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Meta-analysis of genome scans of age-related macular degeneration

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A genetic contribution to the development of age-related macular degeneration (AMD) is well established. Several genome-wide linkage studies have identified a number of putative susceptibility loci for AMD but only a few of these regions have been replicated in independent studies. Here, we perform a meta-analysis of six AMD genome screens using the genome-scan meta-analysis method, which allows linkage results from several studies to be combined, providing greater power to identify regions that show only weak evidence for linkage in individual studies. Results from non-parametric analysis for a broad AMD clinical phenotype (including two studies with quantitative traits) were extracted. For each study, 120 genomic bins of ~30 cM were defined and ranked according to maximum evidence for linkage within each bin. Bin ranks were weighted according to study size and summed across all studies; the summed rank (SR) for each bin was assessed empirically for significance using permutation methods. A high SR indicates a region with consistent evidence for linkage across studies. The strongest evidence for an AMD susceptibility locus was found on chromosome 10q26 where genome-wide significant linkage was observed ($P = 0.00025$). Several other regions met the empirical significance criteria for bins likely to contain linked loci including

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adjacent pairs of bins on chromosomes 1q, 2p, 3p and 16. Several of the regions identified here showed only weak evidence for linkage in the individual studies. These results will help prioritize regions for future positional and functional candidate gene studies in AMD.

INTRODUCTION

Age-related macular degeneration (AMD), with its numerous forms of late stage manifestations, is a progressive disorder primarily affecting the central area of the retina (1,2). Whereas early stages of AMD are characterized by hard and soft drusen [extracellular yellowish deposits between the retinal pigment epithelium (RPE) and the choroids] (3), late manifestations are differentiated by atrophy of the RPE and the photoreceptors or by choroidal neovascularization, both outcomes accompanied by serious vision impairment (4). AMD is a multifactorial trait involving both genetic and environmental effects although the precise aetiology of AMD remains elusive. Age, smoking, and to a lesser extent, diet and sunlight exposure are among the most commonly reported risk factors for disease onset (reviewed in 5). A genetic contribution to AMD is well established by familial aggregation analyses (6,8) and twin studies (9–11).

A number of candidate gene associations have been reported with AMD, but consistent with many other complex disorders, few have been confirmed in subsequent studies. Recently, three independent groups have identified significant association with a common coding variant in the gene encoding complement factor H (*CFH*) on chromosome 1q31 (12–14). This polymorphism, which leads to a tyrosine to histidine change at amino acid 402, increases risk of AMD with an odds ratio of between 2.1 and 5.7 and may contribute to ~50% of AMD cases in these studies. Also reported by some but not all groups is an association with the apolipoprotein E (*APOE*) gene on chromosome 19q13 (15–17). At this locus, the E4 allele appears to confer a protective effect, whereas disease risk is increased by the E2 allele. Disease genes have been identified for a number of monogenic disorders which share phenotypic and pathological similarities with AMD. Many of these have been regarded as candidates for AMD but none has shown consistent association with AMD. For example, the *ABCA4* gene on chromosome 1p21, which is associated with autosomal recessive Stargardt disease, has subsequently been shown to confer susceptibility to AMD (18–20). However, several other studies (21–23) have failed to replicate this result.

To date, nine genome-wide linkage studies in AMD have been published (24–32) and a large number of putative candidate regions for susceptibility genes have been suggested. In particular, evidence for linkage to regions on chromosomes 1q and 10q has been remarkably consistent for a complex disorder, despite the different methodologies utilized for defining the clinical phenotype and performing the statistical analyses. However, several other regions exhibiting significant linkage in individual studies have not been replicated or show only nominal evidence for linkage in other studies. This is not unexpected because the power of individual linkage studies is likely to be low for complex disease genes that may be of weak or moderate effect. Several thousand affected sibling

pairs (ASPs) may be required to provide high power to detect linkage (33,34).

