# Association of Diras2 with attention-deficit / hyperactivity disorder

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## Scientific Background

### Attention-deficit/hyperactivity disorder (ADHD)

- Genetically complex
- Neurodevelopmental disorder
- Heritability of ADHD ~70-80%
- High persistence into adulthood
- Linkage studies and genome-wide association studies → susceptibility loci

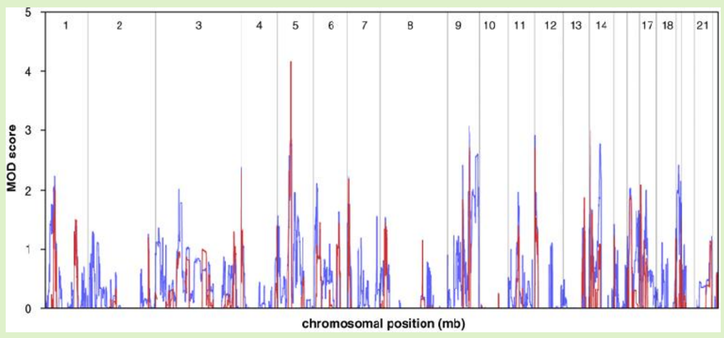


**Fig. 1:** MOD scores achieved in a linkage study. MOD global is shown in red, MOD single in blue. The MOD global on Chr. 9q22 is 3.30. (Romanos et al., 2008)

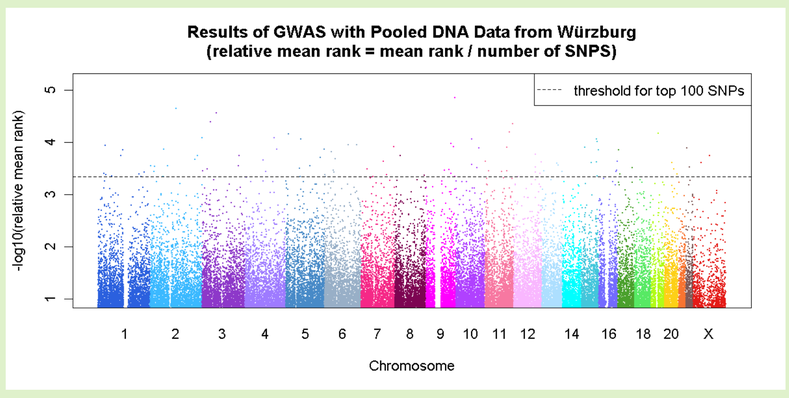


**Fig. 2:** Genome-wide association study in adult ADHD. (Lesch et al., 2008)

## Methods

**Finemapping of 11 potential candidate genes**

- iPLEX™: Mass array based SNP genotyping
- Case-control association studies
  - >1600 adult ADHD patients and >1800 controls from Germany, the Netherlands, Norway and Spain (IMpACT Consortium)
  - Two independent family-based childhood ADHD samples (234 trios, 71 quartets, 15 multi-sibling families)

**Immunocytology: Fluorescence double stainings**

- Mouse hippocampal primary cell culture
- Primary antibodies against Diras2 and cell markers
- Fluorescence labeled secondary antibodies

**Expression analysis during development**

- Total RNA from mouse brains at different embryonic and postnatal developmental stages
- Quantitative real-time PCR
- Calculation of PCR efficiencies using LinRegPCR
- Determination of normalized expression levels by the CFX Manager™ software (Biorad)

## Results: Association studies

**Association of *DIRAS2* with ADHD**

aADHD: 6 SNPs (p=0.0098- 0.04), 2 haplotypes, replication in IMpACT sample, pooled analysis p=0.0552 (corrected)

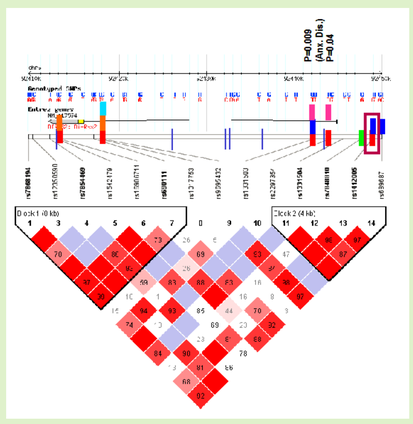


**Fig. 3:** LD blot of the *DIRAS2* gene. Locations of associated SNPs are marked with boxes. Red: German aADHD sample; Green: German family sample from Würzburg; Orange: German family sample from Homburg;Pink: Dutch aADHD sample; Light blue: Norwegian aADHD sample; Blue: association with personality traits in the German aADHD sample; Magenta frame: pooled analyses of the IMpACT sample

