Autism and Genome-Wide Association Studies

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Autism is one of the "most genetic" psychiatric diseases. Its high heritability points towards a possible near complete genetic pathogenesis. However, despite enormous efforts during last years, that sometimes were even criticized the understanding of the disease causes and progress is still missing.

The first study looking at the genome level for genetic factors of autism in 99 affected families identified a region on chromosome 7q already in 1998 (1998). The results from the International molecular genetic study of the autism consortium were confirmed in the next year by French investigators (Philippe *et al* 1999). However, this study also found other 10 associated regions spread though the genome. Interestingly, one of the regions was localized at the chromosome Xp. The X chromosome associations could explain the gender difference in autism prevalence. But findings of parental inheritance of autism in some families argue against the association with gonosomal regions. Other mechanisms probably related to the effect steroid hormones on brain development might be responsible for the gender-related skewness of autism prevalence.

A genome-wide association study targeted at the Asperger syndrome revealed some overlap loci between this entity and autism (3p14-24), but more interestingly an overlap between Asperger syndrome, autism and schizophrenia (1q21-22) (Ylisaukko-oja *et al* 2004). Further loci were found to be associated with autism on the chromosomes 12q14, 16p, 17q11.2 and 19p13 without identifying specific genes or variants (Philippi *et al* 2005; McCauley *et al* 2005; Ma *et al* 2007). In contrast, recent studies using analytic methods with higher resolution showed associations with genes and their polymorphisms like in the case of paired-like homeodomain transcription factor 1 (PITX1) on chromosome 5q31 (Philippi *et al* 2007). The PITX1 gene product is a regulator of hypothalamic and pituitary production of hormones making it functional candidate gene potentially explaining the endocrine differences of autistic patients (Baron-Cohen *et al* 2005).

In studies analyzing samples from patients coming from one specific population the results brought new susceptibility loci including 3q25-27 (Auranen *et al* 2003). One large family with several affected individuals was screened and the recently published results showed once more several significant loci associations, indicating that even related subjects might have a complex genotypic background (Allen-Brady *et al* 2009). On the other hand a similar case reports analyzing one family led to the conclusion that uniparental disomy of chromosome 1 or trisomy of 8p are causing autism (Wassink *et al* 2005; Papanikolaou *et al* 2006). These thorough analyses of small scale studies involving a small number of patients bring results that are mostly not confirmed in larger population-based studies (Ylisaukko-oja *et al* 2005). Their importance lies more in shedding light on the potential mechanisms behind cerebral development like in the case of neuroligin 4 encoded by the X-linked NLGN4 gene (Lawson-Yuen *et al* 2008) and its binding partner SHANK3 (Durand *et al* 2007). Some results of large studies can even be explained by the results of family-based analyses.

Some studies have concentrated on specific autism symptoms like the delay in language acquisition (Alarcon *et al* 2005). There are indices in the literature that especially the several times reported locus on chromosome 7q might have an important function in language development. Interestingly, this locus has been found to be expressed only from the paternal allele (Ashley-Koch *et al* 1999). The association with 7q35 region is valid beyond autism spectrum disorders. Other studies have concentrated on small isolated populations that might be interesting from a genetic point of view, but the significance of the results for a general population is rather small (Lauritsen *et al* 2006). They are more oriented towards the understanding of the pathogenesis, than to a diagnostic purpose.

There are at least two published meta-analysis taken together results from several previous studies and the main outcome was the significant association with 7q. Other loci were far behind reaching statistical significance only under specific analytic conditions (Trikalinos *et al* 2006; Badner & Gershon 2002). The 7q locus is reported in association with autism in nearly all published studies. One study is of special interest, as it shows that the tight association loosens as the diagnostic criteria are weaker (Schellenberg *et al* 2006). The locus has been named AUTS1. However, to shed light on the pathogenesis a closer look at the 7q region is needed. One of the candidate genes is surely RELN coding for the neuroguidance molecule reelin. At least in one study the associations between autism and polymorphisms of this gene were proved to be significant (Skaar *et al* 2005). Other genes located in this region might also be involved. Studies analyzing polymorphisms of the Forkhead Box P2 and Protein Tyrosine Phosphatase Receptor-type Zeta 1 have not confirmed these assumptions. The GRM8 gene encoding a glutamate receptor is also both, a functional and positional candidate gene, but in contrast to the RELN gene, an association with autism has not been confirmed at least in Chinese population (Li *et al* 2008). The RELN gene failed on the other hand to be associated with autism in the Indian population as shown in a recently published study (Dutta *et al* 2008).

