

Autism is not a wrinkled pea: a new study for gene finding in a multifactorial neurodevelopmental disorder



Literature exercise

Mark Patrick Roeling
mp.roeling@psy.vu.nl
2023806
GENE HUNTING

This literature exercise evaluates the recent findings concerning the etiology and genetic architecture of autism in three parts; phenotype, genetics and a new study design.

1. PHENOTYPE

Clinical definition

Autism is a developmental neuropsychiatric disorder in which the behavioral pattern is characterized by a three main criteria (APA., DSM-IV-TR, p. 70)

Qualitative impairment in social interaction, as manifest by impairment in the use of nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures, failure to develop appropriate peer relationships, and lack of social sharing or reciprocity.

Patients have **impairments in communication**, such as a delay in, or total lack of, the development of spoken language. In patients who do develop adequate speech, there remains a marked impairment in the ability to initiate or sustain a conversation, as well as stereotyped or idiosyncratic use of language.

Patients also exhibit restricted, **repetitive and stereotyped patterns of behavior, interests, and activities**, including abnormal preoccupation with certain activities and inflexible adherence to routines or rituals.

The phenotypic variation is enormous. So large that in fact, autism cannot as a disorder on its own but has to be considered as a spectrum disorder. Therefore, the literature often refers to autism as ‘Autism Spectrum Disorders’ (ASD). Figure 1 provides an overview of the spectrum.

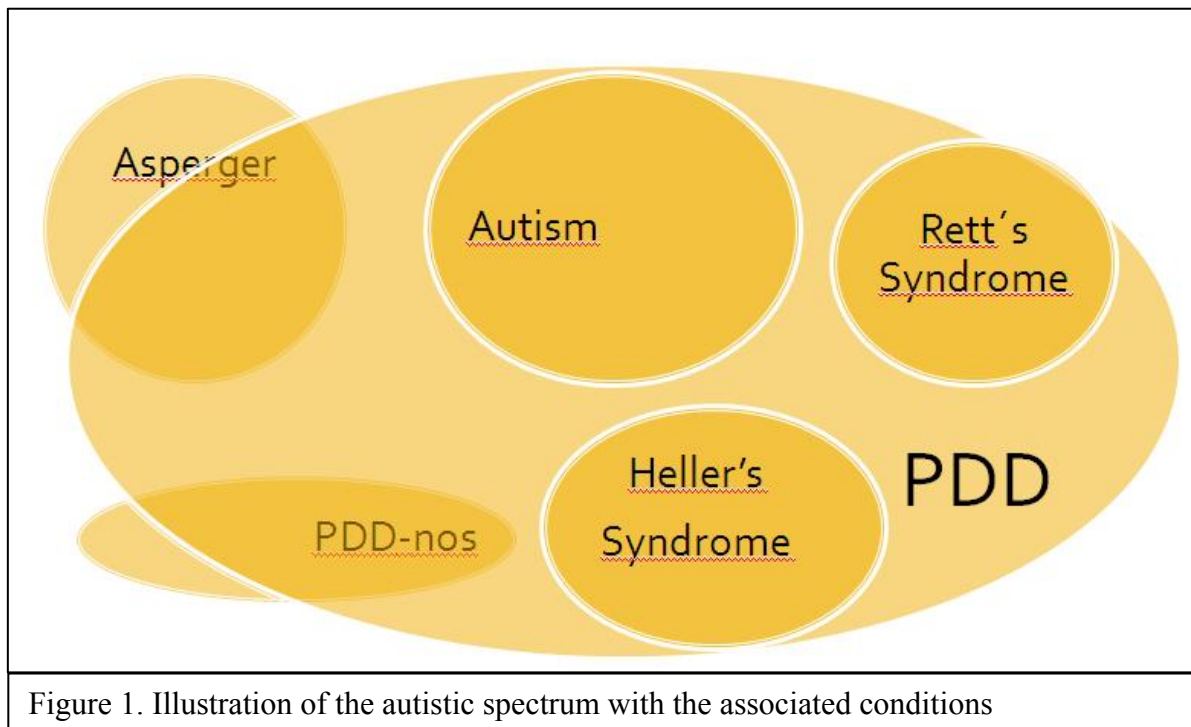


Figure 1. Illustration of the autistic spectrum with the associated conditions

Because of the phenotypic variation of autism, many studies have focused not primarily on the diagnosis of autism, Aspergers's Syndrome or other sole conditions but on autistic traits and broad spectrum definitions of the pathology. Good examples of this indirect attenuation of the disease are found in the twin studies that have been published, which I will explain later on. Mainly, the phenotypic variation is related to the severity of the symptoms. The more severe the symptoms, the more severe the diagnosis. To illustrate this, please see figure 2.



