Identification of candidate genes for developmental colour agnosia in a single unique family

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Abstract

Colour agnosia is a disorder that impairs colour knowledge (naming, recognition) despite intact colour perception. Previously, we have identified the first and only-known family with hereditary developmental colour agnosia. The aim of the current study was to explore genomic regions and candidate genes that potentially cause this trait in this family. For three family members with developmental colour agnosia and three unaffected family members CGH-array analysis and exome sequencing was performed, and linkage analysis was carried out using DominantMapper, resulting in the identification of 19 cosegregating chromosomal regions. Whole exome sequencing resulted in 11 rare coding variants present in all affected family members with developmental colour agnosia and absent in unaffected members. These variants affected genes that have been implicated in neural processes and functions (CACNA2D4, DDX25, GRINA, MYO15A) or that have an indirect link to brain function, development or disease (MAML2, STAUL, TMED3, RABEPK), and a remaining group lacking brain expression or involved in non-neural traits (DEPDC7, OR1J1, OR8D4).

Although this is an explorative study, the small set of candidate genes that could serve as a starting point for unravelling mechanisms of higher level cognitive functions and cortical specialization, and disorders therein as developmental colour agnosia.

Introduction

Genetics of developmental disorders of the human brain has been a main strategy to uncover biological mechanisms of brain development and functioning. In particular, understanding of the development of the cerebral cortex has benefited from the recognition of specific cortical abnormalities and the elucidation of causative gene defects [1–3]. Genetic aberrations in
cortical specializations may be a treasure trove to find genetic dominators and mechanisms of cognitive functions [4, 5].

Different disorders of cortical specialization have been described (reviewed by [4]): prosopagnosia (i.e. inability to recognise faces/people despite normal intelligence), synesthesia (i.e. stimulation of one sensory or cognitive pathway leads to automatic, involuntary experiences in a second sensory or cognitive pathway, for example, letters or numbers are perceived as inherently coloured), dyslexia (i.e. disability in reading despite normal intelligence), and congenital amusia (i.e. defect in processing pitch, but may also include musical memory and recognition).

In this study we focussed on a very rare disorder of cortical specialization, namely developmental colour agnosia. Disorders of colour processing have been reported at different levels, ranging from wavelength processing deficits up to very specific impairments in object-colour associations [6]. One of the most intriguing disorders may be colour agnosia. In colour agnosia, people have intact colour perception, yet have severe difficulties in naming, categorising, and recognising colours and form adequate object-colour associations [7-9]. In most cases, colour agnosia is acquired after brain damage, either bilateral or left hemisphere lesions [10], mostly in the occipitotemporal lobe [6].

Over a decade ago we reported the first and only case of developmental colour agnosia [9]. Additionally, a familial factor in developmental colour agnosia was described when family members in three generations also showed difficulties in colour naming and recognition [7], indicating a genetic origin of the trait. The affected individuals of this family all display normal cognition and intact colour perception at a visuosensory level, e.g. matching of equiluminant colours. However, they could not name colours, match colour names to colours, categorise hues into general clusters of colour or point a colour that is mentioned by the examiner [11]. This trait likely is a disorder in cortical specialization [4]. Such disorders may have a genetic origin resulting in abnormal cortical development.

In order to identify genetic origins and to gain more insight into the developmental mechanisms related to these disorders, we analyzed all participating members of this family by CGH-arrays and whole-exome sequencing. The results reveal a small set of extremely rare variants that are candidate to cause developmental colour agnosia within the limits of this study.

Subjects and methods
Subjects and DNA isolation
Informed and written consent were obtained from the family members to perform DNA analysis and sequencing, and to publish results in a scientific paper as approved by the Medical Ethical Committee of the University Medical Center Utrecht by METC protocol number 12–222. Four members were phenotyped earlier [7]. For this study, two additional family members were tested and diagnosed with or without developmental colour agnosia. Phenotyping has been described by [7, 11]. In total three affected (age 78, 51 and 13) and three unaffected family members (46, 12 and unknown adult) were available for this study (Fig 1). The individuals were recruited in this study in 2010 and 2011. Genomic DNA of the six family members was isolated from saliva using the QIAamp® DNA Blood Mini Kit (Qiagen) with the supplementary protocol for isolation of genomic DNA from saliva and mouthwash as provided by the company. The DNA concentration was determined by using Qubit Quant-IT (Invitrogen). The authors TCWN, EVSH, GWvH, MJvZ, EHB and JPHB had access to information that could identify individual participants during or after data collection. Data contacts are g.van-haaften@umcutrecht.nl and onderzoekdiviesieherzisenen@umcutrecht.nl.
Array-based comparative genomic hybridization and linkage analysis

