>G (p.Gln119Arg)) in ZMYM3 was reported in the index patient of a family with XLID with four affected males in two generations. However, the patient also carries a missense change in the established XLID gene HCF1 [43].

The ZMYM3 gene is also referred to as ZNF261 and DXS6673E. ZMYM3 belongs to the MYM family. This gene contains 5 tandem repeats of a Cys-X2-Cys-X19-missense change in the established XLID gene males in two generations. However, the patient also carries a

Web resources

The URLs for data presented are as follows:
- dbSNP, http://www.ncbi.nlm.nih.gov/SNP/
- ConSeq, http://conseq.tau.ac.il/
- PolyPhen-2, genetics.bwh.harvard.edu/pph2/
- SIFT, sift.jcvi.org
- Phd-SNP, http://snps.biofold.org/phd-snp/phd-snp.html
- RefSeq, http://www.ncbi.nlm.nih.gov/refseq/
- SNPs &GO, http://snps.biofold.org/snps-and-go/snps-and-go.html
- PANTHER, http://www.pantherdb.org/tools/csnpScore-Form.jsp?
- HOPE, http://www.cmbi.ru.nl/hope/input
- MERAP, http://www.sourceforge.net/projects/merap/files/
- MERAP20131101/
- OMIM, http://www.ncbi.nlm.nih.gov/omim/
- NHLBI Exome Sequencing Project (ESP)
- Exome Variant Server, http://evs.gs.washington.edu/EVS/
Acknowledgements

We thank all the patients and their families for their active participation and cooperation. The work was funded by the Sigrid Jusélius Foundation, Finnish Medical Association and by the Project GENCODYS (241995), which is funded by the European Union Framework Program 7 (FP7). Nathalie Fiemaners and Melanie Bieneck are acknowledged for their excellent technical assistance.

Authors details

1Department of Medical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland. 2Outpatient Clinic for Persons with Intellectual Disabilities, Tampere University Hospital, Tampere, Finland. 3Norio Centre, Department of Medical Genetics, Helsinki, Finland. 4Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland. 5Child Neurology, Päijät-Häme Central Hospital, Lahti, Finland. 6National Institute for Health and Welfare, Helsinki, Finland. 7Center for Human Genetics, University Hospital Leuven, Leuven, Belgium. 8Human Genome Laboratory, Department of Human Genetics, VIB Center for the Biology of Disease, KU Leuven, Leuven, Belgium. 9Department of Computational Molecular Biology, Max Planck Institute for Molecular Genetics, Berlin, Germany. 10Department of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany.

