A structural variant of the C-terminal prion-like domain of TDP-43 causes vacuolar muscle degeneration

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Neuronal TDP-43-positive inclusions are a hallmark lesion found in frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), present in 45% and 97% of cases, respectively. Missense mutations in the C-terminal prion-like domain (PrLD) of TDP-43 are the main type of disease-causing mutations reported for ALS. This domain mediates self-interaction, and mutations of this region are thought to lead to aggregate formation. However, the scope of TDP-43 proteinopathies goes beyond neural tissues, with reports showing TDP-43 pathology in vacuolar myopathies. Nevertheless, genetic evidence for a primary role for TDP-43 in myopathies is still amiss. Here we identified a multigenerational family with an autosomal dominant rimmed vacuole myopathy. Whole exome sequencing and genome-wide linkage analysis mapped the disease to an 11bp deletion in TARDBP (LOD-score of 3.6), causing a frameshift mutation in the C-terminal domain (CTD) of TDP-43 (TDP-43p.Trp385IlefsTer10). This constitutes a novel type among described TDP-43 mutations, which are predominantly missense mutations. Patient-derived muscle biopsies revealed the presence of p62/TDP-43-positive sarcoplasmic inclusions and nuclear depletion of TDP-43. Additionally, we verified higher numbers of autophagosomes and a transcriptomic signature indicative of reduced mitochondrial and lipid metabolism, alongside a switch in sarcomeric protein isoforms suggesting increased muscle regeneration. Together with these observations, functional assays in D. melanogaster showed that TDP-43p.Trp385IlefsTer10 retains normal function but has reduced toxic gain-of-function properties. By studying this unique variant of TDP-43 it is our goal to clarify the importance of the CTD of TDP-43 and how its remodelling can affect the formation of aggregates. Furthermore, these results genetically link TDP-43 to vacuolar muscle degeneration for the first time. This not only highlights the importance of the PrLD in pathological conditions in a tissue-specific manner, but it also expands the implications of TDP-43 proteinopathies, from a nearly neuronal-exclusive context into a broader spectrum encompassing myopathies as well.

CLEC16A mislocalization and impaired interaction with the retromer underly a recessive severe neurodevelopmental disorder.

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Background: CLEC16A is a C-type lectin (CLEC) transmembrane protein that recognizes and guides antigens to the cell surface and has been localized to the late endosomes of antigen presenting cells. CLEC16A functions as E3 ubiquitin ligase which prevents autophagy and promotes mitophagy. GWAS studies have associated CLEC16A SNVs to autoimmune disorders like multiple sclerosis and type-1 diabetes. However its role in physiological development is unexplored.

Methods: We identified bi-allelic loss-of-function variants in CLEC16A, in siblings from unrelated families, with a severe neurodevelopmental disorder, progressive microcephaly, brain atrophy, corpus callosum dysgenesis, growth delay, hypotonia and early demise. We studied the cellular CLEC16A properties in vitro and in zebrafish embryos.
Results: Exogenous expression in HEK293T cells shows that CLEC16A prominently localizes to early endosomes, while the protein bearing a human C-terminal deletion loses it physiological localization. Proteomics of CLEC16A interactome shows binding to the retromer heterotrimer components VPS35, VPS26 , and to TRIM27, an interaction which is partially lost for the C-terminal truncated protein. Targeted knock-down of Clec16a by CRISPR-Cas9 in zebrafish embryos resulted in the accumulation of acidic/phagolysosome compartments and abnormal staining of mitochondria, both in neuronal and microglial lineages. This phenotype could be rescued with WT but not with mutant mRNA.
Conclusion: This study reveals a constitutional function of CLEC16A during human brain development. Retromer is a crucial component of the endosomal network, mediates retrograde transport to the trans-Golgi network and plasma membrane, and regulates autophagy and mitophagy. The discovery of interactions between CLEC16A and retromer show the importance of (retromer dependent) endosomal trafficking during brain development.

