

# Absolute pitch exhibits phenotypic and genetic overlap with synesthesia

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**Absolute pitch (AP) and synesthesia are two uncommon cognitive traits that reflect increased neuronal connectivity and have been anecdotally reported to occur together in an individual. Here we systematically evaluate the occurrence of synesthesia in a population of 768 subjects with documented AP. Out of these 768 subjects, 151 (20.1%) reported synesthesia, most commonly with color. These self-reports of synesthesia were validated in a subset of 21 study subjects, using an established methodology. We further carried out combined linkage analysis of 53 multiplex families with AP and 36 multiplex families with synesthesia. We observed a peak NPL LOD = 4.68 on chromosome 6q, as well as evidence of linkage on chromosome 2, using a dominant model. These data establish the close phenotypic and genetic relationship between AP and synesthesia. The chromosome 6 linkage region contains 73 genes; several leading candidate genes involved in neurodevelopment were investigated by exon resequencing. However, further studies will be required to definitively establish the identity of the causative gene(s) in the region.**

## INTRODUCTION

Absolute pitch (AP), also known as ‘perfect pitch’, is a rare cognitive trait characterized by the ability to instantly recognize and name the pitch of a musical note or ambient sound without the use of a reference pitch (1,2). It is most easily recognized in musicians who work within a standardized system of pitch naming, although AP can also be found rarely in individuals without musical training (3). The population prevalence of AP in musically educated individuals is highly variable. Rates of  $\geq 15\%$  have been observed in music conservatories or professional orchestras (4), whereas only  $\sim 4\%$  of music students in a liberal arts college environment report AP (5); the true prevalence in the general population is not well documented but estimates of 1 of 1500 in school age children have been suggested (6). Subsequent to the first description of multiplex families with AP (6), we

and others have documented familial aggregation of AP that is highly likely to reflect a genetic component (5,7,8).

Synesthesia is another uncommon cognitive trait in which stimuli in one sensory realm, such as hearing a sound, stimulates a perceptual experience in another sensory realm, such as color (9,10). The most common forms of synesthesia involve words or numbers triggering color, so-called grapheme-color synesthesia. Like AP, synesthesia has been shown to run in families, and a recent study of multiplex families with synesthesia provided some evidence of linkage (11).

A possible relationship between AP and synesthesia is suggested by scattered reports of ‘colored hearing’ or other synesthetic phenomena in subjects with AP (12,13). Brain imaging studies in both AP and synesthesia provide support for alterations in both structural and functional brain connectivity as the neural substrate to explain these phenotypes (14–17). In this report, we demonstrate that there is also substantial genetic as well as phenotypic overlap between AP and synesthesia.

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## RESULTS

### AP is associated with synesthesia

Anecdotal reports from AP subjects with colored hearing or other synesthetic experiences suggested that these two traits may occur together, but a formal analysis of this association has been lacking. We recruited 1518 subjects who reported the presence of AP, 284 (18.7%) of whom also reported synesthetic experiences. In 842 of those reporting AP, the presence of AP was formally tested (see Materials and Methods), with 768 of 842 (91.2%) showing robust evidence of AP ability. Thus, self-report is a quite reliable indicator of AP. Of the 768 subjects with documented AP on formal testing, 155 (20.1%) reported synesthesia, a much higher rate than the ~4% reported in the general population (18). Associations between pitch and color were most common (84%), with fewer numbers of subjects having synesthesia involving smell, shapes or other more complex sensory experiences. As shown in Table 1, there was no difference in the prevalence of synesthesia between Caucasian and Asian AP subjects in our data set; there was no gender bias observed with any of these phenotypes.