Array-CGH microarray analysis was performed using 105K (Agilent amadid 019015, hg18) microarray slides from Agilent Technologies (Santa Clara, CA) following manufacturer’s protocols and a mixed pool of either 50 healthy males or females was used as a reference. Scanned images were analyzed by Feature Extraction software (Agilent Technologies). Data analysis was performed using DNA analytics 4.76 Software from Agilent Technologies using the ADM-2 algorithm. Interpretation of CNV-data was performed as described in the guidelines of [12]. Copynumber variations and sequence homologies flanking the breakpoints were confirmed using the Database of Genomic Variation (http://www.projects.tcag.ca/variation/) and the UCSC Genome Browser (http://www.genome.ucsc.edu/index) [13].

Linkage analysis was performed by using the array CGH chromosome profiles and mapping of the disease loci was calculated with DominantMapper [14]. This program calculated which chromosomal regions are present in all affected family members and not present in all non-affected family members based on SNP genotyping data.

Library preparation and whole exome sequencing

DNA (2ug) was used for the preparation of a barcoded fragment library [15]. This was followed by multiplexed enrichment and sequencing on the SOLiD Next-generation sequencing platform containing the exonic sequences of ~18,000 genes and covering a total of ~37Mb of genomic sequence [15, 16]. In brief, genomic DNA was fragmented using the Covaris S2 System (Applied Biosystems) to 100–150-bp fragments with a mean size of 125 bp using the following settings: number of cycles, 3; bath temperature, 4˚C; bath temperature limit, 8˚C; mode, frequency sweeping; duty cycle, 20%; intensity, 5; cycles/burst, 200; and time per cycle, 1 min 45 seconds. After fragmentation, the DNA was end repaired and phosphorylated at the
5' end using the End-It DNA End-Repair Kit (EpiCenter) and purified with the Agencourt AMPure XP system (Beckman Coulter Genomics). DNA was then ligated to double-stranded truncated adaptors compatible with the SOLiD next-generation sequencing platform using the Quick Ligation Kit (NEB). After purification, each sequencing library was nick translated, barcode and amplified in a single PCR assay. The intensity of library bands was examined on a 2% agarose gel (Lonza FlashGel System). Amplified library fragments in the range of 175–225 bp in size were selected on a 4% agarose gel and purified using a QIAquick Gel Extraction Kit (Qiagen). Libraries from the family members were pooled in equimolar concentrations and were enriched using the Agilent SureSelect Human All Exon 50Mb Kit (Agilent Technologies). Enriched library pool fragments were amplified using 12 PCR cycles and elongated to a full-length adaptor sequence required for SOLiD sequencing. SOLiD sequencing was performed according to the instructions in the SOLiD 4 manual to produce enough 50-bp reads to obtain sufficient coverage for a single allele in each library.

**Variant detection and exome sequencing analysis**

Raw sequencing reads were mapped against the human reference genome GRCh37/hg19 using our custom pipeline based on the Burrows-Wheeler Alignment (BWA) algorithm. Sequence data were submitted to the EMBL-EBI Sequence Read Archive (see URLs). Single-nucleotide variants (SNVs) and small indels (≤7 nt) were called by our custom analysis pipeline as described [17]. All scripts are available upon request. The criteria for variant detection were set to enable discovery of heterozygous variants, since we assume dominant inheritance. We required that variants should minimally be supported by two read seeds (first 25 bp, the higher quality portion of a read), and we set the cut-off for coverage to a minimum of ten reads and the cutoff for non-reference allele percentage to 15%. Since it is probably a dominant inherited phenotype, we set the cut-off for non-reference allele percentage to a maximum of 80%. A maximum of five clonal reads (defined as reads with an identical start site) were included in the analysis. Only truncating variants including nonsense frameshifting insertions or deletions, or missense and splice site alterations were selected. All polymorphisms with an allele frequency < 0.002 in the different databases in EVS database (http://evs.gs.washington.edu/EVS/), Exac (http://exac.broadinstitute.org/) or GoNL (http://www.nlgenome.nl/) were excluded, and variants present in our in-house database > 0,1,2 x present were excluded from analysis; For each variant, the genomic location, amino-acid change, effect on protein function, conservation score and output from prediction programs (Polyphen, Polyphen-2, SIFT and Condel) were compiled [15]. Alamut was used to study the variants in more detail. Prioritization and variant selection is described in Fig 2. Identified variants were confirmed with Sanger sequencing.

