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Supplemental Data

Heterozygous Variants in *KDM4B* Lead to Global Developmental Delay and Neuroanatomical Defects

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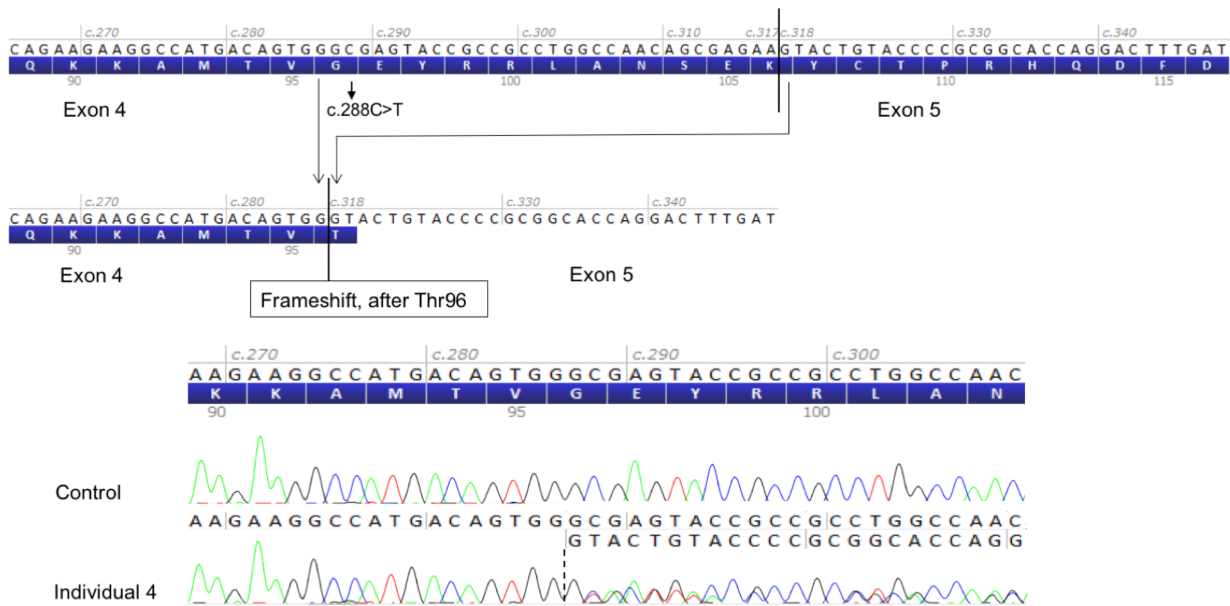
SUPPLEMENTAL DATA

Supplemental Figure:

Figure S1: Synonymous variant leading altered splicing in Individual 4

Individual 4: c.(288C>T), r.(287_317del), p.(Glu97Thrfs*66)



RT PCR and sequencing data for the variant in Individual 4. RNA was extracted from peripheral blood. Variant c.288C>T creates an ectopic splice site resulting in a 31 nucleotide deletion in the mRNA r.(287_317del), and a frameshift p.(Glu97Thrfs*66).

Supplemental Table

Table S1: Dysmorphic physical features present in the clinical cohort

Individual	Dysmorphic Physical Features
1	Turricephaly, protruding metopic ridge, short philtrum, large hyperangulated ears, down-turned corners of mouth, low adipose tissue in forearms and feet, dystrophic hair
2	Mild facial asymmetry, epicanthal folds, high palate
3	Synophrys, upslanting palpebral fissures, upturned tip of nose, tent shaped mouth, clinodactyly of 5th fingers, short thumbs
4	Metopic ridge present, short philtrum, sparse temporal hair, broad nasal bridge, full upper lip
5	Low posterior hair line, wide nasal bridge, downturned corners of mouth, Short hands and feet, bilateral clinodactyly of the 5th fingers and fourth toes
6	Bitemporal narrowing, narrow palpebral fissures, lowered inner canthus, large ears, Mild microstomia with tented upper lip vermilion
7	Positional plagiocephaly, short broad forehead, round face with prominent micrognathia, sparse hair, horizontal palpebral fissures, markedly thinned eyebrows, small low set posteriorly rotated ears with over-folded helices and cupped shape, short nose with anteverted nares, microstomia, long philtrum, prominent calcanei with deep-set nails
8	Anteverted nares, short philtrum
9	Synophrys, bulbous tip of nose, Robin syndrome, single palmar crease, clinodactyly of 5th finger with P2 hypotrophy

Supplemental Methods:

Human subjects

Individual 2 was enrolled in a research study at the Manton Center for Orphan Disease Research at Boston Children's Hospital (BCH) and exome sequencing (ES) was performed by GeneDx. The study and collection of this cohort data was approved by the BCH Institutional Review Board (IRB). The *KDM4B* variant was added to GeneMatcher and 6 additional individuals were identified with *KDM4B* variants. Data for these individuals was shared in accordance with their home IRB/ REC (Research Ethics Committee).