Pooling of raw genotype data across studies provides an optimal strategy to maximize power to detect linkage, although this can be difficult due to differences in genotyped markers, family structure and phenotype definition. The genome-scan meta-analysis (GSMA) method (35,36) allows linkage results from several studies to be combined, providing greater power to identify regions which show only weak evidence for linkage in individual studies, as confirmed by simulation studies (36). This method is robust to differences in study design and analysis methods and has been successfully applied to several other complex diseases including rheumatoid arthritis (37), schizophrenia (38), type 2 diabetes (39), coronary heart disease (40), hypertension (41), cleft lip and palate syndrome (42) and inflammatory bowel disease (43). Here, we apply the GSMA method to six published AMD genome-wide linkage scans.

RESULTS

Summed ranks (SRs) for each bin $c.n$ (the n th bin on chromosome c) from weighted analysis are shown in Figure 1A, where each study contributes SRs scaled by the square root of the number of affected individuals. Significant thresholds of 95 and 99% are shown where, for example, each bin has a 5% probability of attaining a SR higher than the 95% limit. Bins of nominal significance ($P < 0.05$) are also listed in Table 1. In total, 15 bins were observed with a SR above the 95% limit when compared with the six bins expected to exceed this value if no linkage was present.

The most significant results were obtained for two adjacent bins of chromosome 10 (bin 10.6: $P = 0.00025$, bin 10.5: $P = 0.0057$), which showed significant and suggestive genome-wide evidence for linkage, respectively. A total of 13 additional bins on chromosomes 1, 2, 3, 4, 12 and 16 were nominally significant, including adjacent pairs of bins 1.7/1.8 (1q23.3–q32), 2.2/2.3 (2p25.1–p16.2), 3.2/3.3 (3p25.3–p14.1) and 16.2/16.3 (16p13–q23.1). Clustering of significant results in adjacent bins is commonly found in the GSMA as linkage peaks under multipoint linkage analysis can extend across a region of 30–50 cM (44), and ranks in adjacent bins are therefore correlated.

The 20 most significant bins are shown in Figure 1B, ordered by SR (solid line). The empirical distribution of the SRs for the highest value (second highest, third highest, etc.) is shown as a boxplot, with the ordered rank (OR) P -value displayed below. Bin 10.6 (rank 120) and bins ranked 101–115 all have nominally significant OR P -values ($P_{OR} < 0.05$), implying that these bins, when considered as a group, have higher SRs than expected. Although the SRs in these bins provide only nominal evidence for linkage, the presence of significant OR P -values strengthens the evidence for linkage in all bins

