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Deep sequencing reveals 50 novel genes for recessive cognitive disorders

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Common diseases are often complex because they are genetically heterogeneous, with many different genetic defects giving rise to clinically indistinguishable phenotypes. This has been amply documented for early-onset cognitive impairment, or intellectual disability, one of the most complex disorders known and a very important health care problem worldwide. More than 90 different gene defects have been identified for X-chromosome-linked intellectual disability alone, but research into the more frequent autosomal forms of intellectual disability is still in its infancy. To expedite the molecular elucidation of autosomal-recessive intellectual disability, we have now performed homozygosity mapping, exon enrichment and next-generation sequencing in 136 consanguineous families with autosomal-recessive intellectual disability from Iran and elsewhere. This study, the largest published so far, has revealed additional mutations in 23 genes previously implicated in intellectual disability or related neurological disorders, as well as single, probably disease-causing variants in 50 novel candidate genes. Proteins encoded by several of these genes interact directly with products of known intellectual disability genes, and many are involved in fundamental cellular processes such as transcription and translation, cell-cycle control, energy metabolism and fatty-acid synthesis, which seem to be pivotal for normal brain development and function.

Early-onset cognitive impairment, or intellectual disability, is an unresolved health care problem and an enormous socio-economic burden. Most severe forms of intellectual disability are due to chromosomal abnormalities or defects in specific genes. For many years, research into the genetic causes of intellectual disability and related disorders has focused on X-chromosome-linked intellectual disability (XLID). It has become clear, however, that X-linked forms account for only 10% of intellectual disability cases, which means that the vast majority of the underlying genetic defects must be autosomal[1]. For severe forms of intellectual disability, autosomal-dominant inheritance is rare because most affected individuals do not reproduce, but recent observations suggest that in outbred Caucasian populations, a significant portion of the sporadic cases may be due to dominant *de novo* mutations[2–4]. So far, relatively little is known about the role of autosomal recessive intellectual disability (ARID), because in Western societies, where most of the research takes place, its investigation has been hampered by infrequent parental consanguinity and small family sizes.

In most Northern African countries, and also in the Near and Middle East, parental consanguinity and large families are common; for example, in Iran, 40% of the families are consanguineous and about two-thirds of the population is 30 years of age or younger. Since 2004, we have performed systematic array-based consanguinity mapping in 272 consanguineous Iranian families. In several dozen families, we have defined single linkage intervals and mapped the underlying gene defects[5,6], and by subsequent mutation screening of candidate genes from these intervals, we and others identified several novel ARID genes (for review see refs 1, 7).

Recently, exome enrichment and next-generation sequencing have been introduced as a cost-effective and fast strategy for comprehensive mutation screening and disease-gene identification in the coding portion of the human genome[8–10]. To unravel the molecular basis of ARID in a systematic fashion, we have now used a related, but more targeted, approach. Instead of sequencing entire exomes in consanguineous families, we have focused on the exons from homozygous linkage intervals known to carry the genetic defect. Before sequencing, these exons were enriched by hybrid capture using custom-made oligonucleotide arrays as baits. All patients had cognitive impairment (mostly moderate or severe, see Supplementary Table 1), and in a subset of the families there were signs of autism spectrum disorder. More information about the families and their clinical features, quality controls performed to validate the sequence variants observed and to assess their pathogenicity, as well as other methodological details are provided in Supplementary Information.

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Mutations in known and novel intellectual disability genes

In 115 out of 136 families studied, plausible causal defects were observed, and in 78 of these, a single, apparently disease-causing mutation could be identified (see Supplementary Fig. 1, Tables 1 and 2 and Supplementary Table 2). Twenty-eight protein-truncating changes were found, including frameshift, splice-site and nonsense mutations, as well as whole-exon deletions, plus several smaller in-frame deletions of varying size. In 26 families listed in Table 1, we identified known, mostly syndromic forms of ARID, including rare metabolic defects and storage disorders, such as an atypical form of Tay–Sachs' disease and Sanfilippo's syndrome (mucopolysaccharidosis IIIb), as well as intellectual disability with congenital abnormalities, such as a Joubert-like syndrome resulting from *AHI1* mutations, observed in two unrelated families. Two families were also found with allelic *PRKCG* mutations, implicated previously in spinocerebellar ataxia, and two families carried different allelic mutations in the *SRD5A3* gene, associated with Kahrizi's syndrome, a recently elucidated congenital glycosylation disorder[11,12].

Two mutations involving the adaptor protein complex 4 were observed, namely in the *AP4M1* and *AP4E1* genes, which encode different AP-4 subunits. AP-4 is involved in the recognition and sorting of cargo protein transported from the trans-Golgi network to the endosomal–lysosomal system. Another possibly pathogenic change was found in the *AP4B1* gene, but its effect may be obscured by a *PEX6* mutation in the same family, which causes a severe peroxisome biosynthesis disorder[13] and probably accounts for most of the clinical features. In highly inbred families, coexistence of two different recessive defects is not unexpected and is the most plausible explanation for the complex phenotypes in at least two families with novel forms of ARID (M154 and M189, see Table 2).

Mutations in the *SLC2A1* gene, which encodes a glucose transporter, the *PRKRA* gene with a role in dysautonomia, and the *MED13L* gene, previously associated with intellectual disability and cardiac symptoms, were the only plausible causes of intellectual disability in three families with non-syndromic intellectual disability. None of the respective families showed signs of dysautonomia or cardiac abnormalities. In all other families, the phenotype was characteristic for the molecular defect, including family M198 with folate receptor deficiency, a rare syndromic form of ARID that can often be treated by oral administration of folinic acid[14]. Further details are provided in Table 1.

Apparently pathogenic changes were also found in 50 genes that had not been previously implicated in ARID (see Table 2). Thirty of the relevant families had non-syndromic forms of intellectual disability, whereas 22 exhibited syndromic forms. Only two of the novel ARID genes were mutated in more than a single family. Two different missense mutations with high pathogenicity scores were detected in *ZNF526*, which encodes a krüppel-type zinc-finger protein. One of these changes was observed in DNA samples collected from two distinct families with non-syndromic intellectual disability, but closer inspection revealed that these families, which live in the same city in the northwestern part of Iran, share a common haplotype and thus must be distantly related. In these families, no other potentially disease-causing and co-segregating change could be identified. Zinc-finger proteins are transcriptional regulators, and other krüppel-type zinc-finger genes have been implicated in intellectual disability before[15]. Recent protein interaction studies have indicated a role for ZNF526 in promoting messenger RNA translation and cell growth (N. Hubner *et al.*, personal communication). Another gene within which disease-causing mutations were found in two families was *ELP2*. It encodes a subunit of the RNA polymerase II elongator complex, which is a histone acetyltransferase component of RNA polymerase II. This gene is involved in the acetylation of histones H3 and probably H4, and it may have a role in chromatin remodelling.

Mutations affecting housekeeping genes

In the *LARP7* gene, we found a frameshift mutation in a family with intellectual disability and microcephaly. *LARP7* is a negative transcriptional regulator of polymerase II genes, acting by means of the 7SK RNP system. Within the 7SK RNP complex, the positive transcription elongation factor b (P-TEFb) is sequestered in an inactive form, preventing RNA polymerase II phosphorylation and subsequent transcriptional elongation. Hitherto, no disease association has been reported for *LARP7*.

Presumably causative homozygous mutations were also found in *KDM5A* and *KDM6B*. These genes encode histone demethylases that specifically demethylate histone H3 at lysine 4 and lysine 27, respectively, and they both have a central role in the histone code. We have

Table 1 | Mutations identified in known genes for intellectual disability or related disorders

Family	Gene	Mutation	LOD score	Length (Mb)	OMIM no.	Diagnosis, clinical features
8500306	*AHI1*	R329X	2.65	10.35	608629	Joubert's syndrome 3
M332	*AHI1*	R495H	3.2	11.1	608629	Joubert's syndrome 3
M254	*AP4E1*	V454fs	2.5	13.57	607244	Microcephaly, paraplegia
M004	*AP4M1*	E193K	1.9	16.75	602296	Microcephaly, paraplegia
M324	*BBS7*	533del2aa	3.24	8.2	209900	Bardet–Biedl's syndrome
M107	*CA8*	R237Q	2.4	4.02	613227	Ataxia, cerebellar hypoplasia
M175	*COL18A1*	L1587fs	2.1	9.8	267750	Knobloch's syndrome (eye and brain development)
G026	*FAM126A*	Splice site*	2.4	15.46	610532	Hypomyelination-cataract
M198	*FOLR1*	Splice site*	2.1	16.95	136430	Folate receptor deficiency
M165	*HEXA*	C58Y	2.7	15.91	272800	Psychomotor delay, mild Tay–Sachs' disease
8600276†	*L2HGDH*	R335X	5.1	13.39	609584	Hydroxyglutaric aciduria
M142	*MED13L*	R1416H	1.9	9.17	608808	Non-syndromic ID, no cardiac involvement
8600486	*NAGLU*	R565Q	2.8	13.25	252920	Sanfilippo's syndrome, MPS IIIB
8500234	*PDHX*	R15H	3.13	35.17	245349	Pyruvate dehydrogenase defect
M331	*PEX6*	L534P	3.8	10.83	601498	Peroxisome biogenesis disorder
8307998	*PMM2*	Y106F	2.67	6.71	212065	Glycosylation disorder CDG Ia
8600273	*PRKCG*	V177fs	2.53	0.72	605361	Spinocerebellar ataxia 14
M146	*PRKCG*	D480Y	2.1	7.45	605361	Spinocerebellar ataxia 14
8600162	*PRKRA*	S235T	2.1	40.02	612067	Non-syndromic ID
8600042	*SLC2A1*	V237M	3.73	16.7	606777	Non-syndromic ID
8700017	*SRD5A3*	Y169C	4.8	10.5	612713	Kahrizi's syndrome, CDG
M069†	*SRD5A3*	A68fs	3.01	10.44	612713	Kahrizi's syndrome, CDG
G008	*SURF1*	W227R	1.8	4.59	185620	Leigh's syndrome, very mild form
8600041	*TH*	R202H	2.1	7.23	605407	infantile parkinsonism, Segawa's syndrome
M017N	*VRK1*	R133C	3.4	3	607596	Pontocerebellar hypoplasia
M196	*WDR62*	G705G	2.1	18.33	600176	Microcephaly, cerebellar atrophy

