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To study the epidemiology of Becker muscular dystrophy (BMD) in the Netherlands, we searched the database of the laboratory for diagnostic genome analysis (LDGA) of the Leiden university medical centre (LUMC), where genetic testing has been concentrated since the 1980's, and the Dutch dystrophinopathy database (DDD) for patients diagnosed with BMD. We expect that together these databases contain the vast majority of BMD patients and can thus be used to approximate the incidence of BMD. We collected 380 BMD patients of whom 80 were found in both the LDGA database and the DDD, 208 were only known in the LDGA and 92 only registered in the DDD. An estimate of the incidence per decade was calculated using the date of birth and seemed stable from 1970 until 2009 at around 3 per 100,000 live births (range 2.81-3.09). Interestingly the incidence between 2000-2009 was calculated at 3.06 per 100,000 live births, while part of the BMD patients in this age category is at the time of writing most likely not yet diagnosed, therefore we expect that this number is an underestimate. This might be due to advances in genetic testing due to the availability of gene panels, resulting in a younger age at diagnosis. Preliminary analysis on the type of mutation showed that the vast majority of 66% is a deletion, followed by 14% of duplications, 10% point mutations. Of these deletions and duplications, 87% are predicted not to shift the reading frame of the *DMD* gene (in-frame). In the majority of out-of-frame mutations it concerns an exon 3-7 deletion. Ongoing analyses will focus on the proportion of *de novo* mutations age at diagnosis, and survival. In conclusion, this preliminary data suggests that advances in and availability of genetic testing may have resulted in a lower age at diagnosis of BMD patients. It also emphasizes that a combination of genetic diagnostic registries and clinical registries is required to obtain a complete overview of the epidemiology of BMD.

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Double the trouble: familial hyperlipidemia and Becker muscular dystrophy with a hemizygous nonsense mutation in the dystrophin *(DMD)* gene

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Becker muscular dystrophy (BMD) is an X-linked recessive disorder caused by the absence of dystrophin. It is characterized by progressive skeletal and cardiac muscle weakness that usually becomes apparent between the ages of 5 and 15. Familial hypercholesterolemia (FH) is an autosomal dominant disorder of lipid metabolism present from birth. It is characterized by remarkably high low-density lipoprotein cholesterol (LDL-C) levels causing premature coronary heart disease. We report a 9-year-old boy with familial hyperlipidemia who presented with creatinine kinase elevation. He was born after uneventful pregnancy and delivery with consanguineous marriage of his parents. The motor developmental milestones were normal. On physical and neurological examination at the age of 9, he had only mild pseudohypertrophy in the gastrocnemius muscles. Gowers' sign was negative. He had no myotonia or muscle weakness. The cardiological evaluation was unremarkable. Serum creatine kinase (CK) level was elevated (2889 UI/L). Moreover, serum triglyceride (183 mg/dl), VLDL cholesterol (37 mg/dl), LDL cholesterol (212 mg/dl), and total cholesterol (289 mg/dl) were elevated. We identified a heterozygous mutation of c.1246 C>T (p.R416W) (paternal) in the *LDLR* gene and a hemizygous nonsense mutation of c.71G>A (p.W24) in the *DMD* gene. Based on the clinical manifestations, examination, and laboratory findings, we diagnosed BMD and familial hyperlipidemia. We described a case with familial hyperlipidemia presented with serum creatinine kinase (CK) elevation and identified hemizygous nonsense mutation of c.71G>A p.W24 in the dystrophin gene.

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DMD - BRAIN

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Expression and localization of dystrophin isoforms transcripts in human adult control brain areas.

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Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular disease due to pathogenic variants in the *DMD* gene. DMD often is associated with cognitive and neuro-behavioural co-morbidities, which pathogenesis and genotype-phenotype relationship are only partially understood. Multiple *DMD* isoforms, differentially affected based on the mutation site, play a role in these co-morbidities, based on their prominent (Dp71) or exclusive (Dp140) brain expression. All known dystrophin isoforms were analysed in 24 normal adult human brain areas using TaqMan assays on TissueScan cDNA array (OriGene). Ct were used to build a heatmap of dystrophin isoforms expression with values ranging from 40 (lowest) to 27 (highest) Ct. A specific BaseScope™ ZZ probe was also used to detect and localize the Dp71 in a formalin-fixed paraffin-embedded cerebellum. Three main dystrophin isoform clusters were identified. Cluster 1 includes Dp116, Dp427p1, Dp260.1 that show low expression in all brain areas. Cluster 2 includes Dp260.2, Dp427m, Dp140 with intermediate expression levels. Cluster 3 consists of Dp427b, Dp427p2, Dp71 that have the overall high expression in most brain areas. Hierarchical clustering also highlighted brain areas with overall low dystrophin expression, such as telencephalon (frontal, temporal and occipital lobe, amygdala, caudate, and choroid plexus), diencephalon (thalamus and hypothalamus), and myelencephalon (medulla). By contrast, the cerebellum showed the highest expression of all DMD isoforms. BaseScope on cerebellum showed Dp71 expression in molecular, granular, and Purkinje layers, white matter and blood vessels. We showed that adult human brain areas have differential enrichments for expression of specific dystrophin isoforms, and that BaseScope has high sensitivity in detecting Dp71 in fixed bran tissues. This information on the regional and cellular pattern of expression of the multiple dystrophin isoforms, may contribute to the understanding of the DMD brain co-morbidities.

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Regulation of the alternative splicing of Dp71, the major brain dystrophin transcript, by splicing factors

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Duchenne muscular dystrophy (DMD) is a fatal childhood genetic disorder, caused by the absence of dystrophin throughout the body. Parallel to muscle degradation, loss of dystrophin in the brain can lead to cognitive impairments and intellectual disabilities. Such DMD brain co-morbidities are linked to the loss of dystrophin protein 71 (Dp71). Dp71 is the most predominant dystrophin protein in the brain, and alternative splicing at exons 71 and 78 produces several Dp71 isoforms with different subcellular locations and functions. Splicing factors regulating *DMD* alternative exons in skeletal muscle have recently been identified but it is not known what complement of splicing factors regulate *DMD* alternative splicing in the brain. Given the importance of Dp71 in the brain, we aim to assess the neuronal splicing factors regulating the alternative splicing of Dp71 exons 71 and 78 in the brain and whether this is disrupted in DMD. We have generated *DMD* minigenes to study the alternative splicing of exons 71 and 78. These are being used to assess the activity of neuronal splicing factors such as the CUG-BP and ETR-3-like factor (CELF) and muscleblind-like (MBNL) families on the alternative splicing of Dp71. Additionally, we are modelling the loss of