**Gene expression analyses**

Comprehensive human brain transcriptome data sets were used to determine the developmental expression profiles of all candidate genes [18]. The set contains the age-related expression in 39 samples of the dorsolateral prefrontal cortex from individuals ranging from 0.1 to 83 years of age (Gene Logic Inc, Gaithersburg, MD, USA, [18, 19]). Raw data of these studies were available online as GEO entry (GSE11882, Fig 3).

For analysis of gene expression single-cell RNAseq datasets provided by the Allen Brain Institute were used in the Allen Brain Map Transcriptomics Explorer (https://portal.brain-map.org). For the human brain on datasets Human-M1-10X and Human-Multiple cortical areas-SMART-seq were used [20]. For the mouse brain the data set Mouse-Whole mouse brain & hippocampus-10X was used. In addition, data of the mouse cortex [21, 22] were mined through the Single Cell Portal (https://singlecell.broadinstitute.org/single_cell) and the
Results
CGH-array and linkage analysis

Segregation patterns of developmental colour agnosia in the family are most consistent with Mendelian dominant inheritance (Fig 1). Array CGH performed on the six family members
showed that there are no large chromosomal rearrangements co-segregating with the phenotype of developmental colour agnosia. Next, we performed linkage analysis on SNP data to identify loci cosegregating with developmental colour agnosia. All family members were included in the linkage analysis assuming an autosomal dominant model of inheritance. The genome wide linkage resulted in the identification of multiple linked chromosomal regions (Table 1). These linked regions were used for the identification of the candidate genes assuming that the causal gene will be present in a linked region.

Exome sequencing and candidate gene selection

We hypothesized that developmental colour agnosia is caused by a single heterozygous rare non-synonymous variant present in all affected family members and absent in unaffected family members. Candidate gene selection was performed by filtering as described in Fig 2. Exome sequencing followed by quality control identified in total in all family members 63795 variants present in exons and canonical splice sites. Of these, 5908 variants were protein truncating with putative loss of function alteration, including nonsense frameshift (indels) or splice site variants, and were de novo or rare in the population (0, 1, 2 times present in the in-house
Of these 5908 variants, 1797 were not present in non-affected family members and 29 were present in all three affected family members. Of these 29 variants we further studied the frequency of these variants, to assume that the variants are rare or de novo in a broader population. Based on frequency of presence in the Exome Variant Server (EVS) database (frequency 0:13000–25:13000 (frequency < 0.002)) (n = 17) and Variance GoNL database (24) (n = 16), and confirmation of the variant in the linked region by Sanger sequencing (n = 11), 11 candidate variants in 11 genes remained (Table 2). These were: GRINA (glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1), OR1J1 (olfactory receptor, family 1, subfamily J, member 1), RABEPK (Rab9 effector protein with Kelch motifs), DEPDC7 (DEP domain containing 7), MAML2 (mastermind-like 2 (Drosophila)), OR8D4 (olfactory receptor, family 8, subfamily D, member 4), DDX25 (DEAD (Asp-Glu-Ala-Asp) box polypeptide 25), CACNA2D4 (calcium channel, voltage-dependent, alpha 2/delta subunit 4), TMED3 (transmembrane emp24 protein transport domain containing 3), IL16 (interleukin 16), MYO15A (myosin XVA), STAU1 (staufen double-stranded RNA binding protein 1). Additional information including allele frequency in the 1000 Genome Project [23] (The 1000 Genomes Project Consortium), Variance GoNL [24, 25] and Wellderly cohort [26] is presented in Table 3.

Effect of the mutation

Prediction about the effect of the mutations were made by three software packages: Polyphen, Polyphen-2, SIFT and Condel (Table 3). Two variants (GRINA and RABEPK) were predicted to be probably damaging (Polyphen) and deleterious (SIFT). Six variants (OR1J1, DEPDC7, MAML2, DDX25, TMED3, STAU1) were predicted as tolerated by SIFT; some of these were benign or possibly damaging by Polyphen (OR1J1, DDX25, DEPDC7, TMED3, STAU1).