Received: 30 December 2013 Accepted: 31 March 2014 Published: 11 April 2014

References

1. Leonard H, Wen X: The epidemiology of mental retardation: challenges and opportunities in the new millennium. Ment Retard Dev Disabil Res Rev 2002, 8:117–134.
2. Schalok RL, Bornthick-Duffy SA, Bradley VI, Buntrine WHE, Couter DL, Craig EM, Gomezc SC, Reeve A, Shogren KA, Snell ME, Sprent SA, Tasse MJ, Thompson JR, Verducho-Alonso MA, Yeager MH: AAIDD’s 11th edition of Intellectual Disability: Definition, Classification, and Systems of Support. 2012.
3. de Brouwer AP, Yntema HG and Graaff MJ: Analysis of family L107. FD and MAH were involved in the mutation analysis study of the family D174 and family D172. SAH, HU and VMK performed X-exome sequencing and bioinformatic analysis.
4. Bassani S, Zapata J, Gerosa L, Moretto E, Murru L, Passafaro M: The neurobiology of X-linked intellectual disability. Neuroscientist 2013, 19:541–552.
5. Willemsen M, Kleefstra T: Making headway with genetic diagnostics of intellectual disabilities. Clin Genet 2013, 85:101–110.
6. Ellison JW, Rosenfeld JA, Shaffer LG: Genetics basis of intellectual disability. Annu Rev Med 2013, 64:441-450.
7. Schuurs-Hoeijmakers JH, Schuurs-Hoeijmakers JH, Vulo-van Silfhout AT, Visser LE, van de Vondervoort IV, van Bon BW, de Ligt J, Gillis CN, Hehir-Kwa JY, Neveling K, Del Rosario M, Hira G, Reitan S, Veltto A, Failla P, Greco D, Fichera M, Galei O, Kleefstra T, Grealy ML, Ockeloen CW, Willemsen MH, Bongers M, Jansen M, Pfundt R, Vetelmann JA, Romanos C, Willemsen MA, van Bokhoven H, Brunner HG, et al: Identification of pathogenic gene variants in small families with intellectually disabled siblings by exome sequencing. J Med Genet 2013, 50:802–811.
8. Najmabadi H, Hu H, Gharabichi M, Zenegot J, Abdeini SS, Chen W, Hosseinim R, Behfati F, Haas E, Jamali P, Zech A, Mohneni S, Puttmann L, Vahid LN, Jensen C, Mohede IA, Bieneck M, Larti F, Mueller I, Weissmann R, Danisch H, Wrogemann K, Hadiavi V, Lipkowitz B, Esmaeili-Neih S, Wieczoreck D, Karminegad R, Firouzabadi SG, Cohen M, Fathah Z, et al: Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature 2011, 478:567–63.
9. de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulo-van Silfhout AT, Koolen DA, de Vries P, Gielis CN, del Rosario M, Hoschein A, Schefee H, de Vries BB, Brunner HG, Vetelmann JA, Visser LE: Diagnostic exome sequencing in persons with severe intellectual disability. N Engl J Med 2012, 367:1921–1929.
10. Rauch A, Wieczoreck D, Graef E, Wieland T, Endele S, Schwarzmayr T, Albrecht B, Bartholdi D, Beygo J, Di Donatno N, Dukfe A, Cremer K, Hempel M, Horn D, Hoyer J, Joset P, Röpke A, Moog U, Riss A, Thiel CT, Tschacho A, Wiesener A, Wohlieber E, Zweier C, Elki AB, Zink AM, Rump A, Meisnger C, Grallert H, Stich T, et al: Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. Lancet 2012, 380:1674–1682.
11. Tarpey PS, Smith R, Pikasance E, Whibley A, Edkins S, Hardy C, O’Meara S, Latimer C, Dicks E, Menzies A, Stephens P, Blow M, Greenman C, Xue Y, Tyler-Smith C, Thompson D, Gray K, Andrews J, Barthorpe S, Buck G, Cole J, Dunmore R, Jones D, Maddison M, Mironenko T, Turner R, Turrell K, Varian J, West S, Widsa S, et al: A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. Nat Genet 2009, 41:535–543.
12. Visser LE, de Ligt J, Gillis CN, Jansen L, Steehouwer M, de Vries P, van Lier B, Arts P, Wiekamp N, del Rosario M, van Bon BW, Hoschein A, de Vries BB, Brunner HG, Vetelmann JA: A de novo paradigm for mental retardation. Nat Genet 2010, 42:1109–1112.
13. Hu H, Wrogemann K, Kalscheuer V, Tschacho A, Richard H, Haas SA, Menzel C, Bieneck M, Froyen G, Raynoud M, Van Bokhoven H, Chelly J, Ropers H, Chen W: Mutation screening in 86 known X-linked mental retardation genes by droplet-biomaniplex PCR and massive parallel sequencing. Hugo J 2009, 3:41–49.
14. Allen RC, Zoghbi HY, Matterey AB, Belmouk M, Shaffer LV: A de novo paradigm for mental retardation. Nat Genet 2009, 41:1229–1239.
15. Ng PC, Henikoff S: SIFT: Predicting amino acid changes that affect protein function. Nucl Acids Res 2003, 31:3812–3814.
16. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR: A method and server for predicting damaging missense mutations. Nat Methods 2010, 7:248–249.
17. Calabrese R, Capriotti E, Farisseli P, Martelli PL, Casadio R: Functional annotations improve the predictive score of human disease-related mutations in proteins. Hum Mol Genet 2009, 18:1237–1244.
18. Capriotti E, Calabrese R, Casadio R: Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. Bioinformatics 2002, 22:2372–2374.
19. Chandonia JM, Brame AJ, Thomas PD: Large scale gene function analysis with the PANTHER classification system. Nat Protocols 2013, 8:1551–1556.
20. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG: The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 2002, 34:4687–4698.
21. Venselaar H, Te Beek TA, Kuipers RK, Hekkelman ML, Vriend G: Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. BMC Bioinformatics 2010, 11:548.
22. Berezn C, Glaser F, Rosenberg J, Paz I, Pupko T, Farisseli P, Casadio R, Ben-Tal N: ConSeq: The Identification of Functionally and Structurally Important Residues in Protein Sequences. Bioinformatics 2004, 20:1322–1324.
Unraveling the modular design of glutamate-gated ion channels.

23. van der Maarel SM, Scholten IH, Huber I, Philippe C, Suijkerbuijk RF, Gilgenkrantz S, Kere J, Cremers FP, Rogers RH: Cloning and characterization of DXS6673E, a candidate gene for X-linked mental retardation in Xq13.1. Hum Mol Genet 1996; 5:887–893.

24. Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S: A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. Am J Hum Genet 2004, 74:168–175.

25. Friese EM, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Friesema EC, Capri Y, Kersseboom S, Touraine R, Monnier A, Eymard-Pierre E, Des Pothier E, Echeverri R, Lubs HA, Voeller K, Simensen RJ, Stevenson RE: Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. Am J Hum Genet 2008, 83:1029–1037.