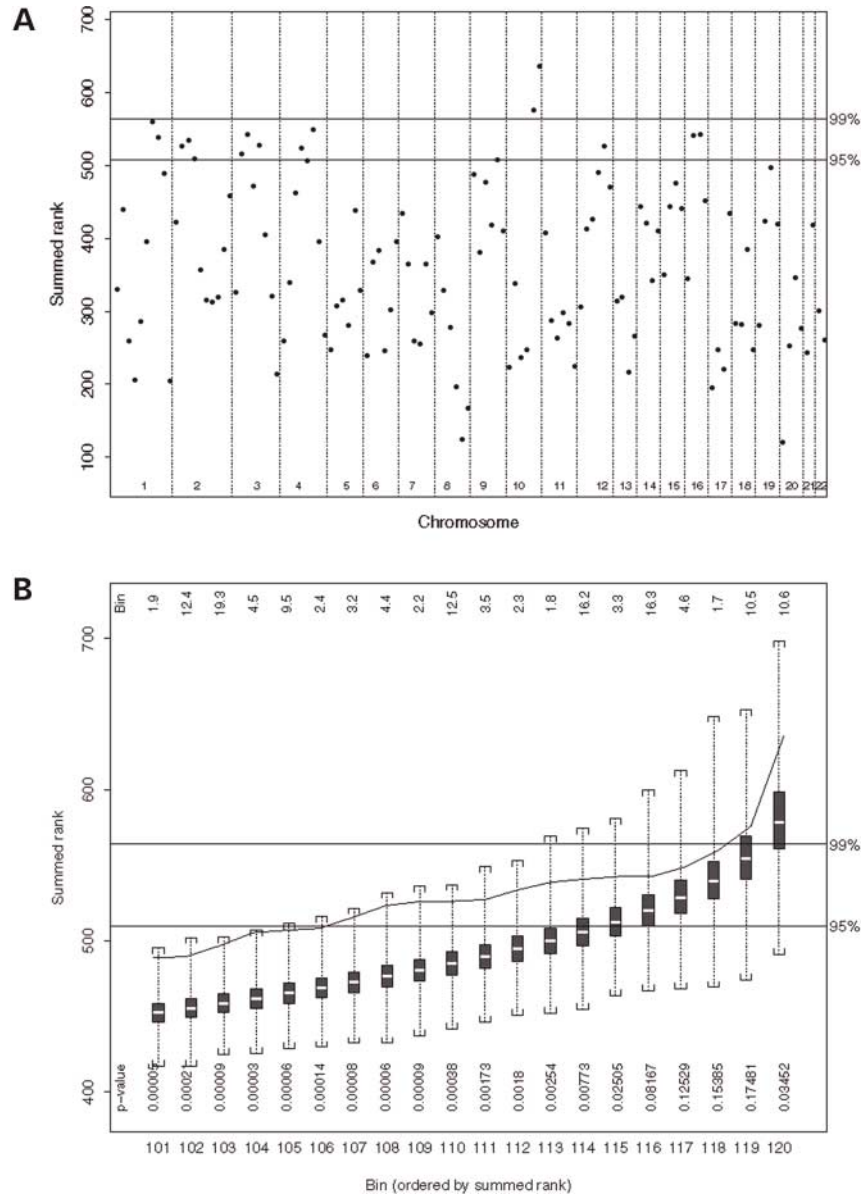


Figure 1. Results from the AMD GSMA. (A) SRs for each bin (weighted by square root of the number of affected individuals in each study); 95 and 99% confidence limits are shown. (B) Highest 20 observed SRs (solid line) with distribution of simulated results (median, interquartile and range of values).

ranked 101–120. This combination of significant SR and OR statistics for a large number of bins is strongly predictive that some of these bins contain susceptibility loci (36).

Heterogeneity P -values for the high-ranking bins are shown in Table 1. Low P -values indicate consistent evidence for linkage in all studies; high P -values indicate genetic heterogeneity with different evidence for linkage attained across studies. No bins showed significant evidence for genetic heterogeneity between studies ($P > 0.975$). Four high-ranking bins showed significantly low heterogeneity ($P < 0.025$). In particular, bin 2.2 attained a heterogeneity P -value of 0.0005, indicating weak but consistent evidence for linkage at this locus.

The average bin width is ~ 30 cM as suggested by Wise *et al.* (35) to meet the assumptions of the GSMA method. To

attempt to refine significant regions further, a secondary analysis was carried out, dividing each bin in half to produce a total of 240 bins. This analysis revealed that bin 10.6a (the proximal half-bin of bin 10.6) showed genome-wide significance ($P_{SR} = 0.00018$), with suggestive evidence for linkage at adjacent half-bin 10.5b ($P_{SR} = 0.0012$). Maximum evidence for linkage in individual studies spans a region of ~ 20 cM. To compare the broad regions of linkage at chromosome 10 identified by individual studies, multipoint non-parametric P -values across the chromosome were superimposed (Fig. 2). All marker positions were scaled to the Marshfield map for comparison. Peaks of linkage in individual studies occurred at up to 25 cM from the boundary of bins 10.5 and 10.6, although the largest study (32) showed maximum evidence for linkage spanning this boundary position.