There is no potential conflict of interest

## Results: Expression studies

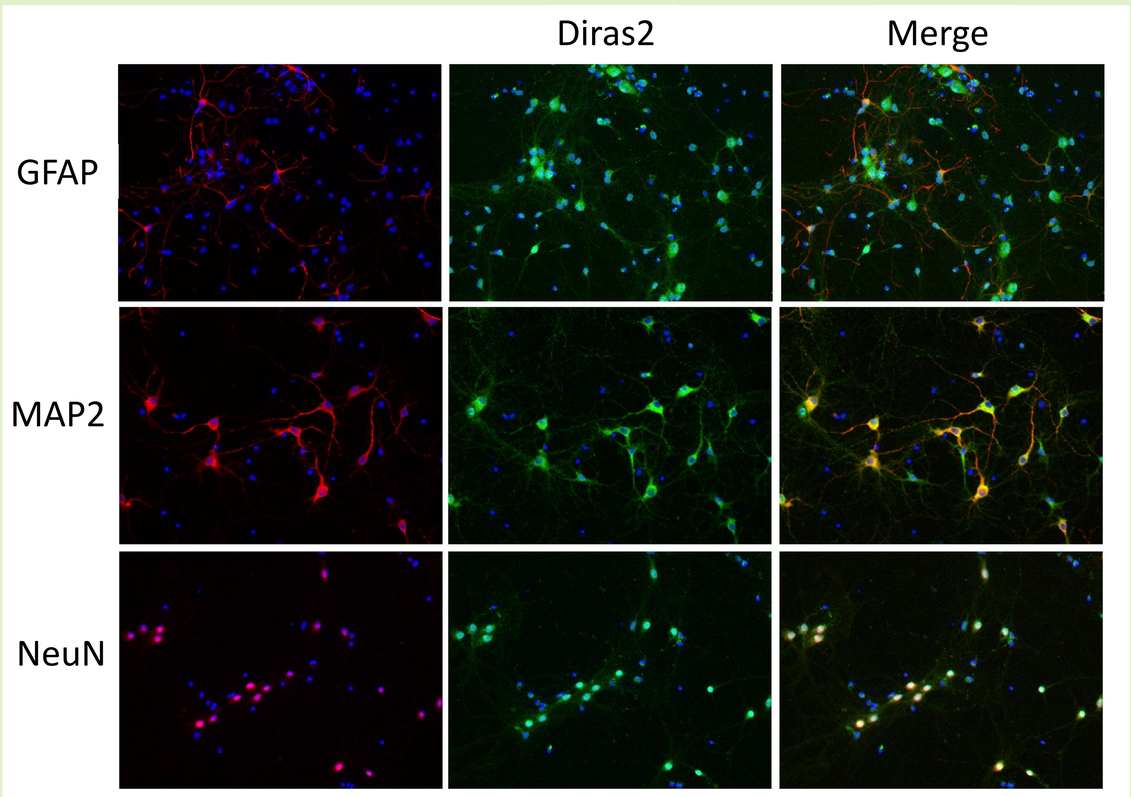


**Fig. 4:** Antibody double stainings of primary hippocampal mouse cells. Diras2 expression is observed in neuronal but not in glial cells. Green = Diras2; Red = cell markers: GFAP = astrocytes; MAP2 = neuronal cells; NeuN = marker of neuronal maturation;