Figure 2. Illustration of the dimensional phenotypic variation

Co-morbidity

The occurrence of other (secondary) pathology in patients with autism can be separated into a comorbid mental condition and a comorbid medical condition

Mental condition	Medical condition
<ul style="list-style-type: none"> - Associated mental retardation (75%) - Abnormalities in the development of cognitive skills - Lower verbal than non-verbal skills - Attention deficit hyperactive disorder - Epilepsy (15-30%) - Schizophrenia - Tics - Obsession / compulsion - Electroencephalographic abnormalities (20-50%) - Hyperlexia - Down syndrome 	<ul style="list-style-type: none"> - Genetic disorder or chromosomal anomaly - Fragile X syndrome - Tuberous sclerosis - 15q duplications - Phenylketonuria - Angelman syndrome - Rett syndrome - Smith-Magenis syndrome - 22q13 deletion syndrome - Cohen syndrome - Adenylosuccinate lyase deficiency - Smith-lemli-opitz syndrome - Several dysmorphic features
<p>APA., 2000; Berney, 2010; Burd et al., 1985; Cohen et al., 2005; Miles et al., 2008; Smalley, 1997.</p>	

Due to the severity of the symptoms, autism is almost always identified as a disorder in the affected patients, leading to a near-to-zero number of undiagnosed patients. The prevalence rate of autistic disorder is 5 cases per 10.000 individuals (APA., 2000). Often, the disorder is recognized before a child reaches the age of 3. Autism spectrum disorders occur in 19/10.000 individuals. Relative risk = occurrence risk / prevalence = 5-10% / (19/10.000) = 25-50 (Piven, 2010). The recurrence risk for siblings with autism is 4.5% (Jorde et al., 1991). In idiopathic autism, the male female ratio is estimated 4-10:1 with an increase in this ratio as the intelligence of the affected individual increases (Folstein and Rosen-Sheidley, 2001).

Brain pathology

The literature concerning brain abnormalities in autism is enormous. Bauman and Kempor suggest various brain abnormalities exist, especially in the temporal lobes and cerebellum, which might correlate with the degree of impairment (Bauman & Kempor, 1994). Impairments in explicit memories might be related to the temporal lobe abnormalities, whereas impairments in implicit memory might be related to the cerebellar abnormalities. Roder suggests that one cause of autism may be an abnormality in the expression of genes in that play a central role in the development of the brainstem (Roder, 2000): An area in the caudal part of the pons is small in autistic subjects and several nuclei in this area including the facial nucleus, which controls facial musculature, are small or missing. In addition, many autistic children have subtle facial abnormalities that may be due to abnormalities of the nervus facialis. In a study of Johansson et al. (2006) the severity of ASD was correlated with both visual and hearing deficits. There are theories that both blindness and profound hearing impairment can cause autistic-like features. Children who are blind and deaf, raised in a spoken-language environment, perform less well in 'autism sensitive' tests measuring 'theory of mind' (Kolb & Whishaw, 2005).

Biomarkers

Several biological markers have been proposed for (the diagnosis) of autism. Recently, an article by Gregg et al. in *Genomics* concluded a difference in blood serum levels (by the measurement of “gene expression” through micro array chipping). Another stream in the literature refers to the use of serotonin as a possible biomarker. The 5-HTT gene has been introduced as a possible mediator in the development of autism (see eg. Freitag, 2007). However eloquent the proposition of biomarkers may be, little studies are well replicated and validated and systematic reviews/meta-analysis often “fail to find an overall association between 5-HTT polymorphisms and autism” (Huang & Santangelo, 2008, p.911).

One published editorial by Geier and Geier (2008) presented a short overview of biomarkers, they are summarized in Table 1.

Porphyrin biomarkers	Derivates of the heme synthesis pathway. Urinary porphyrin profiles are increased in ASD, along with brain mercury levels, mercury levels in baby teeth, and mercury in the urine/fecal samples.
Trans-sulfuration markers	Based on homocysteine. The (abnormal) level of metabolites is said to be correlated with the level of severity in autism, but only one study managed to observe this.
Oxidative stress/inflammation biomarkers	Reactive oxygen is a natural by-product of normal metabolism of oxygen, resulting in unstable atoms that cause disruption to other molecules and subsequent damage to cells. Biomarkers of oxidative stress are increased in ASD. Also, neuroglial activation and neuro-inflammation is also observed in ASD.
Hormonal biomarkers	Because of the sex effect in ASD, hormonal vulnerability is proposed: ASD patients tend to display a hypermasculine profile on many cognitive tasks. Also, abnormal fetal testosterone and fetal estrogen levels are reported. oxidative stress/inflammation biomarker. Other studies have shown elevated blood androgen metabolites in ASD (Geier & Geier, 2007)
Mitochondrial dysfunction biomarkers	A study of Oliviera et al. (2005) has supported a role for a mild mitochondrial dysfunction in ASD
Genetic biomarkers	The most reported chromosome regions are 15q pericentromeric 11-13 region. 17p11, 22q11, 22q13, 16p11.2, 2q37. Array comparative hybridization seems a powerful new tool (but to be honest: the incorporation in ASD is not developed well enough).
More text; see Geier & Geier, 2008.	