26. Schwartz CE, May MM, Carpenter NJ, Rogers RC, Martin J, Bialer MG, Ward J, Namba N, Etani Y, Kitaoka T, Nakamoto Y, Nakacho M, Bessho K, Miyoshi Y, Frints SG, Lenzner S, Bauters M, Jensen LR, Frints SG, Lenzner S, Bauters M, van der Maarel SM, Scholten IH, Huber I, Philippe C, Suijkerbuijk RF, Rogers RH, Cremers FP, Gilgenkrantz S, Kere J, Cremers FP, Rogers RH: Cloning and characterization of DXS6673E, a candidate gene for X-linked mental retardation in Xq13.1. Hum Mol Genet 1996; 5:887–893.

27. Frints SG, Lenzner S, Bauters M, Jensen LR, van Esch H, Des Portes V, Moog U, Macville MV, van Roozendaal K, Schrander-Stumpel CT, Tzschach A, Marique P, Frants RY, Harrell B, van Bokhoven H, Chey J, Beldjord C, Turner G, Geicz J, Moraine C, Raeymaekers N, Ropers HH, Huganir RL, Suzuki E, Gilgenkrantz S, Kere J, Cremers FP, van der Maarel SM, Scholten IH, Huber I, Philippe C, Suijkerbuijk RF, Rogers RH, Cremers FP, Gilgenkrantz S, Kere J, Cremers FP, Rogers RH: Cloning and characterization of DXS6673E, a candidate gene for X-linked mental retardation in Xq13.1. Hum Mol Genet 1996; 5:887–893.

28. Namba N, Etani Y, Kitaoka T, Nakamoto Y, Nakacho M, Bessho K, Miyoshi Y, Frints SG, Lenzner S, Bauters M, Jensen LR, van Esch H, Des Portes V, Moog U, Macville MV, van Roozendaal K, Schrander-Stumpel CT, Tzschach A, Marique P, Frants RY, Harrell B, van Bokhoven H, Chey J, Beldjord C, Turner G, Geicz J, Moraine C, Raeymaekers N, Ropers HH, Huganir RL, Suzuki E, Gilgenkrantz S, Kere J, Cremers FP, van der Maarel SM, Scholten IH, Huber I, Philippe C, Suijkerbuijk RF, Rogers RH, Cremers FP, Gilgenkrantz S, Kere J, Cremers FP, Rogers RH: Cloning and characterization of DXS6673E, a candidate gene for X-linked mental retardation in Xq13.1. Hum Mol Genet 1996; 5:887–893.

29. Capri Y, Friesema EC, Kersseboom S, Touraine R, Monnier A, Eymard-Pierre E, Des Pothier E, Echeverri R, Lubs HA, Voeller K, Simensen RJ, Stevenson RE: Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. Am J Hum Genet 2008, 83:1029–1037.

30. Karapanou O, Papadimitriou A: Thyroid hormone transporters in the human. Hormones 2011, 10:270–279.

31. Geicz J, Barnett S, Liu J, Holloway G, Donnelly A, Eyre H, Ethievaris HS, Baltazar R, Grunn A, Nagaraja R, Gilliam C, Peltonen L, Sutherland GR, Baron M, Mulley JC: Characterization of the human glutamate receptor subunit 3 gene (GRIA3), a candidate for bipolar disorder and nonspecific X-linked mental retardation. Genomics 1999, 62:356–368.

32. Kusunise A, Arvola M, Keinänen K: Molecular dissection of the agonist binding site of an AMPA receptor. EMBO J 1995, 14:6327–32.

33. Wo ZG, Oswald RE: Unraveling the modular design of glutamate-gated ion channels. Trends Neurosci 1995, 18:161–168.

34. Wu Y, Arai AC, Rumbaugh G, Sinavata WC, Des Portes V, De Michele G, Brady AF, Boespflug-Tanguy O, Visser TJ, Vaun-Banier E, Reeser M, Friesema EC, Capri Y, Kersseboom S, Touraine R, Monnier A, Eymard-Pierre E, Des Pothier E, Echeverri R, Lubs HA, Voeller K, Simensen RJ, Stevenson RE: Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. Am J Hum Genet 2008, 83:1029–1037.

35. Chiyonobu T, Hayashi S, Kobayashi K, Morimoto M, Miyanomae Y, Nishimura K, Mushiake S, Mohri I, Arai H, Taniike M, Ozono K: XLID-Causing Mutations and Associated Genes Challenged in Light of Data From Large-Scale Human Exome Sequencing. Am J Hum Genet 2013, 93:368–383.

36. Peltonen L, Jalkanco A, Varlo T: Molecular genetics of the Finnish disease heritage. Hum Mol Genet 1999, 8:1913–1923.

37. Philippe A, Malan V, Jacquemont ML, Boddaert N, Bonnefont JP, Oudin S, Munirich A, Colleaux L, Cormier-Daire V: Xq25 duplications encompassing GRIA3 and STAG2 genes in two families convey recognizable X-linked mental retardation. Am J Med Genet A 2013, 161:1370–1375.

38. Smith SA, Holik P, Stevens J, Mazoyer S, Melis R, Williams B, White R, Albertsen H: Isolation of a gene (DLG3) encoding a second member of the discs-large family on chromosome 17q12-q21. Genomics 1996, 31:145–150.