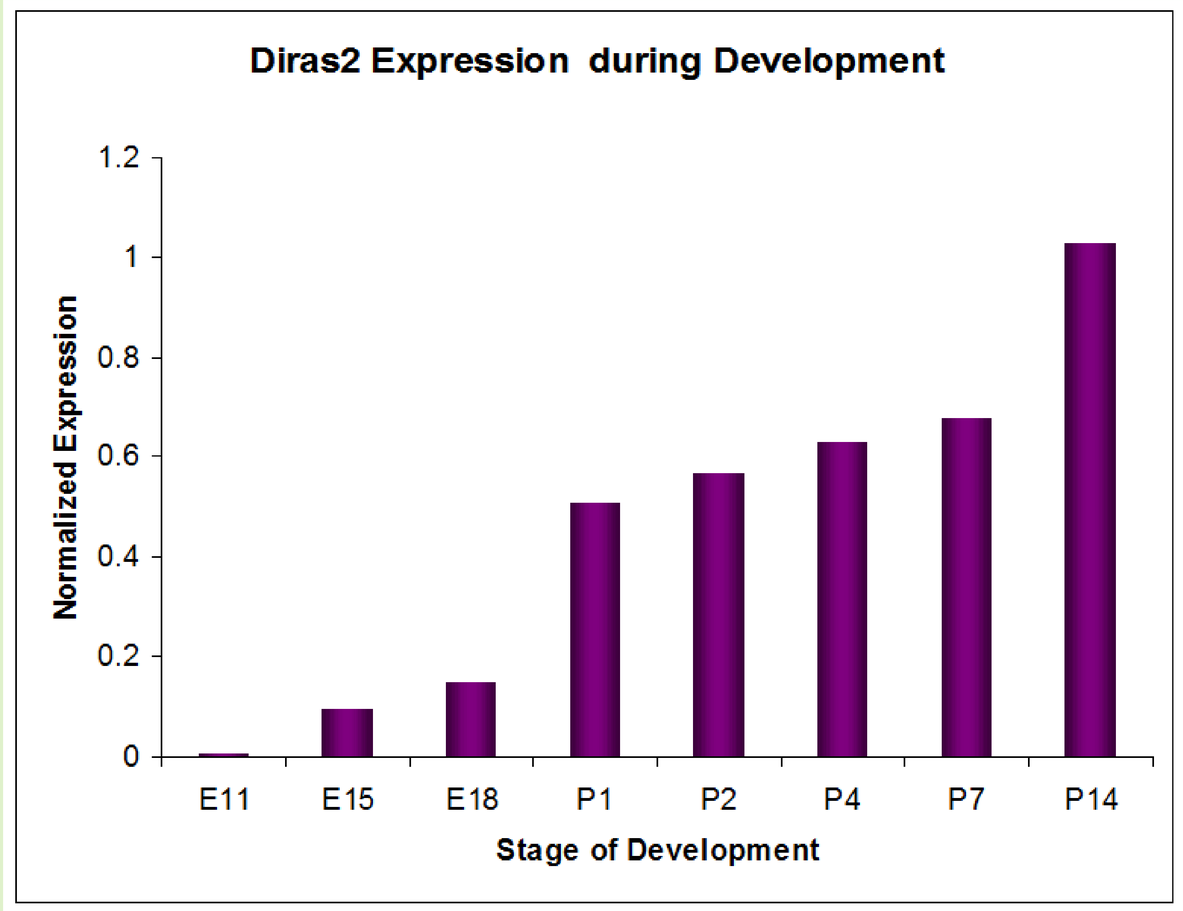


**Fig. 5:** Expression of *Diras2* increases during brain development in mice. Expression levels in mouse brains at embryonic days 11,15 and 18 and postnatal days 1-14 were determined using qPCR.

## Conclusion

The association findings are indicating that *DIRAS2* may play a role in the pathomechanism of ADHD.

The fact, that Diras2 is expressed in neuronal primary cells but not in glial cells and that the expression levels increases during brain development suggests that this gene plays a role during neuronal development.

Re-sequencing of the gene, as well as functional studies like RNA interference induced knock down of Diras2 expression and further expression studies like in situ hybridisations on brain slices are planned or currently underway to explore the role of *DIRAS2* in ADHD.

## References

Lesch et al. (2008) Molecular genetics of adult ADHD: converging evidence from genome-wide association and extended pedigree linkage studies. J Neural Transm.; Zhou et al. (2008) Meta-analysis of genome-wide linkage scans of attention deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet.
Romanos et al. (2008) Genome-wide linkage analysis of ADHD using high-density SNP arrays: novel loci at 5q13.1 and 14q12. Mol Psychiatry.