We validated the self-report for synesthesia in a subset of subjects by carrying out testing for this cognitive trait, based on a published methodology (the 'Test of Genuineness') (19) designed to document the consistency self-report for synesthetic experiences involving color. We chose a random set of 21 subjects with AP who reported color associations with pitch and who were enrolled in our studies relatively recently in order to enhance the likelihood of a response to recontact. Given the time-consuming nature of this validation, we did not attempt to formally test the entire cohort for the reliability of self-report for color synesthesia, but rather to simply test the validity of self-report for color synesthesia. Briefly, subjects are provided with a test kit that contains 99 different auditory experiences on a CD along with a color chart containing 240 different colored squares. Subjects report any color associations (synesthesia) in response to the test auditory conditions. This is repeated with a scrambled version of the auditory conditions several months later (see Materials and Methods). At the time of the first test, subjects are not informed that they will be asked to be retested. As shown in Table 2, 20 of 21 subjects who reported synesthesia showed good correlations between the two time points. As is typical for subjects with AP and color synesthesia, most of these individuals provided very specific descriptions concerning color synesthesia with pitch; examples are provided in Table 2.

In Table 2, one subject (0877) who showed no correlation later indicated a lack of understanding of the questions about synesthetic experience. Indeed, most musicians, especially those who do not have AP, have little awareness of the experience of synesthesia. In a pilot survey of 55 music conservatory students without AP, only 1 (1.8%) of these subjects reported color associations with musical notes (data not shown), a rate which is consistent with the overall background population rates reported for synesthesia (18). Thus, applying the Test of Genuineness to subjects who do not report color synesthesia would be confusing to most individuals.

**Table 1.** Prevalence of synesthesia in subjects with documented AP

Ethnic group <sup>a</sup>	Number of AP subjects	Subjects reporting synesthesia, <i>n</i> (%)
All	768	155 (20.1)
Caucasian	568	125 (22)
Asian	88	17 (19.3)

<sup>a</sup>Ancestry information was not available on 91 subjects.

### Linkage analysis of AP and synesthesia in multiplex families

Previous linkage studies of AP and synesthesia have been inconclusive with only modest evidence of linkage (11,20). We have now carried out linkage analysis in 53 multiplex families with AP, and compared these results with linkage analysis in 36 multiplex families with synesthesia. The synesthesia families have been previously published and analyzed for linkage using microsatellite markers (11). Notably, 8 of the 36 families with synesthesia also reported a family member with AP. We performed genotyping in both AP and synesthesia families, using an SNP linkage panel containing 5735 autosomal SNPs (Illumina, Inc.). Linkage analysis of these data was carried out using MERLIN (v1.1.2), using a non-parametric approach to detect allele sharing. In addition, we carried out parametric analyses as described in Materials and Methods. Figure 1 presents the separate NPL linkage results for our 53 AP families and 36 of the previously published synesthesia families (Fig. 1, top and middle panels). Although evidence for linkage in each independent data set is modest, there are several overlaps in the regions of linkage that achieve an LOD of  $\geq 2$  in both data sets, particularly on chromosomes 2 and 6.

Our population data on AP reveal a strong association between AP and synesthesia; we estimate an OR of ~6.0 for synesthesia in AP subjects, assuming a background population rate of synesthesia of ~4%. This phenotypic overlap between AP and synesthesia is particularly compelling, because the initial linkage data suggested linkage overlap between families with these two traits when analyzed separately, and therefore we combined the linkage results from the AP and synesthesia families. This approach is based on the hypothesis that shared genetic components may underlie patterns of brain connectivity that predispose to both these phenotypes. The NPL analysis of the combined data set is shown in Figure 1 (lower panel), and reveals compelling evidence for linkage on chromosome 6q14.1–6q16.1 (peak LOD = 4.68). This high LOD score results from a close overlap of peaks at this position in both AP and synesthesia (Fig. 2). Interestingly, a previously published linkage study of 45 families of European origin with AP also shows a peak LOD of 1.72 in this region, although this was not highlighted as a major finding in this report and no marker details are given (20). Nevertheless, this earlier linkage provides some independent support for the validity of our linkage results. We also ran the 'simulate' option in MERLIN using 1000 replicates, giving the requirement of an LOD score of 3.26 for meeting the  $P < 0.05$  threshold for genome-wide significance