Table 1. Linked chromosomal regions to colour agnosia.

| Chr. | Locus      | Start       | End         | Length     |
|------|------------|-------------|-------------|------------|
| 2    | 2q22.3-35  | 146360626   | 221800201   | 7539575    |
| 3    | 3p11.2-q11.2 | 87373894   | 94512868    | 7138974    |
| 3    | 3q13.3-q25.31 | 119442572  | 159239866   | 39797294   |
| 4    | 4q13.1-4q22.3 | 60013919   | 96704454    | 36690535   |
| 5    | 5p15.33-5p15.2 | 2346621    | 10752315    | 8405694    |
| 6    | 6q26-27    | 162736476   | 168554222   | 5817746    |
| 8    | 8q24.23-24.3 | 138970102  | 143270729   | 4300627    |
| 8    | 8p22-q13.1 | 14253354    | 68723609    | 54470255   |
| 9    | 9q21.32-q34.11 | 83858558   | 138149166   | 54290608   |
| 10   | 10p15.3-p14 | 135708     | 8638235     | 8502527    |
| 11   | 11p14.3-q24 | 21234023   | 128206410   | 106972387  |
| 12   | 12p13-q23.3 | 661031     | 67262352    | 66601321   |
| 14   | 14q12-q31.2 | 22645683   | 88752821    | 66107138   |
| 15   | 15q15.1-q26.1 | 40509600  | 94077313    | 53567713   |
| 17   | 17p12-q25.3 | 9078327    | 77698582    | 68620255   |
| 19   | 19p12-q21  | 23446279    | 28836326    | 5390047    |
| 20   | 20q13.13-q13.33 | 47050483  | 60111743    | 13061260   |
| 21   | 21q11.2-q22.12 | 14687571 | 38082812    | 23395241   |
| 22   | 22q11.2-q13.2 | 16079545   | 44595591    | 28516046   |

Chromosomal regions linked to developmental colour agnosia (hg19). These haplotypes of these regions are present in all affected individuals and absent in the non-affected individuals.

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MAML2 was predicted as probably damaging by Polyphen. OR8D4 was deleterious on SIFT and benign on Polyphen. MYO15A was probably damaging by Polyphen and undefined by SIFT. The effects of two variants (IL16 and CACNA2D4) could not be predicted, since these are splice site mutations (Table 2).

**Table 2. Properties of selected candidate genes.**

| Genome position (hg19) | Nucleotide change | Gene symbol | Ensemble transcript | Consequence | Amino acid change | SNP id | Allele Frequency (GnomAD v2.1.1) | Protein function |
|-----------------------|-------------------|-------------|---------------------|-------------|------------------|--------|---------------------------------|-----------------|
| 8_145066215           | G/A               | GRINA       | ENST00000313269     | NS          | R221Q            | novel  | 2/282710                        | Glutamate Receptor |
| 9_125239752           | C/T               | OR1J1       | ENST00000259357     | NS          | A152T            | rs143862742  | 4/281994                        | Olfactory receptor |
| 9_127990266           | G/A               | RABEPK      | ENST00000373538     | NS          | G202R            | rs142347258  | 80/278532                       | RAB9 effector protein |
| 11_33052944           | C/T               | DEPDC7      | ENST00000241051     | NS          | A268V            | novel  | 0/249446                        | G-protein signalling DEP domain |
| 11_95712527           | T/C               | MAML2       | ENST00000524717     | NS          | Q1019R           | rs201299551 | 95/279812                       | Transcriptional Coactivator Notch |
| 11_12377296           | G/A               | OR8D4       | ENST00000321355     | NS          | R53H             | rs201007236 | 224/282640                      | Olfactory receptor |
| 11_125786960          | C/G               | DDX25       | ENST00000263576     | NS          | D284E            | rs370049057 | 3/241854                        | DEAD box RNA helicase |
| 12_1949897            | G/A               | CACNA2D4    | ENST00000382722     | SS          | NA               | rs76597005  | 9/280408                        | Calcium Channel |
| 15_79614380           | C/T               | TMED3       | ENST00000299705     | NS          | H160Y            | novel  | 3/282836                        | Transmembrane protein |
| 17_18043906           | C/T               | MYO15A      | ENST00000205890     | NS          | R1763W           | rs200146361 | 513/280690                      | Actin based motor molecule |
| 20_47740994           | T/C               | STAU1       | ENST00000371856     | NS          | R264G            | rs201180807 | 32/282876                       | RNA binding protein |

All variants segregate in the heterozygous state in all affected color agnosia individuals and absent in family members without color agnosia. NS: non-synonymous mutation. SS: mutation changing a splice site.

