Fragile X syndrome

Randi J. Hagerman[1,2], Elizabeth Berry-Kravis[3], Heather Cody Hazlett[4], Donald B. Bailey Jr[5], Herve Moine[6–9], R. Frank Kooy[10], Flora Tassone[1,11], Ilse Gantois[12,13], Nahum Sonenberg[12,13], Jean Louis Mandel[6–9] and Paul J. Hagerman[1,11]

Abstract | Fragile X syndrome (FXS) is the leading inherited form of intellectual disability and autism spectrum disorder, and patients can present with severe behavioural alterations, including hyperactivity, impulsivity and anxiety, in addition to poor language development and seizures. FXS is a trinucleotide repeat disorder, in which >200 repeats of the CGG motif in *FMR1* leads to silencing of the gene and the consequent loss of its product, fragile X mental retardation 1 protein (FMRP). FMRP has a central role in gene expression and regulates the translation of potentially hundreds of mRNAs, many of which are involved in the development and maintenance of neuronal synaptic connections. Indeed, disturbances in neuroplasticity is a key finding in FXS animal models, and an imbalance in inhibitory and excitatory neuronal circuits is believed to underlie many of the clinical manifestations of this disorder. Our knowledge of the proteins that are regulated by FMRP is rapidly growing, and this has led to the identification of multiple targets for therapeutic intervention, some of which have already moved into clinical trials or clinical practice.

Fragile X syndrome (FXS) is caused by the deficiency or absence of fragile X mental retardation 1 protein (FMRP; also known as synaptic functional regulator FMR1), an RNA binding protein with a prominent role in the regulation of a large number of mRNAs in post-synaptic neurons. FXS is most commonly caused by a trinucleotide repeat expansion of CGG in the promoter region of *FMR1*, located at Xq27.3, leading to methylation, transcriptional silencing and the absence or deficiency of FMRP. Individuals with *FMR1* containing >200 CGG repeats have the full mutation, and those with between 55 and 200 CGG repeats carry the premutation and have excessive transcription of *FMR1* (BOX 1). In addition, some individuals with FXS have mosaicism of CGG repeat lengths, with some cells harbouring full mutation alleles and others harbouring premutation alleles. Other individuals with FXS have methylation mosaicism, with some cells containing methylated *FMR1* alleles and others with unmethylated *FMR1* alleles. Both types of mosaicism will support the production of some FMRP, so those individuals might have less-severe cognitive and behavioural defects than someone with a full mutation that is completely methylated, in whom FMRP is absent. With the frequent use of high-throughput targeted screening techniques and whole-exome sequencing in clinical practice, an increasing number of individuals with a deletion or point mutation in *FMR1* have been reported[1,2]. These mutations lead to a dysfunction or absence of FMRP, and individuals have features of FXS, which can be variable or similar to those of patients with FXS and the full mutation. These patients represent <1% of individuals with FXS, although this might increase as more individuals are tested with whole-exome sequencing.

In general, infants with FXS are often hypotonic, with an initial poor suck and frequent regurgitation[3], but most patients present with delays in language development and emerging hyperactivity, anxiety and sensory over-reactivity in the second year of life[3–6] (FIG. 1). The physical features of FXS include prominent ears, a long face, flat feet, hyperextensible finger joints, double-jointed thumbs, soft skin and macro-orchidism that develops at the time of puberty[3,7,8], although altered physical features are not present in all patients (FIG. 2). These features are related to growth and connective tissue changes, including abnormalities of elastin fibres[3,9]. Other manifestations of FXS related to loose connective tissue include hernias, joint dislocations and flat feet with pronation[3,7]. Some individuals with a high-end premutation (that is, those with >130 repeats) demonstrate a mild deficit of FMRP and some features of FXS, such as prominent ears, attention-deficit/hyperactivity disorder (ADHD), problems with motor coordination, anxiety and social deficits[10].

The manifestations of FXS are variable and depend on sex, age, background genetic effects, environmental influences and molecular variations (such as the level of methylation or the presence of mosaicism of repeat size or methylation), which lead to differences in FMRP production[11–13]. In addition, the level of FMRP correlates with the activation ratio (that is, the percentage of cells with the normal *FMR1* allele on the active X chromosome)

Correspondence to R.J.H. MIND Institute, UC Davis Health, University of California, Davis, 2826 50th Street, Sacramento, California 95817, USA.
rjhagerman@ucdavis.edu

Article number: 17065
doi:10.1038/nrdp.2017.65
Published online 29 Sep 2017

Author addresses

[1]MIND Institute, UC Davis Health, University of California, Davis, 2826 50th Street, Sacramento, California 95817, USA.
[2]Department of Pediatrics, School of Medicine, University of California, Davis, Sacramento, California, USA.
[3]Department of Pediatrics, Neurological Sciences, Biochemistry, Rush University Medical Center, Chicago, Illinois, USA.
[4]Carolina Institute for Developmental Disabilities and Department of Psychiatry, University of North Carolina, Chapel Hill, North Carolina, USA.
[5]RTI International, Research Triangle Park, North Carolina, USA.
[6]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France.
[7]Centre National de la Recherche Scientifique, Illkirch, France.
[8]Institut National de la Santé et de la Recherche Médicale, Illkirch, France.
[9]Université de Strasbourg, Strasbourg, France.
[10]Department of Medical Genetics, University of Antwerp, Antwerp, Belgium.
[11]Department of Biochemistry and Molecular Medicine, School of Medicine, University of California, Davis, California, USA.
[12]Department of Biochemistry, McGill University, Montréal, Québec, Canada.
[13]Rosalind and Morris Goodman Cancer Research Centre, McGill University, Montréal, Québec, Canada.

in blood, and this also correlates with the clinical severity of FXS[14]. Consequently, females typically have less-severe manifestations than males, as *FMR1* on the other X chromosome can produce FMRP. It is rare for an individual to be completely unaffected by the full mutation; the mildest clinical involvement is associated with a complete lack of *FMR1* methylation in males and with a very favourable (that is, >0.75) activation ratio in females.

This Primer discusses the epidemiology, molecular pathophysiology, human neuroimaging, diagnosis and management of FXS. The animal studies described in the molecular pathophysiology section set the stage for understanding the use of targeted treatments in FXS, which is the dominant section of this Primer as this is of primary importance to clinicians and families. The quality of life (QOL) of the families is also a substantial concern for clinicians and is addressed.

Epidemiology

The prevalence of the FXS full mutation in the general population is estimated as 1 in 5,000 in males and as 1 in 4,000 to 1 in 8,000 in females. In males, two large studies have been carried out in neonates; a prevalence of 1 in 5,161 for the full mutation was found after the screening of 36,124 newborn males in one study in the United States[15], and a prevalence of 1 in 6,209 males was found after the screening of 24,449 neonates in Québec, Canada[16]. Other studies have found a higher prevalence of the full mutation in males but were carried out on much smaller sample sizes, precluding sufficient power for reliable estimates[17,18]. Moreover, the prevalence can vary in different parts of the world because of founder effects or racial or ethnic differences in haplotypes that might predispose to CGG expansions (reviewed in REF. 19). For example, a very high prevalence of FXS (1 in 19 males and 1 in 46 females) has been reported in Ricaurte, a small town in Colombia (R.J.H. and F.T., unpublished observations); this finding is likely a consequence of strong positive founder effects. One meta-analysis of >50 prevalence studies applied a statistical model that accounted for the characteristics of the populations (for example, individuals with or without intellectual disability), and determined that the prevalence of the full mutation was 1 in 7,143 males and 1 in 11,111 females[20]. Estimates based on the screening of individuals with special needs likely overestimate the true frequency of FXS in the general population. In the largest screening study carried out in newborn females in Québec, full mutations were not found in 12,032 individuals[16].

The prevalence of the premutation ranges from ~1 in 250 to 1 in 813 males and ~1 in 110 to 1 in 270 females[20,21]. Similar results were found in a meta-analysis that included data from >90,000 females and 50,000 males who underwent screening[20,21]. The variations in prevalence across studies can be attributed to several factors, including the use of different allele sizes for the CGG repeat number, particularly for the intermediate and the premutation categories, the use of different detection systems for allele sizing and variations in ethnic background (reviewed in REF. 21).

Mechanisms/pathophysiology

Molecular pathophysiology

FMRP is an RNA binding protein[22,23] with a role in the translational control of several mRNAs in the postsynaptic compartment of neurons, which is linked to group 1 metabotropic glutamate receptor (mGluRI) activation status[24,25]. Other cellular functions of FMRP have been proposed, including activation of the potassium channels KCNT1 (also known as Slack) and BK[26,27], a chromatin-dependent role in the DNA damage response[28] and a role in RNA editing[29]. FMRP can also regulate neuronal activity[30], including hippocampal-dependent learning[31] and the endocannabinoid system.

The FXS full mutation leads to epigenetic silencing of *FMR1*, which is characterized by DNA methylation of the promoter region and modification of histone marks[32]. The threshold effect and the mechanism of epigenetic *FMR1* silencing are not well understood but can be studied using stem cells from embryos carrying the full mutation that were identified during preimplantation genetic diagnosis[33]. *FMR1* silencing occurs at ~11 weeks of gestation and seems to be related to histone H3 dimethylation marking, which is mediated by DNA–RNA duplex formation between the trinucleotide repeat region of *FMR1* DNA and its mRNA counterpart[34]. The mechanism underlying the CGG expansion is not fully understood, but it might be due to an alteration in replication origin usage, together with a stalling of the replication fork at the CGG repeats, which could promote repeat instability[35]. An alternative and not necessarily mutually exclusive mechanism involves abnormal DNA repair[36]; indeed, the DNA mismatch repair complex MutSβ is necessary for the repeat expansion in the germ line of a mouse model[37].

FMRP is ubiquitous, although expression is normally highest in the brain and testicles; indeed, FMRP has been found in all neurons in the mouse brain, across developmental stages[38]. The finding that a loss of FMRP function causes FXS was confirmed by the identification of conventional truncating mutations in patients who have a clinical phenotype similar to those with the FXS

full-mutation and non-expanded CGG repeats (see REF. 2 for a compilation of such mutations) and validates the use of *Fmr1*-knockout animal models, which were first created in 1994, to study FXS pathophysiology[39,40]. These mice have a mild cognitive deficit, hyperactivity, macro-orchidism and increased sensitivity to auditory stimuli, leading to epileptic seizures, which is comparable with symptoms in patients with FXS, in addition to a diminished acoustic startle reflex[39,41]. Similar to patients with FXS, *Fmr1*-knockout mice have dendritic spines that are seemingly immature, and with an increased density, but, unlike in patients, the mice have decreased anxiety symptoms when assessed using the open-field test in some studies, and anxiety levels are not altered in the elevated plus maze[39]. Despite the subsequent generation of point mutation and conditional repeat expansion mouse models, the original knockout model is still the most frequently used as it is the best characterized and recapitulates many of the features of the human syndrome.

Studies using animal models have revealed several alterations in the brain that could underlie the clinical manifestations of FXS. This includes discrete alterations in neuronal plasticity in specific brain regions, including an increase in long-term depression (LTD) in the hippocampus[42] and cerebellum[43], and a decrease in long-term potentiation in the hippocampus, amygdala and several cortical areas[44,45], in addition to decreased γ-aminobutyric acid (GABA) signalling, and increased protein translation[46,47]. These alterations probably account for the increased excitability of neuronal circuits observed in FXS[48], which could relate to the hypersensitivity or over-reactivity to stimuli in patients. Indeed, a deficit in habituation that correlates to the FMRP deficit has been noted in children with FXS[49] and, accordingly, repetitive sensory stimuli have been shown to lead to sympathetic hyperarousal and anxiety in patients[6,49].

Glutamatergic signalling. Excessive glutamate signalling is thought to underlie several of the clinical manifestations of FXS. Indeed, mGluRI-dependent LTD (mGluRI-LTD) is increased in the hippocampus[42] and the cerebellum of *Fmr1*-knockout mice[43]. The implication of excessive mGluRI signalling was confirmed when genetic reduction or pharmacological block of these receptors corrected several phenotypes of the *Fmr1*-knockout mouse, including seizures, hyperactivity and neuronal structural changes[50]. These data highlighted mGluRI antagonists as a potential treatment for FXS[51,52], but clinical trials have shown mixed success (see Management, below). The negative outcomes of the clinical trials call into question the putative major role attributed to the mGluR hypothesis of FXS, although general support exists for this model through data from *Fmr1*-knockout mice[42,53].

Ultimately, one of the main outcomes of mGluRI activation is the translation of postsynaptic proteins that are proposed to potentiate synaptic plasticity, specifically mGluRI-LTD[54], by remodelling the protein content of dendritic spines (notably, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor availability by promoting their internalization)[55]. Downstream effectors of mGluRI signalling are altered in the absence of FMRP; for example, phosphorylation of eukaryotic translation initiation factor 4E (EIF4E) by MAP kinase-interacting serine/threonine-protein kinase (MNK) is increased[56] and phosphorylation of ribosomal protein S6 kinase (S6K) by extracellular-signal-regulated kinase (ERK)[57,58] is increased in post-mortem brain samples from patients with FXS (FIG. 3). Elevated phosphorylation of EIF4E and S6K likely causes the excess protein synthesis of FMRP-target mRNAs observed *in vivo* and *in vitro* in several areas of the *Fmr1*-knockout mouse brain, notably in hippocampal and cortical neurons[46,47]. This increased protein synthesis seems to be of key importance for the pathophysiology of FXS, as several inhibitors of translation have rescuing effects on the mouse phenotypes[24]. The prevailing view, one that agrees with the numerous reports from many laboratories and that largely relies on the mRNA and ribosome binding properties of FMRP[22,23], posits that FMRP is involved in the translational control of a large number of mRNAs in the postsynaptic compartment of neurons[24,25].

Several techniques have been used to identify which gene products are altered by the lack of FMRP (BOX 2). Among the hundreds of proposed deregulated FMRP target mRNAs, only a few have been validated at the protein level through the rescue of some phenotypes of the *Fmr1*-knockout mouse with the experimental manipulation of protein expression or function. The deregulated proteins with a possibly more-prominent role in the pathophysiology of FXS include several $GABA_A$ and $GABA_B$ receptor subunits[59], phosphatidylinositol 3-kinase enhancer (PIKE)[60], matrix metalloproteinase 9 (MMP9)[56,61], glycogen synthase kinase 3 (GSK3)[62], amyloid-β A4 protein (also known as amyloid precursor protein (APP))[63,64] and diacylglycerol kinase-κ (DGKκ; FIG. 3)[65]. The increased expression of PIKE, GSK3 and APP add to the synaptic plasticity deficits in FXS, and inhibiting these molecules might be helpful for the treatment of patients but has not yet been evaluated in human studies[60,63,64].

Box 1 | **Premutation disorder**

The *FMR1* premutation is associated with several distinct disorders that are caused by excessive transcription of *FMR1* (REF. 214), in contrast to the gene silencing caused by the full mutation in individuals with fragile X syndrome (FXS)[215]. The excess *FMR1* mRNA is thought to lead to toxicity through one or more specific mechanisms (reviewed in REFS 21,216), particularly with ageing. Approximately 40% of male premutation carriers and 16% of female premutation carriers who are ≥50 years of age will develop fragile X-associated tremor/ataxia syndrome, a neurodegenerative disorder associated with an intention tremor, cerebellar ataxia, neuropathy and cognitive decline[21,216]. In addition, ~20% of women with the premutation will develop fragile X-associated primary ovarian insufficiency[217]. Moreover, depression, anxiety, hypertension, restless legs syndrome, sleep apnoea, migraine, fibromyalgia and hypothyroidism are more common in premutation carriers than individuals without the premutation (reviewed in REF. 218).

FMRP is associated with *MMP9* mRNA, which encodes an endopeptidase that is important for dendritic spine maturation and synapse formation[66]. MMP9 levels are increased in FXS but can be lowered to normal levels with minocycline treatment[67]. Indeed, in *Fmr1*-knockout mice, treatment with minocycline improves dendritic spine maturation, synapse formation, anxiety levels and performance on a cognitive task (maze performance)[68], as well as ultrasonic vocalizations (which indicates increased language abilities)[69]. In addition, metformin can lower MMP9 levels in the *Fmr1*-knockout mouse. Both of these studies set the stage for trials in patients[69,70].

In mouse cortical neurons, FMRP has been shown to be predominantly associated with *Dgkk* mRNA[65], which encodes a protein that converts diacylglycerol (DAG) to phosphatidic acid (PA)[65]. Indeed, FMRP positively controls DGKκ translation, but how FMRP specifically interacts with *Dgkk* mRNA is unknown. DGKκ is a member of the DGK family[71], which contains 10 isoforms that are mainly expressed in the brain; out of these, only *Dgkk* mRNA can bind to FMRP. DGKs are involved in diverse biological events such as growth factor-dependent or cytokine-dependent cell proliferation and motility, seizure activity, immune responses, cardiovascular responses and glucose metabolism[72]. Thus, DGK proteins have been proposed to contribute to the pathogenesis of a wide variety of diseases, such as cancer, epilepsy, autoimmunity, cardiac hypertrophy, bipolar disorder, Parkinson disease, hypertension, type 2 diabetes mellitus and hypospadias, a common congenital hypoplasia of the penis[73].