**Table 2.** Results of Test of Genuineness for synesthesia in subjects with documented AP

Subject ID	Days (Test 1–Test 2) <sup>a</sup>	Score	Self-report comments
8233	243	36%	C = blue, D = brown, E = white, F = pink, G = black, A = faded white
1140	76	11%	Color with single notes as well as with complex music
1138	132	72%	I have color synesthesia with my AP. F <sup>#</sup> is violet
1111	386	42%	I am a synesthete. All pitches have color and a few evoke taste when they occur in certain chord progressions
1137	61	10%	Color. A and F only at this time. Perhaps more when I was younger
8672	203	73%	Experiences fixed color associations with notes, C = red, G = blue, for example
1056	269	50%	I do associate colors with pitches. I do not literally see colors, but I do think of pitches as being various shades of colors. C = red, B = ocean blue, E = yellow
1040	119	41%	I associate color with nearly everything I do
4862	58	36%	Yes, ever since I was small, I've always associated colors with pitch, also with numbers, letters, smells, and even sounds and instruments
0877 <sup>b</sup>	171	0%	
9772	179	25%	Whenever I hear pitches, I immediately think of colors, smells, places. Sometimes I even have deja-vus, where my whole world seems to be painted by musical patterns
1073	81	27%	D Major is yellow, it is always yellow, does not change
1148	70	61%	I associate colors with the twelve pitches. This has always been for me and has never changed. C = royal blue, F = green, G = indigo, A = fuchsia, B = brown, E = red, D = purple
1162	135	26%	C = white, C <sup>#</sup> = blue, D = burgundy, D <sup>#</sup> = red-orange, E = red, F = pale orange, F <sup>#</sup> = orange, G = yellow, G <sup>#</sup> = dark green, A = green, A <sup>#</sup> = light brown, B = purple
1171	123	21%	I tend to categorize pitches with colors. For example, C and C <sup>#</sup> are different shades of red (major is brighter, minor is darker)
1176	51	83%	
1177	123	46%	Yes. Pitches and key centers all have sensations of color associated with them, influenced also by timbre, register and mode. A-flat has strong deep aqua green feeling to it, whiter, less saturated in higher octaves, darker in lower octaves.
1173	147	35%	Slight key-color thing going on, not something I can see in front of me, just sort of an abstract association, more for certain keys than others. May be tied to the fact that I associate certain letters with colors. C major = yellow, sunny, happy. B = blue
1206	160	20%	C and F are yellow. C <sup>#</sup> and F <sup>#</sup> are black. D is green, E is orange, G is brown, G <sup>#</sup> is purple, A is red, A <sup>#</sup> and B are blue, and D <sup>#</sup> is colourless. Unfortunately, I can't explain what any of this means
1612	70	28%	Definitely color. With individual pitches and associations with keys as well

<sup>a</sup>Days between Test 1 and Test 2.<sup>b</sup>This individual later admitted to misunderstanding the initial questions concerning synesthesia.