CDG, congenital disorder of glycosylation; fs, frameshift; ID, intellectual disability; LOD, logarithm of the odds; MPS, mucopolysaccharidosis; OMIM, Online Mendelian Inheritance in Man.
* See Supplementary Information for further details.
† Remotely related, degree of consanguinity is not clear, analysis performed under conservative assumption of second degree consanguinity.

previously shown that mutations in another lysine-specific histone demethylase, *KDM5C* (also called *JARID1C*), are a relatively frequent cause of X-linked intellectual disability[16]. In two other families, we observed apparently pathogenic mutations that involved histones directly: a frameshift mutation in the *HIST1H4B* gene which belongs to the histone 4 family, and a *HIST3H3* missense mutation with high pathogenicity scores that was the only plausible change in a family with non-syndromic intellectual disability. Together, at least ten of the novel candidate genes for ARID involve histone structure, histone modification, chromatin remodelling or the regulation of transcription, and many of these genes are functionally linked to known and novel intellectual disability genes, as shown in Fig. 1a.

Several other mutated genes are directly or indirectly involved in the regulation of translation. A homozygous frameshift mutation inactivating the *TRMT1* gene was detected in a family with non-syndromic intellectual disability. *TRMT1* is an RNA methyltransferase that dimethylates a single guanine residue at position 26 of most tRNAs. Previously we and others have shown that inactivation of the X-linked gene *FTSJ1*, another RNA methyltransferase, also gives rise to non-syndromic intellectual disability[17,18], and we have recently identified several ARID families with truncating mutations in a third RNA methyltransferase (L.A.M. *et al.*, manuscript in preparation). A large deletion in the *EEF1B2* gene was the only detectable defect in another family with non-syndromic intellectual disability. *EEF1B2* encodes the elongation factor 1β, which is involved in the transport of aminoacyl-tRNAs to the ribosomes. In yet another family with non-syndromic intellectual disability, a missense change was found in *ADRA2B*. This gene encodes a brain-expressed G-protein-coupled receptor that associates with EIF2B, a guanine exchange factor regulating translation[19]; notably, ADRA2B also interacts with the 14-3-3 protein, which in turn associates with RGS7, another novel ARID gene product that regulates G-protein signalling. Finally, in a family with a syndromic form of intellectual disability, a missense change was found in the *POLR3B* gene, involving a nucleotide with a very high conservation score and predicted to be pathogenic by Mutation Taster[20]. *POLR3B* encodes the second-largest core component of RNA polymerase III, which synthesizes small RNAs such as tRNAs and 5S rRNAs[21] and also interacts with ENTPD1, the product of a novel candidate gene for intellectual disability (see GeneCards, http://www.genecards.org/cgi-bin/cardsearch.pl?search=POLR3B and Table 2). Together, these observations indicate that gene defects interfering with transcription and translation are particularly important causes of intellectual disability.

However, we also found pathogenic mutations affecting other fundamental cellular functions and pathways such as cell-cycle control, as illustrated by a mutation inactivating *CCNA2*, and another one truncating *SCAPER*, a specific regulator of the CCNA2–CDK2 complex (see Fig. 1b). The *C11orf46* gene encodes TTI2, a subunit of the Triple T complex, which is required for the establishment of cell-cycle checkpoints and for DNA-damage signalling[22]. Other mutations involved fatty-acid synthesis and turnover (*ACBD6*, *FASN* and *PECR*; see Table 2), protein degradation (*UBR7*), splicing (*ZCCHC8*) and cell migration (*LAMA1*).

Intellectual disability genes with brain-specific functions

Not surprisingly, several mutations involved genes with neuron- or brain-specific functions. For example, we found a frameshift mutation abolishing the function of *CACNA1G*, a T-type calcium channel with a critical role in the generation of $GABA_B$ receptor-mediated spike and wave discharges in the thalamocortical pathway[23,24]. A nonsense mutation inactivated *ZBTB40*, which has a role in glia cell differentiation[25], and other observed changes are expected to interfere with the regulation of neurotransmission, exocytosis or neurotransmitter release. Our study also adds several novel intellectual-disability-associated genes to the Ras and Rho pathway (see Fig. 1c); for example, a convincing missense mutation in the *RALGDS* gene was the only variant detected in one family with non-syndromic intellectual disability. This gene encodes an effector of the Ras-related GTPase Ral, which stimulates the dissociation of GDP from the Ras-related RalA and RalB GTPases, thereby allowing GTP binding and activation of the GTPases[26]. Regulators of small GTPases were among the first genes to be implicated in non-syndromic intellectual disability[27,28]. We also found a homozygous frameshift mutation in *CNKSR1*, which is physically associated with *RALGDS*. Homozygous carriers of this mutation have a severe syndromic phenotype with quadrupedal gait. CNKSR1 binds to rhophilin (Online Mendelian Inheritance in Man (OMIM) 601031), a Rho effector, suggesting that it acts as a scaffold protein and mediates crosstalk between the Ras and Rho GTPase signalling pathways[29]. Neither *RALGDS* nor *CNKSR1* had been implicated in intellectual disability so far; thus, both are novel ARID genes.

Genes without obvious link to intellectual disability

For several of the sequence variants, there is no obvious functional link between the molecular defect and intellectual disability. This applies to *LINS1* and *NDST1*, and it is not easy to understand why

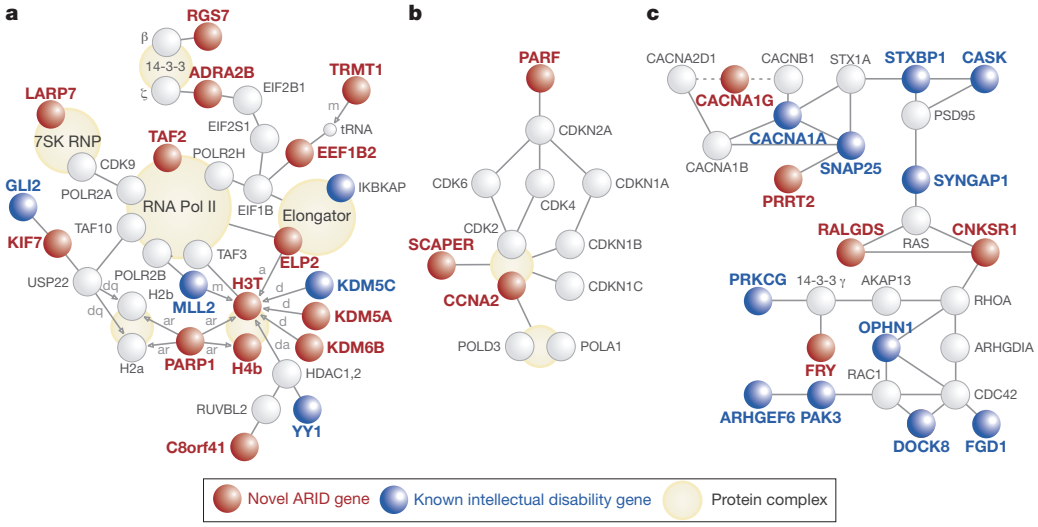

Figure 1 | Known and novel intellectual disability genes form protein and regulatory networks. **a**, Transcriptional/translational network. **b**, Cell-cycle-related network. **c**, Ras/Rho/PSD95 network. Connecting edges in the figure stand for protein–protein interactions. Arrows define direction of post-translational protein modifications: a, acetylation; ar, ADP-ribosylation; d, demethylation; da, deacetylation; dq, deubiquitination; m, methylation. Dotted lines indicate modulation of gene function. Data were obtained in part by using the INGENUITY software package (http://www.ingenuity.com) and by literature mining. More details about these proteins and their interactions are provided in Table 2 and in Supplementary Information.

Table 2 | Apparently causative variants in novel (candidate) genes for intellectual disability