Table 1. Summary of AMD genome scans included in GSMA

Study	Abecasis	Iyengar	Majewski	Schick	Seddon	Weeks
Year of publication	2004	2004	2003	2003	2003	2004
Number of markers	723	371	449	333	393	406
Number of families	113	34	70	105	158	428
Number of affected individuals	331	297	344	258	490	1089
Weighting factor	0.87	0.83	0.90	0.77	1.06	1.58
Phenotype definition	Broad-AMD ^a	AMD ^b	MODEL 3C ^c	AMD ^b	AMD ^d	MODEL B ^c
Analysis method	Non-parametric (Merlin ^f)	Quantitative trait (Sibpal ^g)	Non-parametric (Allegro ^h)	Quantitative trait (Sibpal ^g)	Non-parametric (Genehunter ⁱ)	Non-parametric (Allegro ^h)
Test statistic output	<i>P</i> -value	<i>P</i> -value	LOD (<i>S</i> _{all})	<i>P</i> -value	<i>P</i> -value	LOD (<i>S</i> _{all})

^aAffection status defined by a broad AMD phenotype.

^bAffection status based on 15-level severity score defined by phenotypic criteria and corrected for age.

^cAnalysis included affected and unclassified individuals and only those definitely unaffected individuals classified as unaffected.

^dStatus defined by intermediate or advanced AMD phenotype and individuals <60 years with no disease or minimal maculopathy designated as unknown status.

^ePhenotype classified as affected if individual was clearly or probably affected with age-related maculopathy.

^fMerlin (54).

^gSibpal (S.A.G.E. v4.2).

^hAllegro (51).

ⁱGenehunter (55).

DISCUSSION

The meta-analysis of genome-wide linkage studies in AMD has identified a total of seven chromosomal regions (1q, 2p, 3p, 4q, 10q, 12q and 16q) with evidence for linkage from the SR and OR statistics. The most significant result (chromosome 10q) was identified as a putative locus by several of the independent studies (25,27,29,32) but did not attain a genome-wide level of significance in any of these studies. Sub-division of bins revealed the highest evidence for linkage from the meta-analysis in bin 10.6a (between markers D10S1483 and D10S1222). Linkage to chromosome 10q26 in one study has been confirmed in a fine-mapping candidate linkage study of this region (45) and is therefore a clear candidate region for AMD. This region has shown strong evidence for linkage when analysed by parametric linkage analysis under a dominant genetic model and has also demonstrated a possible interaction with smoking (32) and epistasis with a region on chromosome 3p (27). The second most significant region identified by the GSMA was on chromosome 1q (bins 1.7–1.8) which contains the *CFH* gene. All but one of the individual studies (28) identified this region as a putative susceptibility locus for AMD. However, this region was ranked in the highest 10% of ranks in only two of the studies (24,29) and attained a rank of 108/120 in the largest study (32). Therefore, this locus was less significant in an analysis weighted by study size compared with an unweighted analysis (data not shown). A recent additional genome scan in 110 ASP families (46) has also reported strongest evidence for linkage to this region on chromosome 1q.

To date, no associations between AMD and genes on chromosome 10q have been reported. Currently, the pathophysiology of AMD is not well understood and the chromosome 10q25.2–q26.3 region identified here contains 74 annotated genes, many of these with unknown function. Identification of potential candidate genes for AMD in this region alone, therefore, represents a major challenge. For example, the region of

chromosome 10q26 proximal to bin 10.6 contains the peroxiredoxin 3 (*PRDX3*) gene (Fig. 2), which encodes a protein with antioxidant function. A contribution of oxygen radicals in the pathogenesis of aging disorders, in particular AMD, has been discussed (47,48). The adjacent bin 10.5 contains the *GFRAL* [glial cell line-derived neurotrophic factor (GDNF) family receptor alpha-1] gene. GDNF is a neurotrophic factor that plays a key role in the control of neuronal survival and differentiation.

Chromosome 1q, the region which attained the second most significant result in the meta-analysis, contains the *CFH* gene which has recently been shown to be significantly associated with AMD (12–14). This gene is situated close to the boundaries of bins 1.7 and 1.8. The lack of genome-wide significant linkage at this locus in any of the individual studies may be due to association with a phenotypic subgroup such as neovascular AMD as described in one study (13). It remains to be seen whether susceptibility polymorphisms in this gene fully account for the linkage in this region. Bin 1.7 also contains the *FBLN6* gene encoding hemicentin 1. *FBLN6* belongs to the fibulin family of extracellular matrix proteins with multiple EGF domains and has been implicated in the pathogenesis of ARMD1 (49). A Gln5345Arg mutation was found to segregate with disease in one large family from Oregon, but no effect for this allele was detected in a sample of patients from Michigan (24). The 5345Arg allele appears to be present in ~1% of AMD patients and controls.