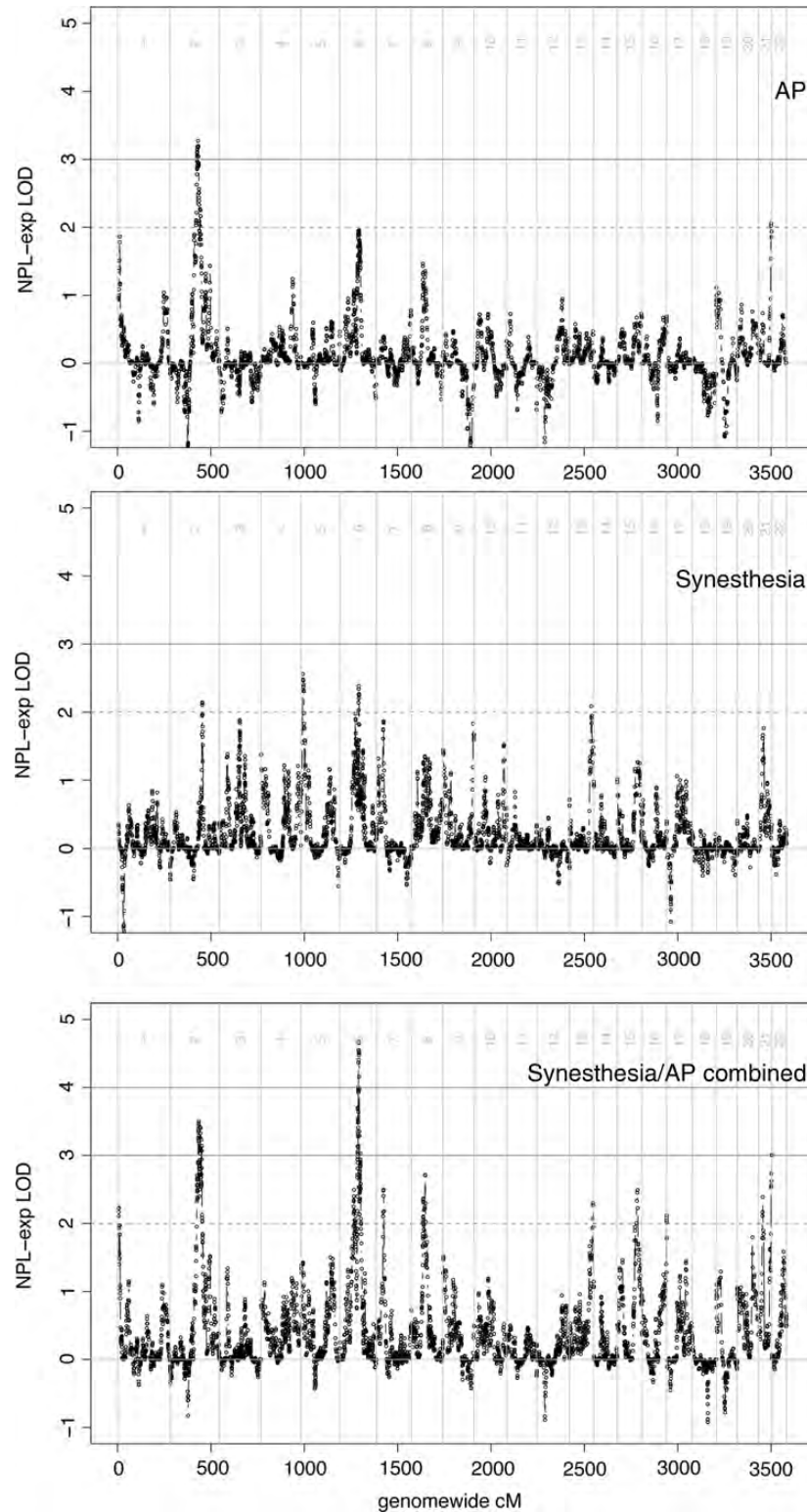
suggested by Lander and Kruglyak (21). The observed peak LOD score of 4.68 on chromosome 6 gives a genome-wide significance of  $P = 0.003$ .

In addition to the region of definite linkage on chromosome 6, the combined linkage analysis provides support for a more complex pattern of linkage on chromosome 2. As shown in Figure 3, AP and synesthesia have somewhat different locations for linkage peaks on chromosome 2, with the combined NPL LOD score hovering at  $\sim 3$  over a 30 Mb region. Interestingly, linkage analysis using a dominant model provides quite robust support for linkage in this region of chromosome 2 with a heterogeneity LOD score (HLOD) of 4.7 at rs1482308 in the combined AP and synesthesia families. A peak HLOD of 3.93 is observed at marker rs6759330 in AP families alone, using a dominant model (Fig. 3).

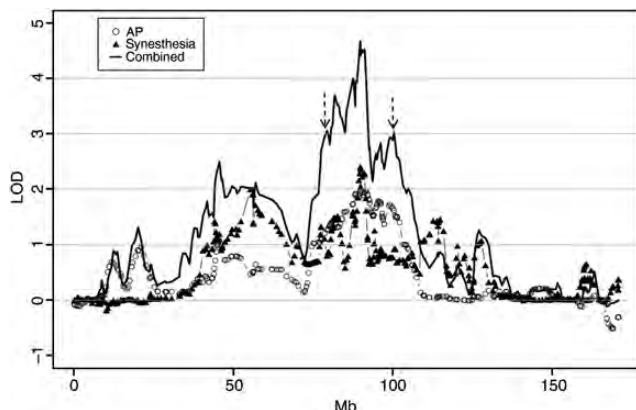
Given the highly significant combined LOD score of 4.68 on chromosome 6, with additional previous evidence of linkage at this same location in an independent data set of AP families (20), we searched this region for likely candidate genes that could plausibly be involved in early cortical brain development and neural connectivity. Of the 73 genes located within the  $\sim 20$  Mb interval flanked by positions with support for linkage of LOD  $> 3$  (Chr6: 79.5–100 Mb, see Supplementary Material, Table S1), a compelling candidate is *EPHA7*, a member of the ephrin family of cell-surface-bound receptor tyrosine kinases. This family of