2. GENETICS

Kanner already proposed a biological relation to the disease in his initial report of autism. But since the review published by Rutter in 1968 and 2000, the genetic research in autism is back on track again. The first twin study was published many decades after.

Three notions have to be made before I address twin studies.

1. All twin and family studies published make use of measures that are often self rated and that capture “autistic traits”. This creates a problem: classical autism is associated with an impaired intellectual ability. The use of self rating scales will only be applicable in higher functioning autism or above. Excluding the severe-end of the autistic spectrum, narrowing down the curve of variance, and making it hard to find genes/good heritability estimates. Moreover, autistic traits do not necessarily generalize to a more advanced diagnosis. If we e.g. would take a recent study presented by Hoekstra et al., 2007, the AQ (autistic spectrum quotient) is used. This is a well validated 50-item Likert scale (study see Hoekstra et al., 2010). But, the scale measures autistic traits. The entire operationalization of the phenotype “autism” lacks any psychiatric background or screening by informants or well validated diagnostic instruments such as the SCID (with Cohen’s kappa to correct for observer-bias). Therefore it is hard to take heritability measures from these studies as examples for general practice.
2. The phenotype in many genetic studies (including twin studies) is often dichotomized. A patient either has or has not a certain diagnosis. At least two statistical complications derive from this method. First, one is creating a 50/50 situation in which the heritability is calculated though a threshold liability model where one “keeps in mind” that there is an underlying continuous scale in the population, throwing away large amounts of power and variance. Second, in many cases the affected patient has been diagnosed because he/she displayed a number of symptoms, high enough to be diagnosed. Often, the sibling or parents do not receive a diagnosis merely because they don’t reach the threshold to get a diagnosis. (eg. you have to display 6 out of 9 symptoms to get diagnosed with depression, suppose a child has 7 symptoms = depression, the parent has only 3 symptoms so isn’t diagnosed). When the phenotype would be displayed on a categorical scale instead of a *all-or-none* scale; many genetic tests would reach a higher power; many con-discordance rates would have been different, and many trio-designed association studies would possibly have been beneficial.
3. Twin studies are often very large epidemiological studies drawn from a “healthy” population. Therefore one often makes use of scales that measure traits instead of real psychiatric diagnoses. The trait-scales used in twin studies are often validated and comparable to psychiatric assessment, but still it lacks sufficient diagnosis of the phenotype. Consequently, the outcomes of twin studies are hard to generalize to the psychiatric community.

Six twin studies have been published to report the heritability of autism. Pairwise concordance rates in MZ twins is 36-96% and 0-30% in DZ twins. Heritability estimates for autism are above 90%. Instead of dominance or epistasis, additive genetic effects are

presumed. No twin studies have been performed on Asperger's syndrome of PDD-NOS. In a recent twin study of Hoekstra et al., 2007 in 18 year old twins and their siblings, the variance of the AQ (see above) was explained by additive genetic factors (57%) and unique environmental factors (43%).

Study	N of twins	Design	Concordance MZ - DZ	Heritability	Additive/Dominant/Shared environmental/Unique Environmental	Measures
Rivo et al., 1974	46 families	Familie study	Not reported	Not reported	segregation ratio of $p = 0.19 \pm 0.07$	Not reported
Folstein & Rutter, 1977	21 same sex pairs	Twin	MZ 4/11 con DZ 0/10 con	> 90%	Psychiatric diagnosis by independent blind diagnosis	Not reported
Steffenburg et al., 1989	21 pairs		MZ 91% DZ 0%	> 90%	Additive genetic and unique environmental (AE)	-ABC-interview -Lotter Checklist -DIPBEC
Bailey et al., 1995	68	Twin study	MZ 92% DZ 10%	82%	Additive genetic and unique environmental (AE)	BAP, ADI
Constantino et al., 2003	219 pairs	Male twins	Not reported	90%	CBCL syndromes account for 43% of the variance in SRS scores	CBCL SRS
Hoekstra et al., 2007	370	Twin-sibling-parent	MZ $r = .55$ DZ $r = .37$	57%	Additive genetic and unique environmental (AE)	AQ
AQ = autism spectrum quotient, CBCL = child behavioral checklist, SRS = social responsiveness scales						

Causal genes

An exhaustive overview of the genes that have been identified as causative for autism has been presented by Freitag in 2007. Another meta-analysis by Trikalinos et al., 2006 also revealed some genes to be (steadily) identified with autism.