Regions identified by individual linkage studies but which were not observed in the GSMA include chromosomes 6q (28), 15q (25,28) and 17q (32). A region on 9q identified by three studies reached marginal significance in the GSMA (bin 9.5: $P_{SR} = 0.051$). The *APOE* gene on chromosome 19q13 lies within bin 19.3; this bin is marginally significant in the GSMA ($P_{SR} = 0.067$). Interestingly, no single study identified linkage to this region despite the replicated association of this gene with AMD. No evidence for linkage was observed for bin 1.5 ($P_{SR} > 0.8$) which contains the *ABCA4*

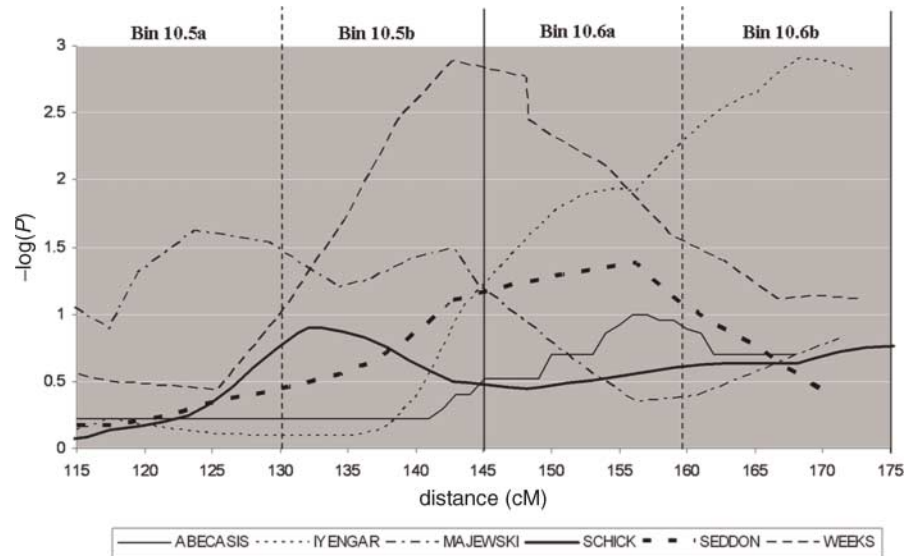


Figure 2. Individual study results for chromosome 10q (bins 10.5–10.6). Comparison of location and evidence of linkage across studies (P -value from each study with 15 cM bin boundaries shown).

gene. The GSMA does not exclude the existence of susceptibility loci in regions not identified by this method, particularly loci that are of weak effect which are very difficult to detect in individual linkage studies.

There are clear differences in AMD prevalence among ethnic groups and geographic location (reviewed in 5). This may indicate differences across populations in the contribution of various susceptibility loci to disease risk. In this case, combining these populations in a GSMA could reduce the power to detect linkage. However, there is no clear source of population differences among these studies, participants being mostly Caucasians of Western European ancestry recruited in the US. Heterogeneity testing revealed low heterogeneity at all seven regions identified by the GSMA, suggesting that a gene or genes at these loci confer susceptibility in at least some families from all populations included in the meta-analysis. Bins showing high heterogeneity (1.10, 5.1, 10.3) may indicate regions where only a subset of studies show linkage to AMD, but none of these regions showed any evidence for linkage under the SR statistic ($P > 0.5$ in each case). Extended studies would be necessary to investigate these regions in detail. Classification of disease is subject to a broad spectrum of signs and severity, and phenotype definition is not consistent across studies. Results extracted from each study for the GSMA are selected for a broad definition according to the majority of studies. A more stringent phenotype definition may reveal regions that contribute to a more severe form of disease. The bin width of ~ 30 cM suggested by the GSMA limits the degree of refinement of linked regions, although 15 cM analysis included here suggests that some additional information can be gained by sub-division of bins. Follow-up studies with additional markers or candidate linkage studies, such as that for chromosome 10q (45), cannot be included in the GSMA.