receptors and their ligands play a prominent role in neural differentiation, migration and functional connectivity in the developing brain (22).

Therefore, we resequenced all 17 exons of *EPHA7* in 96 affected subjects from 39 of our multiplex AP families. One or more of three non-synonymous coding variants were found to be shared among affected subjects in four families exhibiting linkage in this region. All three coding variants were found in pairs of affected subjects in family 0887 (Supplementary Material, Fig. S1), including a previously unreported novel variant at codon 357 (asparagine to serine) in the proband of this family, and her affected sister. Asparagine is highly conserved at this position throughout vertebrate species, with the exception of zebrafish, where threonine is present. Codon 357 is located in one of two fibronectin domains on the extracellular portion of *EPHA7*. The N357S variant was not observed in over 10 000 subjects reported by the Exome Variant Project. Family 887 is of Malaysian origin, and there are no large public population data available concerning the frequency of the N357S variant in Asian populations. We therefore screened 700 local Asian controls as well as 400 local European-American controls; we did not detect this variant, nor did we find the N357S variant in any of the probands of our other AP multiplex families. We were concerned that some of these *EPHA7* variants were found in the few Asian families in our family collection and might bias linkage results. Therefore, NPL-exp was run on the



**Figure 1.** The genome-wide linkage plots are shown for AP (upper plot,  $n = 53$  families) and for synesthesia (middle plot,  $n = 36$  families). The lower plot shows the combined linkage analysis with both AP and synesthesia families. In our AP families, 126 subjects with documented AP were genotyped, and of these, 28 (22%) report the presence of synesthesia in addition to AP. Likewise, among the 36 families with synesthesia, 8 families (22%) include subjects who report the presence of AP, although these individuals have not been formally tested for the AP phenotype. The full family trees are given in Supplementary Material, Figure S2. Analysis of linkage was carried out using the NPL-exp option in MERLIN. LOD scores are shown on the  $Y$ -axis, and genome-wide position (in cM) is given on the  $X$ -axis, with chromosomes as indicated. A maximum NPL-exp LOD score of 4.68 is achieved on chromosome 6q in the combined analysis. The dotted lines indicate LOD = 2 in each plot. The solid lines indicate LOD = 3 and/or LOD = 4 (lower plot).



**Figure 2.** Details of linkage plots (NPL-exp) on chromosome 6 for AP (open circles), synesthesia (closed triangles) and combined AP and synesthesia (solid line). The extent of the linkage region we have considered for investigation of candidate genes is indicated by the dashed arrows.

combined AP and synesthesia data set after excluding the four Asian families in our collection, with minimal change in the main result on chromosome 6 (LOD = 4.57).

Two additional non-synonymous variants in *EPHA7* were shared among affected subjects in four families (Supplementary Material, Fig. S1): rs2278106 (encoding Pro to Ser at codon 278) and rs2278107 (encoding Ile to Val at codon 138). These SNPs are in perfect LD with one another and are found at allele frequencies of ~3% in Caucasian populations, both in the Exome Sequencing Project and in our local controls. The background frequency of these alleles is similar to the frequency we observed in 310 local AP subjects of Caucasian origin. Thus, although these variants exhibit evidence of linkage in these families, these two amino acid changes on their own cannot explain the AP and synesthesia phenotypes in these families.

We also carried out exon sequence of additional candidate genes in the chromosome 6 linkage, including two GABA receptors (*GABRR1* and *GABRR2*) as well as the cannabinoid receptor *CNR1* (23). This did not reveal any variants of interest (data not shown).