DGKκ expression is decreased in the *Fmr1*-knockout mouse[65], which causes an imbalance of DAG (which triggers dendritic spine growth in neurons) and PA (which induces dendritic spine stabilization) levels[65,74] (FIG. 3). DAG levels are increased in *Fmr1*-knockout mice and in the cerebellum of patients with FXS, whereas PA synthesis is impaired in neurons from *Fmr1*-knockout mice, which is consistent with the observed increased dendritic spine growth and reduced stabilization (for review, see REF. 75). Furthermore, reduction of DGKκ expression in the mouse striatum by short hairpin RNA caused autistic behaviours, similar to those observed in the *Fmr1*-knockout mouse, including impaired social interaction, hyperactivity and altered nest-building[65]. In addition, silencing of *Dgkk* in CA1 pyramidal neurons in the hippocampus of wild-type mice caused an increase in the number of immature spines and a decrease in mature spines, similar to the *Fmr1*-knockout mouse[65]. In addition, overexpression of DGKκ in the *Fmr1*-knockout mouse rescued the impaired dendritic spine morphology[65]. Together, these findings introduce a molecular mechanism whereby loss of FMRP leads to an imbalance in DAG and PA levels, which might underlie the increased protein synthesis, autistic behaviours and abnormal dendritic spine morphology in the *Fmr1*-knockout mouse[65]. In this model, FMRP controls general protein translation within dendritic spines by an indirect (DAG-mediated) rather than a direct (RNA-binding) mechanism. Based on these data, targeting DGKκ signalling might provide new therapeutic approaches for FXS.

Endocannabinoid system. The absence of FMRP also dysregulates the endocannabinoid system, which consists of receptors located in the brain and periphery that are involved in numerous processes (such as synaptic plasticity, cognitive performance, anxiety, nociception and seizure susceptibility) and the endogenous cannabinoid ligands (that is, endocannabinoids): anandamide

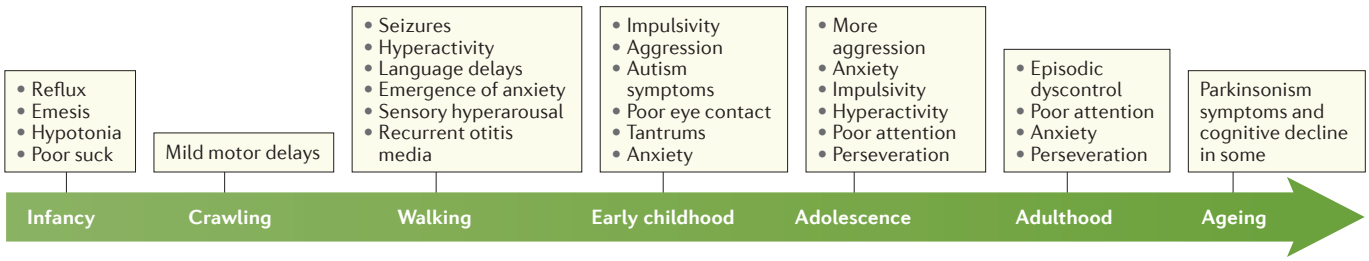

Figure 1 | **Clinical features of FXS.** Most infants with fragile X syndrome (FXS) have an initial poor latch or suck with breastfeeding, and they frequently experience recurrent emesis because of reflux. Recurrent otitis media is observed in >60% of patients in the first few years of life and usually requires the insertion of ventilation tubes (pressure-equalization tubes) to normalize hearing[13]. After the first year of life, tactile defensiveness begins to emerge, individuals have poor eye contact and a tendency to hand-flap with excitement; hand biting or chewing on clothes are also common. Up to 20% of patients have strabismus (that is, crossed eyes or lazy eyes), and if this persists after the first year of life, ophthalmological treatment is needed[3]. Many children with FXS have emerging anxiety and sensory hyperarousal in their second year of life, and once they are able to walk, they typically become hyperactive. Indeed, 80% of boys with FXS have substantial hyperactivity by 3–4 years of age and are diagnosed with attention-deficit/hyperactivity disorder (ADHD), although only 40% of girls with FXS are diagnosed with ADHD by school age[3,246,247]. Children begin overstuffing their mouth with food because of sensory deficits by 3 years of age, and obesity is reported in ~35% of patients by adolescence[162]. If hypotonia is a substantial problem during infancy, motor delays in sitting and walking might occur. Seizures occur in ~8–16% of males and 3–7% of females with FXS, typically present in the first 5 years of life, and are the most substantial medical problem for children with FXS[4,7,248]. Seizures are most commonly partial complex seizures but can also be generalized tonic–clonic or absence seizures[4,248]. Symptoms of autism spectrum disorder (ASD) can develop during early childhood, and ~50–60% of males and 20% of females with FXS also have ASD[43,139,140,233,249]. Intellectual disability is common in males with FXS, although ~15% of males (predominantly those with mosaicism) and 70% of females have an IQ in the borderline to normal range but have learning and emotional problems[12,250]. Many of the initial symptoms of FXS, such as impulsivity, anxiety and poor attention, persist into adulthood, and ~86% of males and 77% of females with FXS meet the diagnostic criteria for an anxiety disorder[6]. During the late stages of adulthood, ~17% of patients with FXS can present with symptoms of parkinsonism and cognitive decline[251]. Individuals with FXS can also have sleep disturbances, mainly waking up in the middle of the night and not being able to go back to sleep, especially in the first 3–4 years of life[3].

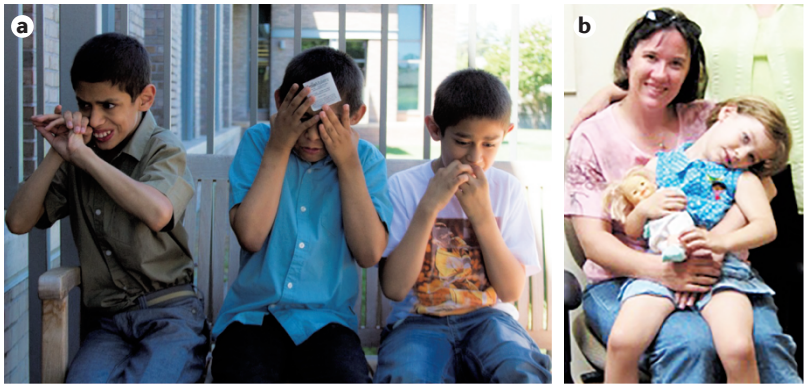

Figure 2 | **Physical features of FXS. a** | Three brothers with fragile X syndrome (FXS) show prominent ears, a physical feature of FXS. The boys have typical behaviour features, such as stereotyped movements of the hands (the boy on the left), avoiding eye contact (the boy in the centre) and hand biting (the boy on the right). **b** | A mother and daughter with FXS who have no obvious physical features.

and 2-arachidonoylglycerol (2-AG). The endocannabinoids bind to the G protein-coupled receptors CB1 and CB2 and modulate synaptic activity[76,77]. 2-AG is the most abundant endocannabinoid in brain and is produced locally within dendritic spines following mGluRI activation (FIG. 3). In the FXS mouse model, endogenous stimulation of 2-AG receptors is altered because the absence of FMRP leads to 2-AG-dependent and mGluRI-dependent synaptic plasticity abnormalities, including enhanced LTD at inhibitory synapses[78–80] and decreased LTD at excitatory synapses[81]. Modifying 2-AG signalling by inhibiting 2-AG degradation[81] or blocking CB1 and CB2 receptors[80] can normalize several phenotypes of the FXS mouse.

Ion channel dysfunction. Voltage-gated ion channels, which have a key role in many aspects of neuronal transmission, are involved in the pathophysiology of FXS. FMRP can directly bind to the sodium-activated potassium channel Slack[26], the large-conductance BK channel[82] and the N-type Ca^{2+} channel $Ca_v2.2$ (REF. 83), and the absence of FMRP in patients with FXS might result from a loss of these protein–protein interactions. FMRP binds mRNAs of several ion channels, including Kv3.1 and Kv4.2 voltage-dependent K^+ channels, non-selective HCN1 channels, and $Ca_v1.3$ Ca^{2+} channels[84–86]. Accordingly, the absence of FMRP is likely to alter the transport and/or translation of the target mRNAs, which could influence the excitability and firing rates of neurons.

GABAergic system. The contribution of abnormalities in the GABAergic system to the disturbance of the excitatory–inhibitory imbalance that is hypothesized to play a central part in the pathophysiology of FXS and autism spectrum disorder (ASD) in general has been explored extensively[48,87]. Several alterations in GABAergic signalling have been detected in the brains of *Fmr1*-knockout mice, including a reduction in the expression of several GABA receptor subunits and a reduction in GABAergic signalling (FIG. 4).

A series of electrophysiological recordings have demonstrated that $GABA_A$ receptor-mediated signalling is compromised in the *Fmr1*-knockout mouse[88–94]. Consistent with the subunit composition measurements in knockout mice, the electrophysiological differences seem to be age-dependent and brain region-dependent. Indeed, defects in phasic (synaptic) and tonic (extrasynaptic) inhibitory signalling and a delay in the transition from excitatory to inhibitory GABAergic signalling during development have been observed in *Fmr1*-knockout mice[95]. Moreover, the oxytocin-mediated, brief transient GABA excitation–inhibition shift that occurs in newborn rodents during delivery is absent from the hippocampal neurons of *Fmr1*-knockout mice[96].

Collectively, the disturbances in GABAergic signalling in *Fmr1*-knockout mice indicate that inhibitory signalling could be targets for novel treatments for FXS. Indeed, preclinical studies in animal models confirmed that the $GABA_A$ receptor is a suitable target[51,97,98]. In a large drug screen in *Fmr1*-deficient *Drosophila*, nine compounds that corrected specific phenotypes of the flies were identified[99] and, of these, three can restore GABA homeostasis. Gaboxadol (also known as THIP), a superagonist of δ-subunit-containing $GABA_A$ receptors, can rescue hyperexcitability of principal neurons of the amygdala of *Fmr1*-knockout mice and can improve some specific behavioural characteristics, including hyperactivity and auditory seizures[100]. Synthetic neurosteroids such as ganaxolone are potent agonists of $GABA_A$ receptors[101] and can prevent audiogenic seizures[102] and correct repetitive and/or perseverative behaviours in the *Fmr1*-knockout mouse[103]. Prenatal treatment of *Fmr1*-knockout mice with bumetanide (which can induce a transient switch from excitatory to inhibitory signalling in GABAergic neurons) restored electrophysiological abnormalities in the mutant offspring as well as hyperactivity and autistic behaviours, consistent with the reported absence of this excitatory–inhibitory switch in these mice[96].

The BMPR2–cofilin pathway. The mRNA encoding bone morphogenetic protein receptor type 2 (BMPR2), which is involved in dendrite formation, is a binding target of FMRP[104]. Depletion of FMRP increases BMPR2 expression and activation of BMP signalling to increase actin polymerization and altered dendritic spine morphology (FIG. 5). Indeed, the amount of full-length BMPR2 and a marker of LIM domain kinase 1 (LIMK1) activity were increased in the prefrontal cortex of patients with FXS compared with post-mortem prefrontal cortex tissue from healthy individuals, suggesting that increased BMPR2 signal transduction is linked to FXS and might be a putative therapeutic target for FXS and possibly ASD. In this regard, an LIMK1 inhibitor reversed the abnormal dendritic spine morphology of *Fmr1*-null neurons to the wild-type phenotype in the knockout mouse, suggesting that LIMK1 inhibitory treatment could ameliorate the abnormally high turnover of dendritic protrusions observed in *Fmr1*-null neurons[104].

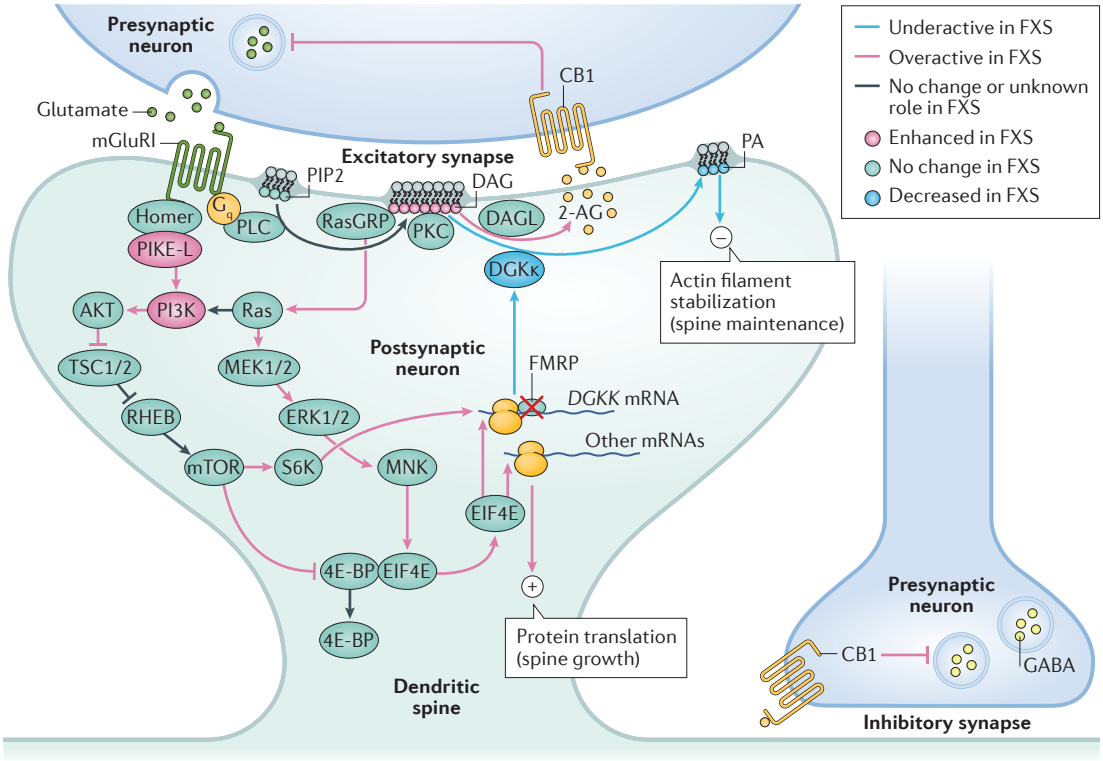

Figure 3 | **Glutamatergic signalling and DGKκ deregulation in FXS.** Group I metabotropic glutamate receptor (mGluRI) signalling is enhanced in fragile X syndrome (FXS). mGluRIs are positively coupled to phospholipase C (PLC) and adenylyl cyclase via G proteins, and mGluRI signalling leads to the production of inositol triphosphate, which causes the release of Ca^{2+} from intracellular stores (not shown). In addition, mGluRI signalling leads to the production of diacylglycerol (DAG), which in turn activates protein kinase C (PKC), the phosphoinositide 3-kinase (PI3K)–AKT–mechanistic target of rapamycin (mTOR) pathway and the extracellular-signal-regulated kinase (ERK) pathway[252,253]. DAG is converted into 2-arachidonoylglycerol (2-AG) by diacylglycerol lipase (DAGL), which suppresses synaptic transmission of glutamate and γ-aminobutyric acid (GABA) via cannabinoid type 1 (CB1) receptors[254]. In the absence of FMRP, several downstream effectors of mGluRI-dependent signalling are over-activated, including PKC[255], the RAS–MEK–ERK pathway[57,256], the PI3K–AKT pathway[60,257,258] and CB1 receptors[78–80], and others are defective (such as RAC–PAK[259]). Enhanced mGluRI signalling alters the expression of several proteins, including diacylglycerol kinase-κ (DGKκ). DGKκ phosphorylates DAG to phosphatidic acid (PA); thus, DGKκ terminates DAG signalling and initiates PA signalling. DAG is the direct effector of a number of pathways, including those involving PKCs, the RAS–MEK–ERK pathway and the PI3K–AKT pathway, and leads to an increase in protein translation by activating eukaryotic translation initiation factor 4E (EIF4E)[253,260]. PA regulates several types of intracellular signalling, including RAC–PAK, mTOR and RAF1-kinase[261–263]. DGKκ expression is reduced in FXS, leading to an increase in DAG signalling, elevated protein translation and dendritic spine growth and reduced dendritic stabilization. 4E-BP, eukaryotic translation initiation factor 4E-binding protein; MNK, MAP kinase-interacting serine/threonine-protein kinase; PIKE-L, phosphatidylinositol 3-kinase enhancer-L; PIP2, phosphatidylinositol-4,5-bisphosphate; RasGRP, RAS guanyl-releasing protein; RHEB, GTP-binding protein Rheb; S6K, ribosomal protein S6 kinase; TSC1/2, tuberous sclerosis proteins 1 and 2.

Neuroimaging data

The effect of FMRP on brain development incorporates the aberrant morphology and growth trajectory observed in FXS. Animal work supports our understanding that brain enlargement at younger ages might be related to processes that result in decreased pruning and/or increased dendritic spine density. More work is needed to study older individuals with fragile X-associated tremor/ataxia syndrome (FXTAS), fragile X-associated primary ovarian insufficiency (FXPOI) and premutation carriers. By doing so, we can examine the developmental and 'dosage' effects of FMRP on brain growth and the consequences that repeat length mosaicism can impart[105]. Much of the work mapping the expression of FMRP in the brain has been performed at the molecular and genetic levels in animal studies. However, the consequence of loss of FMRP on human brain development and functioning has been the focus of numerous neuroimaging investigations.

Structural brain imaging. Neuroimaging studies of individuals with FXS have revealed several key differences in brain morphology compared with healthy individuals. In general, brain overgrowth[106] and increased lateral ventricular size[107] have been observed in individuals with FXS, but these findings are nonspecific and have been observed in patients with other neurodevelopmental disorders, such as ASD. Some of the earliest findings showed abnormalities in the cerebellum, which primarily has a role in motor function but has also been linked

to aspects of cognition, such as attention[108]. Hypoplasia of the cerebellar vermis has been a consistently replicated finding[108–110]. In addition, a reduction in cerebellar size and abnormalities of the cerebellar peduncles have been observed in young boys with FXS[111], and these differences were related to the presence of autism symptoms. A decreased cerebellar volume has been observed in men who have the premutation, with a more prominent reduction noted in those who meet the criteria for FXTAS[112]. Reduced white matter diffusivity has been observed in the middle cerebellar peduncles in women with the premutation, indicating that this is a vulnerable area for both FXS and premutation involvement[113].