Family	Phenotype	Gene	Mutation	LOD score	Length (Mb)	Supporting evidence
M008†	S	*ACBD6*	G22fs	2.65	6.46	P; binds long-chain acyl-CoA molecules, role in fatty acid synthesis or turnover[44].
M173	NS, ASD	*ADK*	H324R	5.1	9.68	S, P; only change in family. Adenosine kinase, regulates adenosine levels in the brain. Overexpression leads to learning impairment in mice[45]; knockout mice develop lethal neonatal liver steatosis[30]. In human, a different gene defect has been found in this condition.
M266-2	NS	*ADRA2B*	R440G	2.53	24.97	S, P; GPCR regulating adrenergic neurons in the CNS. Associates with EIF2B, a GEF regulating translation[19]. Also associates with 14-3-3, which interacts with RGS7, mutated in family 8700136.
M226	NS	*ASCC3*	S1564P	3.2	62.80	S, P, E; helicase that is part of the activating signal co-integrator complex, enhances NF-κB and AP1. Interacts with RARS2, implicated in pontocerebellar hypoplasia 6[46].
M007L‡	NS	*ASCL1*	A41S	2.4	18.13	Encodes the bHLH factor MASH1, critical role in neuronal commitment and differentiation[47,48].
M182	NS	*C11orf46*	R236H	2.1	12.39	P, E; encodes subunit of the Triple T complex, role in regulation of DNA damage response[22].
G001	NS	*C12orf57*	M1V	2.5	11.19	S; function hitherto unknown. May overlap neighbouring *ANT1* (DRPLA) gene (see UCSC Genome Browser, hg18; OMIM 125370).
M100	NS	*C8orf41*	P367L	3.3	6.44	S, P, E; C8orf41 associates with RUVBL2[49], which is involved in regulation of transcription and interacts with HDACs[50].
G015	NS	*C9orf86*	A562P	3.3	2.17	P; encodes Rab-like GTP-binding protein PARF, which interacts with ARF (or CDKN2A). Other Rab has been implicated in ID[49].
8500031	S	*CACNA1G*	S1346fs	2.7	18.76	P, E; encodes a low-voltage-activated calcium channel which may also modulate the firing patterns of neurons[23,24].
8600057	S	*CAPN10*	138ins5aa	2.1	2.09	E; calcium-regulated non-lysosomal endopeptidase with a role in cytoskeletal remodelling and signal transduction, involved in long-term potentiation[51].
8600495	NS	*CASP2*	Q392X	2.5	29.62	P; caspase 2, role in apoptosis, abnormal in CASP2-deficient mice, particularly for motor and sympathetic neurons[52]. Motor abnormalities not observed in family.
M346	NS	*CCNA2*	Splice site*	3.3	52.17	S, P; cyclin A2 is essential for cell cycle control[53]. In mice, targeted deletion of this gene is lethal[54]. Regulated by *SCAPER*, mutated in family 8600277.
8500235‡	S	*CNKSR1*	T282fs	2.53	15.83	P; regulates Raf in the MAPK pathway, acts as scaffold protein linking Ras and Rho signal transduction pathways[29]. Interacts with RALGDS, which is mutated in family 8500155.
M144	NS	*COQ5*	G118S	1.8	15.10	P, E; methyltransferase with pivotal role in coenzyme Q biosynthesis. Interacts with NAB2 which controls length of poly(A) tail (see http://thebiogrid.org/35094/summary/saccharomyces-cerevisiae/coq5.html). The human orthologue of NAB2 is implicated in ARID[32].
M178	NS	*EEF1B2*	Splice site*	2.6	13.84	S, P, E; controls translation by transferring aminoacyl-tRNAs to the ribosome. Interacts with UNC51-like kinase 2 which is involved in axonal elongation translation[55].
G017	NS	*ELP2*	T555P	2.4	14.33	P, E; encodes subunit of the RNA polymerase II elongator complex[56]. ELP3 subunit implicated in motor neuron degeneration. Allelic *ELP2* mutation found in family M8500061.
8500061	NS	*ELP2*	R462L	2.7	16.98	P, E; involved in transcriptional elongation, see also family G017 with allelic *ELP2* mutation.
M263	NS	*ENTPD1*	Y65C	2.65	12.12	P, E; ectonucleoside triphosphate diphosphohydrolase, expressed in CNS; knockout mice display abnormal synaptic transmitter release[57].
M050†	S	*ERLIN2*	R36K	3.73	12.72	S, P,E; involved in the ER-associated degradation of inositol 1,4,5-triphosphate receptors[58].
8500058	NS	*FASN*	R1819W	3.3	4.50	P; gene product synthesizes long-chain fatty acids from acetyl-CoA and malonyl-CoA. Expressed in post-synaptic density. In mice, FASN deficiency leads to embryonic lethality[59].
M269	S	*FRY*	R1197X	2.8	12.68	P; regulates actin cytoskeleton, limits dendritic branching. In HeLa cells, FRY binds to microtubules and localizes on the spindle and is crucial for the alignment of mitotic chromosomes[60].
M251	S	*GON4L*	Splice site*	3.01	40.19	P, E; cloned from brain. Encodes a transcription factor thought to function in cell cycle control[61].
M189‡	S	*HIST1H4B*	K9fs	2.1	48.87	P, E; encodes a member of the histone H4 family; analogy to histone H3 mutation in family G002. Ehlers–Danlos-related symptoms are probably due to *TNXB* mutation.
G002	NS	*HIST3H3*	R130C	2.53	26.74	P; role in spindle assembly and chromosome bi-orientation[62–64]. See also family M189 with *HIST1H4B* mutation.
8500064	NS	*INPP4A*	D915fs	2.4	46.16	P, E; encodes inositol polyphosphate-4-phosphatase, only plausible change in family. Regulates localization of synaptic NMDA receptors, protects neurons from excitotoxic cell death[65]. Knockout mice develop locomotor instability; not observed in this family.
M061	S	*KDM5A*	R719G	2.3	6.06	P, E; encodes histone demethylase specific for Lys 4 of histone H3, role in transcriptional regulation[66]. Other histone demethylase has been implicated in X-linked ID[16]. See also family M8303971 with *KDM6B* mutation.

in humans, adenosine kinase deficiency should lead to intellectual disability, whereas in the mouse, overexpression of *Adk* causes neurological symptoms, and *Adk* deficiency gives rise to early lethal liver steatosis[30]. Nothing is known yet about the function of the *C12orf57* gene, apart from its apparent overlap with *ATN1* (see UCSC Genome Browser, NCBI36/hg18). CAG trinucleotide expansion in the *ATN1* gene is the cause of dentatorubral pallidoluysian atrophy (DRPLA), another syndromic form of intellectual disability. A comprehensive list of families with single, probably disease-causing mutations is shown in Table 2.

Despite exhaustive validation of our data and stringent filtering against all known neutral and pathogenic sequence variants (see Supplementary Information and Supplementary Tables 3–6), it is still possible that not all of these changes will turn out to be causative. Particularly for the numerous missense mutations observed, functional studies will be required to rule out rare polymorphisms that are unrelated to intellectual disability. In a previous study, 1% of the protein-truncating mutations on the X chromosome were found to be unrelated to disease[31], and in our study, 12 observed inactivating mutations did not co-segregate with intellectual disability (see Supplementary Table 4). However, we believe that the vast majority of the changes presented here as probably pathogenic will be confirmed, even if they have been observed only once, because most of the proteins encoded by these novel candidate genes interact with the

Table 2 | Continued

Family	Phenotype	Gene	Mutation	LOD score	Length (Mb)	Supporting evidence
8303971	S	*KDM6B*	P888S	3.1	5.08	S, P; demethylase 6B specifically targeting Lys 27 of histone H3, has a central role in regulation of posterior development by regulating HOX gene expression[67]. Mutation of *KDM5A* gives rise to ID (see family M061).
M154	S	*KIF7*	E758K	2.1	7.46	P, E; knockout mouse model with complex picture involving brain and other neurological abnormalities[68]. Stickler-like clinical features in this family can be explained by co-existing *COL9A1* mutation.
M183	S	*LAMA1*	G1572fs	2.1	5.82	S, P; codes for subunit of laminin, role in attachment, migration and organization of cells during embryonic development. Required for normal retinal development in mice[69].
G030	S	*LARP7*	K276fs	1.93	8.94	S,P; encodes negative transcriptional regulator of polymerase II genes[70].
7903104	S	*LINS1*	H329fs	2.65	7.87	S, P; similar to *lin*, a *Drosophila* gene having important roles in the development of the epidermis and the hindgut. Link with ID unclear.
8600060†	NS	*MAN1B1*	R334C	3.13	2.49	P, E; encodes mannosidase that targets misfolded glycoproteins for degradation. *MAN1B1* frameshift mutation observed in another ARID family by Canadian group (J. Vincent, personal communication).
8600277	NS	*NDST1*	R709Q	2.1	10.18	S, P; only change in family. Encodes heparan *N*-deacetylase/*N*-sulphotransferase, deficiency is lethal in mice due to respiratory distress[71]. No obvious link with ID.
M158	S	*PARP1*	L293F	1.8	16.76	P; poly(ADP-ribose) polymerase involved in histone 1 modification; role in memory stabilization in mice[72].
M194	NS, ASD	*PECR*	L57V	2.5	11.27	P; brain-expressed peroxisomal *trans*-2-enoyl-CoA reductase involved in the biosynthesis of unsaturated fatty acids[73].
8401214	S	*POLR3B*	T199K	1.93	24.89	E; second-largest core component of RNA polymerase III, which synthesizes small RNAs such as tRNAs and 5S rRNAs[21].
8500302	NS	*PRMT10*	G189R	2.65	9.75	P, E; protein arginine methyltransferase 10. Protein arginine methylation affects chromatin remodelling leading to transcriptional regulation, RNA processing, DNA repair and cell signalling[74].
M010	NS	*PRRT2*	A214fs	5.2	25.59	P; interacts with SNAP25 which in turn assembles with syntaxin-1 and synaptobrevin to form exocytotic fusion complex in neurons[55].
8500155	NS	*RALGDS*	A706V	4.0	5.56	S, E; effector of Ras-related RalA and RalB GTPases, role in synaptic plasticity[26]. Interacts with CNKSR1, inactivated in family 8500235.
8700136	NS, ASD	*RGS7*	N304fs	2.53	24.34	P; regulator of G protein signalling. Interacts with 14-3-3 protein, tau and snapin, a component of the SNARE complex required for synaptic vesicle docking and fusion[75]. Indirectly linked with *ADRA2B*, mutated in family M266_2.
8600086	NS	*SCAPER*	Y118fs	3.9	17.45	S, E; interacts with CCNA2/CDK2 complex, transiently maintains CCNA2 in cytoplasm[76]. *CCNA2* is mutated in family M346.
8600012	S	*SLC31A1*	R90G	2.1	13.85	P, E; encodes one of two genes involved in copper import. Deficiency of the SLC31A1 orthologue in mice is early lethal, heterozygotes have progressive neurological disorder[77], similar to patients in this family.
M177	S	*TAF2*	W649R	2.1	19.16	P, E; TATA-box-associated gene is very important regulator of transcription (see OMIM 604912). Other TAF genes have been implicated in X-linked ID (V.K. *et al.*, manuscript in preparation). MAL2 is another, less likely, candidate in this family.
M160	S	*TMEM135*	C228S	2.4	16.89	S, P, E; transmembrane protein involved in fat metabolism and energy expenditure[78].
M300	NS	*TRMT1*	I230fs	3.4	10.34	P, E; encodes dimethylguanosine tRNA methyltransferase[79]. At least two other RNA methyltransferases have been implicated in ID (ref. 17 and L.A.M., manuscript in preparation).
M168	NS, ASD	*UBR7*	N124S	2.5	8.78	P, E; encodes n-regognin 7, a component of E3 ubiquitin ligase[80]. Involved in protein degradation, which has been implicated in ID.
8500320	S	*WDR45L*	R109Q	1.93	2.55	P, E; WD repeat domain, phosphoinositide-interacting protein 3, ILF1-like[81], specific function unknown.
M169	S	*ZBTB40*	Q525X	3.5	14.56	S, P, E; krüppel-type zinc finger, highly expressed in brain. Regulator of glia differentiation[25].
M156	NS	*ZCCHC8*	L90X	2.3	7.64	P; zinc-finger protein, identified in the spliceosome C complex. Interacts with BRCA1 and RBM7[82,83]. RBM10 has been implicated in X-linked ID (V.K. *et al.*, manuscript in preparation).
M025	NS	*ZNF526*	R459Q	4.5	6.13	P; zinc-finger protein, only remaining change in family. Functional relevance supported by 3D modelling. Probable activator of mRNA translation. Allelic *ZNF526* mutation observed in family 8500156.
8500156	NS	*ZNF526*	Q539H	4.04	11.33	P; see family M025 with allelic *ZNF526* mutation.