## DISCUSSION

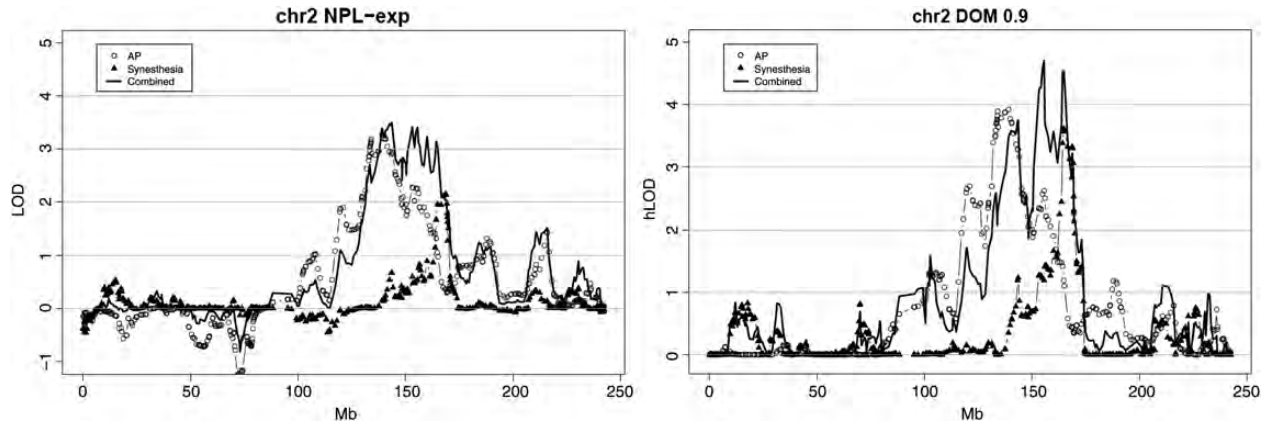
Overall, these data firmly establish that the phenotypes of AP and synesthesia are phenotypically and genetically closely related. Although there have been anecdotal reports of the association of these two phenotypes, this is the first clear demonstration of this fact in a large population sample. This likely reflects an underlying commonality of neurodevelopmental mechanisms involved in these two cognitive traits. It has been convincingly established that both AP and synesthesia exhibit variability in patterns of brain connectivity, using both anatomic as well as functional imaging studies (14–17).

Our data provide strong evidence (LOD = 4.68) for a common region of linkage on chromosome 6q over a ~20 Mb region between ~80 and 100 Mb (Fig. 2 and Supplementary Material, Table S1). In addition, a more complex pattern of linkage on chromosome 2 (Fig. 3) indicates the presence of at least one region of linkage underlying AP, using a

dominant model, and suggests a potential overlap between the two phenotypes at other loci in this broad linkage region. Given the more consistent linkage evidence on chromosome 6, we elected to resequence the exons of several candidate genes in the broad chromosome 6 linkage region as indicated in Figure 2. The region contains 73 genes, and we focused particularly on *EPHA7*, a locus that is involved in many aspects of brain development (see what follows), as well as on the cannabinoid receptor 1 (*CNR1*), which also plays a role in cortical brain development (23). Since disinhibition has also been suggested as a mechanism for synesthesia (10), we also resequenced the exons of two GABA receptors in the region, *GABRR1* and *GABRR2*. These sequencing studies revealed three non-synonymous variants in *EPHA7* that were consistent with linkage (see Supplementary Material, Fig. S1, for examples). One of these, N357S, is completely novel and apparently extremely rare, and we have not observed it in any other AP or normal subjects. Two other *EPHA7* variants occur in ~3% allele frequency in Caucasian populations, which is not significantly different from our AP populations, although sample sizes are still quite limited.