Chr 2	Two studies found an increased risk for autism associated with the haplotype CG = CG consisting of SNIP rs2056202 and rs2292813. However: not replicated in other studies	Segurado et al., 2005 Ramos et al., 2004
Chr 6	Two studies found evidence for association of different SNPs in the glutamate receptor (GluR6). One of the SNPs is suggested to have functional implications, most were found in intronic regions. Glutamate is important in brain development, learning and memory, and linkage has been positive for 6q21. This candidate gene seems relevant in the pathogenesis of AD.	Jamain et al., 2002 Shuang et al., 2004
Chr 7	Linkage on chromosome 7 is best replicated. FOXP2 gene is mentioned in several studies, but not replicated. The Reelin (RELN) gene might be causative as a signaling protein that plays a crucial role in neuronal migration, formation of cortical layers and synaptogenesis. No functional relevance has been established yet. Replication seems difficult. One study associated the rs736707 SNP (not replicated, no functional relevance), which was located in intron 59 of RELN. The Laminin gene (LAMB1) located on 7q31 was predicted to have a damaging effect on protein structure. LAMB1 is an important encoder for a glycoprotein promoter neuronal migration and neurite outgrowth. The neuronal cell adhesion molecule (NRCAM) gene is mentioned also in that study, but not replicated other studies. WNT2 (wingless type mouse mammary tumour virus integration) gene. Lack of the gene reduced social interaction in mice. The gene is found in mutation analysis in two affected siblings with AD, but not replicated and its function isn't proven in human brain development. The Engrailed 2 (EN2) gene in 7q21-36 has been assessed in five samples. EN2 is a homeobox transcription factor important during cerebellar and brainstem development. (intronic rs1861972, rs1861973) A SNP (rs10951154) in HOXA1 (homeobox A-1) was reported in one study but remains unreplicated.	RELN association: Persico et al., 2001 Serajee et al., 2006 Skaar et al., 2005 SNP Serajee et al., 2006 Bonora et al., 2005 Lijam et al., 1997 Benayed et al., 2005 Gharani et al., 2004 Petit et al., 1995 Zhong et al., 2003 Freitag, 2007
Chr 15	15q11-13 has been in important region in idiopathic autism. The GABA genes have been etiologically relevant. Two studies found evidence for an association in intron 3 of the GABRB3. Another study reported an association of a single SNP in the GABRA4 gene on 4p. However the complex arrangement of the 15q11-13 region make it difficult to assess genes in this regard with regard to their relevance for autism.	Buxbaum et al., 2002 Cook et al., 1998 Ma et al., 2005

Chr 17	The serotonin-transporter gene (SLC6A4) has been assessed by several studies. With functional effects on the 5HTTLPR and variable number of tandem repeats in intron 2. However, a systematic meta analysis by Huang and Santangelo found the 5HHT not to be associated with autism. SLC6A4 seems of relevance for the genetics of autism, influencing the phenotype or modulating the severity of autism regarding tot obsessive compulsive symptoms.	see Freitag, 2007 Huang and Santangelo, 2008
Chr X	Several studies found genes like het NLGN3 and NLGN4 genes, the McCP2 gene, and the MAO-A gene. However, little to none association studies have been reported to date, and the effects are not (well) replicated.	See Freitag, 2007 and Bartlett et al., 2005

Numerous novel candidate ASD loci such as SHANK2, SYNGAP1 and DLGAP2 were identified in a recent study of Pinto et al. (2010). Also, maternally inherited X-linked deletions at DDX53–PTCHD1 implicate this locus in ASD. A full list of genes based on evidence of studies of their involvement in ASD is presented in the appendix. The list is separated in three parts:

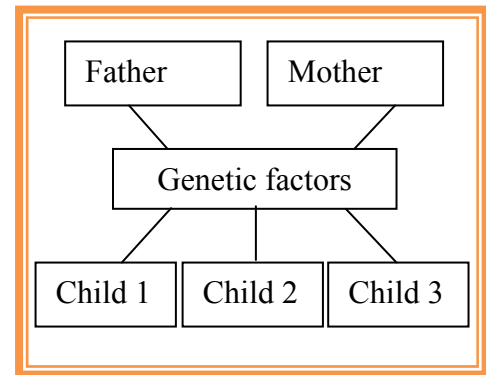
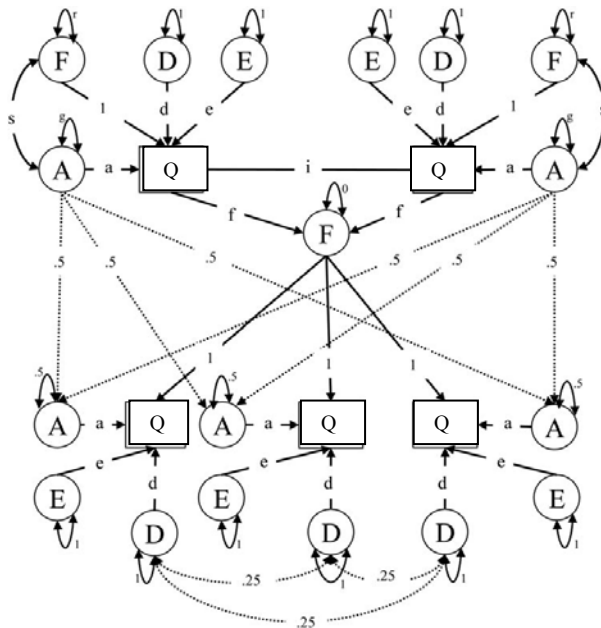
- ASD implicated genes: 36 disease genes and 10 loci strongly implicated in ASD and identified in subjects with ASD or ASD and intellectual disability
 - Intellectual disability genes: 110 disease genes and 17 loci implicated in intellectual disability but not yet in ASD;
 - ASD candidate genes: 103 genes from previous studies of common and rare variants.
- We observed a higher proportion of cases with

Overall, directions lead towards glutamate binding proteins, serotonin mediators and brainstem development (as suggested by studies in brain pathology, see Kolb & Wishaw, 2005). However, no proportion of patients can be explained up to date.