Perhaps the most striking abnormality associated with FXS is observed in the basal ganglia, comprising the caudate nucleus, putamen and globus pallidus (FIG. 6), a region important for many executive functions (such as attention and set-shifting or task shifting) as well as motor planning. The caudate nucleus is significantly enlarged in individuals with FXS[107] and is more pronounced in males than females with FXS[114]. Caudate enlargement is present early in development, within the first 3 years of life, and is specific for FXS, in contrast to other neurodevelopmental disorders such as ASD and global developmental delay[115]. Surface-based morphometry studies have localized the greatest degree of enlargement to the head of the caudate[110], which is where most frontostriatal connections are located. Brain networks in the caudate and amygdala show lower activity in functional MRI studies in children and adults with FXS than in controls[116], and individuals with FXS have reduced metabolism of choline and glutamate in the caudate, which reflects aberrant functioning of these areas[117]. Enlargement of the caudate is negatively correlated with FMRP levels[110,111], suggesting a role for FMRP in preventing overgrowth. In addition, the caudate size has been associated with the repetitive behaviours common in ASD and other neurodevelopmental disorders, and a relationship between caudate enlargement and lower-order repetitive behaviours, such as self-injury measured by parent report checklists, was noted in preschool children with FXS[118].

Temporal lobe abnormalities have also been observed in individuals with FXS, although these findings are more-variable. The hippocampus, which is important for memory and learning, is enlarged in individuals with FXS compared with healthy individuals at younger ages[111,119,120] but not at older ages[121]. Hippocampal enlargement suggests the presence of an atypical developmental trajectory, and it has been hypothesized that loss of FMRP in the hippocampus is linked to the problems with mood and cognition in individuals with FXS[36]. Other temporal lobe structures, such as the fusiform gyrus (which has a role in cognition) are enlarged[111], and the insula (which has a role in emotion processing) and the amygdala (which has a role in fear, emotional processing and memory) are reduced in individuals with FXS compared with healthy individuals[110,115,120]. The uncinate fasciculus, an important white matter tract connecting the hippocampus and amygdala, has an abnormal white matter microstructure in individuals with FXS[122]. Large-scale functional (when the individual is in a resting state) and structural networks show reductions in adolescents and young adults with FXS when compared with controls[123].

Functional brain imaging. Social anxiety is one of the core clinical features of FXS and can be characterized by reduced eye gaze related to aversion to direct gaze. Aversion to eye gaze has been linked to brain regions associated with social anxiety[124]. Sex differences exist in these eye-gaze paradigms, as females have a reduced activation of the insula and fusiform gyrus in response to face stimuli, but males have an increased activation of the amygdala and insula, compared with healthy individuals[125]. Anxiety has a role in gaze aversion and can interfere with other cognitive tasks, resulting in decreased performance. Reduced activation of the superior and medial frontal gyral regions was observed in individuals with FXS during a facial memory task, suggesting poor social cognition might hinder the encoding of facial information[126].

Building on the finding that alterations in frontostriatal connections have a role in some of the executive functioning deficits (such as deficits in working memory) in individuals with FXS, functional studies have also found different activation patterns in these brain regions in patients with FXS. Individuals with FXS have decreased neuronal activity in the frontal cortex on working memory tasks[127,128], as well as aberrant activation patterns when carrying out mathematical operations[129,130]. Reduced activation of orbitofrontal regions during tasks assessing attention and impulse control has been observed in individuals with FXS compared with healthy individuals[131]. In addition, reduced inhibition[132] has been observed in those with FXS, and higher levels of FMRP have been shown to reflect a more-typical pattern of frontostriatal activation. Girls with FXS have abnormal activation patterns in the prefrontal cortex and striatum on tasks that require inhibition, and reduced activation was related to the level of *FMR1* expression[128].

The effect of ASD symptoms on brain function has been an important consideration for functional MRI studies in patients with FXS. Both face-processing and emotion-processing networks are different in individuals with FXS compared with healthy individuals, with similar patterns of activation in the fusiform gyrus for

Box 2 | **Identifying altered gene expression owing to loss of FMRP**

Various high-throughput technologies, including immunoprecipitation-transcriptomic[65,85,219–221] and proteomic approaches[222], have been used to generate a number of partially overlapping lists of fragile X mental retardation 1 protein (FMRP) target mRNAs. Among the hundreds of diverse targets, identifying a common RNA signature that is indicative of a unique mechanism by which FMRP has RNA-specific control of translation has not been possible. For example, the guanine quadruplex (G-quadruplex), the most frequently identified motif, is present in only 40–50% of FMRP target mRNAs[86]. Instead, several other mechanisms by which FMRP can repress translation have been proposed, including cap-dependent initiation repression[223,224], modulation of microRNA activity[225,226] and stalling of translation elongation[219]. Some studies also identified translational activation for specific mRNAs[65,227]. In *Drosophila*, FMRP interacts with 60S ribosomal protein L5 in a way that would interfere with tRNA binding during elongation[228], but how this process relates to the specific alteration of neuronal mRNA translation is still unclear.

individuals with both FXS and ASD; however, individuals with FXS and no ASD have greater activation of the hippocampus, insula, postcentral gyrus and left temporal gyrus. This profile of activation suggests that individuals with FXS have reduced habituation to emotionally laden stimuli when compared with individuals with comorbid ASD.

Diagnosis, screening and prevention

Diagnosis of FXS is made following *FMR1* DNA testing and identification of the CGG expansion (>200 repeats for the full mutation). The FXS DNA diagnostic test involves PCR and Southern blot analysis of patient blood samples, which can identify and size both the premutation and the full mutation alleles. Southern blot analysis can also provide information about the methylation status. Several PCR-based approaches have been developed (reviewed in REFS 102,133) and have enabled the characterization of the complete range of expanded *FMR1* alleles with or without methylation assessment. An evaluation of *FMR1* methylation levels is recommended, as the level of methylation correlates inversely with cognitive abilities. These advanced genotyping tools have reduced the use of the labour-intensive and time-intensive Southern blot analysis, which is still considered the gold standard DNA diagnostic test for FXS. In particular, the use of the triplet-primed PCR assay is the preferred test worldwide, because it detects alleles throughout the expanded range, including the premutation in both males and females, and provides a much more accurate determination of allele size within the premutation range[102,133]. In addition, triplet-primed PCR enables the mapping of AGG interruption sequences, which are interspersed and present within the CGG region of *FMR1*. The AGG interruptions are believed to stabilize *FMR1* during transmission, although the underlying mechanism is unknown[134,135]. Indeed, the presence of AGG interruptions in women helps to predict the risk of CGG expansion to a full mutation during mother-to-child transmission[134,135]. Accordingly, the presence of AGG interruptions, in addition to CGG repeat number and maternal age, has a role in the risk of repeat expansion during transmission[135,136] and must be incorporated into the genetic counselling process.

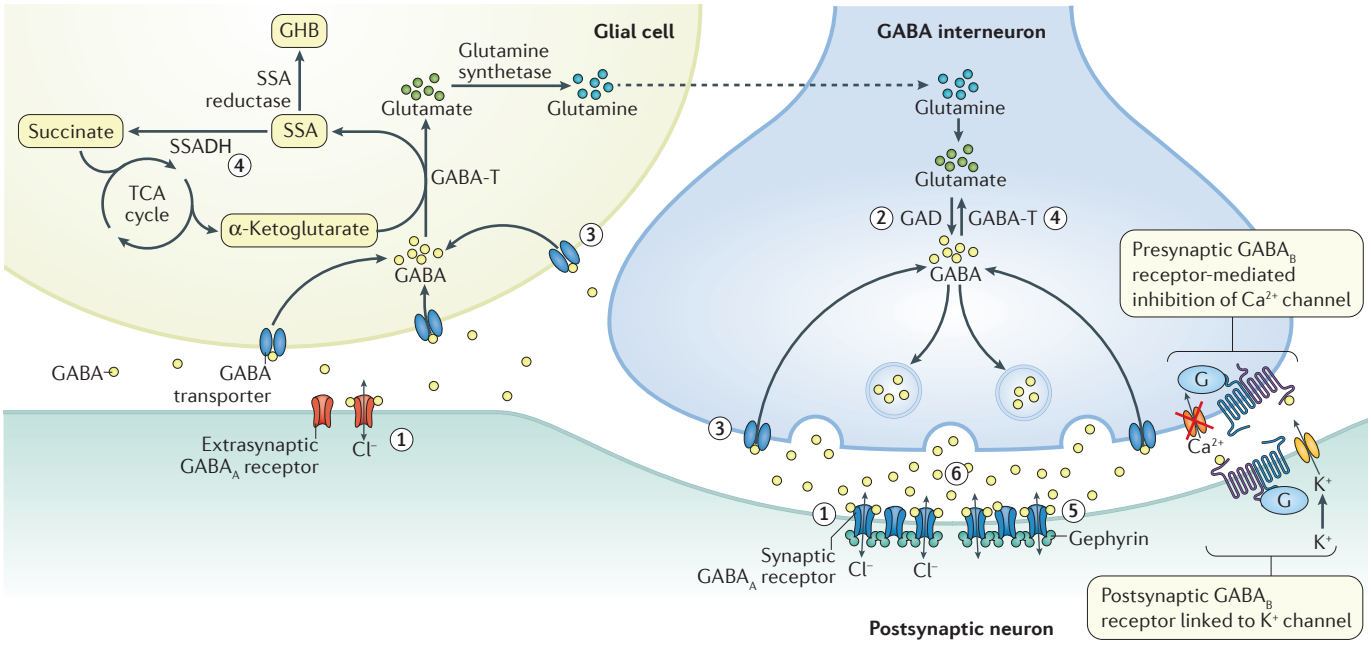

Figure 4 | **Altered GABAergic signalling in *Fmr1*-knockout mice.** The γ-aminobutyric acid (GABA) A receptor (GABA$_A$) is a heteropentameric chloride channel that is assembled as a non-random combination of the receptor subunits α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, θ, π and ρ_{1-3}. Fragile X mental retardation 1 protein (FMRP) binds to the mRNAs of several of these subunits and, accordingly, studies have found reduced expression of approximately half of these subunits in *Fmr1*-knockout mouse and fly models (see 1)[88,264–268]. Age-dependent and brain region-dependent differences in subunit expression have also been reported but are poorly understood[89,103,269,270]. Interestingly, the under-expression of key subunits of the GABA$_A$ receptor could be corrected by genetic rescue of *Fmr1* in knockout mice and flies, underscoring the robustness of the observations[103,271]. GABA$_B$ receptors are composed of two subunits. Expression of the R1a subunit protein, but not its mRNA, is decreased in *Fmr1*-knockout mice and in post-mortem tissue from patients with fragile X syndrome (FXS)[272]. The contribution of the prolonged inhibitory effect of GABA$_B$ receptors, invariably consisting of a dimer of R1a or R1b, and R2 subunits, is less well known. In addition to the receptors themselves, the expression of genes encoding enzymes involved in GABA synthesis (*Gad1* and *Gad2*, which encode glutamate decarboxylase 1 (GAD, see 2)), GABA transporters (*Slc6a1*, *Slc6a12*, *Slc6a13* and *Slc6a11*, which encode the solute carrier family members (see 3)), enzymes involved in GABA degradation (*Aldh5a1*, which encodes a member of the aldehyde dehydrogenase family (SSADH), and *Abat*, which encodes 4-aminobutyrate aminotransferase (GABA-T, see 4)) and in receptor clustering (*Gphn*, encoding gephyrin (see 5)) is reduced in *Fmr1*-knockout mice. These changes have been validated at the protein level for several subunits of the GABA receptor and for enzymes involved in GABA synthesis, reuptake and catabolism[273]. The levels of free GABA are reduced in *Fmr1*-knockout mice (see 6)[90,103,274], and expression of the GABA$_A$ receptor is reduced in patients with FXS[273]. GHB, γ-hydroxybutyrate; SSA, succinic semialdehyde; TCA, tricarboxylic acid. Adapted with permission from REF. 275, Karger.

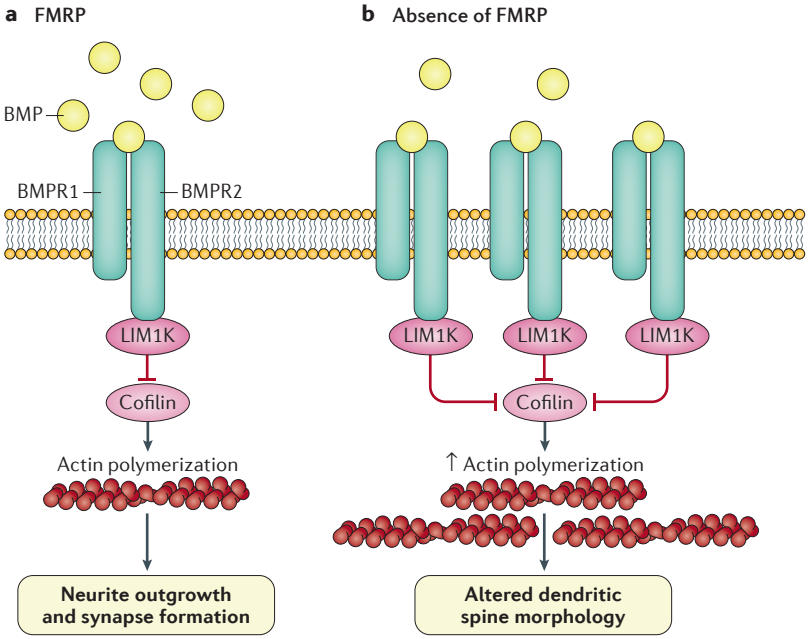

Figure 5 | **The effect of FMRP loss on the BMPR–LIMK–cofilin pathway. a** | Under normal circumstances, bone morphogenic protein receptor (BMPR) signalling leads to the activation of LIM domain kinase 1 (LIMK1), a component of the non-canonical BMP signal transduction pathway, that phosphorylates and inhibits cofilin, leading to actin reorganization, neurite outgrowth and synapse formation. **b** | Fragile X mental retardation 1 protein (FMRP) negatively regulates the production of the bone morphogenic protein receptor 2 (BMPR2) and, as such, loss of FMRP leads to enhanced BMPR signalling, increased actin polymerization and altered dendritic spine and synaptic morphology.

A physician or health care provider should order *FMR1* DNA testing in any child that presents with a substantial developmental delay, intellectual disability or ASD without a known reason. The health care provider should also consider testing for FXS in children who are not talking by 2–3 years of age, as language delays are the initial presentation in most children. Indeed, the mean age of diagnosis in the United States is 3 years[137]. The typical physical features of FXS are variable and might not be present in early childhood or at all (FIG. 2). As ~30% of children with FXS do not have obvious dysmorphic features, the physician often depends on behavioural features to make the clinical diagnosis[3].

Screening and cascade testing

Screening studies have been carried out in neonates using PCR of samples from blood-spot testing, followed by confirmation using PCR and Southern blot analyses of a peripheral blood sample (reviewed in REF. 138). More commonly, high-risk testing has been carried out by screening those who have intellectual disability or ASD. Indeed, ASD is strongly associated with FXS, as ~2–6% of individuals with ASD have FXS, and 50–60% of males with FXS have ASD[43,139,140] (BOX 3). Screening of high-risk individuals who have intellectual disability has been carried out internationally[141–143], and ~2–9% of high-risk individuals have FXS.

The reasons for screening and making the diagnosis of FXS are twofold. First, the diagnosis will lead to new treatment opportunities, which includes targeted treatments (see Management). Second, as FXS is an inherited disorder, diagnosing an individual will FXS will affect other members of their family. The mother of a child with FXS is an obligate carrier of a premutation or full mutation allele, because only a mother can pass on the X-linked full mutation to her children; fathers who are carriers or who have FXS will pass on the premutation to all of their daughters, but not their sons, as mature sperm only carry premutation alleles[144]. The reasons for this are unknown, and this is difficult to study, as mice with the premutation do not recapitulate the transition from premutation to the full methylated mutation (methylation does not occur in the mouse models, even in mice with a CGG repeat length of >200 (REF. 145)). For each child diagnosed with FXS, they usually have many family members with premutation or full mutation alleles[138,141].

As previously discussed, an *FMR1* premutation can have a range of health consequences for individuals (BOX 1). Consequently, once a proband is identified, the family history should include detailed questions about the phenotypes of other family members who might also have FXS or a premutation disorder[21,146]. Given that treatments for the premutation disorders are available, early treatment is likely to be preventive or prophylactic for premutation-associated health problems, such as anxiety, depression, hypertension and hypothyroidism. In addition, counselling for healthy lifestyles, including avoidance of environmental toxins, smoking and excessive alcohol intake, is likely to be preventive for many of the ageing-related problems in individuals with the premutation[146].

When the proband is diagnosed, testing of a sibling who has learning, emotional or behavioural problems is recommended. A sibling with normal development should be tested before adolescence or earlier, as this information regarding the premutation can be more easily accepted by a child than an adolescent or young adult, who might be focused on marriage and reproduction[144]. Occasionally, an individual with apparently normal development and a full mutation might be identified, which precipitates a further work-up that includes neuropsychological testing that usually reveals deficits that are likely to respond to behavioural, educational and medical treatments.

In addition to testing the siblings of individuals with FXS, cascade testing (that is, *FMR1* DNA testing of other extended family members) once a proband is diagnosed is recommended[147]. Indeed, if any of the family members have an expanded *FMR1* allele, they can avoid the development of FXS in their offspring. For example, reproductive technologies allow *in vitro* fertilization and implantation of a healthy embryo, and prenatal diagnostic testing can identify a fetus with FXS, following which a couple can make a decision to terminate or continue with the pregnancy. We hope that in the future, non-invasive prenatal testing will be used to identify a fetus with FXS using a blood test in the expectant mother.