By combining the cohorts of the Autism Genetic Research Exchange project and the Finnish autism project the researchers have found a new candidate gene – the oxytocin receptor gene OXTR (Ylisaukko-oja *et al* 2006). This indicates the importance of sample size in such analyses. The oxytocin pathway is a very interesting potential clue to autism pathogenesis. Previous findings have shown that oxytocin is important for social interaction, especially related to trust and monogamy (Kosfeld *et al* 2005). The lack of empathy in autistic children might be explained by decreased or altered oxytocin receptor signaling. Other studies concentrating on social interaction in autism and its genetic associations did not confirm the oxytocin receptor locus (Duvall *et al* 2007).

Currently, studies looking at the whole genome for variations associated with autism are the most popular way of analyzing genetic factors. One of the main advantages, but also the biggest disadvantage is the lack of an a priori hypothesis. From a scientific view it is quite easy to prepare the design of such a study and there is no bias coming from subjective and selective analysis by the investigators, but the price for these benefits is high. Publications of genome-wide association studies in high impact journals are currently only possible with replication cohorts and analyses. The association with autism has only been published in this way for the CNTNAP2 gene on chromosome 7p35 belonging to a neurexin superfamily (Arking *et al* 2008).

There is a clear problem with the interpretation of results. Statistical analysis suggests that only results confirmed in an independent population or study can be seen as relevant. Moreover, huge numbers of patients and controls are needed leading to higher costs of the so or so expensive analysis. An often overseen fact is that genome-wide association studies only can find high-frequency variants. Thus, variants associated with the disease, but with a low frequency in the population will not be identified by this approach, no matter how large the actual effect of these variants might be.

Some studies indicate that autism might in fact not be associated with nucleotide polymorphism but with structural variations – insertions and deletions like firstly shown by the Cold Spring Harbor Laboratory (Sebat *et al* 2007). Indeed, a copy number variation on chromosome 16p11.2 has been shown to be overrepresented in autistic patients in two independent studies (Weiss *et al* 2008; Marshall *et al* 2008). The largest cohort of 1181 families with at least two affected individuals analyzed by the Autism Genome Project Consortium combined copy number variations and single nucleotide polymorphisms analyses. The result was an association with a newly identified region of the chromosome 11p12-13 (Szatmari *et al* 2007).

Genome-wide association studies are a modern powerful tool for research on both, diagnostic markers and pathogenetic pathways of diseases. Despite their general issues, the results brought by large analyzed cohorts improved our understanding of the pathophysiology of several complex diseases. However, the same analytic tools are more or less powerless in psychiatric diseases. It is very likely that the main cause lies in the heterogeneity of the patients. The diagnostic criteria for mental diseases are subject to bias due to subjectivity, looseness of criteria and lack of clear biomarkers. This makes is difficult to specify the right inclusion criteria for patients involved in such studies leading to increased variability and false negative results of association studies.

Specifically autism is a diagnosis with several different phenotypic subtypes leading to the use of the term autism spectrum disorders. On a molecular level this has been clearly shown in an interesting study analyzing expression profiles of lymphocytes in children with different forms of autism (Nishimura *et al* 2007). The importance of autism heterogeneity has been confirmed in a recent genome-wide association study analyzing on a large cohort separately various subphenotypes. Significant associations have been found with verbal delay but not with quantitative traits like IQ or verbal status (Liu *et al* 2008). Although previous analyses of such subgroups have brought no positive results (Spence *et al* 2006).

The prevalence of autism in developed countries increased more than 5 times during the 90-ties. Although some activists see this as an evidence for an environmental pathogenesis, the truth is that this rapid increase is caused simply by widening of the diagnostic criteria and by heightened awareness. By analyzing heritability and concordance genetic factors are likely to play the dominant role in the pathogenesis. Nevertheless, the interaction between genetic factors and environmental triggers cannot be ruled out (Herbert *et al* 2006). We still do not know the cause of autism. It seems that autism is a disorder with polygenic inheritance each specific variant having only a subtle overall effect. A lot of work must be, thus, done before a complete understanding of the molecular pathogenesis will lead to an improved diagnostics and even more importantly to identification of treatment targets.

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