3. STUDY DESIGN

GWAS study: trio design with broad and narrow phenotype definition

In the study design, with unlimited financial resources and no difficulties finding and pheno/genotyping parents, all options are open. Still I had to come up with a logical study design. Recent GWAS studies were not very robust because of lack of power and probably because of definition of the phenotype. Although the ASD diagnosis is highly standardized through the different consortia, no definite results have been found. Plomin et al. (2008) propose that an approach looking at the separate constructs underlying autism might be more beneficial. Furthermore, since the publication of Folstein and Rosen-Sheidley in 2001, and again by the Engrailed studies (Benayed et al., 2009; Gharani et al., 2006), one has defined two phenotypes: a broad and narrow one. This approach has been very attractive and successful, probably because the narrow approach is a more clinically defined approach in which a patient either has or hasn't a disorder and therefore is not necessarily appropriate for genetic research. A more quantitative trait based approach could be more useful in the study to be performed.



Q = is autistic traits. The (extended) trio design in which the genetic variance is separated in F; specific genes transcended to the child, D; genes that are the same between the parents (eg. the genes coding for the development of skin), and E; specific genes shared with nobody (eg. mutations). We are looking for SNP'S in the affected children that aren't there in the unaffected parents/siblings.

Assumptions:

The disease-related alleles are transmitted in excess of 50% to affected offspring from heterozygous parents. The design has several advantages: first, it controls for population structure and is immune to population stratification. Second, it allows checks for

Mendelian inheritance patterns in genotyping quality control. Third, it is logistically simpler for studies of children's conditions (which is the case in autism), and finally it does not require phenotyping of parents (but will be done). Disadvantages of the design: it may be difficult to assemble both parents and offspring, especially in disorders with older ages of onset (which autism is not) and it is highly sensitive to genotyping error (relatively easy to overcome with good blood serum) (Pearson & Manolio, 2008).

Other methods:

The GWAS approach is selected because of its usefulness in complex non-mendelian heritable conditions as a method to find common genetic variants. A meta-analysis of current studies to look for the effect of genes that have been reported (eg. genes that are always low ranked in variance but consistently found in studies can be important, this could be “discovered” in a meta-analysis). Yet, two recent meta-analyses have been performed in 2006 and there have not been enough GWAS-publications since then to provide a new insight (see Freitag, 2006; Trikalinos et al., 2006). A comparative genomic hybridization was considered, but one good study reported in 2006 already attended this, the study design of that article was comprehensive to the extent that a replication would be difficult and possibly irrelevant (Jacquemont, Sanlaville, Redon, et al. (2006). All other mapping analyses were ruled out because they are proven useful in mendelian or monogenic diseases instead of complex disorders. The relative “contrast” of the GWAS approach, whole-exome sequencing could be used to look at exonic regions which code for functional proteins (eg. how the protein was rendered nonfunctional and *why* a patient might be susceptible to or actually have the disease in question). Moreover, the exome sequencing is able to identify mutations. However, for complex mental disorders GWAS is (at the time) more easily applied to demonstrate the biological relevance of findings by directly functional validation of the effects (Beals, 2010).

Ascertainment strategy

Family members will be collected from autistic patients with both parents, in multiple consortia (as in e.g. the WTCC-designs). The number of participants, as calculated with the genetic power calculator <http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html> for a relative risk of 25-50, a prevalence of 0.1, Odds Ratio of 1.5. This study will need 965 patients with twice that amount of parents. Assuming we will include both parents (and siblings subsequently), the entire study will potentially include 2895 participants.

The diagnosis for autism in the affected child is important, because a more severe phenotype will possibly result in more linkage in the GWAS. After informed consent, the parents are seen by 2 independent psychiatrists or trained psychologists to be diagnosed or not with the structured clinical interview for diagnosis (SCID), the Autism Diagnostic Interview-Revised, and the Autism Diagnostic Observation Schedule-Generic. This will also be done in other first-order family members such as other siblings. The diagnosis will be expressed as a 0-1 variable and forms the narrow defined phenotype. The diagnosis deriving from the two clinicians can be evaluated in overlap and consistency

with Cohen's Kappa (see Drenth & Sijtsma, 2005). Patients with considerable comorbidity will be excluded from the sample.