References 44–83 are listed in Supplementary Information. E, high evolutionary conservation score; P, high pathogenicity score, includes truncating mutations; S, only change found in family. ASD, autism spectrum disorder; GPCR, G-protein-coupled receptor; ID, intellectual disability; NS, non-syndromic; S, syndromic.
* See Supplementary Information for further details.
† Parents are distantly related. LOD scores provided are minimum estimates, calculated on the assumption that they are second cousins.
‡ In ethnically matching healthy controls a single heterozygous carrier was found (for details, see Supplementary Table 3).

products of known or novel genes associated with intellectual disability, as shown in Fig. 1.

Most ARID genes are not synapse specific

We have previously shown that ARID is an extremely heterogeneous disorder[6]. In contrast to non-syndromic hearing impairment or X-linked intellectual disability, common forms of ARID do not seem to exist, although there is evidence for regional clustering of the underlying gene defects[5]. Extrapolating from the number of known X-chromosomal intellectual disability genes argues for the involvement of several hundred genes in non-syndromic ARID, and the total number of ARID genes may well run into the thousands[1]. Identification of most or all of these genes is a prerequisite for early diagnosis, prevention and, eventually, therapy of intellectual disability, but at the present pace, many years would be required to accomplish this task. Here, we have combined homozygosity mapping, targeted exon enrichment and next-generation sequencing to speed up the molecular elucidation of ARID. In 78 out of 136 consanguineous families investigated, we have found apparently pathogenic mutations in single genes. Fifty of these genes had not been implicated in ARID before, and only two of these novel intellectual disability genes were found to be mutated in two independent families. None of the ~10 previously known genes for non-syndromic ARID, including those that were identified in Iranian families[32–36], was observed in our present

cohort, thereby corroborating previous evidence that ARID is extremely heterogeneous.

Much of the research into the molecular causes of intellectual disability has focused on the synapse and synapse-specific genes (for example, see refs 2, 37). In the present study, relatively few of the novel defects identified involve synapse- or neuron-specific genes, and they are vastly outnumbered by ubiquitously expressed genes with indispensable cellular functions, such as DNA transcription and translation, protein degradation, mRNA splicing, energy metabolism as well as fatty-acid synthesis and turnover. Many of these defects were found to be associated with non-syndromic ARID. It is not immediately clear why the clinical consequences of defects involving such a wide spectrum of basic cellular processes should be confined to the brain, but this conceivably reflects the complexity of the central nervous system which may render it particularly vulnerable to damage.

We expect that these findings will have direct implications for the diagnosis and prevention of intellectual disability, and perhaps also for autism, schizophrenia and epilepsy, which often co-exist in intellectual disability patients and are frequently associated with mutations in the same genes (for example, see ref. 38; reviewed in ref. 1). Further investigation of the novel genes and networks presented here should significantly deepen our insight into the pathogenesis of intellectual disability and related disorders. Moreover, this study illustrates the power of large-scale next-generation sequencing in families as a general strategy to shed light on the aetiology of complex disorders and on the function of the underlying genes.

Note added in proof: While this work was in the press, two unrelated groups reported on inactivating ERLIN2 mutations in patients with recessive intellectual disability and progressive motor dysfunction[39,40]. Moreover, syndromic forms of intellectual disability have been described in patients with AP4B1 and AP4E1 (ref. 41) and MAN1B1 (ref. 42) mutations, respectively. Finally, mutations inactivating the *KIF7* gene were identified as the cause of the recessive fetal hydrolethalus and acrocallosal syndromes that include brain malformations[43].

METHODS SUMMARY

Most families studied were from Iran, and less than 10% had a Turkish or Arabic background. Wechsler Intelligence Scales for Children (WISC) and WAIS were used to assess the IQ in children and parents. Many of the pedigrees, as well as the methods used for autozygosity mapping, have been described previously.

Exons from homozygous intervals were enriched with custom-made Agilent SureSelect DNA capture arrays and sequenced on an Illumina Genome Analyser II yielding 76-bp single reads. >98% of the targeted exons were covered by at least four non-redundant sequence reads, each with a PHRED-like quality score of 20 or above (mean, 0.984; median, 0.993; for details, see Supplementary Table 5).

To assess the reliability of this procedure for calling homozygous mutations, we looked up SNP markers from homozygous intervals of five selected families that had been analysed with high-resolution SNP arrays. For 773 out of 776 markers, next-generation sequencing and array-based SNP typing yielded identical results.

To detect single nucleotide variants, high-quality reads were aligned to the human reference genome (hg18) by SOAP2.20 with default settings, typically gap-free. Homozygous exon-spanning deletions were assumed if the sequence coverage of the relevant exon(s) was reduced to <5% of the mean. Details about the detection of smaller deletions and insertions are provided in Methods. All variants were validated by high-resolution array CGH, Sanger sequencing, or both.

Homozygous variants were filtered against dbSNP130/131, whole genomes from 185 healthy individuals studied by the 1000 Genomes Project and exomes from 200 Danish individuals, and found to be absent in at least 100 chromosomes from Iranian controls (see Supplementary Tables 1 and 3). To select and prioritize apparently disease-causing variants, various criteria were used (for more details, see Methods). All putative mutations co-segregated with intellectual disability in the respective families.