In some countries, cascade testing is not carried out after a proband is diagnosed because of cultural beliefs,

concern for disruption of arranged marriages and the potential for wife abuse. In addition, after diagnosis of a child with FXS, the mother might not be identified as the carrier if her safety is at risk, due to cultural issues. The subsequent guidance for the family is dependent on the family circumstances, marital relationship, and education of the parents; thus, guidance will often be delayed while the family dynamics are addressed.

Management

The management of the child diagnosed with FXS is a multidisciplinary endeavour[7,13] and is often led by a paediatrician, neurologist or psychiatrist with expertise in FXS. Individuals with FXS usually require lifelong care by their families and by a medical professional who is knowledgeable about FXS. The medical professional can use new targeted treatments as they become available throughout the patient's childhood and adult life.

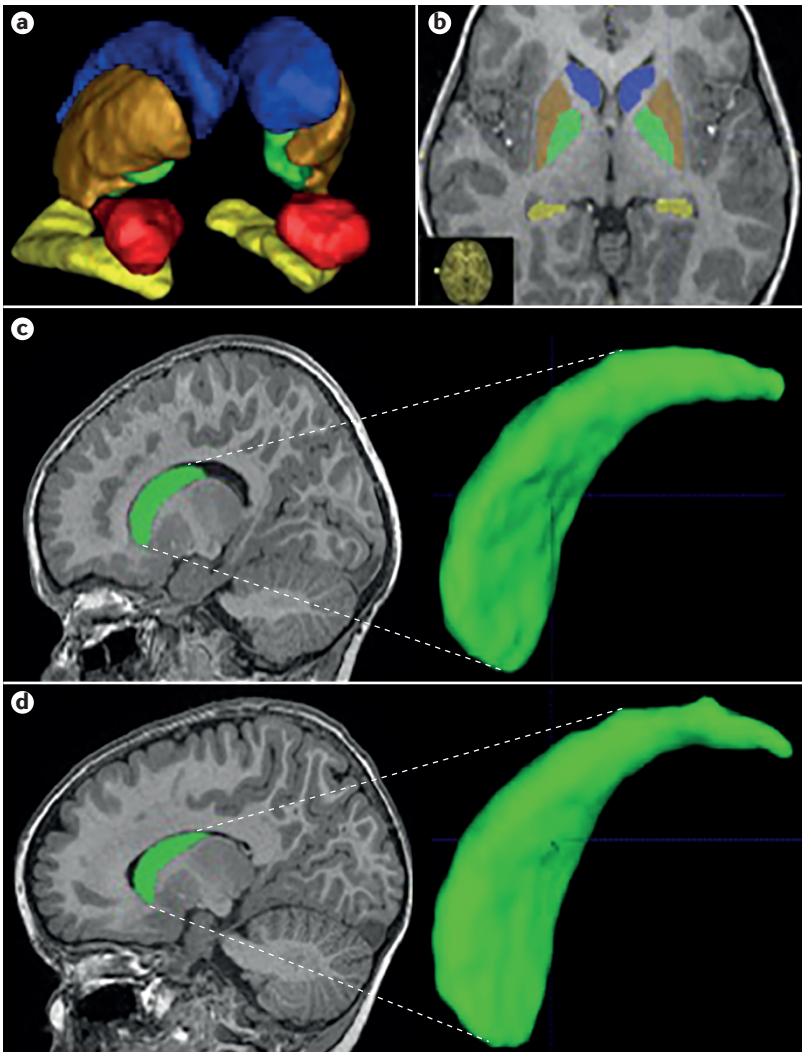

Figure 6 | **Caudate volume in individuals with fragile X syndrome.** Subcortical structures shown segmented (part **a**) and with segmentations overlaid on MRI (part **b**). Blue, caudate nucleus; orange, globus pallidus; green, putamen; red, amygdala; yellow, hippocampus. Structural MRI shows caudate enlargement in an individual with fragile X syndrome (part **c**) compared with a healthy individual (part **d**).

Behavioural alterations

Children with FXS need several therapies, including early speech and language intervention, physical therapy and occupational therapy with a sensory integration approach (to address the often-severe sensory hyper-reactivity)[13]. These interventions are usually included in a special education programme starting from preschool onward, but they can also be performed in an early-start home programme in the first year of life. Additional benefits can be obtained from PROMPT (that is, prompts for restructuring oral muscular phonetic targets) therapy that uses tactile stimulation to the mouth to encourage expressive language[148]. If ASD is diagnosed, which usually occurs by 2 years of age, the child will very likely benefit from the Early Start Denver Model[149,150] or another form of applied behavioural analysis intervention. The Early Start Denver Model aims to promote emotional, social, cognitive and language development in children with ASD, by integrating applied behavioural interventions into everyday family interactions.

Tantrums, irritable behaviour and aggression are common in young children with FXS, and work with a behavioural therapist can be very helpful to the family when these problems arise[151,152]. Often, such intervention can be found in the special education programme through school or through an applied behavioural analysis therapy programme, although work with a private behavioural psychologist can also benefit the family.

The use of special education programmes that are tailored to the pattern of cognitive strengths and weaknesses in individuals with FXS will help academic learning in reading and mathematics and are almost always needed for children with FXS. These interventions combine visual, auditory and tactile presentations and can be provided in a pull-out fashion so that children with FXS can experience mainstream education[151]. These interventions are typically provided by the schools in the United States but not necessarily in other countries. The National Fragile X Foundation has a website (www.fragileX.org) that contains detailed information in several languages on the recommended educational programmes for children with FXS that can be downloaded and given to the school. Other helpful websites include www.fraxa.org, www.fragilex.org.uk and www.fragilex.org.au.

Management of the behavioural manifestations of FXS can also include pharmacological therapies. The conventional psychotropic medications commonly used for treatment of individuals with FXS include stimulants, selective serotonin reuptake inhibitors (SSRIs) and in some cases, atypical antipsychotics. Stimulants are usually beneficial for the treatment of ADHD symptoms in children with FXS who are ≥5 years of age. Indeed, one controlled trial comparing methylphenidate, dextroamphetamine and placebo demonstrated efficacy of the stimulants in improving hyperactivity and inattention[153], although further trials have not been carried out. A positive response to stimulants has been shown ~60% of children with FXS, with a lower response rate in individuals <5 years of age[154]. Often, a long-acting stimulant is used so that a steady blood level can be achieved during the day[154]. For individuals with severe hyperactivity who are <5 years of age,

Box 3 | **Association between FXS and ASD**

Autism spectrum disorder (ASD) and fragile X syndrome (FXS) are strongly associated and are thought to share molecular features. Many of the mRNAs regulated by fragile X mental retardation 1 protein (FMRP) are products of genes that are associated with ASD[219,229] and, accordingly, the deficiency of FMRP leads to a dysregulation of many pathways that are associated with ASD[230]. In addition, some evidence suggests that FMRP is deficient in the brains of individuals with ASD[231,232]. Although many of the behavioural manifestations of FXS with ASD are similar to those of ASD only, including hand mannerisms and poor eye contact, the range of social deficits are generally more severe in individuals with ASD than those with FXS[233]. One of the manifestations of both disorders is substantial anxiety, although compared with individuals who have idiopathic ASD, the anxiety is more severe in individuals with comorbid FXS and ASD and correlates with the severity of the ASD[6,234,235]. Anxiety is associated with greater levels of stress and higher cortisol release in those with FXS than in healthy individuals (reviewed in REFS 236,237).

In general, patients with FXS have a more severe cognitive deficit but better social skills than individuals with idiopathic ASD[233]. When matched for age and IQ, individuals with FXS have better receptive and expressive language abilities than individuals with ASD[238]. In addition, significant differences in plasticity and metaplasticity in individuals with FXS compared with those with ASD have been reported[239].

Confirming a diagnosis of ASD in individuals with FXS is important, as individuals with both diagnoses have a higher rate of seizures, behaviour problems (including aggression and sleep disturbances) and a higher use of atypical antipsychotics than individuals with FXS only[43].

the use of an α-adrenergic agonist, such as guanfacine or clonidine, might be indicated[154]. Administering clonidine at bedtime can also help with sleep disturbances, although melatonin is initially prescribed, as this has been shown to improve sleep disturbances in individuals with FXS in a controlled trial[155]. In addition, the use of L-acetylcarnitine to treat ADHD symptoms in children with FXS has been evaluated in Italy, where stimulants are not legal; one controlled trial has documented positive reports from the parents of these children but not from their teachers[156]. Valproic acid can improve ADHD symptoms in children with FXS[157], but treatment with stimulants seems to be more helpful for these symptoms, whereas valproic acid can be useful for stabilizing mood and decreasing aggression in children and adults with FXS[154].

Anxiety is common in children with FXS who are ≥2 years of age. In children with FXS 2–6 years of age, treatment with low-dose sertraline (an SSRI) significantly improved the visual reception, fine motor coordination and composite T Score on the Mullen Scales of Early Learning, compared with placebo[158]. In addition, in a post hoc analysis, children with ASD and FXS had a significant improvement in expressive language compared with those that received placebo[158]. For children with FXS who demonstrate aggression or severe anxiety that is not improved with an SSRI, use of an atypical antipsychotic, such as aripiprazole or risperidone, can be useful[154]. Indeed, one open-label study showed a significant improvement in social behaviour in children with FXS treated with aripiprazole[159].

Associated medical conditions

As previously mentioned, several conditions are more common in individuals with FXS[3,7] (FIG. 1). These conditions can affect development or behaviour in children, so treatment is important. The child's paediatrician should look for and ask about relevant symptoms at routine, annual well-child visits and should refer to specialists for further evaluation and management if needed[160].

Owing to the expressive language delays in children with FXS, recurrent otitis media might lead to conductive hearing loss and further problems with language and articulation. As such, the prompt and appropriate treatment of otitis media and/or any other otological issues, including hearing monitoring, antibiotics as needed and a relatively low threshold for early ventilation tube (pressure-equalization tube) placement is essential[7]. Children with FXS might not communicate pain well, and behavioural problems such as head banging might be a sign of pain from acute otitis media. If chronic infections of the adenoids and tonsils become a problem, adenoidectomy and/or tonsillectomy can be carried out at the same time as the ventilation tubes are inserted. Children with FXS who have obstructive sleep apnoea might also require a tonsillectomy or adenoidectomy[7].

Obtaining good vision screening for children with FXS can be difficult due to the communication and behavioural problems associated with this condition. As such, evaluation by an ophthalmologist or optometrist is recommended within the first 3–4 years of life to assess for and correct vision problems. If the child has strabismus, an earlier evaluation is recommended, and this should be managed with eye patching, vision therapy or surgery to avoid amblyopia and compounding visual processing problems[7,160].

Seizures are common in children with FXS and should be identified by electroencephalography and neurology referral. Indeed, ambulatory electroencephalogram can be used to distinguish behavioural spells such as staring and seizures. If the child has seizures, or if epileptiform discharges associated with a clinical correlate such as staring spells are present on the electroencephalogram, treatment with an anticonvulsant should be started. In addition, anticonvulsants such as valproate are also mood stabilizers and can help with aggression or episodic dyscontrol in some children[154].

Children, especially those with intellectual disability, require adequate sleep for optimal development, learning and functioning, but sleep disruption is common in children with FXS[161]. Accordingly, monitoring and managing obstructive sleep apnoea and other sleep problems are of particular importance in individuals with FXS, as they are linked to decrements in daytime performance and behaviour. As such, the primary care physician should enquire about potential sleep problems in children with FXS at every well-child visit. As previously mentioned, melatonin can be used for sleep problems in children with FXS and individuals with ASD and can be obtained over the counter in most countries[155].

Owing to the increased risk of obesity in children with FXS[162], encouraging patients to follow a healthy diet, including food restriction when necessary, and partaking in exercise programmes for 30–40 minutes 4–5 times a week is important to minimize the health problems associated with weight gain. Children with FXS who present with the Prader–Willi phenotype (<10% of patients with FXS) develop severe hyperphagia and obesity,

usually by 6–9 years of age, and typically have small genitalia and delayed puberty[163]. Molecular studies have demonstrated a deficit of cytoplasmic FMR1-interacting protein 1 in individuals with the Prader–Willi phenotype of FXS, although patients do not have a deletion at 15q11–13 nor uniparental disomy, which is common in individuals with Prader–Willi syndrome[163]. Treatment of the obesity and hyperphagia of patients with the Prader–Willi phenotype of FXS is difficult[162]. Although treatment with metformin can be beneficial[164], controlled trials have not yet been carried out.

Other complications of FXS include gastro-oesophageal reflux and flat feet. Antacids should be used for the treatment of gastro-oesophageal reflux, when needed to prevent pain, oesophagitis and resulting behavioural decompensation. As individuals with FXS are not always able to describe heartburn, the only sign of gastro-oesophageal reflux might be behavioural outbursts occurring in patterns related to meals, or sleep dysfunction with frequent night awakenings. Some individuals with FXS benefit from orthotics or shoe inserts for the management of foot pronation and flat feet, and this can help with motor development in younger patients and to avoid leg pain and reduce gait problems when older.

Targeted treatments

One of the most exciting aspects of the FXS field is the identification of targeted treatments that have the potential to reverse the neurobiological aspects of FXS (TABLE 1). Most of the targeted treatments for FXS are supported by animal studies involving knockout of the *Fmr1* gene homologues (reviewed in REFS 51,60,159,165).

The use of targeted treatments began with the articulation of the mGluR hypothesis of intellectual disability in FXS[42,53]. An initial phase I/II trial of the mGluR5 negative modulator AFQ056 showed improvement in behavioural problems including hyperactivity, stereotypic behaviour and inappropriate speech in patients with FXS who had

Table 1 | **Targeted treatments in development for FXS**

Mechanism or pathway target	Phase of development			
	Preclinical	Phase I or open label	Phase II	Phase III
Block excess mGluRI signalling directly by inhibiting mGluR5 receptor	• MPEP • Fenobam • CTEP • AFQ056 • STX107	• AFQ056 • Fenobam • STX107	• AFQ056 • RO4917526	• None
Block excess mGluRI signalling by inhibiting pathway leading from receptor activation to protein translation	• Lithium* • PAK inhibitors • Lovastatin* • GSK3β inhibitors • PIKE inhibitors • PI3K inhibitors • NNZ-2256 • Metadoxine • Bryostatin	• Lithium* • Lovastatin*	• NNZ-2256 • Metadoxine • Lovastatin*	• None
Block excess activity of protein produced to excess in absence of FMRP	• Minocycline* • STEP inhibitors • Rolipram • PDE inhibitors	• Rolipram • Minocycline*	• Minocycline*	• None
Increase deficient AMPA receptor activity	• CX516	• None	• CX516	• None
Regulate signalling through GABA or other non-glutamate receptors to reduce or balance mGluRI or other abnormal receptor signalling resulting from absence of FMRP	• Baclofen* • Arbaclofen • Acamprosate* • Ganaxolone • Metadoxine	• Acamprosate* • Ganaxolone • Donepezil* • Gaboxadol	• Arbaclofen • Acamprosate* • Ganaxolone • Metadoxine • Donepezil*	• Arbaclofen
Block excess synthesis of specific proteins with miRNAs	• miR-125a	• None	• None	• None
Regulate abnormal channel activity in absence of FMRP	• BK channel blockers • Slack channel blockers	• None	• None	• None
Regulate abnormal insulin signalling in the absence of FMRP	• Metformin*	• Metformin*	• None	• None
Regulate abnormal endocannabinoid signalling	• Cannabidiol • Endocannabinoid blockers	• None	• None	• None

Mechanisms and development of targeted treatments in this chart have been reviewed in Gross *et al.*[276], and/or Hoffmann and Berry-Kravis[277], as well as in specific references in the text. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; FMRP, fragile X mental retardation 1 protein; GABA, γ-aminobutyric acid; GSK3β, glycogen synthase kinase-3β; mGluRI, group 1 metabotropic glutamate receptor; miRNA, microRNA; MPEP, 2-methyl-6-(phenylethynyl)pyridine; PAK, serine/threonine-protein kinase PAK; PDE, phosphodiesterase; PIKE, PI3K enhancer; STEP, striatum-enriched protein-tyrosine phosphatase.*Denotes drugs that are approved by the US FDA for another disorder.

a fully methylated full mutation[166], but subsequent larger phase IIb trials of AFQ056 and a similar mGluR5 modulator (RO4917523) did not significantly improve behavioural problems in adolescents and adults with FXS over a 3-month treatment period[165]. Possible reasons for this might relate to a different balance of pathological mechanisms in the *Fmr1*-knockout mouse than in patients. In addition, studies have suggested a role for dysregulation of dopamine signalling[167] or endocannabinoid signalling[78–81] that interacts with mGluR signalling in the mouse model. Moreover, the behavioural outcome measures might have been inadequate and susceptible to large placebo effects[168] that obscured drug effects over short time periods, and more objective behavioural measures directed at the core features of FXS, including cognitive testing paradigms and testing in younger children, might be needed[165,169].

GABA receptor agonists can reverse some of the FXS-like traits in animal models of FXS[59,170]. In a phase II, placebo-controlled trial, treatment with the $GABA_B$ receptor agonist arbaclofen improved social withdrawal and parent-nominated problem behaviours in patients with FXS, compared with placebo, and, in a post hoc analysis, improved several parameters in a subgroup of patients with social deficits or ASD[171]. In two subsequent phase III trials, adolescents and adults with FXS that received arbaclofen did not show behavioural improvement, compared with patients who received placebo, but children 5–11 years of age had a significant improvement in the ABC-C_{FX} (Aberrant Behaviour Checklist-Community Edition, factored for FXS[172]) Irritability Subscale scores and in the Parenting Stress Index, suggesting QOL benefits, compared with children that received placebo[173]. In addition, the children had a trend for improvement in the ABC-C_{FX} Social Avoidance and Hyperactivity Subscales[173]. Acamprosate (a US FDA-approved therapy for alcohol withdrawal) is an agonist of both $GABA_A$ and $GABA_B$ receptors and has been shown to improve hyperactivity and social functioning in individuals with FXS in an open-label trial[174] and normalized elevated APP levels[175]. Acamprosate, ganaxolone (a $GABA_A$ receptor positive allosteric modulator) and metadoxine (an indirect GABA activator) have been or are being tested in small placebo-controlled trials in individuals with FXS. Gaboxadol, a $GABA_A$ receptor δ-selective agonist, is being explored in a phase I trial. Although only limited early results are available, patients with FXS who were treated with metadoxine showed an improvement in a secondary outcome, the Vineland Daily Living Skills subscale[176], and in inhibition scores on an executive function task (the KiTAP, which has good reliability and clinical validity in FXS), compared with individuals who received placebo[177]. A controlled trial of ganaxolone did not show efficacy in the primary outcome measure (improvement at the Clinical Global Improvement Scale) but did show improvement in anxiety in a post hoc analysis of a subgroup of more-anxious participants[178].