The broad defined phenotype will consist of the Autism spectrum quotient (AQ). The AQ measures personal preferences and habits with 50-item. The items are self-reported and scored on a 4-point Likert scale. The AQ is available in many languages as it is a well-known scale in the field to measure autistic traits (Hoekstra et al., 2010). The AQ is in line with the 'extreme male theory' in autism, has good reliability and moderate internal consistency: $\alpha = .63 - .79$.

The items of the AQ will be oblimin rotated with principal component analysis into 3 factors: Restrictive patterns of behavior; impaired social interaction; impaired social communication.



The three factors are expressed as continuous variables, providing more power. The three factors are also allowed to interact. Rather than testing for interaction per se, we are interested in allowing for interaction with genetic factors when testing for association at a given locus. Regression analysis and structural equation modeling allows for this interaction. The three factors above do have some phenotypic overlap (like the NEO-FFI), however the correlation estimates are only small and therefore I think it's possible to see them as whole entities with their own (and maybe minor overlapping) genetic influences.

In the running of both scans, the narrow and broad. Age and sex will be estimated covariates. Age because autism is a disorder in which the importance of underlying genetic factors seems to increase over time (see dissertation Hoekstra). In early youth there are also shared environmental influences explaining the variance in autistic traits. However, when the child reaches 18years, the variance is explained by additive genetic and unique environmental effects only. Sex is a covariate because autism is more frequent in males compared to females (see above).

The broader definition could also include other variables such as biomarkers and neuroendophenotypes. However, no good biomarkers exist that can be linked unequivocally to autism. MRI usage as a possible phenotype can be used but is in this study is nearly impossible (500€ per scan x 2895 persons = expensive) and the brain regions implemented are difficult to scan (brain stem/anterior cingulated nucleus). Although the prevalence and incidence of autism is small and many countries require the available resources and material, future studies (but other designs) could quite possibly use MRI as a good endophenotype.

Genotyping strategies

The study will make use of 1.000.000 Affymetrix SNP markers. The use of 500.000 and 1 million has been widely reported in the recent literature (see e.g. Ponti et al., 2010). The half-million is able to tap 79% of the genome respectively and the million SNP array is able to measure over 85%. For a (Western Europe/North America) population this array will suffice in terms of observable SNPs, furthermore the usage of more SNPs as high as 3 million is reported in genome scans. However, this has not been implemented yet in complex mental disorders. Blood serum (peripheral blood) will be used as it provides high quality DNA (Kaplan & Saddock).

For the identification of the responsible gene and variant we will implement the results in Hapmap to look for already known functions of specific genes in the suggested region. Especially genes that have to do with cognitive function and the production of enzymes responsible for neuronal- and brainstem development as reported. Furthermore, the available regions will be compared to the outcomes of earlier studies (after 2001) to search for replication in our study. With specific exon-sequencing more detailed associations could be found between protein-coding regions of the genome and the available phenotype. When genes are found that have not been reported and to which functioning is not known yet, the possible findings can be implemented in further models (such as a mouse model to look study the phenotype after silencing/deletion).

Conclusion:

As mentioned above, this study aims to detect common genetic variants in a complex non-mendelian heritable disorder. This study is new because it is the first trio-design GWAS that uses a broad and narrow definition if the phenotype. Also, it is the first GWAS to include underlying separate autistic traits in a dimensional fashion that represent the main characteristics of the pathology. The separate dimensions of the AQ are allowed to interact in the model. These three considerations create a very powerful study as more variance is included in the dimensional model and the power is increased by including a family and by the allowance for interaction (Cordell, 2009). Furthermore, the broader definition (indirectly) allows the finding and replication of more genes. Conclusively, the next step in this study is the identification of rare genetic variants which could be done by detection and analysis of copy number variants. The study design and acquired data allow for those analyses. Although a recent CNV study in autism was published in Nature by the WTCC (Ponti et al., 2010), this study could be clinically superior and could prove to be a good replication or alternative to find the remaining genes.