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**Gene expression during human brain development**

We have attempted to prioritize the 11 candidate genes for a possible role in developmental colour agnosia. For all these genes, we have analyzed (developmental) brain expression (Fig 3). We hypothesised cortical expression during development for a gene linked to developmental colour agnosia and possible increased expression during postnatal brain maturation. For that reason we first studied expression of the 11 candidate genes in the human brain of subjects free of neurological disorders, in the age range of 0.1 to 83 years, represented by the Ingenuity data set. All genes seem to be expressed in the brain, while two genes, TMED3 and MAML2, displayed an increased expression during development (TMED3, 0.4–2.4 years old and MAML2, 0.1–2.4; Fig 3). The other nine genes do not show a clear expression profile during a specific developmental age window.

Mining of single-cell transcriptomic data of the adult human cortex and mouse brain showed that MAML2 was the only gene that maintained expression at high levels, albeit restricted to only a limited number of cell types (Fig 4). The other genes displayed a low to very low level of expression (GRINA, RABEPK, DDX25, CACNA2D4, STAU1) or no expression. In the human M1 motor cortex MAML2 was expressed by three types of inhibitory interneurons, one type of excitatory neuron, and by non-neuronal cells: astrocytes, an olfactory progenitor neuron and microglia (Fig 4). In this respect it was de only gene that was expressed by non-neuronal cells. In the mouse cortex and hippocampus Maml2 was also expressed in a very restricted set of neurons (dentate gyrus and CA3) and in non-neuronal cells. Grina and Stau1 displayed wide, but low expression in inhibitory and excitatory neurons (Fig 4).
In an attempted to prioritize the 11 identified candidate genes for a possible role in colour agnosia, we have analysed gene function, link to visual perception, link to cortical specialization, conservation of the nucleotide or amino acid, frequency worldwide or in the Dutch population (Table 3) and its presence in previously linked regions for other cortical disorders for example prosopagnosia or dyslexia [4].

None of the identified genes or its variants was present amongst previously identified genes or loci related to dyslexia, dyscalculi or prosopagnosia. Linked regions were studied on OMIM.

Table 3. Variant frequencies and mutation prediction.

| Gene_name | Frequencies | Effect | Polyphen_s | Polyphen_p | SIFT_s | SIFT_p | Conservation (Condel) |
|-----------|-------------|--------|------------|------------|--------|--------|----------------------|
| EVS       | GoNL        | Exac   | Effect     | Polyphen_s | Polyphen_p | SIFT_s | SIFT_p | Conserved Nucl | Conserved AA |
| GRINA     | 1/13005     | 0      | 0/121000   | NS         | 1       | probably damaging | 0 | deleterious | highly | highly |
| OR1J1     | 0           | 0      | 1/120930   | NS         | 0.001   | benign             | 0.56 | tolerated | weakly | highly |
| RABEPK    | 11/12995    | 2/996  | 36/120704  | NS         | 1       | probably damaging | 0 | deleterious | highly | highly |
| DEPDC7    | 0           | 0      | 0/120000   | NS         | 0.896   | possibly damaging | 0.07 | tolerated | moderately | moderately |
| MAML2     | 1/12217     | 0      | 50/119130  | NS         | 0.998   | probably damaging | 0.06 | tolerated | moderately | highly |
| OR8D4     | 6/12996     | 1/996  | 102/121352 | NS         | 0       | benign             | 0 | deleterious | weakly | weakly |
| DDX25     | 1/12537     | 1/996  | 3/76840    | NS         | 0       | benign             | 0.49 | tolerated | weakly | highly |
| CACNA2D4  | 0           | 0      | 3/120548   | SS         | undef   | undef             | undef | undef | na (splicesite) | na (splicesite) |
| TMED3     | 0           | 0      | 0/121000   | NS         | 0.773   | possibly damaging | 0.06 | tolerated | moderately | highly |
| MYO15A    | 17/12365    | 4/996  | 199/119810 | NS         | 0.999   | probably damaging | undef | undef | weakly | highly |
| STAU1     | 3/13003     | 0      | 16/121402  | NS         | 0.911   | possibly damaging | 0.33 | tolerated | weakly | highly |

Candidate variants and their frequency in different databases. Polyphen and SIFT predict the effect of the mutation. Effect NS is non-synonymous. SS splice site. Conserved nucleotide (Nucl) and conserved amino acids (AA) determined the conservation of the altered amino acid (AA). Condel stands for CONsensus DELeteriousness score of non-synonymous single nucleotide variants (SNVs) according to González-Pérez & López-Bigas [57].

Discussion

In an attempted to prioritize the 11 identified candidate genes for a possible role in colour agnosia, we have analysed gene function, link to visual perception, link to cortical specialization, conservation of the nucleotide or amino acid, frequency worldwide or in the Dutch population (Table 3) and its presence in previously linked regions for other cortical disorders for example prosopagnosia or dyslexia [4].