In summary, a meta-analysis of genome-wide linkage studies in AMD has confirmed several candidate regions. These results provide a basis for dense genotyping with

additional markers and families. Refinement of these regions will assist in the identification of positional and functional candidate genes for association studies in AMD.

MATERIALS AND METHODS

Genome-scan meta-analysis

Briefly, the GSMA method divides the genome into N chromosomal bins of approximately equal size, where bin $c.n$ denotes the n th bin on chromosome c . For each study, the most significant test statistic observed in each bin is recorded. Bins are then ranked in order of significance, with the most significant bin assigned rank N , and the rank of each bin is summed across studies. The summed rank (SR) of each bin forms a test statistic which is assessed empirically against the distribution of SRs. The null hypothesis is that no susceptibility loci exist within each bin and that ranks are therefore randomly assigned. A bin with a high SR indicates evidence for linkage across several studies.

Application of GSMA to AMD

From a total of nine published genome-wide linkage studies, six were identified as eligible for inclusion in the GSMA (Table 2). An early study (26) was excluded because the original genome scan for candidate regions utilized a DNA pooling method for a single large family rather than a LOD score method. Two other studies (30,31) were excluded because the families in these studies formed a part of a subsequent study (32). Investigators from each study were invited to contribute full multipoint non-parametric results across the genome; all six eligible study groups agreed to participate in the meta-analysis. Only original genome-scan results were included in the GSMA; any follow-up studies in candidate regions were excluded.

One population-based study (28) ascertained participants through the Beaver Dam census (50). Briefly, a private

Table 2. Regions with $P_{SR} < 0.05$ identified by the AMD GSMA

Chromosome	Bin	Rank	Boundary markers		Region	P_{SR}	P_{OR}	P_{HET}
1	1.7	3	D1S2705	D1S202	1q23.3–q31.1	0.0104	0.1231	0.141
	1.8	8	D1S202	D1S425	1q31.1–q32	0.0209	0.0015	0.313
2	2.2	12	D2S297	D2S2312	2p25.1–p23.2	0.0308	4×10^{-5}	0.0005
	2.3	9	D2S2312	D2S2251	2p23.2–p16.2	0.0244	0.0008	0.262
	2.4	15	D2S2251	D2S139	2p16.2–p12	0.0495	0.0001	0.344
3	3.2	14	D3S3589	D3S3521	3p25.3–p22.1	0.0410	3×10^{-5}	0.285
	3.3	6	D3S3521	D3S1287	3p22.1–p14.1	0.0187	0.0176	0.018
	3.5	10	D3S3655	D3S1290	3q12.3–q22.1	0.0295	0.0008	0.201
4	4.4	13	D4S1543	D4S3026	4q13.3–q24	0.0331	2×10^{-5}	0.254
	4.6	4	D4S422	D4S2980	4q28.3–q32.1	0.0152	0.0991	0.172
10	10.5	2	D10S1690	D10S1483	10q23.33–q26.13	0.0057	0.1456	0.104
	10.6	1	D10S1483	qter	10q26.13–10qter	0.00025	0.0295	0.017
12	12.5	11	D12S318	D12S1349	12q23.2–q24.31	0.0305	0.0002	0.167
16	16.2	7	D16S3103	D16S415	16p13–q12.2	0.0195	0.0051	0.012
	16.3	5	D16S415	D16S516	16q12.2–q23.1	0.0187	0.0616	0.049

$P_{SR} < 0.0083$ denotes suggestive linkage; $P_{SR} < 0.00042$ denotes significant linkage. Bin ranked 1 indicates the bin with the highest summed rank.