LITERATURE

- American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders*. 4th ed. TR. Washington, DC: American Psychiatric Press
- Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E. et al. (1995). Autism as a strongly genetic disorder: evidence from a British twin study. *Psychological Medicine*, 25, 63-77.
- Bartlett, C.W., Gharani, N., Millonig, J.H., Brzustowicz, L.M. (2005). Three autism candidate genes: a synthesis of human genetic analysis with other disciplines. *International journal of developmental neuroscience*, 23, 221-234.
- Bauman, M.L., & Kempor, T.L. (1994). *The neurbiology of autism*. Baltimore: John Hopkins University Press
- Beals, J. (2010). GWAS vs Whole-Exome Sequencing: What's the Difference and Why We Should Care. Retrieved online from: <http://www.medscape.com/viewarticle/728457>
- Benayed, R., Gharani, N., Rossman, I., Mancuso, V., Lazar, G., Kamdar, S. et al. (2005). Support for the homeobox transcription factor gene ENGRAILED 2 as an autism spectrum disorder susceptibility locus. *Am J Hum Genet*, 77, 851–868.
- Berney, T. (2010). Comorbidity in autism. Retrieved online from: www.autismhellas.gr/files/el/tmcda.ppt
- Bonora, E., Lamb, J.A., Barnby, G., Sykes, N., Moberly, T., Beyer, K.S. et al. (2005). Mutation screening and association analysis of six candidate genes for autism on chromosome 7q. *Eur J Hum Genet*, 13, 198–207
- Burd, L., Kerbeshian, J., Fisher, W. (1985). Inquiry into the incidence of hyperlexia in a statewide population of children with pervasive developmental disorder. *Psychol. Rep.* 57, 236-238.
- Buxbaum, J.D., Silverman, J.M., Smith, C.J., Greenberg, D.A., Kilifarski, M., Reichert, J. et al. (2002). Association between a GABRB3 polymorphism and autism. *Molecular Psychiatry*, 7, 311–316.
- Cohen, D., Pichard, N., Tordjman, S., Baumann, C., Burglen, L., Excoffier, E., Lazar, G., Mazet, P., Pinquier, C., Verloes, A., Heron, D. (2005). Specific genetic disorders and autism: clinical contribution towards their identification. *J. Autism Dev. Disord*, 35, 103-116.
- Constantino, J.N., Hudziak, J.J., Todd, R.D. (2003). Deficits in reciprocal social behavior in male twins: evidence for a genetically independent domain of psychopathology. *Journal of the American Academy for Child Adolescent Psychiatry*, 42, 458–467.
- Cook, Jr E.H., Courchesne, R.Y., Cox, N.J., Lord, C., Gonen, D., Guter, S.J. et al. (1998). Linkage-disequilibrium mapping of autistic disorder, with 15q11–13 markers. *Am J Hum Genet*, 62, 1077–1083.
- Cordell, H.J. (2009). Detecting gene-gene interactions that underlie human diseases. *Nature review genetics*, 10, 392-404.
- Drenth, P.J.D., & Sijtsma, K. (2005). Testtheorie, inleiding in de theorie van de psychologische test en zijn toepassingen. Houten: Bohn Stafleu van Loghum.
- Folstein, S., & Rosen-Sheidley, B. (2001). Genetics of autism: complex aetiology for a heterogeneous disorder. *Nature Review Genetics*, 2, 943-955.
- Miles, J. H., Takahashi, T. N., Hong, J., Munden, N., Flournoy, N., Braddock, S. R., Martin, R. A., Spence, M. A., Hillman, R. E., Farmer, J. E. (2008). Development and validation of a measure of dysmorphology: useful for autism subgroup classification. *Am. J. Med. Genet.* 146A: 1101-1116.

- Folstein, S., & Rutter, M. (1977). Infantile autism: a genetic study of 21 twin pairs. *Journal of Child Psychology and Psychiatry*, 18, 297-321.
- Freitag, C.M. (2007). The genetics of autistic disorders and its clinical relevance: a review of the literature. *Molecular Psychiatry*, 12, 2-22.
- Geier, D.A., Geier, M.R. (2008). Autism spectrum disorder-associated biomarkers for case evaluation and management by clinical genetics (editorial). *Expert rev. mol. diagn*, 8(6), 671-674.
- Gharani, N., Benayed, R., Mancuso, V., Brzustowicz, L.M., Millonig, J.H. (2004). Association of the homeobox transcription factor, ENGRAILED 2, 3, with autism spectrum disorder. *Mol Psychiatry*, 9, 474-484.
- Huang, C.H., Santangelo, S.L. (2008). Autism and Serotonin Transported Gene Polymorphisms: A systematic review and meta-analysis. *American Journal of Medical Genetics B*, 147B, 903-913.
- Hoekstra, R.A., Bartels, M., Verweij, C.J.H., Boomsma, D.I. (2007). Heritability of autistic traits in the general population. *Archives of pediatric adolescent medicine*, 161, 372-377.
- Hoekstra, R.A., Vinkhuyzen, A.A.E., Wheelwright, S., Bartels, M., Boomsma, D.I., Baron-Cohen, S., Posthuma, D., Van der Sluis, S. (2010). The Construction and validation of an abridged version of the Autism Spectrum Quotient (AQ-short). *Journal of autism and developmental disorders* doi: 10.1007/s10803-010-1073-0
- Jacquemont, M.L., Sanlaville, D., Redon, R., Raoul, O., Cormier-Daire, C., Lyonnet, S. et al. (2006). Array-based comparative genomic hybridization identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. *Journal of Medical Genetics*, 30, 843-849.
- Jamain, S., Betancur, C., Quach, H., Philippe, A., Fellous, M., Giros, B. et al. (2002). Linkage and association of the glutamate receptor 6 gene with autism. *Molecular Psychiatry*, 7, 302-310
- Johansson, M., Rastam, M., Billstedt, E., Danielsson, S., Strömland, K., Miller, M., & Gillberg, C. (2006). Autism spectrum disorders and underlying brain pathology in CHARGE association. *Developmental Medicine & Child Neurology*, 48, 40-50.
- Jorde, L.B., Hasstedt, S.J., Ritvo, E.R., Mason-Brothers, A., Freeman, B.J., Pingree, C. et al. (1991). Complex segregation analysis of autism. *American Journal of Human Genetics*, 49, 932-938.
- Kolb, B., & Whishaw, I.Q. (2005). *Fundamentals of human neuropsychology: fifth edition*. Worth Publishers.
- Lijam, N., Paylor, R., McDonald, M.P., Crawley, J.N., Deng, C.X., Herrup, K., et al. (1997). Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. *Cell*, 90, 895-905.
- Ma, D.Q., Whitehead, P.L., Menold, M.M., Martin, E.R., Ashley-Koch, A.E., Mei, H. et al. (2005). Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. *Am J Hum Genet*, 77, 377-388.
- Pearson T.A., & Manolio, T.A. (2008). How to interpret a Genome-wide Association Study. *JAMA*, 299(11), 1335-1344.
- Petit, E., Hérault, J., Martineau, J., Perrot, A., Barthelemy, C., Hameury, L. et al. (1995). Association study with two markers of a human homeogene in infantile autism. *J Med Genet*, 32, 269-274.
- Persico, A.M., D'Agruma, L., Maiorano, N., Totaro, A., Militeri, R., Bravaccio, C. et al. (2001). Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Molecular Psychiatry*, 6, 150-159.
- Pinto, D., Pagnamenta, A.T., Klei, L., Anney, R., Merico, D., Regan, R., Conroy, J. et al. (2010). Functional impact of global rare copy number variation in autism spectrum disorders. *Nature*, 466, 368-372.