None of the identified genes or its variants was present amongst previously identified genes or loci related to dyslexia, dyscalculi or prosopagnosia. Linked regions were studied on OMIM.
GO-analysis identified that the calcium channel, voltage-dependent, alpha 2/delta subunit 4 (Cav1.4) gene CACNA2D4 and MYO15A were involved in visual perception, which makes CACNA2D4 and MYO15A candidate genes of interest.

CACNA2D4 encodes the α2δ4 subunit of the photoreceptor voltage-gated calcium channels (Cav1.4), which modulate glutamate release from the photoreceptors to the bipolar cells. The CACNA2D4 mutation is a splice-site mutation and its role on the Cav1.4 protein function is unclear. Mutations in the human CACNA2D4 gene are involved in recessive cone dystrophy [27, 28]. Mice with a premature stop mutation in Cacna2d4 show abnormal morphology of ribbon synapses in the rods and cones [29]. In mice loss of Cacna2d4 abolished rod synaptogenesis and synaptic transmission and disrupted mGluR6 clustering [30]. This suggests that CACN2D4 is involved in visual perception by the eye, but it does not provide an argument for its involvement in higher order cortical specialisation and visual perception. There was no indication that the family members with developmental colour agnosia suffered from an ophthalmic problem. This may also suggest that the splice-site mutation that we identified is not deleterious. Furthermore, CACNA2D4 has been implicated in autism spectrum disorders [31], and a deletion in the CACNA2D4 gene was found in a patient with bipolar disorder, but it is unknown if this patient suffers with vision problems [32]. Our attempts to trace this patient for developmental colour agnosia testing failed. Studies of other CACNA subtypes showed that thermal pain and tactile stimulation in the Cacna2d3 mutant mice triggered strong cross-activation of brain regions involved in vision, olfaction, and hearing. This study suggested that this knockout model could be a model for synaesthesia [33], which is a disorder related to developmental colour agnosia [34].

MYO15A mutations are associated with non-syndromic hearing loss. Deafness due to its critical role for the formation of stereocilia in cochlear hair cells [35]. Recessive mutations lead to autosomal recessive non-syndromic hearing loss [36]. MYO15A mutations are associated with the Usher syndrome having affected hearing [37]. Visual perception is also affected in Usher syndrome from the age of 10 years onwards. Vision loss is caused by retinopathy which is due to degeneration of retina cells, usually the rods cells.

Since no direct link to visual perception was found for the other genes, their possible function in the (developing) brain was considered for these genes. It has been indicated that glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1 (glutamate binding) (GRINA) is involved in nervous system development and disease mainly through signalling mechanisms of neuronal survival [38]. Upregulation of GRINA transcripts was observed in post-mortem brains of patients with major depressive disorder, supporting a role in brain functions [39]. Grina knock-out (KO) mice do not display an obvious phenotype [40]. This KO study showed that the effects of the GRINA gene are probably subtle. Since it is likely that effects on higher order visual perception will not be evident in mice, GRINA remains a potentially interesting candidate gene for consideration of a role in developmental colour agnosia. The effect of the mutation is probably damaging or deleterious (SIFT) and the site of mutation is highly conserved for nucleotide sequence and amino acid motif.

DDX25 (DEAD (Asp-Glu-Ala-Asp) box polypeptide 25) encodes a member of the large DEAD-box RNA helicases. Members of this protein family share high structural conservation and are generally involved in developmental processes such as gametogenesis, germ cell specification, and stem cell biology. Data on roles of DDX proteins in the brain are scarce. However, recently two members have been implicated in brain processes. Firstly, in a family with orofaciodigital syndrome type V a homozygous frameshift deletion in DDX59 (c.185del: p. Phe62fs*13) has been reported [41]. The neurological phenotypes of this syndrome include developmental delay, cognitive impairment, diffuse white matter abnormalities associated with cortical and subcortical ischemic events [41]. Further research in Drosophila showed
impaired development of peripheral and central nervous system upon loss-of-function of \textit{DDX59}, supporting a conserved role of this DEAD-box RNA helicase in neurological function. Secondly, mutations in \textit{DDX3X} cause neurodevelopmental abnormalities in females, including intellectual disability, cortical malformations, autism and epilepsy \cite{42}. In this study it was shown that in vivo depletion of Ddx3x in mice reduced neuronal differentiation and impaired corticogenesis \cite{42}.