Although our sequencing results clearly do not yet establish *EPHA7* as a causative locus, this gene remains a compelling candidate gene for phenotypes related to differences in neural connectivity, since we have not assessed potential regulatory non-coding regions of this gene. *EPHA7* has been specifically related to the formation of cortical thalamic connectivity involving the auditory cortex (24), as well as other cortical regions (22). *EPHA7* regulates apoptosis of neural progenitors, and knock-out animals for *EPHA7* exhibit increased cortical thickness. Levels of expression of ephrins are tightly regulated and are likely to be critical for neural development (25), suggesting that regulatory variants could play a role in cognitive phenotypes such as AP and synesthesia. The *EPHA7* gene is relatively large (180 kb), and substantial resequencing will be required to definitively address this question. The current data provide ample justification for making that effort, as well as for pursuing the analysis of other candidate genes in the linkage peaks we have identified on chromosomes 2 and 6.

The approaches to phenotyping for synesthesia and AP deserve comment. Currently, the synesthesia phenotype can be assessed only using self-report, with supplemental tests such as the Test of Genuineness in order to confirm that subjects are stable in their perception over time, and not just ‘making it up’. It may be possible in the future to detect synesthetic experience, using functional brain imaging, but clearly this is not suitable for large population studies. In contrast, the phenotype of AP is testable with the standard note-naming approaches that we have utilized here. However, this phenotypic assessment relies on subjects having some knowledge of note names. We have recently developed a freely downloadable iOS App, PitchMatch!, which is based on a testing paradigm developed by Ross *et al.* (3) and does not require knowledge of musical note names (see [www.absolutepitchstudy.com/pitchmatch.html](http://www.absolutepitchstudy.com/pitchmatch.html) for information on PitchMatch!). This may facilitate a broader approach to assessing the prevalence of the AP phenotype in the population in the future.



**Figure 3.** Details of linkage plots on chromosome 2 for AP (open circles), synesthesia (closed triangles) and combined AP and synesthesia (solid line). The left panel shows results using the NPL-exp option in MERLIN. The right panel shows results using a dominant model with penetrance set at 0.9. Note that in addition to two prominent peaks (HLOD > 4) in the combined analysis, a linkage peak of HLOD = 3.93 is also observed for AP families alone.

Finally, it is intriguing that AP may occur with increased frequency in subjects with autistic spectrum disorder (26), and a recent report suggests normal subjects with AP exhibit a spectrum of traits that have been related to autism, although this does not reach the level of autistic spectrum disorder *per se* in these subjects (27). There is some evidence that synesthesia may also occur with increased frequency in autistic subjects (Baron-Cohen, unpublished data). Increased short-range neural connectivity has been proposed as a neural feature of autism (28), and this may explain an association among these apparently disparate traits. Defining the genetic basis of AP and synesthesia may conceivably provide insights into the genetics and neurobiology of autistic spectrum disorders.

## MATERIALS AND METHODS

### Study subjects

Multiplex families with AP as well as singleton subjects with AP were recruited over a 10-year period using a variety of ascertainment methods including flyers being sent to music schools around the USA, direct mailings and website promotion, word of mouth and personal contacts of the authors (P.K.G. and E.K.). Of the 53 multiplex families utilized for linkage in the current study, there were 4 Asian families, and the remaining 49 families were of European origin. Multiplex families with synesthesia have been described previously (11). Full family trees are given in Supplementary Material, Figure S2.

### Genotyping and linkage analysis

Genotyping was carried out using the Illumina SNP linkage panel which contains 5735 autosomal SNP markers. The quality of the genotyping in 36 synesthesia pedigrees was checked using both PEDCHECK (v1.1) and PEDSTATS (v0.6.10) programs. Approximately 8000 Mendelian inconsistent errors were detected or roughly 0.8% of all typings. No individuals and no SNPs had disproportionately higher error rates than others, and we deleted SNPs with Mendelian error for linkage analysis instead of manually correcting them.

The second set of pedigrees has 53 families containing 245 persons genotyped (132 are AP subjects), plus 21 untyped pedigree-connecting individuals (2 of them with AP). The genotyping of the AP data set was pre-processed to eliminate poor-quality SNP calls, yielding 5305 SNPs across 22 autosomal chromosomes; only 60 Mendelian errors were present in the data set.