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1. Ropers, H. H. Genetics of early onset cognitive impairment. *Annu. Rev. Genomics Hum. Genet.* **11,** 161–187 (2010).
2. Hamdan, F. F. *et al.* Excess of *de novo* deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. *Am. J. Hum. Genet.* **88,** 306–316 (2011).
3. Hamdan, F. F. *et al.* Mutations in SYNGAP1 in autosomal nonsyndromic mental retardation. *N. Engl. J. Med.* **360,** 599–605 (2009).
4. Vissers, L. E. *et al.* A *de novo* paradigm for mental retardation. *Nature Genet.* **42,** 1109–1112 (2010).
5. Najmabadi, H. *et al.* Homozygosity mapping in consanguineous families reveals extreme heterogeneity of non-syndromic autosomal recessive mental retardation and identifies 8 novel gene loci. *Hum. Genet.* **121,** 43–48 (2007).
6. Kuss, A. W. *et al.* Autosomal recessive mental retardation: homozygosity mapping identifies 27 single linkage intervals, at least 14 novel loci and several mutation hotspots. *Hum. Genet.* **129,** 141–148 (2011).
7. Kaufman, L., Ayub, M. & Vincent, J. B. The genetic basis of non-syndromic intellectual disability: a review. *J. Neurodev. Disord.* **2,** 182–209 (2010).
8. Ng, S. B. *et al.* Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nature Genet.* **42,** 790–793 (2010).
9. Ng, S. B. *et al.* Exome sequencing identifies the cause of a mendelian disorder. *Nature Genet.* **42,** 30–35 (2010).
10. Lupski, J. R. *et al.* Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. *N. Engl. J. Med.* **362,** 1181–1191 (2010).
11. Cantagrel, V. *et al.* SRD5A3 is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder. *Cell* **142,** 203–217 (2010).
12. Kahrizi, K. *et al.* Next generation sequencing in a family with autosomal recessive Kahrizi syndrome (OMIM 612713) reveals a homozygous frameshift mutation in *SRD5A3*. *Eur. J. Hum. Genet.* **19,** 115–117 (2011).
13. Raas-Rothschild, A. *et al.* A PEX6-defective peroxisomal biogenesis disorder with severe phenotype in an infant, versus mild phenotype resembling Usher syndrome in the affected parents. *Am. J. Hum. Genet.* **70,** 1062–1068 (2002).
14. Steinfeld, R. *et al.* Folate receptor alpha defect causes cerebral folate transport deficiency: a treatable neurodegenerative disorder associated with disturbed myelin metabolism. *Am. J. Hum. Genet.* **85,** 354–363 (2009).
15. Shoichet, S. A. *et al.* Mutations in the *ZNF41* gene are associated with cognitive deficits: identification of a new candidate for X-linked mental retardation. *Am. J. Hum. Genet.* **73,** 1341–1354 (2003).
16. Jensen, L. R. *et al.* Mutations in the *JARID1C* gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. *Am. J. Hum. Genet.* **76,** 227–236 (2005).
17. Freude, K. *et al.* Mutations in the *FTSJ1* gene coding for a novel S-adenosylmethionine-binding protein cause nonsyndromic X-linked mental retardation. *Am. J. Hum. Genet.* **75,** 305–309 (2004).
18. Ramser, J. *et al.* A splice site mutation in the methyltransferase gene *FTSJ1* in Xp11.23 is associated with non-syndromic mental retardation in a large Belgian family (MRX9). *J. Med. Genet.* **41,** 679–683 (2004).
19. Klein, U., Ramirez, M. T., Kobilka, B. K. & von Zastrow, M. A novel interaction between adrenergic receptors and the α-subunit of eukaryotic initiation factor 2B. *J. Biol. Chem.* **272,** 19099–19102 (1997).
20. Schwarz, J. M., Rodelsperger, C., Schuelke, M. & Seelow, D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nature Methods* **7,** 575–576 (2010).
21. Geiduschek, E. P. & Kassavetis, G. A. The RNA polymerase III transcription apparatus. *J. Mol. Biol.* **310,** 1–26 (2001).
22. Hurov, K. E., Cotta-Ramusino, C. & Elledge, S. J. A genetic screen identifies the Triple T complex required for DNA damage signaling and ATM and ATR stability. *Genes Dev.* **24,** 1939–1950 (2010).
23. Zamponi, G. W., Lory, P. & Perez-Reyes, E. Role of voltage-gated calcium channels in epilepsy. *Pflugers Arch.* **460,** 395–403 (2010).
24. Singh, B. *et al.* Mutational analysis of *CACNA1G* in idiopathic generalized epilepsy. *Hum. Mutat.* **28,** 524–525 (2007).
25. Södersten, E., Lilja, T. & Hermanson, O. The novel BTB/POZ and zinc finger factor Zbtb45 is essential for proper glial differentiation of neural and oligodendrocyte progenitor cells. *Cell Cycle* **9,** 4866–4875 (2010).
26. Zhu, X. *et al.* Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects. *Proc. Natl Acad. Sci. USA* **99,** 10293–10298 (2002).
27. Billuart, P. *et al.* Oligophrenin-1 encodes a rhoGAP protein involved in X-linked mental retardation. *Nature* **392,** 923–926 (1998).
28. D'Adamo, P. *et al.* Mutations in GDI1 are responsible for X-linked non-specific mental retardation. *Nature Genet.* **19,** 134–139 (1998).
29. Jaffe, A. B., Aspenstrom, P. & Hall, A. Human CNK1 acts as a scaffold protein, linking Rho and Ras signal transduction pathways. *Mol. Cell. Biol.* **24,** 1736–1746 (2004).
30. Boison, D. *et al.* Neonatal hepatic steatosis by disruption of the adenosine kinase gene. *Proc. Natl Acad. Sci. USA* **99,** 6985–6990 (2002).
31. Tarpey, P. S. *et al.* A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. *Nature Genet.* **41,** 535–543 (2009).
32. Pak, C. *et al.* Mutation of the conserved polyadenosine RNA binding protein, ZC3H14/dNab2, impairs neural function in *Drosophila* and humans. *Proc. Natl Acad. Sci. USA* **108,** 12390–12395 (2011).
33. Garshasbi, M. *et al.* A defect in the *TUSC3* gene is associated with autosomal recessive mental retardation. *Am. J. Hum. Genet.* **82,** 1158–1164 (2008).
34. Mir, A. *et al.* Identification of mutations in *TRAPPC9*, which encodes the NIK- and IKK-β-binding protein, in nonsyndromic autosomal-recessive mental retardation. *Am. J. Hum. Genet.* **85,** 909–915 (2009).
35. Moheb, L. A. *et al.* Identification of a nonsense mutation in the very low-density lipoprotein receptor gene (VLDLR) in an Iranian family with dysequilibrium syndrome. *Eur. J. Hum. Genet.* **16,** 270–273 (2008).

36. Motazacker, M. M. *et al.* A defect in the ionotropic glutamate receptor 6 gene (*GRIK2*) is associated with autosomal recessive mental retardation. *Am. J. Hum. Genet.* **81,** 792–798 (2007).
37. Laumonnier, F., Cuthbert, P. C. & Grant, S. G. The role of neuronal complexes in human X-linked brain diseases. *Am. J. Hum. Genet.* **80,** 205–220 (2007).
38. Ullmann, R. *et al.* Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. *Hum. Mutat.* **28,** 674–682 (2007).
39. Yildirim, Y. *et al.* A frameshift mutation of ERLIN2 in recessive intellectual disability, motor dysfunction and multiple joint contractures. *Hum. Mol. Genet.* **20,** 1886–1892 (2011).
40. Alazami, A. M., Adly, N., Al Dhalaan, H. & Alkuraya, F. S. A nullimorphic *ERLIN2* mutation defines a complicated hereditary spastic paraplegia locus (SPG18). *Neurogenetics* doi:10.1007/s10048-011-0291-8 (2011).
41. Abou Jamra, R. *et al.* Adaptor protein complex 4 deficiency causes severe autosomal-recessive intellectual disability, progressive spastic paraplegia, shy character, and short stature. *Am. J. Hum. Genet.* **88,** 788–795 (2011).
42. Rafiq, M. A. *et al.* Mutations in the alpha 1,2-mannosidase gene, *MAN1B1*, cause autosomal-recessive intellectual disability. *Am. J. Hum. Genet.* **89,** 176–182 (2011).
43. Putoux, A. *et al.* KIF7 mutations cause fetal hydrolethalus and acrocallosal syndromes. *Nature Genet.* **43,** 601–606 (2011).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions H.H.R. and H.N. initiated and directed this study. H.H., M.G., W.C., S.H., K.W., V.K., R.U., K.K. and A.W.K. contributed to its design and coordination. H.N., K.K., A.T., P.J., V.H., D.W., M.C., I.R., F.M., C.H., A.D., A.R., M.J.S.B., M.F. and H.D. recruited patients and families, and K.K. and A.T. were responsible for the clinical investigations. F.B., S.G.F. and R.K. did the karyotyping. M.G. performed the linkage analyses and together with R.W. and H.H., he established data management tools. R.U. and I.M. performed exon enrichments, and C.J. and M.B. did the deep sequencing experiments. H.H. analysed the sequencing data and provided bioinformatics support. M.G., S.S.A., M.H., A.Z., M.M., L.P., L.N.V., L.A.M., F.L., B.L., S.E.-N., Z.F., J.H., L.M. and A.W.K. participated in the validation of the results and the segregation analyses. T.Z. performed the pathway analyses. H.H., M.G., A.Z., L.P., R.W., T.Z., L.M., A.W.K., A.T., K.K., H.N. and H.H.R. evaluated and interpreted the results, and H.H.R., H.H. and M.G. wrote the manuscript.

Author Information Raw sequencing data can be retrieved from the Sequence Read Archive (SRA), accession number SRA036250. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to H.H.R. (ropers@molgen.mpg.de) or K.K. (kkahrizi@uswr.ac.ir).

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Materials and Methods

Recruitment of families, clinical examination and autozygosity mapping

Families analysed in this study were recruited between 2004 and 2010, often in rural areas of Iran through a network of counsellors or genetic nurses that had been established by H.N. and co-workers before this study. Less than 10% of these families had a Turkish or Arabic background and were recruited elsewhere. Initially, we focused on non-syndromic forms of ARID, but since 2005, all families with consanguineous parents and at least two mentally handicapped children were included, because it proved impossible to distinguish strictly between non-syndromic and syndromic intellectual disability. All families were examined by experienced clinical geneticists; Wechsler Intelligence Scales for Children (WISC) and WAIS were used to assess the IQ in children and parents, and standard clinical assessment forms were used to document the findings. In only few families, IQ testing could not be performed due to lack of parental co-operation. These families were only included if clinical findings left no doubt about the mental impairment of the patients, and if they could be distinguished unambiguously from their healthy siblings. When undergoing their first examination, many of the patients were already in their twenties, and it was often difficult to assess the history and course of their disorder, particularly in rural areas. In some of the patients, the initial diagnosis had been non-syndromic intellectual disability, before sequencing revealed mutations in known genes for syndromic forms of intellectual disability. In all of these cases, clinical re-examination of the (now older) patients confirmed the molecular diagnosis. Numerous pedigrees, as well as the methods used for autozygosity mapping, have been described previously[5,6].

Exon enrichment and high-throughput sequencing

Custom-made Agilent SureSelect DNA Capture Arrays were used for the enrichment of exons from homozygous intervals, including, on average, 60 bp of flanking sequences on either side of the exon. Enriched exons were sequenced on an Illumina Genome Analyzer II, generating 76-bp single reads, which were aligned to the human reference genome (hg18).

Sequence coverage of targeted exons

More than 98% of the targeted exons were covered by at least four non-redundant sequence reads, each with a PHRED-like quality score of 20 or above (mean, 0.984; median, 0.993), which we deemed sufficient to detect homozygous sequence variants reliably. Details for each of the 136 ARID samples are given in Supplementary Table 5. In only eleven samples, including five from patients with putative mutations in novel candidate genes, the coverage

was lower than 95%. More than 90% of the 'missing' exons were part of repetitive reads that could not be unambiguously mapped back to the human genome.

Detection of homozygous variants by NGS

To assess further the reliability of targeted exon enrichment and Next Generation Sequencing (NGS) for calling homozygous mutations, we looked up all known genetic markers in these sequences which had been typed with SNP arrays upon homozygosity mapping. To maximize the power of this comparison, five families were chosen that had been analysed with high-resolution SNP arrays. In these families, the sequence coverage ranged from 91% to almost 100%. As shown in Supplementary Table 6, a total of 776 homozygous SNP markers were called in the respective intervals of the five selected families, and for 773 of these markers, identical genotypes were determined by NGS.

Calling of single nucleotide variants and exon-spanning deletions

As a first step, we removed low-quality sequencing reads flagged as such by the Illumina Genome Analyzer. Remaining reads were then aligned by SOAP2.20[84] with default settings, typically gap-free. Aligned reads were used to call single nucleotide variants (SNVs). Homozygous variants in the relevant linkage intervals were considered real if there were at least four non-identical supporting reads with a PHRED-like quality score of 20 or above, and if at least 80% of the reads showed the mutation.