ATRIP-deficient patient expands molecular and clinical spectrum of Seckel syndrome

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Background/Objectives:
We report the second ATRIP-deficient patient clinically diagnosed with Seckel Syndrome (SS). The ATRIP protein is required for ATR stabilization by complex formation and is crucial for localization of the ATR-ATRIP complex to regions of DNA damage and ATR activation. Besides the typical clinical SS characteristics (primary dwarfism, facial dysmorphia, skeletal abnormalities, microcephaly and mental retardation), our patient suffers from an immunodeficiency. However a link between ATRIP and the immune system was not previously reported.
Methods:
Whole exome sequencing (WES), transcriptomics, western blot, micronucleus assays, flow cytometry and single cell RNA-Sequencing.
Results:
The patient is homozygous for a splice variant (c.829+5G>T) in ATRIP leading to out-of-frame exon 5 skipping. Western blot showed absence of ATRIP protein and analysis of micronuclei in response to DNA damage by mitomycin C and ionizing radiation revealed defective DNA repair. Downstream substrates of the ATR-ATRIP complex are currently investigated.
WES ruled out a pathogenic variant in 460 genes linked to inborn errors of the immune system. Immunophenotyping reveals low absolute B cell numbers, aberrant T cell subsets, decreased plasmacytoid dendritic cells, low CD56dimCD16+ natural killer cells and increased low density neutrophils. Additionally, a first look at the single cell RNA-Seq data suggests a recombination deficiency during B and T cell development, as was published for ATR-deficient SS patients (1), and allows to further elaborate the immune phenotype.
Conclusion:
We expanded the molecular and clinical spectrum of SS and further validations will provide insights into the link with the immune system and will contribute to the disease mechanism.
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Novel mRNA therapy restores GALT protein and enzyme activity in young classic galactosemia zebrafish

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Messenger RNA (mRNA) has emerged as a novel therapeutic approach for inborn errors of metabolism. Classic galactosemia (CG) is an inborn error of galactose metabolism caused by a severe deficiency of galactose-1-phosphate uridylyltransferase (GALT) activity leading to neonatal illness and chronic impairments affecting the brain and female gonads. In this proof of concept study, we used our zebrafish model for CG to evaluate the potential of human GALT mRNA (hGALT mRNA) packaged in two different lipid nanoparticles to restore GALT expression and activity at early stages of development. Both, one cell-stage and intravenous single-dose injections resulted in hGALT protein expression and enzyme activity in the CG zebrafish (galt knockout) at 5 days post fertilization (dpf). Moreover, the levels of galactose-1-phosphate (Gal-1-P) and galactonate, metabolites that accumulate because of the deficiency, showed a decreasing trend. LNP-packaged mRNA was effectively translated and processed in the CG zebrafish without signs of toxicity. This study shows that mRNA therapy restores GALT protein and enzyme activity in the CG zebrafish model, and that the zebrafish is a suitable system to test this approach. Further studies are warranted to assess whether repeated injections safely mitigate the chronic impairments of this disease.

FiberFISH mapping of 22q11.2 rearrangements shows locus heterogeneity

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Background: The 22q11.2 deletion syndrome (22q11.2DS) is with an estimated incidence of 1 in 1500 to 3000 live births about ten-fold more frequent than any other genomic disorder. The driver of the rearrangements is thought to be non-allelic homologous recombination (NAHR) between two of several low copy repeats (LCRs) on chromosome 22 (LCR22-A until LCR22-H). The full sequence nor the breakpoints of these LCR22s have been charted due to the genetically complex nature of the LCR22s. Another layer of complicity lies within the huge inter-individual variability of LCR22A.

Methods: Cell lines from patients and parents were established with an LCR22-ADdel (n=12), LCR22-ABdel (n=2), LCR22-ACdel (n=2), LCR22-BDdel (n=1), LCR22-CDdel (n=1). The fiber-FISH technique was used to map the LCR22s and rearrangements at subunit level and provided a tool to determine the parent of origin.

Results: Multiple specific haplotypes could be discovered for all duos. In patients a rearranged locus ranging from 20 kb to 160 kb could be detected. We identify rearrangements in different subunits.

Conclusion: Fiber-FISH confirms and provided an excellent tool to detect the variation of different haplotypes for LCR22s in the human population. We demonstrate variability in the position of the NAHR subunits, suggesting that rearrangements within the LCRs occur at different positions. In addition, we enlarge the spectrum of LCRA variation in the human population and, for the first time, identify the rearrangements in LCRs-B and C.