A role of DDX25 in the brain has not been studied. So far DDX25 has been implicated in spermatogenesis and primordial germ cell development \cite{43, 44}. Polymorphisms in the \textit{DDX25} gene have been found that alter protein function \cite{45}. During our expression analyses of DDX25 we found significant expression in the brain. The Allen Brain Atlas Data Portal (http://casestudies.brain-map.org/celltax#section_explora) showed expression of Ddx25 in mouse cerebral cortex, nucleus accumbens, several thalamic and hypothalamic nuclei, locus coeruleus, raphe nucleus, inferior olivary complex, and absence in globus pallidus, and most of the midbrain and brain stem. Further exploration of mouse cortical gene expression of the data of \cite{22} through the Single Cell Portal (https://portals.broadinstitute.org/single_cell) showed expression of Ddx25 in all subtypes of interneurons and pyramidal cells. Transcripts were absent in glial cells (not shown). Mining of more recent data from single-cell transcriptomics \cite{20} showed that expression of DDX25 in human and mouse cortex is relatively low and is predominating in somatostatin- and parvalbumin-expressing interneurons, and in several types of pyramidal neurons, mostly located in layers 5–6 (dataset human M1; Fig 4A). In the dataset ‘Multiple cortical areas’ no evident expression was detected. There was no clear age-related change in human brain samples (Fig 3). In mouse single-cell data sets there was very low expression in parvalbumin-expressing interneurons and layer 5 pyramidal neurons (Fig 4B). Overall, DDX25 appears a gene with low and restricted expression in the adult cortex. Since it is part of a little-studied family causing severe neurological problems, as in the case of \textit{DDX3A} and \textit{DDX59} mutations, DDX25 should still be considered a potential candidate gene for involvement in developmental colour agnosia.

\textit{Staufen double-stranded RNA binding protein 1} gene \textit{STAU1} carried a possibly damaging (Polyphen) and tolerated (SIFT) mutation in a highly conserved amino acid motif. Staufen1 is a key protein involved in the local translation of mRNA in the dendrite and is involved in protein synaptic transmission. Further, Staufen 1 was indispensable for self-renewal and differentiation of neuronal precursor cells in vitro. Cultured neurons from \textit{Stau1} knockout mice showed reduced dendritic trees and developed fewer synapses, but the mice did not show any obvious defects in brain development \cite{46}. We note that recently a molecular and functional relationship between Stau1 and DEAD-box RNA helicases (Ddx5/17) was established in a complex with the Kruppel-like factor Klf4 \cite{47}. It was shown that this Klf4/Stau1/Ddx5/7 protein complex regulates neurogenesis-associated mRNAs and plays a role in mammalian corticogenesis. Furthermore, local regulatory networks containing \textit{STAU1} have been identified in cognitive decline in older adults \cite{48}. In accord with a neuronal role, \textit{STAU1} appeared expressed in virtually all types of cortical neurons in mouse and man, but not in non-neuronal cells of the cerebral cortex.

The \textit{Mastermind-like 2} (\textit{MAML2}) gene, encoding a transcriptional activator of the Notch signalling pathway \cite{49}, has been implicated in cancers, but has no known function in the nervous system. The effect of the found mutation is probably damaging in Polyphen and tolerated in SIFT. The nucleotide and amino acid sequence is moderate and highly conserved, respectively. In this study \textit{MAML2} is notable since its brain expression is increased during development (age 0.1–0.5 years) in comparison to older ages (Fig 3). Interestingly, it displayed highest expression by several non-neuronal cell types. In the human cortex these were: oligodendrocyte progenitor cell L1-6 PDGFRA COL20A1 (trimmed mean expression level 10.2), several
types of astrocytes (level 9.8–10.3) and microglia (level 10.9) (Fig 4). Neuronal expression of MAML2 at appreciable level occurred in three subtype of inhibitory neurons: inhibitory neuron L1 Pax6 Mir 101–1 (level: 8.2), inhibitory neuron L1-2 VIP WNT4 (level: 8.54), inhibitory neuron L1-6 SST NPY (level 8.67). Two excitatory cell types expressed MAML2: L3 LINC00507 and L3 FEZF2 (respectively at level 8.58 and 7.08). In a single- dataset of the mouse cortex and hippocampus, Maml2 gene was highest expressed in cells of the dentate gyrus and CA region (Fig 4). The Allen Mouse Brain Atlas confirmed the selective high expression of Maml2 in the dentate gyrus.

RABEPK (Rab9 effector protein with Kelch motifs) belonged to a small set of genes emerging in genome-wide association studies of opioid use disorder [50]. However, the gene has not been studied further to any extent. It is thought to participate in protein transport in the trans-Golgi network. The effect of the mutations is a probably damaging in Polyphen and deleterious in SIFT and the conservation is high for both sequences. Expression of the RABEPK gene is not detectable in single-cell data sets of the human cortex and in very low level in several types of interneurons and pyramidal cells in the mouse cortex and hippocampus (Fig 4).