One of the lessons from both the animal and human studies is that reversing phenotypes of the *Fmr1*-knockout mouse is easier than changing the disease in patients with FXS. Part of the difficulty with human studies is that a new medication must first be evaluated in adults with FXS before the FDA will approve trials in children. However, reversing the symptoms of FXS might be easier when treatment is started at younger ages, as demonstrated with low-dose sertraline studies[158]. Thus, therapies targeting cognition and learning, such as mGluR5 antagonists, would best be tried in younger children with FXS, using outcomes that measure cognition and learning, rather than maladaptive behaviour. Indeed, one study is in progress through the NIH-funded NeuroNEXT network (NCT02920892), in which AFQ056 treatment is combined with intensive parent-implemented language intervention through Skype (which can prompt language learning in young children with FXS[179–181]), to examine whether targeted learning in children with FXS who are 3–6 years of age can be facilitated by AFQ056, compared with placebo. The use of quantitative outcome measures to assess language improvements and brain processing with event-related potentials[182] and eye-tracking measures of facial processing[183] can be more sensitive at detecting improvements than behavioural questionnaires that have a high variability[184]. Indeed, in the AFQ056 trials that did not show behavioural improvements in adults and adolescents with FXS, two objective phenotype-based outcome measures conducted in a two-site substudy[184] showed improvement in face gaze with eye tracking and in inhibition scores on the KiTAP in these patients.

Minocycline can lower MMP9 levels, which can improve the maturity and strength of synaptic connections in *Fmr1*-knockout mice[68,185,186], and a controlled trial of minocycline demonstrated significant behavioural benefits in children with FXS measured by the Clinical Global Impressions Scale and the Visual Analogue Scale[187]. In addition, a novel event-related potential assessment showed improvements in a habituation paradigm in patients with FXS treated with minocycline compared with those that received placebo[182]. Thus, minocycline is used in the clinic to help with behaviour problems, anxiety and attention in children with FXS. In addition to the MMP9-lowering effects, minocycline can stall translation and has antioxidant and anti-apoptotic properties[187]. However, adverse effects of minocycline can include darkening of the permanent teeth (when the drug is started in children who are <8 years of age) or nails (when used chronically). In addition, minocycline treatment can increase antinuclear antibody levels, which can (albeit rarely) cause a lupus-like syndrome, with a rash or swollen joints. However, this can be reversed when minocycline is discontinued[68]. Thus, the antinuclear antibody titre should be measured on a 6-monthly or yearly basis in children who are treated with minocycline.

Other targeted treatments include metformin, lovastatin and trofinetide (TABLE 1). Metformin[188] has been used in clinical practice, and patients with FXS who have been treated with metformin have shown improvements in behaviour and language, but this has not yet been studied in a controlled trial[164]. Metformin is used for the treatment of type 2 diabetes mellitus, and can be beneficial for the treatment of obesity, which is a common problem in individuals with FXS, especially for those treated with

Box 4 | **Factors that can affect quality of life in patients or families with FXS**

Patients

Four characteristics can affect quality of life (QOL) in patients with fragile X syndrome (FXS):

- Psychosocial challenges, including anxiety, autism spectrum disorder, hypersensitivities and associated behavioural problems (such as self-injury and aggression)
- Health problems, such as seizures, sleep problems, food sensitivities and gastrointestinal issues
- Cognitive and executive function limitations, including problems with attending to relevant stimuli
- Social isolation and limitations in functional and adaptive behaviour

Families

Having a family member with FXS can alter one's QOL. Four main factors can alter the QOL of family members:

- The functional, cognitive and adaptive limitations of their child, which can create the need for specialized services and potentially lifelong caregiving responsibilities[240]
- The behavioural and psychosocial characteristics of their child that can limit social acceptance, create a need to constantly manage environmental stimuli, adversely affect the ability to participate in community settings and experiences or — in the case of aggression — behaviours that can harm parents or peers[241]
- The heritability features of FXS that can implicate parents and grandparents as carriers, which can stimulate the need to test other children in the family — potentially leading to additional members of the family being diganosed with FXS full mutation or premutation. In addition, the heritability is a factor in deciding when and how to tell siblings, and it often leads to major discussions across extended family members about the need for or desirability of cascade testing[242,243]
- The growing evidence that a subset of premutation carriers are also at risk of several adverse health outcomes, which can lead to concerns about reproductive risk, depression or other mood disorders, and other potential health problems, including late-onset disorders such as fragile X-associated tremor/ataxia syndrome or fragile X-associated primary ovarian insufficiency[203,244,245]

antipsychotics[154]. The mechanism of action of metformin is the regulation of insulin signalling, but it also reduces ERK signalling and decreases levels of phosphorylated EIF4E and MMP9 in *Fmr1*-knockout mice[70]. Treatment with lovastatin, which indirectly suppresses ERK activity and reverses some phenotypes in the FXS mouse model[189], has been shown to improve behavioural symptoms, including hyperactivity in an open-label trial[190], that were correlated with normalization of ERK activity in platelets[191]. Trofinetide is an analogue of the terminal tripeptide of insulin-like growth factor I and can decrease abnormal ERK and AKT activity and normalizes phenotypes in the FXS mouse[192], in addition to reversing the oxidative damage in neurons with the full mutation[193]. Trofinetide has shown promising results in a phase II trial, with an improvement in a novel composite measure of the FXS phenotype[194]. All of these novel agents need further study in controlled trials that are designed to demonstrate functional improvements that link to QOL to be considered by the FDA for drug registration.

Quality of life

QOL is a complex and multidimensional construct that is heavily dependent on context and experience and, as such, cannot be viewed as a stable trait within an individual but rather as a state that can vary across time and contexts. Although objective measures can be used to determine the assumed QOL, ultimately, that QOL is an inherently subjective phenomenon must be recognized. QOL is difficult to assess in individuals with intellectual and developmental disabilities because their cognitive and communication limitations often influence their ability to conceptualize and accurately report perceived QOL. Nonetheless, as the ultimate goal of treatments and services is to improve QOL for individuals with FXS and their families, describing factors that contribute to reductions or impairments in QOL is essential.

Patients

Only scant research has directly assessed QOL in individuals with FXS, but an extensive set of studies of the fragile X phenotype suggest four highly interrelated characteristics which, if addressed, could lead to a significant improvement in QOL (BOX 4).

As part of a series of surveys of >1,000 families that had at least one child with FXS, parents were asked to rate their child's overall QOL on a 5-point scale (excellent, very good, good, fair or poor). Interestingly, 61% of parents who had a son with FXS and 70% of parents who had a daughter with FXS rated their child's QOL as 'very good' or 'excellent' and only 9% of sons and 8% of daughters were rated as having a 'fair' or 'poor' QOL[195]. In a multi-country study in Europe, mean health-related QOL scores of patients with FXS were generally lower than scores of the general population, and in at least one country (Sweden), scores were lower than those of patients with other disorders, including diabetes mellitus or hypertension[196].

QOL is associated with the number and severity of phenotypic features of FXS. Indeed[197], the presence of more co-occurring conditions (for example, attention problems, hyperactivity, aggressiveness, self-injury, ASD, seizures, anxiety or depression) has been shown to be associated with lower parental estimates of their child's QOL[197]. In addition, in France, health-related QOL was significantly lower for individuals with FXS who had lower ratings on an index of activities of daily living and mobility[198].

Families

The QOL of families that have a member with FXS has been studied more extensively than the QOL of patients with FXS, given that parents are more able to report on this than their children, as well as the extensive ramifications of FXS that could adversely affect family QOL (BOX 4).

Despite these considerable challenges, studies have suggested that parents (primarily mothers) who have a child with FXS are not likely to report a lower QOL than parents in the general population[199,200]. In fact, in one study, 53% of parents rated the effect of having a child with FXS as somewhat or mostly positive, and most families (78%) characterized their overall life situation as good or very good[201]. However, a more nuanced view of various aspects of QOL suggests some aspects of a family's QOL that are clearly challenged by having a member with FXS. Among these include social supports and social life[201], caregiver injuries as a result of aggressive behaviour[202], high levels of parenting stress[199]

and a higher prevalence of major depressive disorder that is persistent in mothers[203]. Raising a child with FXS has a significant effect on employment and financial burden on both society[196,198] and families[202,204], and in some cases the effect is greater than that experienced by families of children with other disorders[205]. Behaviour problems in the child with FXS are consistently associated with lower ratings of family QOL[199], and parents rate the control of behaviour problems as their most desired outcome for treatment[206]. Higher levels of QOL are associated with hope and optimism[199,200], social supports and social lives[201].

Outlook

Reversing the cognitive and behavioural abnormalities of FXS will not be easy, as this will require more than one targeted treatment, in addition to the use of simultaneous educational and behavioural interventions, most optimally, in a young child with FXS. Animal models have ignited the field of targeted treatments, and we have learned much over the past decade after the failure of many clinical trials. Indeed, advances in our understanding of the underlying neurobiology and of the ubiquitous nature of FMRP, or lack thereof, have fuelled the development of many more targeted treatments that could potentially modify the underlying disease. However, demonstrating the effectiveness of these agents in patients with FXS will require the development and use of better, more objective measures of outcomes that assess core FXS phenotypes, markers both of target engagement and of disease modification. Such objective measures will avoid the bias of behavioural questionnaires that are completed by family members who want a cure, which can influence both the placebo and drug effects. In addition, changing models of drug development so that studies in children are performed earlier in the developmental process and for longer time periods is required. Thus, potential treatments that modify synaptic function in animal models but have a presumed lack of effectiveness due to the failure to show short-term behavioural change in adults are not abandoned. We need to develop models for measuring learning within medication trials, as this is the key problem in FXS.

Much work has been done on outcome measures to develop and validate more objective, direct testing and observation-based assessments such as expressive language sampling[207] and computer tablet-based cognitive tasks[208]. Methods have been developed for normalizing standardized cognitive tests for individuals with intellectual disability or FXS to avoid floor effects, so that the effect of cognitive level on treatment effects can truly be studied[209]. More work is needed in this area to identify and validate the best measures for target engagement (such as event-related potentials), short-term changes and long-term disease modification. A natural history study (FORWARD, Fragile X Online Registry With Accessible Research Database) has been implemented at Fragile X Clinical and Research Consortium clinics in the United States with the Centers for Disease Control and Prevention (CDC) funding[210] to characterize key gaps and needs in FXS therapy and to define the course of the disease, enabling future very long-term studies to assess whether disease modification has occurred from the baseline trajectory. Trials in young children have been initiated (for example, low-dose sertraline[158]; and the AFQ056 NeuroNEXT trial) and work is ongoing to push for drug development models that involve trials in children earlier in the development process. Indeed, this approach seems to be meeting conceptual success and will hopefully be included in the next wave of trials of new targeted treatments for FXS. Advances have been made regarding optimal approaches for therapy, including language interventions (PROMPT and parent-implemented language intervention[179–181]) and educational or behavioural interventions beginning early in life[211,212]. Studies are now evaluating the combination of targeted treatments with educational or behavioural interventions (for example, AFQ056 with parent-implemented language intervention). Such research will lead to earlier and better-targeted treatments for FXS. However, that more than one targeted treatment will be needed to correct the multiple dysregulated pathways that occur in the absence of FMRP is likely. Fortunately, the agents currently in development target a broad range of cellular functions that are affected by the loss of FMRP.

Advances in DNA technology, including CRISPR-Cas9, have been used to correct the expanded CGG repeat in induced pluripotent stem cells[213], and eventually, it might be possible to add these corrected stem cells into the central nervous systems of those with FXS. Additionally, work is ongoing to identify a gene reactivation strategy in FXS (http://www.fulcrumtx.com/programs/). However, many hurdles remain before stem cell therapies and/or gene reactivation or editing would be acceptable for treating patients with FXS or other neurodevelopmental disorders.

1. Myrick, L. K. *et al.* Fragile X syndrome due to a missense mutation. *Eur. J. Hum. Genet.* **22**, 1185–1189 (2014).
2. Quartier, A. *et al.* Intragenic *FMR1* disease-causing variants: a significant mutational mechanism leading to fragile-X syndrome. *Eur. J. Hum. Genet.* **25**, 423–431 (2017).
This paper provided an account of new intragenic mutations in *FMR1* and an excellent review of the phenotypes associated with previously reported mutations in *FMR1*.
3. Hagerman, R. J. in *Fragile X Syndrome: Diagnosis, Treatment and Research* (eds Hagerman, R. J. & Hagerman, P. J.) 3–109 (Johns Hopkins Univ. Press, 2002).
4. Berry-Kravis, E. *et al.* Seizures in fragile X syndrome: characteristics and comorbid diagnoses. *Am. J. Intellect. Dev. Disabil.* **115**, 461–472 (2010).
5. Hogan, A. L. *et al.* Autism spectrum disorder symptoms in infants with fragile X syndrome: a prospective case series. *J. Autism Dev. Disord.* **47**, 1628–1644 (2017).
6. Cordeiro, L., Ballinger, E., Hagerman, R. & Hessl, D. Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: prevalence and characterization. *J. Neurodev. Disord.* **3**, 57–67 (2011).
7. Kidd, S. A. *et al.* Fragile X syndrome: a review of associated medical problems. *Pediatrics* **134**, 995–1005 (2014).
This was the first paper to compare frequency of medical problems in a large cohort with FXS with frequencies in the general paediatric population, defining those problems that are more common in FXS; it suggested medical screening and management of these problems for patients with FXS.
8. Heulens, I. *et al.* Craniofacial characteristics of fragile X syndrome in mouse and man. *Eur. J. Hum. Genet.* **21**, 816–823 (2013).
9. Waldstein, G. *et al.* Fragile X syndrome: skin elastin abnormalities. *Birth Defects Orig. Artic. Ser.* **23**, 103–114 (1987).
10. Pretto, D. *et al.* Clinical and molecular implications of mosaicism in *FMR1* full mutations. *Front. Genet.* **5**, 318 (2014).
11. Dyer-Friedman, J. *et al.* Genetic and environmental influences on the cognitive outcomes of children with fragile X syndrome. *J. Am. Acad. Child Adolesc. Psychiatry* **41**, 237–244 (2002).
12. Loesch, D. Z., Huggins, R. M. & Hagerman, R. J. Phenotypic variation and FMRP levels in fragile X. *Ment. Retard. Dev. Disabil. Res. Rev.* **10**, 31–41 (2004).