Objective 3D facial phenotyping in Cri-du-Chat Syndrome

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Background: Cri-du-Chat Syndrome (CdCS) is a genetic disorder caused by deletions on the short arm of chromosome 5. The phenotypic spectrum is broad and multiple critical regions have been defined. We report five patients from three families with a small 2.7Mb 5p15.33-15.32 deletion overlapping with a proposed critical region for facial dysmorphism. We objectively assess the facial phenotype of these individuals by 3D imaging and we use 3D facial images of a large cohort of individuals with CdCS as a reference. We aim to study facial genotype-phenotype correlations in CdCS and to evaluate the involvement of the 5p15.33-15.32 region in facial dysmorphism in CdCS.

Methods: We modelled the 3D facial shape of our patients (n=5), a large cohort of individuals with CdCS (n=97) from a dataset with various genetic disorders (n=3313) and controls (n=40) using dense surface registration. To objectively model shape variation, we calculated facial signatures using craniofacial growth curves. We applied principal component analysis and cosine-based distance metrics to these signatures to compare the facial phenotype of our patients with the reference CdCS group, with other genetic disorders and with controls.

Results: All 5p15.33-15.32 deletion carriers clustered away from CdCS patients. The cosine distances to the average CdCS phenotype independently indicated that facial features in both groups are distinct (range: 0.77-1.30). The within-group variance for our patients was high, objectively supporting the clinical observation of a heterogeneous facial phenotype in these patients.

Conclusion: Facial dysmorphism in patients with a small 5p15.33-15.32 deletion is distinct from the facial dysmorphism in a large cohort of CdCS patients. We present 3D facial phenotyping as a tool to objectively study genotype-phenotype correlations.

Deciphering the genetic cause of recurrent and spontaneous pregnancy loss

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Introduction
The prevalence of miscarriage is approximately 15.3% of all recognized pregnancies. 80% of pregnancy losses occur in the first trimester. In 60% of cases chromosomal abnormalities are found, whereas in livebirths this number is less than 1% when no prenatal diagnosis was used. No study previously performed a SNP-based haplotyping analysis on miscarried products of conception biopsies from distinct locations, comparing early (~7 weeks gestational age) SPL and RPL in a moderately large (n=86) study population. In this study we investigated the prevalence and nature of (mosaic) de novo genomic aberrations in RPL and SPL.

Materials and Methods
Samples from 86 families with pregnancy loss (n=42 RPL cohort, n=44 SPL cohort) with normal karyotyping results in both parents were analyzed in this study. DNA was isolated from blood of both parents and placental tissues from the miscarried products of conception. The placenta tissues were sampled from two distinct extraembryonic and embryonic germ layers, i.e., the extraembryonic mesoderm and the chorionic villi cytotrophoblast, respectively. We performed SNP-genotyping and applied haplarithmisis to delineate allelic architecture of fetal tissues.

Results
We analyzed 175 fetal DNA samples. Within the RPL cohort, we found aberrations in 24 tissues (14 families), including genome-wide and chromosomal abnormalities. Within the SPL group, we found aberrations in 16 tissues (8 families), including genome-wide, chromosomal, and segmental abnormalities, suggesting that mosaic alterations may cause pregnancy loss.

Conclusions
Multiple genome wide aberrations are found in the RPL group but only one in the SPL group, indicating an association between genome wide mosaic aberrations and RPL. While aberrations in the RPL group were more maternal in origin, in the SPL group they were more paternal in origin. Our findings can lead to a better understanding of causative factors for SA and RPL and the need for a SNP-based non-invasive prenatal testing.