Homozygous exon-spanning deletions were assumed if the sequence coverage of the relevant exon(s) was reduced to less than 5% of the mean, and if they were not present in the relevant databases of the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=195061365&c=chrX&g=wgEncodeHudsonalphaCnv; http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=195061365&c=chrX&g=dgv; http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=195061365&c=chrX&g=cnp). Such observations were validated by high-resolution array CGH, either by referring to SNP typing data obtained upon homozygosity mapping or, if the resolution of these SNP arrays was too low, by using custom-made high-resolution Agilent chips and/or by Sanger sequencing.

Algorithm for calling smaller indels

To detect smaller insertions and deletions (indels), we collected all 76-nucleotide single-end reads that could not be aligned to the human reference genome in a gap-free manner. If present in multiple copies, we retained only the read with the highest median quality score. Thirty-two-nucleotide strings, including the ends of these reads, were aligned to the human genome.

If both ends could be aligned at unique positions in the genome, we recorded their observed distance (D_O) and orientation (O_O) and compared it to the expected distance (D_E) and orientation (O_E). Insertions or deletions were called if $D_O < D_E$ or $D_O > D_E$, respectively. If O_O differed from O_E, or if the two ends of 76-nucleotide reads mapped to different chromosomes, this was taken as evidence for more complex rearrangements, such as inversions and translocations.

If only one of the two 32-bp strings could be aligned at a unique genomic position, we used it as an anchor and extracted a 10-nucleotide string from the other end of the read. Taking into consideration the orientation of the anchor, we then screened the adjacent 10,000 nucleotides for this 10-nucleotide string. If the 10-nucleotide string had the same orientation as the anchor, we recorded its position and compared its D_O with the D_E. For $D_O > D_E$ and $D_O < D_E$, deletions and insertions were called, respectively.

The exact breakpoints of insertions, deletions and other rearrangements were defined by pattern growth, allowing no mismatches.

Filtering out polymorphisms and selection of disease-causing variants

To eliminate previously reported, apparently non-pathogenic changes, all sequence variants were filtered against dbSNP130/131, whole genomes from 185 healthy individuals made available by the 1000 Genomes Project[85], and exomes from 200 Danish individuals[86]. In addition, the OMIM catalogue (http://www.ncbi.nlm.nih.gov/omim) and the Human Gene Mutation Database (HGMD, http://www.hgmd.org/) were used as a filter to identify all previously described pathogenic changes. We also excluded recurrent variants, even if they were observed only twice. A flowchart for this protocol is presented in Supplementary Fig. 1.

Among a total of 392 novel sequence variants (~3 per family, see Supplementary Table 2), protein-truncating changes were identified in 54 families, but 12 of these variants did not co-segregate with intellectual disability in the respective families, including recurrent *FOXD1* and *H6PD* frameshifts (see Supplementary Table 4). Several different criteria were used to rank and select probable disease-causing missense changes or small in-frame deletions, but only one of these criteria ((1) two or more families with the same disease phenotype carrying different, apparently pathogenic mutations of the same gene) was taken as sufficiently convincing to establish a role of the relevant gene in intellectual disability. Other criteria included: (2) the presence of a single non-polymorphic variant in the family; (3) the evolutionary conservation of the relevant nucleotide, as defined by the $\text{PhyloP}_{44\text{allvertebrate}}$ score; (4) the pathogenicity of these variants, as predicted by PolyPhen2, SIFT and eventually,

MutationTaster (http://www.mutationtaster.org/); and (5) the available biological and medical evidence supporting or refuting a role of this gene in brain function, including disease links in humans and animal models and a variety of other functional clues. All putative mutations were confirmed by Sanger sequencing, and all showed co-segregation with intellectual disability in the respective families.

Mutations in novel candidate genes are no stratification artefacts

To rule out the possibility that some of the 52 putative mutations in novel intellectual disability genes are common polymorphisms that are specific to the respective Iranian sub-population, we have screened between 100 and 468 chromosomes from Iranian controls (see Supplementary Table 3). Because there are several sub-populations in Iran, we have also specified the ethnic roots whenever this information could be obtained. All parents and healthy controls with a given ethnic background had grandparents that came from the same region. Only three of the 52 putative mutations were observed in controls, each in a single heterozygous carrier, including a missense change and two frameshift mutations (see Supplementary Table 3). Homozygosity was not observed for any of these variants, and carrier frequencies ranged between 1/53 and 1/96, indicating that even in ethnically matching—and possibly distantly related—controls, these variants are not common.

Data retrieval

Raw sequencing data can be retrieved from the Sequence Reads Archive (SRA), accession number SRA036250.

Supplementary Figures

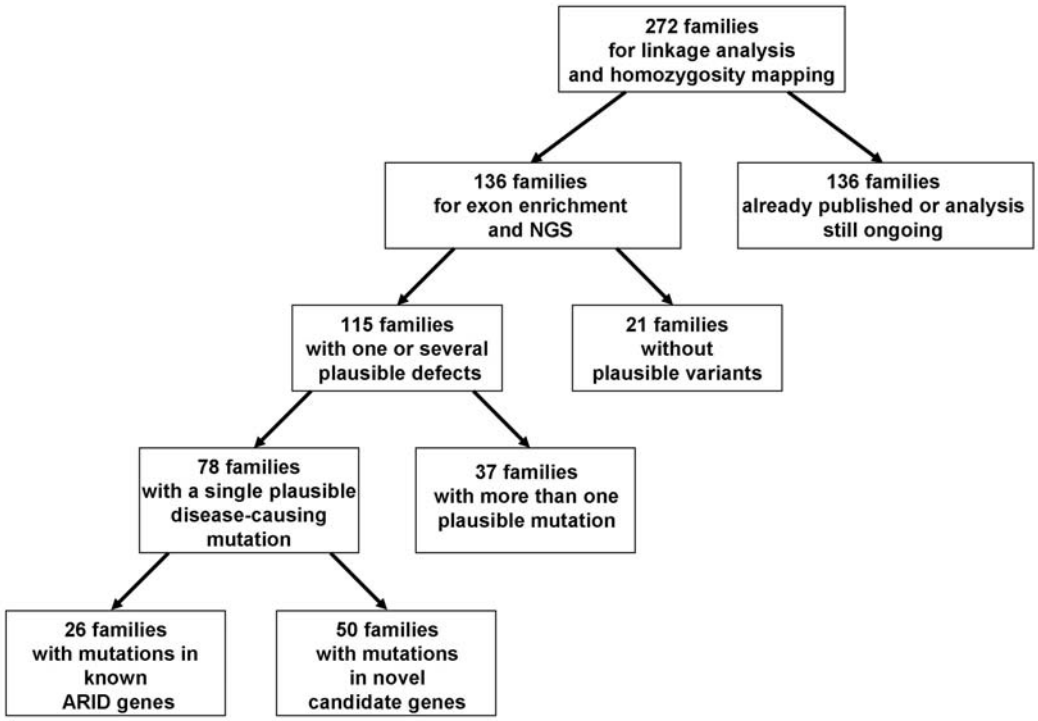

Figure S1: Unravelling autosomal recessive ID by targeted exon enrichment and NGS; overview and results. All of the 406 homozygous mutations identified by NGS were confirmed by Sanger sequencing (Table-S2). As a further test to assess the accuracy of massive parallel sequencing for calling homozygous mutations, SNP typing data, which had been generated upon homozygosity mapping, were used as controls. For five samples tested, more than 99.5% homozygous SNP markers defined by a high resolution Affymetrix SNP array in the targeted exons were confirmed by NGS (Table-S). For example, family 8700136 was chosen because in this family, sequencing covered all 162 SNP markers in the exonic portion of its sole homozygous 24 Mb interval (chr1: 219,421,375-243,764,133). As expected, all of these markers were found to be homozygous for the same allele as determined by array-based SNP typing, i.e., the results of SNP typing and NGS were completely identical.

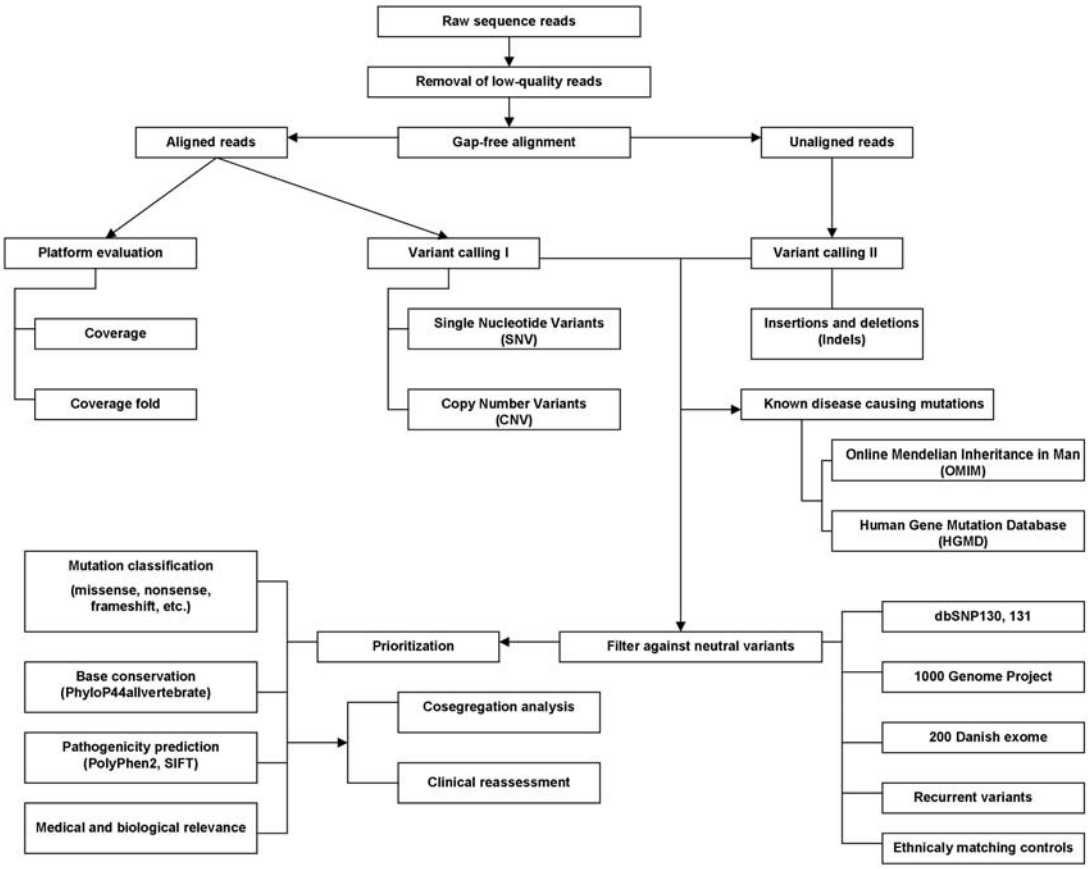

Figure S2: Variant calling, filtering and prioritization scheme.