RNA analysis of Individual 4

RNA was extracted from peripheral blood drawn in PAXgene blood tubes (Qiagen) according to the manufacturers recommendations. Reverse transcriptase was performed using Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen) and random hexamers. Specific amplification of KDM4B mRNA was done using the gene specific primers, EXON3F ATCATGACGTTTCGCCCAAC and EXON5R ATCGGGGAGACAAAGGTGAG. The PCR product was sequenced using Brilliant Dye terminator v1.1 chemistry (Nimagen) and an ABI sequencer 3730 Genetic Analyzer (Illumina). Obtained sequences were analyse using CodonCode Aligner (CodonCode Corporation).

Kdm4b mouse model

The mouse model was generated by homologous recombination in embryonic stem cells using the Knockout-first allele method[1], producing the $Kdm4b^{tm1a(EUCOMM)Wtsi}$ allele which were then phenotyped (Figure 3A). Mice were phenotyped by the Mouse Genetics Project (MGP) pipeline at the Wellcome Sanger Institute (UK) and were given a HFD (high fat diet) from 4 weeks of age (Western RD, 829100, 21.4% crude fat content, 42% kcal as fat, 0.2% cholesterol Special Diet Services, Witham, UK). Animal care was as previously described[2].

After weaning, animals were housed three to four mice per cage with WT controls housed separately, in a specific-pathogen-free environment in individually ventilated cages under 12/12 light/dark cycle with temperature-controlled conditions and free

access to food and water with hardwood bedding. All animals were regularly monitored for health and welfare concerns and were additionally checked prior to and after procedures.

Behavioral analysis

Behavioral experiments were conducted at the Wellcome Sanger Institute (UK) by a small group of operators. The open-field test encompasses studying basic locomotor activity, hyperactivity, exploratory behavior, and anxiety in mice. The mice were placed at the edge of the arena which was illuminated with dispersed light measured between 166 and 175lx. The arena measured 45.7 × 45.7 × 50cm and comprised of infrared transparent, black acrylic walls and a sandblasted grey acrylic floor (TSE Systems, Bad Hombery, Germany). The mice were monitored for 10 minutes by the ActiMot system (version 7.01) which uses a grid of 32 IR beams on each axis to locate the mice, as well as a further 32 beams on one axis, 8cm above the base of the arena, to measure rearing activity. Within the software, the arena was split into two zones: a peripheral zone of 8cm diameter from each wall and a central zone that was 42% of the area of the arena.

Data were analyzed using Acti-Track software (version 7.01). Speed and distance covered by the mice were then quantified as well as the percentage of time spent in defined zones of the field (periphery, intermediate or center). Seven male and seven female heterozygous mice were tested at 9 weeks of age. Data were analyzed using a

linear mixed model to determine whether a behavioral parameter is significantly different between the experimental groups.

Neuroanatomical studies

Neuroanatomical studies were carried out using 3 heterozygous *Kdm4b*$^{+/-}$ and a baseline set of 40 WT mice at 16-week old as previously described[3]. Mice were anaesthetized with Ketamine (100 mg/kg, intraperitoneally) and Xylazine (10 mg/kg, i.p.), blood collected via the retroorbital route and death confirmed before the brains were dissected and fixed in 4% buffered formalin for a week, then transferred to 70% ethanol. Samples were embedded in paraffin using an automated embedding machine (Sakura Tissue-Tek VIP) and cut at a thickness of 5µm with a microtome in order to obtain coronal brain region at Bregma +0.98 mm, Bregma -1.34 mm and Bregma -2.06 mm. The sections were then stained with 0.1% Luxol Fast Blue (Solvent Blue 38; Sigma-Aldrich) and 0.1% Cresyl violet acetate (Sigma-Aldrich) and scanned using Nanozoomer 2.0HT, C9600 series at 20× resolution.

Seventy-two brain parameters, made up of area and length measurements as well as cell level features, were taken blind to the genotype across the three coronal sections. Data were analyzed using a linear mixed model to determine whether a brain region is associated with neuroanatomical defect or not.

Ethical considerations in animal use

The care and use of mice in the Wellcome Sanger Institute study was carried out in accordance with UK Home Office regulations, UK Animals (Scientific Procedures) Act of 1986 under UK Home Office license (80/2076) that approved this work, which was reviewed regularly by the Wellcome Sanger Institute Animal Welfare and Ethical Review Body.

Supplemental References

1. Skarnes, W.C., Rosen, B., West, A.P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A.O., Thomas, M., Harrow, J., Cox, T., et al. (2011). A conditional knockout resource for the genome-wide study of mouse gene function. Nature 474, 337-342.

2. White, J.K., Gerdin, A.K., Karp, N.A., Ryder, E., Buljan, M., Bussell, J.N., Salisbury, J., Clare, S., Ingham, N.J., Podrini, C., et al. (2013). Genome-wide generation and systematic phenotyping of knockout mice reveals new roles for many genes. Cell 154, 452-464.

3. Mikhaleva, A., Kannan, M., Wagner, C., and Yalcin, B. (2016). Histomorphological Phenotyping of the Adult Mouse Brain. Curr Protoc Mouse Biol 6, 307-332.