Identification of potential core functions in human immune responses of genes with previously unknown function

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Inborn errors of immunity (IEI) is a group of genetic disorders with both heterogeneous genetic causes and phenotypic manifestations, complicating its diagnosis. Many genes known to cause IEIs have a function in the innate immune response, also known as the “first line of defence”. Exome and genome sequencing leads to a diagnosis in 15-30% of IEI cases, indicating a potential for improvement, in part through the identification of novel genes with a role in the immune system and, in turn, in IEIs. In order to identify core genes in the human innate immune response, we applied QuantSeq 3’ mRNA sequencing in in vitro pathogen-stimulated peripheral blood mononuclear cells (PBMCs) from 5 healthy donors. PBMCs were stimulated with LPS, S. Aureus, PolyIC and C. Albicans for 4 and 24 hours to mimic early and late innate immune responses to gram-negative and gram-positive bacteria, viruses and fungi, respectively. Overlapping genes differentially expressed in stimulated PBMCs compared to unstimulated cells allows for the identification of genes that likely have core functions in the immune response. As a proxy for undescribed genes, we extracted “Corf” and “KIAA” genes, of which four are differentially expressed in all stimuli - strongly implicating a role in human host-pathogen responses; KIAA0040, C11orf21, C1orf122 and C15orf48, of which only the latter has recently been found to be implicated in the regulation of inflammation and immunity. These results indicate the potential for characterising the innate immune response, which provides candidate genes that may play a role in the pathogenesis of IEIs. We will reanalyse the genetic variation in these uncharacterised genes with a potential role in the immune response in exomes of IEI patients in our in-house database, potentially contributing to the growing number of genes implicated in IEIs and for the diagnosis of patients using previously missed disease-causing variation.

Variant effect prediction based on custom long-read transcriptomes

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Our knowledge of transcript annotations is still incomplete and may result in a failure to detect disease-causing variants. For example, in patients with primary immunodeficiencies it could be valuable to annotate transcripts that are only expressed under certain conditions such as host-pathogen interactions. Current variant annotation software uses only precomputed pathogenicity prediction scores based on reference transcripts. The pipeline presented here is designed to annotate variants with custom transcript annotations for downstream prioritization.
The input of the pipeline is a sample-specific/non-reference long-read transcriptome in fasta format obtained from peripheral blood nuclear cells of a healthy individual that was stimulated with various pathogens, variant file(s) derived from unsolved exome data of patients with suspected inborn errors of immunity (IEI) in VCF format, and a reference genome build. The Ensembl Variant Effect Predictor is used in conjunction with Polyphen-2 to provide custom variant annotations. Our pipeline is available at https://github.com/cmbi/VEP_custom_annotations.
The input long-read transcriptome contained 37,434 novel transcripts detected through PacBio IsoSeq on peripheral blood cells exposed to various immune stimuli. The re-annotation pipeline was tested on 148 undiagnosed IEI patient's exomes. Out of a total of 802,352 variants, 6.2% had a more severe effect in the novel transcript annotation than in the reference.
Genetic variant annotation may benefit from long-read sequencing approaches that discover novel transcripts. This benefit can be reaped without extensive bioinformatic knowledge using this pipeline. Our pipeline outputs crucial information for further prioritization of potentially disease-causing variants, and will become increasingly useful due to the rising number of long-read RNAseq datasets.

Diagnostic yield of partial exome sequencing in 872 children with neurodevelopmental disorders

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Neurodevelopmental disorders (NDD) are genetically and phenotypically heterogenous conditions, currently best investigated by agnostic sequencing of a large number of genes(1). Partial exome sequencing, focusing only on disease-associated genes, is less costly, while also enabling a higher sequencing depth than WES or WGS(2).
We evaluated the diagnostic yield of an in-house designed, regularly updated, partial exome sequencing panel (Mendeliome), containing 4867 disease-associated genes in its most recent iteration. We retrospectively reviewed 872 patients that underwent Mendeliome sequencing for NDD, done with at least one parent, between 2016 and 2021 in our departments. We investigated diagnostic yield based on phenotypic sub-groups, defined by the presence or absence of a given set of HPO terms, and on phenotypic complexity.
Overall yield of Mendeliome-sequencing was 26%. Best results were observed in syndromic (37%) and epilepsy- associated intellectual disability (ID) (39%). Yield for Autism spectrum disorders (ASD) was 19% (ASD without ID: 15%, ASD with ID: 26%). Yield was 23% in severe- and 36% in mild to moderate ID. In epileptic patients, overall yield was 27% (focal epilepsy: 19%, non-focal epilepsy: 28%). Concerning phenotype complexity, we observed yields as low as 14% for patients with only 1 associated HPO term, and up to 42% for patients with over 10 terms. A decrease in overall diagnostic yield from 31% to 17% was observed between 2016 and 2021, probably due to the gradual inclusion of less syndromic patients. Boys were overrepresented in our cohort (60% vs 40%). Diagnostic yield in boys was 22%, while being 32% in girls. For duo- and trio-sequencing we observed a diagnostic yield of 23% and 27%, respectively. Observed yields were comparable to those described in a recent meta-analysis(3).