census of the population of Beaver Dam, Wisconsin, was performed from Autumn 1987 to Spring 1988. All 5924 people who were 43–86 years identified as living in the township were eligible and were invited to participate in the study from Spring 1988 to the end of Autumn 1990. Of those eligible, 4926 people participated; participants were re-evaluated at 5 and 10 years after the initial visit. In all remaining studies, patients and families were recruited in the US from local clinics and referral centres; the majority of families were Caucasians of Western European ancestry. As phenotype definition of AMD is not straightforward, some studies defined disease status under several alternative diagnostic criteria, and in two cases, a quantitative scoring system was utilized. Analysis methods across studies included both model-free and parametric approaches as well as quantitative trait analysis. To provide the most uniform disease classification and design across studies, where several phenotype definitions and/or analysis methods were used, results from nuclear families with a broad clinical definition of AMD were selected, as this was most compatible with a single quantitative score used in two studies (Table 2).

Marker sets and maps differed across studies so markers were assigned to bins according to their Marshfield map position (<http://research.marshfieldclinic.org/genetics/>). Any markers not found in the Marshfield map were positioned at a distance from the previous marker relative to that in the study-specific map. In studies which did not contain markers in a telomeric bin, the linkage statistic in the adjacent bin was assumed. For comparison of evidence for linkage across studies, P -values were obtained directly from analysis output in all but two studies (27,32). In these, non-parametric S_{all} LOD scores were obtained from ALLEGRO (51) and can therefore be converted to P -values where $2 \ln(10) \text{LOD} \sim \chi^2_{(1)}$.

In total, 120 bins of ~ 30 cM using boundary markers suggested by Wise *et al.* (35) were defined and were positioned according to the Marshfield map. The highest attainable SR value across all six studies was therefore 720. The GSMA program (<http://mmg.umsd.ac.uk/GSMA>) was used to rank

bins and calculate SR values across studies. Equal test statistics for several bins within a study were assigned tied ranks. OR statistics were also obtained, defined by the conditional probability of the SR value for a specific bin given the position of the bin when SRs are ordered by magnitude. For example, if the third highest bin has a SR of X , the SR P -value gives the probability that an arbitrary bin achieves a value of X or higher, whereas the OR P -value gives the probability that the third most significant result in a GSMA has SR of X or higher. SR analysis assesses the significance of each bin independently, whereas OR analysis considers the distribution of SRs across all bins simultaneously. Empirical significance values for SRs (P_{SR}) and ORs (P_{OR}) were obtained from 100 000 permutations. For 120 bins, values of $P_{SR} < 0.00042$ (expected once by chance in 20 meta-analyses) and $P_{SR} < 0.0083$ (expected once by chance per single meta-analysis) correspond to the genome-wide significant/suggestive evidence for linkage, as defined by Lander and Kruglyak (52). Equivalent values for 240 bins are $P_{SR} < 0.00021$ (significant linkage) and $P_{SR} < 0.0042$ (suggestive linkage).

In an unweighted analysis, studies contribute linkage results equally to the GSMA. A weighted analysis was carried out to allow results to reflect the relative contribution of each study such that smaller, lower-powered studies would have less influence on the overall results. An AMD study-weighting factor was defined by the square root of the number of affected individuals in each study (Table 2) which was considered as an adequate reflection of the different designs across studies (ASPs, nuclear families, extended pedigrees).

AMD results were tested for heterogeneity between studies, using the Q statistic proposed by Zintzaras and Ioannidis (53). For bin j ,

$$Q_j = \sum_{i=1}^N w_i (R_{i,j} - \bar{R}_j)^2$$

where $R_{i,j}$ is the rank of study i in bin j and \bar{R}_j is the mean rank for bin j . Both tails of the distribution of Q are used for testing: high values indicate heterogeneity of linkage evidence between

studies; low values indicate consistent linkage evidence across studies. To test for significance, the joint distribution of SR and Q was simulated for 1×10^6 bins from the ranks of each study. High SRs have high study ranks and less potential to show genetic heterogeneity. Therefore, the P -value for Q in each bin, P_{HET} , was assessed from the subset of 20 000 simulations with SR closest to the observed SR for that bin.

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