Supplementary information to Fig. 1

Known and novel ID genes form protein and regulatory networks

As many as 22 of the novel candidate genes encode members of three protein networks. 14 of these proteins belong to a transcription/translation network, as illustrated in Fig. 1A. TAF2 (novel ARID gene) is a core component of RNA polymerase II complex. Trimethylation of histone H3 (H3T, novel ARID gene) at lysine 4 (H3K4me3) facilitates interaction with TAF3, linking histone modifications with the RNA Pol II-mediated transcription [87]. KDM6B (novel ARID gene) specifically demethylates Lys-27 of histone H3, whereas KDM5A (novel ARID gene) and KDM5C (known ID gene) demethylate Lys-4 of histone H3. Histone methyltransferase MLL2 (known ID gene) methylates 'Lys-4' of histone H3 (H3K4me) and associates with a core component of RNA Pol II, POLR2B, and activates transcription [88]. YY1 (known ARID gene) acts as transcriptional repressor by recruiting histone deacetylases (HDACs) [89-90].. C8ORF41 (novel ARID gene) associates with RUVBL2 [91], which is involved in the regulation of transcription and interacts with HDACs [50]. In yeast, RUVBL2 and a C8ORF41 homolog, TTI2, are part of the ASTRA complex, which may play a role in telomere maintenance [92]. PARP1 (novel ARID gene) covalently ADP-ribosylates the amino-terminal histone tails of all core histones [93]. KIF7 (novel ARID gene), a core regulator of Shh signaling, has been shown to associate with deubiquitinase USP22 [94] which deubiquitinates core histones H2A and H2B [95], and KIF7 interacts with the Gli2 transcription factor (known ID gene) controlling its proteolysis and stability in the Hedgehog signaling pathway [96]. ELP2 (novel ARID gene) and IKBKAP are components of RNA polymerase II elongator complex, which associates with RNA Pol II [97]. Mutations in IKBKAP were reported to result in familial dysautonomia, a severe neurodevelopmental disorder. Elongation factor EEF1B2 (novel ARID gene) is involved in the transfer of aminoacyl-tRNAs to the ribosome [98]. RNA methyltransferase TRMT1 dimethylates (m) tRNAs [79]. ADRA2B (novel ARID gene) links G protein–mediated signaling and cellular control of protein synthesis by interacting with EIF2B1 [19] and 14-3-3 zeta [99]. 14-3-3 interacts with RGS7 (novel ARID gene) which functions as GTPase-activating protein (GAP) stimulating the inactivation of heterotrimeric G proteins [100]. RGS7 acts to down-regulate $G_{\alpha q}$-mediated calcium mobilization [101]. CDK9 phosphorylates POLR2A [102] and associates with LARP7 (novel ARID gene) in 7SK RNP complex [70]. LARP7 negatively affects transcription of polymerase II genes.

Several novel candidate genes play a role in cell cycle control (see Fig.1b). Parf (C9ORF86, novel ARID gene) interacts with the cyclin-dependent kinase inhibitor, CDKN2A (ARF), contributing to CDKN2A-mediated growth arrest and normal cellular proliferation [103]. Cyclin A2 (CCNA2, novel ARID gene) binds and activates cyclin-dependent kinase 2 (CDK2) which promotes cell cycle G1/S and G2/M transitions [104]. The Cyclin A-Cdk2 complex phosphorylates the N-terminus of POLA1 and POLD3, leading to an inhibition of DNA polymerase-primase [105]. S-phase cyclin A-associated protein SCAPER (novel ARID gene) is a substrate and regulator of cyclin A-Cdk2 during different phases of the cell cycle, including S and G2/M [76].

5 novel ID candidate genes belong to the Ras/Rho/PSD-95 network (Fig. 1c)**.** 14-3-3 γ binds to AKAP13, which is induced by PKA. It suppresses the activation of Rho [106] which regulates the cytoskeletal architecture. 14-3-3 gamma interacts with, and is phosphorylated by, a protein kinase C isoform, PRKCG (known ID gene). FRY (novel ARID gene), which regulates the actin cytoskeleton and plays a key role in patterning sensory neuron dendritic fields in *Drosophila*, binds to 14-3-3 gamma [106]. CNKSR1 (novel ARID gene), a scaffold protein, links Rho and Ras signaling pathways [29]. RALGDS regulates RAS signal transduction by acting as a guanine nucleotide dissociation stimulator [107]. SCAPER (novel ARID gene) specifically interacts with the cyclin A/Cdk2 complex and regulates cell cycle progression [108]. PRRT2 (novel ARID gene) was found to interact with SNAP25 (known ID gene), a Q-SNARE protein contributing two α-helices in the formation of the exocytotic fusion complex in neurons where it assembles with syntaxin-1 (Stx1a), in a large scale protein-protein interaction study [55]. SNAP25 interacts with the calcium channel CACNA1A, which is linked to spinocerebellar

ataxia. CACNA1A-interacting L-type calcium channel beta1b (CACNB1) and CACNA2D1 modulate T-type, low voltage-activated calcium channel CACNA1G (novel ARID gene) [109].

References (continued)

44 Soupene, E., Serikov, V. & Kuypers, F. A. Characterization of an acyl-coenzyme A binding protein predominantly expressed in human primitive progenitor cells. *J Lipid Res* **49**, 1103-1112, (2008).

45 Yee, B. K., Singer, P., Chen, J. F., Feldon, J. & Boison, D. Transgenic overexpression of adenosine kinase in brain leads to multiple learning impairments and altered sensitivity to psychomimetic drugs. *Eur J Neurosci* **26**, 3237-3252, (2007).

46 Namavar, Y. *et al.* Clinical, neuroradiological and genetic findings in pontocerebellar hypoplasia. *Brain* **134**, 143-156, (2011).

47 Horton, S., Meredith, A., Richardson, J. A. & Johnson, J. E. Correct coordination of neuronal differentiation events in ventral forebrain requires the bHLH factor MASH1. *Mol Cell Neurosci* **14**, 355-369, (1999).

48 Yun, K. *et al.* Modulation of the notch signaling by Mash1 and Dlx1/2 regulates sequential specification and differentiation of progenitor cell types in the subcortical telencephalon. *Development* **129**, 5029-5040, (2002).

49 Giannandrea, M. *et al.* Mutations in the small GTPase gene RAB39B are responsible for X-linked mental retardation associated with autism, epilepsy, and macrocephaly. *Am J Hum Genet* **86**, 185-195, (2010).

50 Kim, J. H. *et al.* Transcriptional regulation of a metastasis suppressor gene by Tip60 and beta-catenin complexes. *Nature* **434**, 921-926, (2005).

51 Wang, X., Bao, X., Pal, R., Agbas, A. & Michaelis, E. K. Transcriptomic responses in mouse brain exposed to chronic excess of the neurotransmitter glutamate. *BMC Genomics* **11**, 360, (2010).

52 Bergeron, L. *et al.* Defects in regulation of apoptosis in caspase-2-deficient mice. *Genes Dev* **12**, 1304-1314, (1998).

53 Gong, D. & Ferrell, J. E., Jr. The roles of cyclin A2, B1, and B2 in early and late mitotic events. *Mol Biol Cell* **21**, 3149-3161, (2010).

54 Murphy, M. *et al.* Delayed early embryonic lethality following disruption of the murine cyclin A2 gene. *Nat Genet* **15**, 83-86, (1997).

55 Stelzl, U. *et al.* A human protein-protein interaction network: a resource for annotating the proteome. *Cell* **122**, 957-968, (2005).

56 Fellows, J., Erdjument-Bromage, H., Tempst, P. & Svejstrup, J. Q. The Elp2 subunit of elongator and elongating RNA polymerase II holoenzyme is a WD40 repeat protein. *J Biol Chem* **275**, 12896-12899, (2000).

57 Schaefer, U., Machida, T., Broekman, M. J., Marcus, A. J. & Levi, R. Targeted deletion of ectonucleoside triphosphate diphosphohydrolase 1/CD39 leads to desensitization of pre- and postsynaptic purinergic P2 receptors. *J Pharmacol Exp Ther* **322**, 1269-1277, (2007).

58 Pearce, M. M., Wormer, D. B., Wilkens, S. & Wojcikiewicz, R. J. An endoplasmic reticulum (ER) membrane complex composed of SPFH1 and SPFH2 mediates the ER-associated degradation of inositol 1,4,5-trisphosphate receptors. *J Biol Chem* **284**, 10433-10445, (2009).