- Piven, J. (2010). Towards Defining the Autism Phenotype. Retrieved online from: <http://www.youtube.com/watch?v=IrrNXC8nOcc&feature=channel>
- Plomin, R., DeFries, J.C., McClearn, G.E., & McGuffin, P. (2008). *Behavioral genetics*, fifth edition. New York: Worth Publishers.
- Ramoz, N., Reichert, J.G., Smith, C.J., Silverman, J.M., Beshpalova, I.N., Davis, K.L. et al. (2004). Linkage and association of the mitochondrial aspartate/glutamate carrier SLC25A12 gene with autism. *American Journal of Psychiatry*, *161*, 662–669.
- Ritvo, E.R., Spence, M.A., Freeman, B.J., Mason-Brothers, A., Mo, A., Marazita, M.I. (1985). Evidence for autosomal recessive inheritance in 46 families with multiple incidences of autism. *American Journal of Psychiatry*, *142*, 187-192.
- Roder, P.M. (2000). The early origins of autism *Scientific American* *282*(2), pp 56-63.
- Rutter, M. (1968). Concepts of autism: a review of research. *J. Child Psychol. Psychiatry*, *9*, 1–25.
- Rutter, M. (2000). Genetic studies of autism: from the 1970s into the millennium. *Abnormal child psychology*, *28*, 3-14.
- Segurado, R., Conroy, J., Meally, E., Fitzgerald, M., Gill, M., Gallagher, L. (2005). Confirmation of association between autism and the mitochondrial aspartate/glutamate carrier SLC25A12 gene on chromosome 2q31. *American Journal of Psychiatry*, *162*, 2182–2184.
- Serajee, F.J., Zhong, H., Mahbulul Huq, A.H. (2006). Association of Reelin gene polymorphisms with autism. *Genomics*, *87*, 75–83.
- Shuang, M., Liu, J., Jia, M.X., Yang, J.Z., Wu, S.P., Gong, X.H., et al. (2004). Family based association study between autism and glutamate receptor 6 gene in Chinese Han trios. *Am J Med Genet B* *131*: 48–50.
- Skaar, D.A., Shao, Y., Haines, J.L., Stenger, J.E., Jaworski, J., Martin, E.R. et al. (2005). Analysis of the RELN gene as a genetic risk factor for autism. *Molecular Psychiatry*, *10*, 563–571.
- Smalley, S. (1997). Behavioral genetics '97; Genetic Influences in Childhood-Onset Psychiatric Disorders: Autism and Attention-Deficit/Hyperactivity Disorder. *American Journal of Human genetics*, *60*, 1276-1282.
- Steffenburg, S., Gillberg, C., Hellgren, L., Andersson, L., Gillberg, I.C., Jakobsson, G., et al. (1989). A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. *Journal of Child Psychology and Psychiatry*, *30*, 405–416.
- Trikalinos, T.A., Karvouni, A., Zintzaras, E., Ylisaukko-oja, T., Peltonen, L., Järvelä, I., Loannidis, J.P.A. (2006). A heterogeneity based genome search meta-analysis for autism-spectrum disorders. *Molecular Psychiatry*, *11*, 29-36.
- Zhong, H., Serajee, F.J., Nabi, R., Huq, A.H. (2003). No association between the EN2 gene and autistic disorder. *J Med Genet*, *40*, e4.