13. Oostra, B. A. & Hoogeveen, A. in *Fragile X Syndrome: Diagnosis, Treatment and Research* (eds Hagerman, R. J. & Hagerman, P. J.) 169–190 (Johns Hopkins Univ. Press, 2002).
14. Tassone, F. *et al.* FMRP expression as a potential prognostic indicator in fragile X syndrome. *Am. J. Med. Genet.* **84**, 250–261 (1999).
15. Coffee, B. *et al.* Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. *Am. J. Hum. Genet.* **85**, 503–514 (2009).
16. Levesque, S. *et al.* Screening and instability of *FMR1* alleles in a prospective sample of 24,449 mother-newborn pairs from the general population. *Clin. Genet.* **76**, 511–523 (2009).
17. Fernandez-Carvajal, I. *et al.* Screening for expanded alleles of the *FMR1* gene in blood spots from newborn males in a Spanish population. *J. Mol. Diagn.* **11**, 324–329 (2009).
18. Song, F. J., Barton, P., Sleightholme, V., Yao, G. L. & Fry-Smith, A. Screening for fragile X syndrome: a literature review and modelling study. *Health Technol. Assess.* **7**, 1–106 (2003).
19. Maia, N. *et al.* Contraction of fully expanded FMR1 alleles to the normal range: predisposing haplotype or rare events? *J. Hum. Genet.* **62**, 269–275 (2017).
20. Hunter, J. *et al.* Epidemiology of fragile X syndrome: a systematic review and meta-analysis. *Am. J. Med. Genet. A* **164A**, 1648–1658 (2014).
This was the largest meta-analysis study conducted to assess prevalence estimates of the full mutation and premutation in the total population.
21. Tassone, F. & Hall, D. A. *FXTAS, FXPOI, and Other Premutation Disorders* (Springer International Publishing, 2016).
This comprehensive book discussed the clinical, epidemiological and molecular issues involved in the premutation disorders that can affect carriers of a premutation throughout their lifespan.
22. Khandjian, E. W., Corbin, F., Woerly, S. & Rousseau, F. The fragile X mental retardation protein is associated with ribosomes. *Nat. Genet.* **12**, 91–93 (1996).
23. Tamanini, F. *et al.* FMRP is associated to the ribosomes via RNA. *Hum. Mol. Genet.* **5**, 809–813 (1996).
24. Richter, J. D., Bassell, G. J. & Klann, E. Dysregulation and restoration of translational homeostasis in fragile X syndrome. *Nat. Rev. Neurosci.* **16**, 595–605 (2015).
25. Darnell, J. C. & Klann, E. The translation of translational control by FMRP: therapeutic targets for FXS. *Nat. Neurosci.* **16**, 1530–1536 (2013).
26. Brown, M. R. *et al.* Fragile X mental retardation protein controls gating of the sodium-activated potassium channel Slack. *Nat. Neurosci.* **13**, 819–821 (2010).
27. Deng, P. Y. *et al.* FMRP regulates neurotransmitter release and synaptic information transmission by modulating action potential duration via BK channels. *Neuron* **77**, 696–711 (2013).
28. Alpatov, R. *et al.* A chromatin-dependent role of the fragile X mental retardation protein FMRP in the DNA damage response. *Cell* **157**, 869–881 (2014).
29. Shamay-Ramot, A. *et al.* Fmrp interacts with Adar and regulates RNA editing, synaptic density and locomotor activity in zebrafish. *PLoS Genet.* **11**, e1005702 (2015).
30. Akins, M. R., Leblanc, H. F., Stackpole, E. E., Chyung, E. & Fallon, J. R. Systematic mapping of fragile X granules in the mouse brain reveals a potential role for presynaptic FMRP in sensorimotor functions. *J. Comp. Neurol.* **520**, 3687–3706 (2012).
31. Guo, W. *et al.* Ablation of Fmrp in adult neural stem cells disrupts hippocampus-dependent learning. *Nat. Med.* **17**, 559–565 (2011).
32. Nelson, D. L., Orr, H. T. & Warren, S. T. The unstable repeats — three evolving faces of neurological disease. *Neuron* **77**, 825–843 (2013).
33. Vershkov, D. & Benvenisty, N. Human pluripotent stem cells in modeling human disorders: the case of fragile X syndrome. *Regen. Med.* **12**, 53–68 (2017).
34. Colak, D. *et al.* Promoter-bound trinucleotide repeat mRNA drives epigenetic silencing in fragile X syndrome. *Science* **343**, 1002–1005 (2014).
35. Gerhardt, J. *et al.* The DNA replication program is altered at the FMR1 locus in fragile X embryonic stem cells. *Mol. Cell* **53**, 19–31 (2014).
36. Mirkin, S. M. Expandable DNA repeats and human disease. *Nature* **447**, 932–940 (2007).
37. Zhao, X. N. *et al.* Mutsβ generates both expansions and contractions in a mouse model of the fragile X-associated disorders. *Hum. Mol. Genet.* **24**, 7087–7096 (2015).
38. Gholizadeh, S., Halder, S. K. & Hampson, D. R. Expression of fragile X mental retardation protein in neurons and glia of the developing and adult mouse brain. *Brain Res.* **1596**, 22–30 (2015).
39. Bakker, C. E. & Oostra, B. A. Understanding fragile X syndrome: insights from animal models. *Cytogenet. Genome Res.* **100**, 111–123 (2003).
40. The Dutch-Belgian Fragile X Consortium. *Fmr1* knockout mice: a model to study fragile X mental retardation. *Cell* **78**, 23–33 (1994).
41. Kooy, R. F. Of mice and the fragile X syndrome. *Trends Genet.* **19**, 148–154 (2003).
42. Huber, K. M., Gallagher, S. M., Warren, S. T. & Bear, M. F. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc. Natl Acad. Sci. USA* **99**, 7746–7750 (2002).
This paper established that loss of FMRP causes exaggerated protein synthesis-dependent mGluRI signalling in the *Fmr1*-knockout mouse model.
43. Kaufmann, W. E. *et al.* Autism spectrum disorder in fragile X syndrome: cooccurring conditions and current treatment. *Pediatrics* **139**, S194–S206 (2017).
44. Li, J., Pelletier, M. R., Perez Velazquez, J. L. & Carlen, P. L. Reduced cortical synaptic plasticity and GluR1 expression associated with fragile X mental retardation protein deficiency. *Mol. Cell. Neurosci.* **19**, 138–151 (2002).
45. Sidorov, M. S., Auerbach, B. D. & Bear, M. F. Fragile X mental retardation protein and synaptic plasticity. *Mol. Brain* **6**, 15 (2013).
46. Qin, M., Kang, J., Burlin, T. V., Jiang, C. & Smith, C. B. Postadolescent changes in regional cerebral protein synthesis: an *in vivo* study in the *FMR1* null mouse. *J. Neurosci.* **25**, 5087–5095 (2005).
47. Dolen, G. *et al.* Correction of fragile X syndrome in mice. *Neuron* **56**, 955–962 (2007).
48. Contractor, A., Klyachko, V. A. & Portera-Cailliau, C. Altered neuronal and circuit excitability in fragile X syndrome. *Neuron* **87**, 699–715 (2015).
49. Miller, L. J. *et al.* Electrodermal responses to sensory stimuli in individuals with fragile X syndrome: a preliminary report. *Am. J. Med. Genet.* **83**, 268–279 (1999).
50. Santoro, M. R., Bray, S. M. & Warren, S. T. Molecular mechanisms of fragile X syndrome: a twenty-year perspective. *Annu. Rev. Pathol.* **7**, 219–245 (2012).
51. Braat, S. & Kooy, R. F. Fragile X syndrome neurobiology translates into rational therapy. *Drug Discov. Today* **19**, 510–519 (2014).
52. Pop, A. S., Gomez-Mancilla, B., Neri, G., Willemsen, R. & Gasparini, F. Fragile X syndrome: a preclinical review on metabotropic glutamate receptor 5 (mGluR5) antagonists and drug development. *Psychopharmacology (Berl.)* **231**, 1217–1226 (2014).
53. Bear, M. F., Huber, K. M. & Warren, S. T. The mGluR theory of fragile X mental retardation. *Trends Neurosci.* **27**, 370–377 (2004).
54. Pfeiffer, B. E. & Huber, K. M. Current advances in local protein synthesis and synaptic plasticity. *J. Neurosci.* **26**, 7147–7150 (2006).
55. Sutton, M. A. & Schuman, E. M. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* **127**, 49–58 (2006).
56. Gkogkas, C. G. *et al.* Pharmacogenetic inhibition of eIF4E-dependent Mmp9 mRNA translation reverses fragile X syndrome-like phenotypes. *Cell Rep.* **9**, 1742–1755 (2014).
57. Sawicka, K., Pyronneau, A., Chao, M., Bennett, M. V. & Zukin, R. S. Elevated ERK/p90 ribosomal S6 kinase activity underlies audiogenic seizure susceptibility in fragile X mice. *Proc. Natl Acad. Sci. USA* **113**, E6290–E6297 (2016).
58. Hoeffer, C. A. *et al.* Altered mTOR signaling and enhanced CYFIP2 expression levels in subjects with fragile X syndrome. *Genes Brain Behav.* **11**, 332–341 (2012).
59. Braat, S. & Kooy, R. F. Insights into GABAAergic system deficits in fragile X syndrome lead to clinical trials. *Neuropharmacology* **88**, 48–54 (2015).
60. Gross, C. *et al.* Increased expression of the PI3K enhancer PIKE mediates deficits in synaptic plasticity and behavior in fragile X syndrome. *Cell Rep.* **11**, 727–736 (2015).
61. Sidhu, H., Dansie, L. E., Hickmott, P. W., Ethell, D. W. & Ethell, I. M. Genetic removal of matrix metalloproteinase 9 rescues the symptoms of fragile X syndrome in a mouse model. *J. Neurosci.* **34**, 9867–9879 (2014).
62. Guo, W. *et al.* Inhibition of GSK3β improves hippocampus-dependent learning and rescues neurogenesis in a mouse model of fragile X syndrome. *Hum. Mol. Genet.* **21**, 681–691 (2012).
63. Pasciuto, E. *et al.* Dysregulated ADAM10-mediated processing of APP during a critical time window leads to synaptic deficits in fragile X syndrome. *Neuron* **87**, 382–398 (2015).
64. Westmark, C. J. *et al.* Reversal of fragile X phenotypes by manipulation of AβPP/Aβ levels in Fmr1KO mice. *PLoS ONE* **6**, e26549 (2011).
65. Tabet, R. *et al.* Fragile X mental retardation protein (FMRP) controls diacylglycerol kinase activity in neurons. *Proc. Natl Acad. Sci. USA* **113**, E3619–E3628 (2016).
This paper identified the dysregulation of the DAG/PA homeostasis in neurons lacking FMRP as the possible molecular cause of mGluR signalling alteration.
66. Michaluk, P. *et al.* Influence of matrix metalloproteinase MMP-9 on dendritic spine morphology. *J. Cell Sci.* **124**, 3369–3380 (2011).
67. Dziembowska, M. *et al.* High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. *Am. J. Med Genet. A* **161A**, 1897–1903 (2013).
68. Bilousova, T. V. *et al.* Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *J. Med. Genet.* **46**, 94–102 (2009).
69. Rotschafer, S. E., Trujillo, M. S., Dansie, L. E., Ethell, I. M. & Razak, K. A. Minocycline treatment reverses ultrasonic vocalization production deficit in a mouse model of fragile X syndrome. *Brain Res.* **1439**, 7–14 (2012).
70. Gantois, I. *et al.* Metformin ameliorates core deficits in a mouse model of fragile X syndrome. **23**, 674–677 (2017).
71. Imai, S., Kai, M., Yasuda, S., Kanoh, H. & Sakane, F. Identification and characterization of a novel human type II diacylglycerol kinase, DGKκ. *J. Biol. Chem.* **280**, 39870–39881 (2005).
72. Sakane, F., Imai, S., Kai, M., Yasuda, S. & Kanoh, H. Diacylglycerol kinases as emerging potential drug targets for a variety of diseases. *Curr. Drug Targets* **9**, 626–640 (2008).
73. van der Zanden, L. F. *et al.* Common variants in DGKK are strongly associated with risk of hypospadias. *Nat. Genet.* **43**, 48–50 (2011).
74. Kim, K., Yang, J. & Kim, E. Diacylglycerol kinases in the regulation of dendritic spines. *J. Neurochem.* **112**, 577–587 (2010).
75. Hall, S. S., Lightbody, A. A., Hirt, M., Rezvani, A. & Reiss, A. L. Autism in fragile X syndrome: a category mistake? *J. Am. Acad. Child Adolesc. Psychiatry* **49**, 921–933 (2010).
76. Mouslech, Z. & Valla, V. Endocannabinoid system: an overview of its potential in current medical practice. *Neuro Endocrinol. Lett.* **30**, 153–179 (2009).
77. Pacher, P., Batkai, S. & Kunos, G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* **58**, 389–462 (2006).
78. Zhang, L. & Alger, B. E. Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. *J. Neurosci.* **30**, 5724–5729 (2010).
79. Maccarrone, M. *et al.* Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. *Neuropsychopharmacology* **35**, 1500–1509 (2010).
80. Busquets-Garcia, A. *et al.* Targeting the endocannabinoid system in the treatment of fragile X syndrome. *Nat. Med.* **19**, 603–607 (2013).
81. Jung, K. M. *et al.* Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. *Nat. Commun.* **3**, 1080 (2012).
82. Myrick, L. K. *et al.* Independent role for presynaptic FMRP revealed by an FMR1 missense mutation associated with intellectual disability and seizures. *Proc. Natl Acad. Sci. USA* **112**, 949–956 (2015).
83. Ferron, L., Nieto-Rostro, M., Cassidy, J. S. & Dolphin, A. C. Fragile X mental retardation protein controls synaptic vesicle exocytosis by modulating N-type calcium channel density. *Nat. Commun.* **5**, 3628 (2014).
84. Darnell, J. C. *et al.* Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* **107**, 489–499 (2001).
85. Brown, V. *et al.* Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell* **107**, 477–487 (2001).