Conclusion: Diagnostic yield of Mendeliome sequencing is highly dependent on the patient's phenotype and phenotype-complexity, with syndromic NDD ranking highest and isolated ASD lowest.

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The performance of GS as a first-tier test for neurodevelopmental disorders

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Background: Genome sequencing (GS) can identify novel diagnoses for patients who have exhausted routine diagnostic procedures. We tested whether GS is a better first-tier genetic diagnostic test than current exome-based standard of care (SOC) by assessing the technical and clinical validity of GS for patients with neurodevelopmental disorders (NDD).
Methods: Using a prospective parallel design, we performed both GS and exome sequencing in 150 consecutive NDD patient-parent trios. We developed a strategy for the filtering, prioritization, and interpretation of genomic variants, based on variant type and mode of inheritance. Diagnostic yield was calculated from disease-causing variants affecting exonic sequence of known NDD genes as primary outcome measure.
Results: GS (30%, n=45) and SOC (28.7%, n=43) had similar diagnostic yield. All 43 conclusive diagnoses obtained in SOC were also identified by GS. These 43 conclusive diagnoses included a mixture of single nucleotide variants (SNVs, n=26) , insertion-deletion variants (InDels, n=13), copy number variants (CNVs, n=3), and a repeat expansion (n=1). In addition, all 31 possible diagnoses obtained in SOC were also identified by GS. SOC, however, required integration of multiple test results (average 1.5; range 1-6) to obtain these conclusive and possible diagnoses. GS yielded two more conclusive diagnoses, and four more possible diagnoses than ES-based SOC (35 vs 31). Interestingly, all six likely pathogenic variants detected only by GS were CNVs. These CNVs were not identified in ES due to limitations in CNV calling or the absence of targets in the ES enrichment procedure.
Conclusion: Our data provide the technical and clinical validity of GS to serve as routine first-tier genetic test for patients with NDD. The additional diagnostic yield from GS is limited. Still, GS comprehensively identified all variants in a single experiment, suggesting that GS could constitute a more efficient genetic diagnostic workflow for patients with NDD.

Mutational processes in childhood acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is marked by low mutational burden. Nevertheless, a subset of children with ALL has a substantially elevated number of mutations, a phenomenon known as hypermutation. We previously found that hypermutation occurs more frequently in ALL relapses, suggesting an effect of therapy, but a small subset of cases is already hypermutated from initial diagnosis. To study the causes and consequences of hypermutation in childhood ALL, we performed whole exome/whole genome sequencing (WES/WGS) on normal and leukemic DNA. We included 26 patients with multiple relapses and 48 patients with single relapse, identifying a mutational process in 11 (42%) and 10 (21%) patients, respectively. We discerned two mutational signatures exclusively present in relapse, one of which is known to result from thiopurine treatment. Remarkably, in six relapsed ALL patients, we observed a mutational signature strongly resembling a pattern of UV-induced mutations typically found in melanomas. This pattern was always detected from the earliest stages of the disease, but the etiology and consequences for outcome are still unknown. Finally, we observed aberrant APOBEC mutagenesis, an antiviral defence system targeting single-stranded DNA, in patients at diagnosis and (continuing in) relapse. To reveal the frequency of APOBEC-associated hypermutation at diagnosis, we subsequently filtered an RNA sequencing dataset of 214 ALL samples at diagnosis for potential somatic mutations that resembled the APOBEC-associated signatures. WGS-based validation eventually confirmed APOBEC mutagenesis in six patients, of which three were ETV6-RUNX1-fusion positive, a good-prognosis ALL subtype. In conclusion, we have shown that multiple distinct mutational mechanisms contribute to hypermutation in ALL. Two of these mechanisms occur already at diagnosis, suggesting a role in the earliest stages of ALL development. Whether these mechanisms can cause ALL, and what the consequences are for therapy and outcome requires further studies.