59 Chirala, S. S. *et al.* Fatty acid synthesis is essential in embryonic development: fatty acid synthase null mutants and most of the heterozygotes die in utero. *Proc Natl Acad Sci U S A* **100**, 6358-6363, (2003).

60 Chiba, S., Ikeda, M., Katsunuma, K., Ohashi, K. & Mizuno, K. MST2- and Furry-mediated activation of NDR1 kinase is critical for precise alignment of mitotic chromosomes. *Curr Biol* **19**, 675-681, (2009).

61 Kuryshev, V. Y. *et al.* An anthropoid-specific segmental duplication on human chromosome 1q22. *Genomics* **88**, 143-151, (2006).
62 Kelly, A. E. *et al.* Survivin reads phosphorylated histone H3 threonine 3 to activate the mitotic kinase Aurora B. *Science* **330**, 235-239, (2010).
63 Wang, F. *et al.* Histone H3 Thr-3 phosphorylation by Haspin positions Aurora B at centromeres in mitosis. *Science* **330**, 231-235, (2010).
64 Yamagishi, Y., Honda, T., Tanno, Y. & Watanabe, Y. Two histone marks establish the inner centromere and chromosome bi-orientation. *Science* **330**, 239-243, (2010).
65 Sasaki, J. *et al.* The PtdIns(3,4)P(2) phosphatase INPP4A is a suppressor of excitotoxic neuronal death. *Nature* **465**, 497-501, (2010).
66 Lopez-Bigas, N. *et al.* Genome-wide analysis of the H3K4 histone demethylase RBP2 reveals a transcriptional program controlling differentiation. *Mol Cell* **31**, 520-530, (2008).
67 Agger, K. *et al.* UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature* **449**, 731-734, (2007).
68 Liem, K. F., Jr., He, M., Ocbina, P. J. & Anderson, K. V. Mouse Kif7/Costal2 is a cilia-associated protein that regulates Sonic hedgehog signaling. *Proc Natl Acad Sci U S A* **106**, 13377-13382, (2009).
69 Edwards, M. M. *et al.* Mutations in Lama1 disrupt retinal vascular development and inner limiting membrane formation. *J Biol Chem* **285**, 7697-7711, (2010).
70 Markert, A. *et al.* The La-related protein LARP7 is a component of the 7SK ribonucleoprotein and affects transcription of cellular and viral polymerase II genes. *EMBO Rep* **9**, 569-575, (2008).
71 Fan, G. *et al.* Targeted disruption of NDST-1 gene leads to pulmonary hypoplasia and neonatal respiratory distress in mice. *FEBS Lett* **467**, 7-11, (2000).
72 Fontan-Lozano, A. *et al.* Histone H1 poly[ADP]-ribosylation regulates the chromatin alterations required for learning consolidation. *J Neurosci* **30**, 13305-13313, (2010).
73 Gloerich, J. *et al.* Peroxisomal trans-2-enoyl-CoA reductase is involved in phytol degradation. *FEBS Lett* **580**, 2092-2096, (2006).
74 Fisk, J. C. *et al.* TbPRMT6 is a type I protein arginine methyltransferase that contributes to cytokinesis in Trypanosoma brucei. *Eukaryot Cell* **9**, 866-877, (2010).
75 Ilardi, J. M., Mochida, S. & Sheng, Z. H. Snapin: a SNARE-associated protein implicated in synaptic transmission. *Nat Neurosci* **2**, 119-124, (1999).
76 Tsang, W. Y. & Dynlacht, B. D. Double identity of SCAPER: a substrate and regulator of cyclin A/Cdk2. *Cell Cycle* **7**, 702-705, (2008).
77 Lee, J., Prohaska, J. R. & Thiele, D. J. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. *Proc Natl Acad Sci U S A* **98**, 6842-6847, (2001).
78 Exil, V. J. *et al.* Stressed-induced TMEM135 protein is part of a conserved genetic network involved in fat storage and longevity regulation in Caenorhabditis elegans. *PLoS One* **5**, e14228, (2010).
79 Liu, J. & Straby, K. B. The human tRNA(m(2)(2)G(26))dimethyltransferase: functional expression and characterization of a cloned hTRM1 gene. *Nucleic Acids Res* **28**, 3445-3451, (2000).
80 Tasaki, T. *et al.* Biochemical and genetic studies of UBR3, a ubiquitin ligase with a function in olfactory and other sensory systems. *J Biol Chem* **282**, 18510-18520, (2007).
81 Proikas-Cezanne, T. *et al.* WIPI-1alpha (WIPI49), a member of the novel 7-bladed WIPI protein family, is aberrantly expressed in human cancer and is linked to starvation-induced autophagy. *Oncogene* **23**, 9314-9325, (2004).
82 Matsuoka, S. *et al.* ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* **316**, 1160-1166, (2007).
83 Gustafson, M. P., Welcker, M., Hwang, H. C. & Clurman, B. E. Zcchc8 is a glycogen synthase kinase-3 substrate that interacts with RNA-binding proteins. *Biochem Biophys Res Commun* **338**, 1359-1367, (2005).

84 Li, R. *et al.* SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics* **25**, 1966-1967, (2009).
85 Durbin, R. M. *et al.* A map of human genome variation from population-scale sequencing. *Nature* **467**, 1061-1073, (2010).
86 Li, Y. *et al.* Resequencing of 200 human exomes identifies an excess of low-frequency non-synonymous coding variants. *Nat Genet* **42**, 969-972, (2010).
87 Vermeulen, M. *et al.* Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4. *Cell* **131**, 58-69, (2007).
88 Hughes, C. M. *et al.* Menin associates with a trithorax family histone methyltransferase complex and with the hoxc8 locus. *Mol Cell* **13**, 587-597, (2004).
89 Yang, W. M., Inouye, C., Zeng, Y., Bearss, D. & Seto, E. Transcriptional repression by YY1 is mediated by interaction with a mammalian homolog of the yeast global regulator RPD3. *Proc Natl Acad Sci U S A* **93**, 12845-12850, (1996).
90 Yang, W. M., Yao, Y. L., Sun, J. M., Davie, J. R. & Seto, E. Isolation and characterization of cDNAs corresponding to an additional member of the human histone deacetylase gene family. *J Biol Chem* **272**, 28001-28007, (1997).
91 Jeronimo, C. *et al.* Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme. *Mol Cell* **27**, 262-274, (2007).
92 Shevchenko, A. *et al.* Chromatin Central: towards the comparative proteome by accurate mapping of the yeast proteomic environment. *Genome Biol* **9**, R167, (2008).
93 Messner, S. *et al.* PARP1 ADP-ribosylates lysine residues of the core histone tails. *Nucleic Acids Res* **38**, 6350-6362.
94 Sowa, M. E., Bennett, E. J., Gygi, S. P. & Harper, J. W. Defining the human deubiquitinating enzyme interaction landscape. *Cell* **138**, 389-403, (2009).
95 Zhao, Y. *et al.* A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. *Mol Cell* **29**, 92-101, (2008).
96 Ben-Shachar, S. *et al.* Microdeletion 15q13.3: A locus with incomplete penetrance for autism, mental retardation, and psychiatric disorders. *J Med Genet*, jmg.2008.064378, (2009).
97 Hawkes, N. A. *et al.* Purification and characterization of the human elongator complex. *J Biol Chem* **277**, 3047-3052, (2002).
98 Le Sourd, F. *et al.* eEF1B: At the dawn of the 21st century. *Biochim Biophys Acta* **1759**, 13-31, (2006).
99 Prezeau, L., Richman, J. G., Edwards, S. W. & Limbird, L. E. The zeta isoform of 14-3-3 proteins interacts with the third intracellular loop of different alpha2-adrenergic receptor subtypes. *J Biol Chem* **274**, 13462-13469, (1999).
100 Benzing, T. *et al.* 14-3-3 interacts with regulator of G protein signaling proteins and modulates their activity. *J Biol Chem* **275**, 28167-28172, (2000).
101 Shuey, D. J., Betty, M., Jones, P. G., Khawaja, X. Z. & Cockett, M. I. RGS7 attenuates signal transduction through the G(alpha q) family of heterotrimeric G proteins in mammalian cells. *J Neurochem* **70**, 1964-1972, (1998).
102 Fu, T. J., Peng, J., Lee, G., Price, D. H. & Flores, O. Cyclin K functions as a CDK9 regulatory subunit and participates in RNA polymerase II transcription. *J Biol Chem* **274**, 34527-34530, (1999).
103 Tompkins, V., Hagen, J., Zediak, V. P. & Quelle, D. E. Identification of novel ARF binding proteins by two-hybrid screening. *Cell Cycle* **5**, 641-646, (2006).
104 Gong, D. & Ferrell, J. E., Jr. The roles of cyclin A2, B1, and B2 in early and late mitotic events. *Mol Biol Cell* **21**, 3149-3161.
105 Schub, O. *et al.* Multiple phosphorylation sites of DNA polymerase alpha-primase cooperate to regulate the initiation of DNA replication in vitro. *J Biol Chem* **276**, 38076-38083, (2001).
106 Jin, J. *et al.* Proteomic, functional, and domain-based analysis of in vivo 14-3-3 binding proteins involved in cytoskeletal regulation and cellular organization. *Curr Biol* **14**, 1436-1450, (2004).

107 Hofer, F., Fields, S., Schneider, C. & Martin, G. S. Activated Ras interacts with the Ral guanine nucleotide dissociation stimulator. *Proc Natl Acad Sci U S A* **91**, 11089-11093, (1994).
108 Tsang, W. Y., Wang, L., Chen, Z., Sanchez, I. & Dynlacht, B. D. SCAPER, a novel cyclin A-interacting protein that regulates cell cycle progression. *J Cell Biol* **178**, 621-633, (2007).
109 Dubel, S. J. *et al.* Plasma membrane expression of T-type calcium channel alpha(1) subunits is modulated by high voltage-activated auxiliary subunits. *J Biol Chem* **279**, 29263-29269, (2004).