The expression of transmembrane emp24 protein transport domain containing 3 (TMED3) is noticeable in its increased between the age of 0.3–8 years in the human prefrontal cortex (Fig 3). TMED3 expression is assigned to all interneurons and pyramidal cells in the mouse cortex (Single Cell Portal; [22]), but displays very low to no expression in more recent human and mouse datasets. TMED3 participates in tumor progression, but functions of TMED3 in the brain have not been described. Interestingly, it has been proposed that in tumors TMED3 promotes endogenous Wnt-Tcf activity, which is an important pathway in brain development, and Il11/Stat3 signalling [51, 52]. The effect of the mutation is possibly damaging in Polyphen and tolerated in SIFT and conservation is moderate for nucleotide and highly conserved for amino acid sequence (Table 2).

The expression of transmembrane emp24 protein transport domain containing 3 (TMED3) is noticeable in its increased between the age of 0.3–8 years in the human prefrontal cortex (Fig 3). TMED3 expression is assigned to all interneurons and pyramidal cells in the mouse cortex (Single Cell Portal; [22]), but displays very low to no expression in more recent human and mouse datasets. TMED3 participates in tumor progression, but functions of TMED3 in the brain have not been described. Interestingly, it has been proposed that in tumors TMED3 promotes endogenous Wnt-Tcf activity, which is an important pathway in brain development, and Il11/Stat3 signalling [51, 52]. The effect of the mutation is possibly damaging in Polyphen and tolerated in SIFT and conservation is moderate for nucleotide and highly conserved for amino acid sequence (Table 2).

The effect of the mutation found in the DEPDC7 (DEP domain-containing 7) gene is a possibly damaging one in Polyphen and tolerated in SIFT (Table 2). Brain expression of DEPDC7 was not detectable. However, it has been suggested that DEPDC7 DNA hypomethylation may be associated with depression [53]. The biological function of the protein encoded by the DEPDC7 (DEP domain-containing 7) gene is poorly understood. The DEPDC7 protein participates in gene regulation by NF-κB [54]. It has been shown that it inhibits tumor growth [55]. The gene has been detected amongst nine genes in a microdeletion in a patient with cryptorchidism and azoospermia [56].

No data are available on the function of olfactory receptors OR1J1 (olfactory receptor, family 1, subfamily J, member 1) and OR8D4. Expression of ORs has been observed in other tissues than olfactory neurons. OR8D4 has been encountered as a risk gene of systemic lupus erythematosus. The mutation for OR1J1 is benign for Polyphen and tolerated for SIFT. The mutation in OR8D4 is benign in Polyphen and deleterious in SIFT.

Taken together, this study is the first attempt to pinpoint genomic regions and genetic variants in developmental colour agnosia, a cognitive trait affecting the understanding of colours. This study was made possible by the finding and characterization of an unique family carrying this trait [7, 9]. We assumed that developmental colour agnosia is an extremely rare trait. Additional subjects were not found since initiation of this study in 2012. Since developmental colour agnosia is not a disorder that requires medical or psychological attention, it will generally stay unnoticed, which hampers the estimation of prevalence. Consequently, the small number of affected subjects is a main limitation of this study.

In addition to number of subjects, this study is also limited by the assumption that developmental colour agnosia is inherited in an autosomal dominant manner with complete penetrance, based on the segregation pattern in the family. Furthermore, the exomes were analysed
in 2012 and since then the quality of capture and sequencing have improved. In addition, we cannot exclude the involvement of genomic rearrangement or mutations in noncoding parts of the genome which we would not have captured using exome sequencing. Within these limitations this study indicates 11 genes that link to the trait. Amongst these there are candidate that have been implicated in neural processes and functions (CACNA2D4, MYO15A, DDX25, GRINA), that have an indirect link to brain function or development (STAU1, MAML2, TMED3, RABEPK), while others remain unlikely due to lack of brain expression, involvement of non-neural traits (OR1J1, OR8D4, DEPDC7)

In summary, we identified 19 genomic regions and 11 coding variants in hereditary colour agnosia by exome sequencing. Since replication is not possible yet due to the uniqueness of this identified family we cannot pinpoint the causal variant at this stage. Further research is required to identify the genetic origins of developmental colour agnosia for which the identification of new families with this trait is a prerequisite.

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