We ran two non-parametric linkage analyses and three parametric ones. Only autosomal loci were analyzed. The two non-parametric linkage analyses are the NPL (merlin -npl) and NPL-exp options (merlin -npl -exp). The NPL-exp option is designed to detect larger increases of allele sharing among affected subjects in a small number of pedigrees, as versus small increases of allele sharing in a large number of pedigrees in the NPL option. In other words, NPL-exp is more appropriate if there is locus heterogeneity among affected subjects or among pedigrees. The three parametric linkage analyses use the models with penetrances of 0.0001, 0.9, 0.9 (DOM09); 0.0001, 0.65, 0.65 (DOM065); and 0.0001, 0.0001, 0.75 (REC075). The first two are dominant disease models and the third one recessive.

To estimate the probability that high LOD scores are obtained by chance, we ran the MERLIN 'simulate' option, which uses the gene-dropping method to randomly generate marker data. In this simulation, the pedigree structure and phenotype are preserved, and random marker data are consistent with the original marker data in allele frequency and recombination pattern. One thousand replicates were simulated, with the maximum of HLOD ranging from 0.95 to 5.28. The 95 percentile of the max(HLOD) is 3.26, which would be the empirical LOD for 'genome-wide significant linkage' defined by Lander and Kruglyak (21). The median (50 percentile) of max(HLOD) in the 1000 simulated data sets is 2.11.

### AP Testing

In order to ascertain the AP phenotype, subjects were asked to complete a pitch identification test online or a nearly identical version recorded on a compact disc (CD). Target stimuli were 90 computerized tones (48 piano tones and 42 pure tones), presented in a randomized order and spanning a 4-octave range.

Subjects tested online were pre-assigned a unique coded number and password combination with which to gain access to the test website. The online test requirement was to listen to each tone and identify the pitch by choosing the key or note name on the image of a keyboard. For the CD test, subjects orally named each tone to the test administrator (E.K.). Tones were presented at 3 s intervals on the CD version and at 4 s intervals on the online version, to allow additional time to click on the keyboard image.

Subjects listened to the test once only and no warm-up test was permitted. Responses were scored modified from a previously reported scoring system (4): absolutely correct response = 1; responses correct within a half-tone = 0.75; responses more than a half tone off, but correct within a whole tone = 0.5; responses equal to or more than a whole tone away from the target tone were given a score of 0. AP possessors were designated AP1 if the pure tone score was  $\geq 30$ . Individuals whose piano score was  $\geq 36$  but did not achieve a pure score consistent with the AP1 designation were designated AP2.

### Synesthesia testing

A subset of 21 subjects who reported synesthesia that involved color associations underwent objective testing using a modification of a previously published method (19). Briefly, a testing kit containing a CD with 99 sound conditions (51 words and 48 non-words) was sent by mail to the subject, along with a standardized color chart, the Cambridge Synesthesia Chart, containing 240 different colored squares. Subjects took the test by listening to the sound conditions and picking a color (or colors) that matched their synesthetic experience, if present. Subjects responded with a numbered code assigned to each colored square. After returning the test materials, the subjects were contacted again to repeat the test, using a scrambled version of the original 99 sound conditions on a CD. A mean of 142.5 days (range 51–269 days; see Table 2) elapsed between the two testing events. The color response on the second test was scored with regard to the similarity in color to the initial response, if present for that condition, with scores of 3, 2, 1 or 0 reflecting decreasing similarity to the first color response. Individuals were scored according to what percentage of the maximum total similarity of scores between the first and the second response. Results are given in Table 2.

### Sequencing and genotyping for association

DNA samples of 96 subjects from 39 multiplex families with AP were sequenced at Polymorphic DNA Technologies, Inc. (Alameda, CA, USA). All exon sequences for *EPHA7*, *GABRR1*, *GABRR2* and *CNRI* were specifically amplified by nested PCR and used as templates for bidirectional Sanger sequencing on ABI 3730XL DNA sequencers. Ten microliter TaqMan assays for *EPHA7* snps rs2278106 and rs2278107 were purchased from ABI as predesigned genotyping assays and run on ABI Viia7 according to the manufacturer's standard protocol. A custom TaqMan was designed by ABI for the N357S variant and run using the standard 10  $\mu$ l TaqMan

protocol. All genotyping assays were validated using the original 96 resequenced samples.

### SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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*Conflict of Interest statement.* None declared.

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