86. Suhl, J. A., Chopra, P., Anderson, B. R., Bassell, G. J. & Warren, S. T. Analysis of FMRP mRNA target datasets reveals highly associated mRNAs mediated by G-quadruplex structures formed via clustered WGGA sequences. *Hum. Mol. Genet.* **23**, 5479–5491 (2014).
87. Braat, S. & Kooy, R. F. The $GABA_A$ receptor as a therapeutic target for neurodevelopmental disorders. *Neuron* **86**, 1119–1130 (2015).
This paper highlighted that the GABAergic system is compromised in a range of related neurodevelopmental disorders, including FXS.
88. Curia, G., Papouin, T., Seguela, P. & Avoli, M. Downregulation of tonic GABAergic inhibition in a mouse model of fragile X syndrome. *Cereb. Cortex* **19**, 1515–1520 (2009).
89. Sabanov, V. *et al.* Impaired GABAergic inhibition in the hippocampus of Fmr1 knockout mice. *Neuropharmacology* **116**, 71–81 (2017).
90. Olmos-Serrano, J. L. *et al.* Defective GABAergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. *J. Neurosci.* **30**, 9929–9938 (2010).
91. Vislay, R. L. *et al.* Homeostatic responses fail to correct defective amygdala inhibitory circuit maturation in fragile X syndrome. *J. Neurosci.* **33**, 7548–7558 (2013).
92. Centonze, D. *et al.* Abnormal striatal GABA transmission in the mouse model for the fragile X syndrome. *Biol. Psychiatry* **63**, 963–973 (2008).
93. Paluszkiewicz, S. M., Olmos-Serrano, J. L., Corbin, J. G. & Huntsman, M. M. Impaired inhibitory control of cortical synchronization in fragile X syndrome. *J. Neurophysiol.* **106**, 2264–2272 (2011).
94. Gibson, J. R., Bartley, A. F., Hays, S. A. & Huber, K. M. Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. *J. Neurophysiol.* **100**, 2615–2626 (2008).
95. He, Q., Nomura, T., Xu, J. & Contractor, A. The developmental switch in GABA polarity is delayed in fragile X mice. *J. Neurosci.* **34**, 446–450 (2014).
96. Tyzio, R. *et al.* Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science* **343**, 675–679 (2014).
97. D'Hulst, C. & Kooy, R. F. The $GABA_A$ receptor: a novel target for treatment of fragile X? *Trends Neurosci.* **30**, 425–431 (2007).
98. Lozano, R., Hare, E. B. & Hagerman, R. J. Modulation of the GABAergic pathway for the treatment of fragile X syndrome. *Neuropsychiatr. Dis. Treat.* **10**, 1769–1779 (2014).
99. Chang, S. *et al.* Identification of small molecules rescuing fragile X syndrome phenotypes in *Drosophila*. *Nat. Chem. Biol.* **4**, 256–263 (2008).
100. Olmos-Serrano, J. L., Corbin, J. G. & Burns, M. P. The $GABA_A$ receptor agonist THIP ameliorates specific behavioral deficits in the mouse model of fragile X syndrome. *Dev. Neurosci.* **33**, 395–403 (2011).
101. Reddy, D. S. & Estes, W. A. Clinical potential of neurosteroids for CNS disorders. *Trends Pharmacol. Sci.* **37**, 543–561 (2016).
102. Tassone, F. Advanced technologies for the molecular diagnosis of fragile X syndrome. *Expert Rev. Mol. Diagn.* **15**, 1465–1473 (2015).
103. Braat, S. *et al.* The GABAA receptor is an FMRP target with therapeutic potential in fragile X syndrome. *Cell Cycle* **14**, 2985–2995 (2015).
104. Kashima, R. *et al.* Augmented noncanonical BMP type II receptor signaling mediates the synaptic abnormality of fragile X syndrome. *Sci. Signal.* **9**, ra58 (2016).
105. Hagerman, P. Fragile X-associated tremor/ataxia syndrome (FXTAS): pathology and mechanisms. *Acta Neuropathol.* **126**, 1–19 (2013).
106. Hazlett, H. C. *et al.* Trajectories of early brain volume development in fragile X syndrome and autism. *J. Am. Acad. Child Adolesc. Psychiatry* **51**, 921–933 (2012).
107. Reiss, A. L., Abrams, M. T., Greenlaw, R., Freund, L. & Denckla, M. B. Neurodevelopmental effects of the *FMR-1* full mutation in humans. *Nat. Med.* **1**, 159–167 (1995).
108. Reiss, A. L., Patel, S., Kumar, A. J. & Freund, L. Preliminary communication: neuroanatomical variations of the posterior fossa in men with the fragile X (Martin–Bell) syndrome. *Am. J. Med. Genet.* **31**, 407–414 (1988).
109. Mostofsky, S. H. *et al.* Decreased cerebellar posterior vermis size in fragile X syndrome: correlation with neurocognitive performance. *Neurology* **50**, 121–130 (1998).
110. Gothelf, D. *et al.* Neuroanatomy of fragile X syndrome is associated with aberrant behavior and the fragile X mental retardation protein (FMRP). *Ann. Neurol.* **63**, 40–51 (2008).
111. Hoeft, F. *et al.* Morphometric spatial patterns differentiating boys with fragile X syndrome, typically developing boys, and developmentally delayed boys aged 1 to 3 years. *Arch. Gen. Psychiatry* **65**, 1087–1097 (2008).
112. Wang, J. Y. *et al.* Abnormal trajectories in cerebellum and brainstem volumes in carriers of the fragile X premutation. *Neurobiol. Aging* **55**, 11–19 (2017).
113. Shelton, A. L. *et al.* White matter microstructure, cognition, and molecular markers in fragile X premutation females. *Neurology* **88**, 2080–2088 (2017).
114. Eliez, S., Blasey, C. M., Freund, L. S., Hastie, T. & Reiss, A. L. Brain anatomy, gender and IQ in children and adolescents with fragile X syndrome. *Brain* **124**, 1610–1618 (2001).
115. Hazlett, H. C. *et al.* Teasing apart the heterogeneity of autism: same behavior, different brains in toddlers with fragile X syndrome and autism. *J. Neurodev. Disord.* **1**, 81–90 (2009).
116. Bruno, J. L. *et al.* Altered brain network segregation in fragile X syndrome revealed by structural connectomics. *Cereb. Cortex* **27**, 2249–2259 (2017).
117. Bruno, J. L. *et al.* Aberrant basal ganglia metabolism in fragile X syndrome: a magnetic resonance spectroscopy study. *J. Neurodev. Disord.* **5**, 20 (2013).
118. Wolff, J. J., Hazlett, H. C., Lightbody, A. A., Reiss, A. L. & Piven, J. Repetitive and self-injurious behaviors: associations with caudate volume in autism and fragile X syndrome. *J. Neurodev. Disord.* **5**, 12 (2013).
119. Reiss, A. L., Lee, J. & Freund, L. Neuroanatomy of fragile X syndrome: the temporal lobe. *Neurology* **44**, 1317–1324 (1994).
120. Kates, W. R., Abrams, M. T., Kaufmann, W. E., Breiter, S. N. & Reiss, A. L. Reliability and validity of MRI measurement of the amygdala and hippocampus in children with fragile X syndrome. *Psychiatry Res.* **75**, 31–48 (1997).
121. Jakala, P. *et al.* Fragile-X: neuropsychological test performance, CGG triplet repeat lengths, and hippocampal volumes. *J. Clin. Invest.* **100**, 331–338 (1997).
122. Hall, S. S., Dougherty, R. F. & Reiss, A. L. Profiles of aberrant white matter microstructure in fragile X syndrome. *Neuroimage Clin.* **11**, 133–138 (2016).
123. Hall, S. S., Jiang, H., Reiss, A. L. & Greicius, M. D. Identifying large-scale brain networks in fragile X syndrome. *JAMA Psychiatry* **70**, 1215–1223 (2013).
124. Garrett, A. S., Menon, V., MacKenzie, K. & Reiss, A. L. Here's looking at you, kid: neural systems underlying face and gaze processing in fragile X syndrome. *Arch. Gen. Psychiatry* **61**, 281–288 (2004).
125. Watson, C., Hoeft, F., Garrett, A. S., Hall, S. S. & Reiss, A. L. Aberrant brain activation during gaze processing in boys with fragile X syndrome. *Arch. Gen. Psychiatry* **65**, 1315–1323 (2008).
126. Holsen, L. M., Dalton, K. M., Johnstone, T. & Davidson, R. J. Prefrontal social cognition network dysfunction underlying face encoding and social anxiety in fragile X syndrome. *Neuroimage* **43**, 592–604 (2008).
127. Kwon, H. *et al.* Functional neuroanatomy of visuospatial working memory in fragile X syndrome: relation to behavioral and molecular measures. *Am. J. Psychiatry* **158**, 1040–1051 (2001).
128. Menon, V., Leroux, J., White, C. D. & Reiss, A. L. Frontostriatal deficits in fragile X syndrome: relation to *FMR1* gene expression. *Proc. Natl Acad. Sci. USA* **101**, 3615–3620 (2004).
129. Rivera, S. M., Menon, V., White, C. D., Glaser, B. & Reiss, A. L. Functional brain activation during arithmetic processing in females with fragile X syndrome is related to FMR1 protein expression. *Hum. Brain Mapp.* **16**, 206–218 (2002).
130. Klabunde, M. *et al.* Examining the neural correlates of emergent equivalence relations in fragile X syndrome. *Psychiatry Res.* **233**, 373–379 (2015).
131. Tamm, L., Menon, V., Johnston, C. K., Hessl, D. R. & Reiss, A. L. fMRI study of cognitive interference processing in females with fragile X syndrome. *J. Cogn. Neurosci.* **14**, 160–171 (2002).
132. Hoeft, F. *et al.* Fronto-striatal dysfunction and potential compensatory mechanisms in male adolescents with fragile X syndrome. *Hum. Brain Mapp.* **28**, 543–554 (2007).
133. Rajan-Babu, I. S. & Chong, S. S. Molecular correlates and recent advancements in the diagnosis and screening of FMR1-related disorders. *Genes* **7,** E87 (2016).
134. Yrigollen, C. M. *et al.* AGG interruptions within the maternal *FMR1* gene reduce the risk of offspring with fragile X syndrome. *Genet. Med.* **14**, 729–736 (2012).
135. Nolin, S. L. *et al.* Fragile X AGG analysis provides new risk predictions for 45–69 repeat alleles. *Am. J. Med Genet. A* **161A**, 771–778 (2013).
136. Yrigollen, C. M. *et al.* AGG interruptions and maternal age affect *FMR1* CGG repeat allele stability during transmission. *J. Neurodev. Disord.* **6**, 24 (2014).
This large study aimed to determine the predicted risk to expansion to a full mutation during maternal transmission, and it identified CGG repeat number, AGG interruptions and maternal age as the main players.
137. Bailey, D. B. Jr, Raspa, M., Bishop, E. & Holiday, D. No change in the age of diagnosis for fragile X syndrome: findings from a national parent survey. *Pediatrics* **124**, 527–533 (2009).
138. Tassone, F. *et al. FMR1* CGG allele size and prevalence ascertained through newborn screening in the United States. *Genome Med.* **4**, 100 (2012).
139. Harris, S. W. *et al.* Autism profiles of males with fragile X syndrome. *Am. J. Ment. Retard.* **113**, 427–438 (2008).
140. Kaufmann, W. E. *et al.* Autism spectrum disorder in fragile X syndrome: communication, social interaction, and specific behaviors. *Am. J. Med Genet. A* **129A**, 225–234 (2004).
141. Yuhas, J. *et al.* High-risk fragile X screening in Guatemala: use of a new blood spot polymerase chain reaction technique. *Genet. Test. Mol. Biomarkers* **13**, 855–859 (2009).
142. Winarni, T. I. *et al.* Identification of expanded alleles of the *FMR1* gene among high-risk population in Indonesia by using blood spot screening. *Genet. Test. Mol. Biomarkers* **16**, 162–166 (2012).
143. Kanwal, M. *et al.* Molecular diagnosis of fragile X syndrome in subjects with intellectual disability of unknown origin: implications of its prevalence in regional Pakistan. *PLoS ONE* **10**, e0122213 (2015).
144. McConkie-Rosell, A. *et al.* Recommendations from multi-disciplinary focus groups on cascade testing and genetic counseling for fragile X-associated disorders. *J. Genet. Couns.* **16**, 593–606 (2007).
145. Berman, R. F. *et al.* Mouse models of the fragile X premutation and fragile X-associated tremor/ataxia syndrome. *J. Neurodev. Disord.* **6**, 25 (2014).
146. Polussa, J., Schneider, A. & Hagerman, R. Molecular advances leading to treatment implications for fragile X premutation carriers. *Brain Disord. Ther.* **3,** 1000119 (2014).
147. Visootsak, J. *et al.* Climbing the branches of a family tree: diagnosis of fragile X syndrome. *J. Pediatr.* **164**, 1292–1295 (2014).
148. Rogers, S. J. *et al.* Teaching young nonverbal children with autism useful speech: a pilot study of the Denver Model and PROMPT interventions. *J. Autism Dev. Disord.* **36**, 1007–1024 (2006).
149. Dawson, G. *et al.* Randomized, controlled trial of an intervention for toddlers with autism: the Early Start Denver Model. *Pediatrics* **125**, e17–23 (2010).
150. Dawson, G. *et al.* Early behavioral intervention is associated with normalized brain activity in young children with autism. *J. Am. Acad. Child Adolesc. Psychiatry* **51**, 1150–1159 (2012).
151. Braden, M. L. *Fragile, Handle with Care: More About Fragile X Syndrome, Adolescents and Adults* (Spectra Publishing Co., 2000).
152. Hills Epstein, J. L., Riley, K. & Sobesky, W. in *Fragile X Syndrome: Diagnosis, Treatment, and Research* (eds Hagerman, R. J. & Hagerman, P. J.) 339–362 (Johns Hopkins Univ. Press, 2002).
153. Hagerman, R. J., Murphy, M. A. & Wittenberger, M. D. A controlled trial of stimulant medication in children with the fragile X syndrome. *Am. J. Med. Genet.* **30**, 377–392 (1988).
154. Hagerman, R. J. *et al.* Advances in the treatment of fragile X syndrome. *Pediatrics* **123**, 378–390 (2009).
155. Wirojanan, J. *et al.* The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X syndrome. *J. Clin. Sleep Med.* **5**, 145–150 (2009).
156. Torrioli, M. G. *et al.* Double-blind, placebo-controlled study of L-acetylcarnitine for the treatment of hyperactive behavior in fragile X syndrome. *Am. J. Med. Genet.* **87**, 366–368 (1999).

157. Torrioli, M. *et al.* Treatment with valproic acid ameliorates ADHD symptoms in fragile X syndrome boys. *Am. J. Med Genet. A* **152A**, 1420–1427 (2010).
158. Greiss Hess, L. *et al.* A randomized, double-blind, placebo-controlled trial of low-dose sertraline in young children with fragile X syndrome. *J. Dev. Behav. Pediatr.* **37**, 619–628 (2016).
This paper used a low dose of sertraline in young children (2–6 years of age) with FXS and found significant improvement compared with placebo in development, including visual reception, fine motor coordination, composite cognitive score and, in those with FXS plus ASD, overall expressive language score. This work suggested that early treatment with low-dose sertraline is beneficial and can be used clinically.
159. Erickson, C. A., Stigler, K. A., Posey, D. J. & McDougle, C. J. Aripiprazole in autism spectrum disorders and fragile X syndrome. *Neurotherapeutics* **7**, 258–263 (2010).
160. Hersh, J. H. & Saul, R. A. Health supervision for children with fragile X syndrome. *Pediatrics* **127**, 994–1006 (2011).
161. Kronk, R. *et al.* Prevalence, nature, and correlates of sleep problems among children with fragile X syndrome based on a large scale parent survey. *Sleep* **33**, 679–687 (2010).
162. McLennan, Y., Polussa, J., Tassone, F. & Hagerman, R. Fragile X syndrome. *Curr. Genomics* **12**, 216–224 (2011).
163. Nowicki, S. T. *et al.* The Prader–Willi phenotype of fragile X syndrome. *J. Dev. Behav. Pediatr.* **28**, 133–138 (2007).
164. Dy, A. B. C. *et al.* Metformin as targeted treatment in fragile X syndrome. *Clin. Genet.* http://dx.doi.org/10.1111/cge.13039 (2017).
165. Berry-Kravis, E. *et al.* Mavoglurant in fragile X syndrome: results of two randomized, double-blind, placebo-controlled trials. *Sci. Transl Med.* **8**, 321ra325 (2016).
This was hitherto the largest phase IIb clinical trial and illustrated the complexity of FXS pathology and the difficulty of the translation of human treatments validated in mouse models.
166. Jacquemont, S. *et al.* Epigenetic modification of the *FMR1* gene in fragile X. syndrome is associated with differential response to the mGluR5 antagonist AFQ056. *Sci. Transl Med.* **3**, 64ra61 (2011).
167. Wang, H. *et al.* FMRP acts as a key messenger for dopamine modulation in the forebrain. *Neuron* **59**, 634–647 (2008).
168. Curie, A. *et al.* Placebo responses in genetically determined intellectual disability: a meta-analysis. *PLoS ONE* **10**, e0133316 (2015).
169. Budimirovic, D. B. *et al.* Updated report on tools to measure outcomes of clinical trials in fragile X syndrome. *J. Neurodev. Disord.* **9**, 14 (2017).
170. Henderson, C. *et al.* Reversal of disease-related pathologies in the fragile X mouse model by selective activation of $GABA_B$ receptors with arbaclofen. *Sci. Transl Med.* **4**, 152ra128 (2012).
171. Berry-Kravis, E. M. *et al.* Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: a randomized, controlled, phase 2 trial. *Sci. Transl Med.* **4**, 152ra127 (2012).
172. Sansone, S. M. *et al.* Psychometric study of the Aberrant Behavior Checklist in fragile X syndrome and implications for targeted treatment. *J. Autism Dev. Disord.* **42**, 1377–1392 (2012).
173. Berry-Kravis, E. *et al.* Arbaclofen in fragile X syndrome: results of phase 3 trials. *J. Neurodev. Disord.* **9**, 3 (2017).
174. Erickson, C. A. *et al.* Impact of acamprosate on behavior and brain-derived neurotrophic factor: an open-label study in youth with fragile X syndrome. *Psychopharmacology (Berl.)* **228**, 75–84 (2013).
175. Erickson, C. A. *et al.* Impact of acamprosate on plasma amyloid-beta precursor protein in youth: a pilot analysis in fragile X syndrome-associated and idiopathic autism spectrum disorder suggests a pharmacodynamic protein marker. *J. Psychiatr. Res.* **59**, 220–228 (2014).
176. Berry-Kravis, E., Rubin, J., Harary, E. & Daniely, Y. A 6-week, randomized, multicenter, double-blind, parallel, flexed- and fixed-dose study of MDX (metadoxine extended-release; MG01CI) compared with placebo in adolescents and adults with fragile X syndrome. *AACAP* http://files.shareholder.com/downloads/AMDA-1SVKDP/0x0x858015/E85D78F1-33D2-46F4-B4C7-96472D1F9A22/AACAP_AL014_poster_final.pdf (2015).
177. Knox, A. *et al.* Feasibility, reliability, and clinical validity of the Test of Attentional Performance for Children (KiTAP) in fragile X syndrome (FXS). *J. Neurodev. Disord.* **4**, 2 (2012).
178. Ligsay, A. *et al.* A randomized double-blind, placebo-controlled trial of ganaxolone in children and adolescents with fragile X syndrome. *J. Neurodev. Disord.* **9**, 26 (2017).
179. McDuffie, A. *et al.* Distance video-teleconferencing in early intervention. *Top. Early Childhood Special Educ.* **33**, 172–185 (2013).
180. McDuffie, A. *et al.* A spoken-language intervention for school-aged boys with fragile X syndrome. *Am. J. Intellect. Dev. Disabil.* **121**, 236–265 (2016).
181. McDuffie, A. *et al.* Early language intervention using distance video-teleconferencing: a pilot study of young boys with fragile X syndrome and their mothers. *Am. J. Speech Lang. Pathol.* **25**, 46–66 (2016).
182. Schneider, A. *et al.* Electrocortical changes associated with minocycline treatment in fragile X syndrome. *J. Psychopharmacol.* **27**, 956–963 (2013).
183. Farzin, F., Scaggs, F., Hervey, C., Berry-Kravis, E. & Hessl, D. Reliability of eye tracking and pupillometry measures in individuals with fragile X syndrome. *J. Autism Dev. Disord.* **41**, 1515–1522 (2011).
184. Berry-Kravis, E. *et al.* Sensitivity of the KiTAP executive function battery and an eye tracking paradigm to effects of AFQ056 in fragile X syndrome. *Ann. Neurol.* **80**, S413 (2016).
185. Paribello, C. *et al.* Open-label add-on treatment trial of minocycline in fragile X syndrome. *BMC Neurol.* **10**, 91 (2010).
186. Utari, A. *et al.* Side effects of minocycline treatment in patients with fragile X syndrome and exploration of outcome measures. *Am. J. Intellect. Dev. Disabil.* **115**, 433–443 (2010).
187. Leigh, M. J. *et al.* A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile x syndrome. *J. Dev. Behav. Pediatr.* **34**, 147–155 (2013).
188. Monyak, R. E. *et al.* Insulin signaling misregulation underlies circadian and cognitive deficits in a *Drosophila* fragile X model. *Mol. Psychiatry* **22**, 1140–1148 (2017).
189. Osterweil, E. K. *et al.* Lovastatin corrects excess protein synthesis and prevents epileptogenesis in a mouse model of fragile X syndrome. *Neuron* **77**, 243–250 (2013).
190. Caku, A., Pellerin, D., Bouvier, P., Riou, E. & Corbin, F. Effect of lovastatin on behavior in children and adults with fragile X syndrome: an open-label study. *Am. J. Med Genet. A* **164A**, 2834–2842 (2014).
191. Pellerin, D. *et al.* Lovastatin corrects ERK pathway hyperactivation in fragile X syndrome: potential of platelet's signaling cascades as new outcome measures in clinical trials. *Biomarkers* **21**, 497–508 (2016).
192. Deacon, R. M. *et al.* NNZ-2566, a novel analog of (1–3) IGF-1, as a potential therapeutic agent for fragile X syndrome. *Neuromolecular Med.* **17**, 71–82 (2015).
193. Deacon, R. M. *et al.* Nrf2, a novel therapeutic target in fragile X syndrome is modulated by NNZ2566. *Genes Brain Behav.* http://dx.doi.org/10.1111/gbb.12373 (2017).
194. Berry-Kravis, E. *et al.* The treatment of fragile X syndrome with Trofinetide (NNZ-2566). *Ann. Neurol.* **80**, S412 (2016).
195. Bailey, D. B., Raspa, M. & Olmsted, M. G. Using a parent survey to advance knowledge about the nature and consequences of fragile X syndrome. *Am. J. Intellect. Dev. Disabil.* **115**, 447–460 (2010).
196. Chevreul, K. *et al.* Social/economic costs and health-related quality of life in patients with fragile X syndrome in Europe. *Eur. J. Health Econom.* **17** (Suppl. 1), 43–52 (2016).
This paper described the burden and costs of FXS and suggested key outcomes that should change as a function of appropriate treatment.
197. Bailey, D. B. Jr., Raspa, M., Olmsted, M. & Holiday, D. B. Co-occurring conditions associated with *FMR1* gene variations: findings from a national parent survey. *Am. J. Med Genet. A* **146A**, 2060–2069 (2008).
198. Chevreul, K., Berg Brigham, K., Brunn, M., des Portes, V. & Network, B.-R. R. Fragile X syndrome: economic burden and health-related quality of life of patients and caregivers in France. *J. Intellect. Disabil. Res.* **59**, 1108–1120 (2015).
199. Bailey, D. B. Jr, Sideris, J., Roberts, J. & Hatton, D. Child and genetic variables associated with maternal adaptation to fragile X syndrome: a multidimensional analysis. *Am. J. Med Genet. A* **146A**, 720–729 (2008).
200. Wheeler, A. C., Skinner, D. G. & Bailey, D. B. Perceived quality of life in mothers of children with fragile X syndrome. *Am. J. Ment. Retard.* **113**, 159–177 (2008).
201. Raspa, M., Bailey, D. B. Jr, Bann, C. & Bishop, E. Modeling family adaptation to fragile X syndrome. *Am. J. Intellect. Dev. Disabil.* **119**, 33–48 (2014).
202. Bailey, D. B. Jr. *et al.* Health and economic consequences of fragile X syndrome for caregivers. *J. Dev. Behav. Pediatr.* **33**, 705–712 (2012).
203. Roberts, J. E. *et al.* Trajectory and predictors of depression and anxiety disorders in mothers with the *FMR1* premutation. *Biol. Psychiatry* **79**, 850–857 (2016).
This paper provided the first insights into the longitudinal effects of premutation carrier status on depression and anxiety disorders.
204. Ouyang, L., Grosse, S., Raspa, M. & Bailey, D. Employment impact and financial burden for families of children with fragile X syndrome: findings from the National Fragile X Survey. *J. Intellect. Disabil. Res.* **54**, 918–928 (2010).
205. Ouyang, L. *et al.* A comparison of family financial and employment impacts of fragile X syndrome, autism spectrum disorders, and intellectual disability. *Res. Dev. Disabil.* **35**, 1518–1527 (2014).
206. Cross, J. *et al.* Caregiver preferences for the treatment of males with fragile X syndrome. *J. Dev. Behav. Pediatr.* **37**, 71–79 (2016).
207. Berry-Kravis, E. *et al.* Development of an expressive language sampling procedure in fragile X syndrome: a pilot study. *J. Dev. Behav. Pediatr.* **34**, 245–251 (2013).
208. Hessl, D. *et al.* The NIH Toolbox Cognitive Battery for intellectual disabilities: three preliminary studies and future directions. *J. Neurodev. Disord.* **8**, 35 (2016).
209. Sansone, S. M. *et al.* Improving IQ measurement in intellectual disabilities using true deviation from population norms. *J. Neurodev. Disord.* **6**, 16 (2014).
210. Sherman, S. *et al.* FORWARD: a registry and longitudinal clinical database to study fragile X syndrome. *Pediatrics* **139**, S3 (2017).
211. Rogers, S. J. *et al.* Autism treatment in the first year of life: a pilot study of infant start, a parent-implemented intervention for symptomatic infants. *J. Autism Dev. Disord.* **44**, 2981–2995 (2014).
212. Au, J. *et al.* A feasibility trial of Cogmed working memory training in fragile X syndrome. *J. Pediatr. Genet.* **3**, 147–156 (2014).
213. Park, C. Y. *et al.* Reversion of *FMR1* methylation and silencing by editing the triplet repeats in fragile X iPSC-derived neurons. *Cell Rep.* **13**, 234–241 (2015).
214. Tassone, F. *et al.* Elevated *FMR1* mRNA in premutation carriers is due to increased transcription. *RNA* **13**, 555–562 (2007).
215. Tassone, F. *et al.* Elevated levels of *FMR1* mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. *Am. J. Hum. Genet.* **66**, 6–15 (2000).
216. Hagerman, R. J. & Hagerman, P. Fragile X-associated tremor/ataxia syndrome — features, mechanisms and management. *Nat. Rev. Neurol.* **12**, 403–412 (2016).
217. Sherman, S., Pletcher, B. A. & Driscoll, D. A. Fragile X syndrome: diagnostic and carrier testing. *Genet. Med.* **7**, 584–587 (2005).
218. Hagerman, R. & Hagerman, P. Advances in clinical and molecular understanding of the *FMR1* premutation and fragile X-associated tremor/ataxia syndrome. *Lancet Neurol.* **12**, 786–798 (2013).
219. Darnell, J. C. *et al.* FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell* **146**, 247–261 (2011).
220. Ascano, M. Jr *et al.* FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature* **492**, 382–386 (2012).
221. Miyashiro, K. Y. *et al.* RNA cargoes associating with FMRP reveal deficits in cellular functioning in *Fmr1* null mice. *Neuron* **37**, 417–431 (2003).
222. Tang, B. *et al. Fmr1* deficiency promotes age-dependent alterations in the cortical synaptic proteome. *Proc. Natl Acad. Sci. USA* **112**, E4697–E4706 (2015).
223. Schenck, A., Bardoni, B., Moro, A., Bagni, C. & Mandel, J. L. A highly conserved protein family interacting with the fragile X mental retardation protein (FMRP) and displaying selective interactions with FMRP-related proteins FXR1P and FXR2P. *Proc. Natl Acad. Sci. USA* **98**, 8844–8849 (2001).