Impact of common genetic variants on cytokine response heterogeneity upon BCG vaccination in infants from Guinea-Bissau

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Background: Mycobacterium tuberculosis (TB) continues to be one of the leading causes of mortality worldwide. Over 25% of TB deaths occur in the African Continent. Bacillus Calmette-Guerín (BCG) being TB vaccine also provides non-specific protective effects against other infections through “trained innate immunity”. Several studies have demonstrated that the host genetic variation has a strong influence on the immune response, e.g to influenza, Hepatitis B or measles vaccination. However, which genetic variants affect cytokine responses to secondary infections upon BCG vaccination is unknown. Moreover, while studies in European populations have demonstrated the role of genetic polymorphisms in the inter-individual variability in cytokine responses upon stimulation, it is not clear whether these findings are transferable to non-Europeans.

Materials and Methods: We utilized an African trial cohort (Guinea-Bissau) of low-birth-weight (<2.5 kg) infants (~400 samples) randomized to BCG vaccination or no BCG-vaccination. In vitro stimulation of whole blood was done using five different stimuli followed by seven different cytokine measurements. We performed genome-wide SNP cytokine QTL (cQTL) mapping followed by pathway enrichment and functional annotation. The results were compared using a European BCG-vaccinated adult cohort (n=300).

Results: In the African samples, we identified 9 independent cQTLs ($P < 5 \times 10^{-8}$) affecting cytokine responses specifically in the BCG group but not in the control group. Interestingly, these cQTLs show pleiotropic effects. Also, nominal cQTLs ($p < 0.05$) between European and African samples showed very limited overlap (1.4% to 1.5%), indicating either age or ethnicity-associated genetic effects. We identified several causal genes at these loci and implicated complement pathway in regulating cytokine response after BCG vaccination, which was confirmed via functional validation.

Conclusions: Our study shows that distinct genetic loci affect cytokine response in BCG-vaccinated African infants; the same associations were not seen in otherwise similar BCG-unvacciantes infants or in European BCG-vaccinated adults.

Genome-wide and pathway-specific polygenic risk scores in an Alzheimer's disease case-control cohort

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Genome-wide association studies identified over 100 loci significantly associated with AD, with APOE the strongest. Polygenic risk scores (PRS) combining the effect of AD-associated variants offer great possibilities for risk prediction. However, there is no consensus on the optimal p-value threshold (pT) for SNP inclusion, the role of APOE in these scores, nor whether pathway-specific scores (pPRS) would be better than genome-wide scores.

Using PRSice and the summary statistics from Kunkle et al.(1), we calculated PRS for different pTs (PRS-AD) in a well-characterized AD case-control cohort (N-case=179, N-control=98). We also built PRS excluding the APOE region (PRS-noAPOE), including only the APOE region (PRS-APOE) or the two main APOE variants (rs429358-rs7412, APOEε2+ε4), and the weighted sum of PRS-noAPOE + APOEε2+ε4. pPRS were built for pathways defined by Kunkle et al.(1) and Tesi et al.(2)

We found the best model fit with APOEε2+ ε4 with an R^2 of 37.5% for discriminating cases from controls. For PRS-AD, relaxing the pT results in a huge drop of R2: from 29.3% at pT=5E-8 (N-SNP=65, p=1.82E-11) to 1.4% at pT=0.05 (N-SNP=45,125, p=9.24E-02). A similar trend is observed for the other scores, except for the PRS-APOE score where also the number of SNPs is relatively stable when relaxing pT (N-SNP=35 at pT=5E-08, N-SNP=67 at pT=0.05). Removing APOE from PRS-AD resulted in a drop in R^2 from 29.3% to 5.0% (pT=5E-8). All pPRS except for the angiogenesis pathway are significantly associated with AD, mainly because of APOE being included.

Our analyses show that AD PRS are largely driven by APOE, and especially the two main APOE variants. Our results further are in line with recent research suggesting that AD has an oligogenic architecture rather than a polygenic. The exact role of AD-associated variants beyond APOE, but also of other variants within the APOE region, needs to be further explored.

1. Kunkle et al [Nat Genet. 2019;51(3):414–30]
2. Tesi et al [Transl Psychiatry 10, 332 (2020)]