224. Napoli, I. *et al.* The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell* **134**, 1042–1054 (2008).
225. Jin, P. *et al.* Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nat. Neurosci.* **7**, 113–117 (2004).
226. Muddashetty, R. S. *et al.* Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. *Mol. Cell* **42**, 673–688 (2011).
227. Bechara, E. G. *et al.* A novel function for fragile X mental retardation protein in translational activation. *PLoS Biol.* **7**, e16 (2009).
228. Chen, E., Sharma, M. R., Shi, X., Agrawal, R. K. & Joseph, S. Fragile x mental retardation protein regulates translation by binding directly to the ribosome. *Mol. Cell* **54**, 407–417 (2014).
229. Iossifov, I. *et al. De novo* gene disruptions in children on the autistic spectrum. *Neuron* **74**, 285–299 (2012).
230. Boland, M. J. *et al.* Molecular analyses of neurogenic defects in a human pluripotent stem cell model of fragile X syndrome. *Brain* **140**, 582–598 (2017).
231. Fatemi, S. H., Folsom, T. D., Rooney, R. J. & Thuras, P. D. mRNA and protein expression for novel GABAA receptors theta and rho2 are altered in schizophrenia and mood disorders; relevance to FMRP–mGluR5 signaling pathway. *Transl Psychiatry* **3**, e271 (2013).
232. Fatemi, S. H., Kneeland, R. E., Liesch, S. B. & Folsom, T. D. Fragile X mental retardation protein levels are decreased in major psychiatric disorders. *Schizophr. Res.* **124**, 246–247 (2010).
233. McDuffie, A., Thurman, A. J., Hagerman, R. J. & Abbeduto, L. Symptoms of Autism in males with fragile X syndrome: a comparison to nonsyndromic ASD using current ADI-R scores. *J. Autism Dev. Disord.* **45**, 1925–1937 (2015).
234. Thurman, A. J., McDuffie, A., Hagerman, R. & Abbeduto, L. Psychiatric symptoms in boys with fragile X syndrome: a comparison with nonsyndromic autism spectrum disorder. *Res. Dev. Disabil.* **35**, 1072–1086 (2014).
235. Talisa, V. B., Boyle, L., Crafa, D. & Kaufmann, W. E. Autism and anxiety in males with fragile X syndrome: an exploratory analysis of neurobehavioral profiles from a parent survey. *Am. J. Med Genet. A* **164A**, 1198–1203 (2014).
236. Hardiman, R. L. & Bratt, A. Hypothalamic–pituitary–adrenal axis function in fragile X syndrome and its relationship to behaviour: a systematic review. *Physiol. Behav.* **167**, 341–353 (2016).
237. Hessl, D., Rivera, S. M. & Reiss, A. L. The neuroanatomy and neuroendocrinology of fragile X syndrome. *Ment. Retard. Dev. Disabil. Res. Rev.* **10**, 17–24 (2004).
238. Thurman, A. J., McDuffie, A., Hagerman, R. J., Josol, C. K. & Abbeduto, L. Language skills of males with fragile X syndrome or nonsyndromic autism spectrum disorder. *J. Autism Dev. Disord.* **47**, 728–743 (2017).
239. Oberman, L. M. *et al.* Abnormal mechanisms of plasticity and metaplasticity in autism spectrum disorders and fragile X syndrome. *J. Child Adolesc. Psychopharmacol.* **26**, 617–624 (2016).
240. Hartley, S. L. *et al.* Exploring the adult life of men and women with fragile X syndrome: results from a national survey. *Am. J. Intellect. Dev. Disabil.* **116**, 16–35 (2011).
241. Wheeler, A. C., Raspa, M., Bishop, E. & Bailey, D. B. Jr. Aggression in fragile X syndrome. *J. Intellect. Disabil. Res.* **60**, 113–125 (2016).
242. Raspberry, K. A. & Skinner, D. Negotiating desires and options: how mothers who carry the fragile X gene experience reproductive decisions. *Soc. Sci. Med.* **72**, 992–998 (2011).
243. Raspa, M., Edwards, A., Wheeler, A. C., Bishop, E. & Bailey, D. B. Jr. Family communication and cascade testing for fragile X syndrome. *J. Genet. Couns.* **25**, 1075–1084 (2016).
244. Wheeler, A. C. *et al.* Associated features in females with an *FMR1* premutation. *J. Neurodev. Disord.* **6**, 30 (2014).
245. Wheeler, A. C. *et al.* Health and reproductive experiences of women with an *FMR1* premutation with and without fragile X premature ovarian insufficiency. *Front. Genet.* **5**, 300 (2014).
246. Cornish, K., Cole, V., Longhi, E., Karmiloff-Smith, A. & Scerif, G. Mapping developmental trajectories of attention and working memory in fragile X syndrome: developmental freeze or developmental change? *Dev. Psychopathol.* **25**, 365–376 (2013).
247. Cornish, K., Scerif, G. & Karmiloff-Smith, A. Tracing syndrome-specific trajectories of attention across the lifespan. *Cortex* **43**, 672–685 (2007).
248. Musumeci, S. A. *et al.* Epilepsy and EEG findings in males with fragile X syndrome. *Epilepsia* **40**, 1092–1099 (1999).
249. Roberts, J. E. *et al.* Autistic behavior in boys with fragile X syndrome: social approach and HPA-axis dysfunction. *J. Neurodev. Disord.* **1**, 283–291 (2009).
250. de Vries, B. B. *et al.* Mental status of females with an FMR1 gene full mutation. *Am. J. Hum. Genet.* **58**, 1025–1032 (1996).
251. Utari, A. *et al.* Aging in fragile X syndrome. *J. Neurodev. Disord.* **2**, 70–76 (2010).
252. Abe, T. *et al.* Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca^{2+} signal transduction. *J. Biol. Chem.* **267**, 13361–13368 (1992).
253. Banko, J. L., Hou, L., Poulin, F., Sonenberg, N. & Klann, E. Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. *J. Neurosci.* **26**, 2167–2173 (2006).
254. Tanimura, A. *et al.* The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. *Neuron* **65**, 320–327 (2010).
255. Deng, P. Y. & Klyachko, V. A. Increased persistent sodium current causes neuronal hyperexcitability in the entorhinal cortex of Fmr1 knockout mice. *Cell Rep.* **16**, 3157–3166 (2016).
256. Osterweil, E. K., Krueger, D. D., Reinhold, K. & Bear, M. F. Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. *J. Neurosci.* **30**, 15616–15627 (2010).
257. Sharma, A. *et al.* Dysregulation of mTOR signaling in fragile X syndrome. *J. Neurosci.* **30**, 694–702 (2010).
258. Gross, C. *et al.* Excess phosphoinositide 3-kinase subunit synthesis and activity as a novel therapeutic target in fragile X syndrome. *J. Neurosci.* **30**, 10624–10638 (2010).
259. Chen, L. Y. *et al.* Physiological activation of synaptic Rac > PAK (p-21 activated kinase) signaling is defective in a mouse model of fragile X syndrome. *J. Neurosci.* **30**, 10977–10984 (2010).
260. Almena, M. & Merida, I. Shaping up the membrane: diacylglycerol coordinates spatial orientation of signaling. *Trends Biochem. Sci.* **36**, 593–603 (2011).
261. Ghosh, S. & Bell, R. M. Regulation of Raf-1 kinase by interaction with the lipid second messenger, phosphatidic acid. *Biochem. Soc. Trans.* **25**, 561–565 (1997).
262. Stace, C. *et al.* PA binding of phosphatidylinositol 4-phosphate 5-kinase. *Adv. Enzyme Regul.* **48**, 55–72 (2008).
263. Avila-Flores, A., Santos, T., Rincon, E. & Merida, I. Modulation of the mammalian target of rapamycin pathway by diacylglycerol kinase-produced phosphatidic acid. *J. Biol. Chem.* **280**, 10091–10099 (2005).
264. Gantois, I. *et al.* Expression profiling suggests underexpression of the $GABA_A$ receptor subunit delta in the fragile X knockout mouse model. *Neurobiol. Dis.* **21**, 346–357 (2006).
265. D'Hulst, C. *et al.* Decreased expression of the $GABA_A$ receptor in fragile X syndrome. *Brain Res.* **1121**, 238–245 (2006).
This was the first paper to demonstrate convincingly that GABAergic abnormalities underlie FXS.
266. Hong, A., Zhang, A., Ke, Y., El Idrissi, A. & Shen, C. H. Downregulation of $GABA_A$ beta subunits is transcriptionally controlled by Fmr1p. *J. Mol. Neurosci.* **46**, 272–275 (2011).
267. El Idrissi, A. *et al.* Decreased $GABA_A$ receptor expression in the seizure-prone fragile X mouse. *Neurosci. Lett.* **377**, 141–146 (2005).
268. Gatto, C. L., Pereira, D. & Broadie, K. GABAergic circuit dysfunction in the *Drosophila* fragile X syndrome model. *Neurobiol. Dis.* **65**, 142–159 (2014).
269. Adusei, D. C., Pacey, L. K., Chen, D. & Hampson, D. R. Early developmental alterations in GABAergic protein expression in fragile X knockout mice. *Neuropharmacology* **59**, 167–171 (2010).
270. Kratovac, S. & Corbin, J. G. Developmental changes in expression of inhibitory neuronal proteins in the fragile X syndrome mouse basolateral amygdala. *Brain Res.* **1537**, 69–78 (2013).
271. D'Hulst, C. *et al.* Positron emission tomography (PET) quantification of GABAA receptors in the brain of fragile X patients. *PLoS ONE* **10**, e0131486 (2015).
272. Kang, J. Y. *et al.* Deficits in the activity of presynaptic γ-aminobutyric acid type B receptors contribute to altered neuronal excitability in fragile X syndrome. *J. Biol. Chem.* **292**, 6621–6632 (2017).
273. D'Hulst, C. *et al.* Expression of the GABAergic system in animal models for fragile X syndrome and fragile X associated tremor/ataxia syndrome (FXTAS). *Brain Res.* **1253**, 176–183 (2009).
274. Davidovic, L. *et al.* A metabolomic and systems biology perspective on the brain of the fragile X syndrome mouse model. *Genome Res.* **21**, 2190–2202 (2011).
275. Paluszkiewicz, S. M., Martin, B. S. & Huntsman, M. M. Fragile X syndrome: the GABAergic system and circuit dysfunction. *Dev. Neurosci.* **33**, 349–364 (2011).
276. Gross, C., Hoffmann, A., Bassell, G. J. & Berry-Kravis, E. M. Therapeutic strategies in fragile X syndrome: from bench to bedside and back. *Neurotherapeutics* **12**, 584–608 (2015).
This was a comprehensive review of all preclinical work and targeted treatments in clinical trials for FXS, including outcome measures used and measures showing change.
277. Hoffmann, A. & Berry-Kravis, E. in *Neuronal and Synaptic Dysfunction in Autism Spectrum Disorder and Intellectual Disability* (eds Sala, C. & Verpelli, C.) 325–346 (Academic Press, 2016).

Acknowledgements
This work was supported by the following grants. R.J.H., P.J.H. and F.T. were funded by US Health Resources and Services Administration (HRSA) grant R40MC27701, National Institute of Child Health and Human Development grant R01HD036071, HRSA grant R40MC22641, Department of Defense grant PR101054, MIND Institute Intellectual and Developmental Disability Research Center U54HD07912. H.M. and L.M. are funded by Agence Nationale de la Recherche (ANR-12-BSV8-0022), Fondation Jérôme Lejeune funding, FRAXA Research Foundation and grant ANR-10-LABX-0030-INRT, a French State fund managed by the Agence Nationale de la Recherche under the frame programme Investissements d'Avenir ANR-10-IDEX-0002-02 to H.M. and J.L.M. R.F.K. is funded by grants from the Research Foundation Flanders, FRAXA Research Foundation and the Fondation Jérôme Lejeune. H.C.H. was funded by NICHD R01HD059854. The authors thank L. Makhoul for help with preparation of this manuscript.

Author contributions
Introduction (R.J.H. and P.J.H.); Epidemiology (F.T.); Mechanisms/pathophysiology (J.L.M., H.M., R.F.K., N.S., H.C.H. and P.J.H.); Diagnosis, screening and prevention (R.J.H. and F.T.); Management (E.B.-K. and R.J.H.); Quality of life (D.B.B.); Outlook (R.J.H., E.B.-K. and P.J.H); Overview of Primer (R.J.H. and P.J.H.).

Competing interests
R.J.H. has received funding from Novartis, Roche, Alcobra, Neuren and Marinus for clinical trials in FXS and has consulted with Zynerba for clinical trials in FXS. E.B.-K. has received funding from Novartis, Roche, Alcobra, Neuren, Cydan, Fulcrum, GW, Marinus, Edison and Neurotrope Pharmaceuticals to consult on trial design and development strategies and/or conduct clinical trials in FXS, and from Asuragen Inc. to develop testing standards for *FMR1* testing. D.B.B. received funding from The John Merck Fund to plan a fragile X newborn screening study. R.F.K. has received logistic support and ganaxolone treatment funding from Marinus Pharmaceuticals to conduct a clinical trial in FXS. F.T. has received funding for molecular studies in FXS from Asuragen. All other authors declare no competing interests.

How to cite this article
Hagerman, R. J. *et al.* Fragile X syndrome. *Nat. Rev. Dis. Primers* **3**, 17065 (2017).