08 - 34_Molecular_Genetics

- 54 - Social phobia
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01 - 1. Cell cycle

1. Cell cycle

02 - 2. Cell division

2. Cell division

03 - Meiotic division

Meiotic division

1. Cell cycle Each cell undergoes a natural cycle in terms of its replication and nucleic acid synthetic activity. The cell cycle consists of four separate phases: G1 phase, S phase, G2 phase and M phase. G1 stands for growth phase 1, S for the synthetic phase, G2 for growth phase 2 and M phase for mitosis phase. Cells in the quiescent G0 phase of the cycle are stimulated by the growth factors (e.g. EGF, epithelial growth factor; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor) and result in activation of transcription factors and lead to the initiation of DNA synthesis, followed by mitosis and cell division. Thus from G0 the cell moves on to G1 when the chromosomes are prepared for replication. This is followed by the synthetic (S) phase, when the 46 chromosomes are duplicated into chromatids, followed by another gap phase (G2), which eventually leads to mitosis (M). Note that while certain cells pause or freeze the cycle temporarily and stay in G0, e.g. liver cells, neurons remain in G0 indefinitely.
2. Cell division Cell division is a process by which cells reproduce. During cell division, a sequence of steps enables the replicated genetic material in a parent cell to be equally distributed to two daughter cells. Before a dividing cell enters mitosis, it undergoes a period of growth called interphase. Interphase can be termed as the "holding" stage occurring between two consecutive cell divisions. Replication of cellular genetic material and organelles occurs during interphase in preparation for the next division. It is the longest phase, and all steps in the cell cycle (i.e. G0, G1, G2 and S) except stage (M) constitute interphase. Mitotic division Mitosis is composed of several stages. Prophase: Condensation of chromatin to discrete chromosomes, accompanied by a breakdown of the nuclear envelope and the formation of spindles at opposite cellular "poles". Metaphase: Alignment of chromosomes at the metaphase plate (a plane that is equidistant from the two spindle poles) – equatorial alignment. Anaphase: Separation of paired chromosomes (sister chromatids) followed by migration to opposite ends of the cell. This separation of chromatids preserves the chromosomal numbers in daughter cells. Telophase: In this last stage, the chromosomes are packed into distinct new nuclei in the emerging daughter cells. Cytokinesis (division of cytoplasm) also starts at this time. Meiotic division Meiosis is divided into two parts: meiosis I and meiosis II. At the end of the meiotic process, four daughter cells are produced (only two are produced at the end of the mitotic process), each with onehalf of the number of chromosomes as the parent cell, unlike mitosis where each cell has the same number of chromosomes as the parent. Meiosis 1 is a reduction division.

04 - 3. Chromosomal Numbers

3. Chromosomal Numbers:

© SPMM Course The main differences are the occurrence of synapsis (crossing over) in the prolonged prophase phase and non-separation of sister chromatids during anaphase 1, leading to reduced (half) chromosomal numbers in daughter cells. Meiosis 2 is same as a normal mitosis. 3. Chromosomal Numbers: Chromosomes are intranuclear structures containing one linear molecule of DNA. Human cells are called diploid as they have 46 chromosomes, 23 inherited from each parent; thus there are 23 'homologous' pairs of chromosomes (22 pairs of 'autosomes' and two 'sex chromosomes'). The sex chromosomes, called X and Y, are not homologous but are different in size and shape. Males have an X and a Y chromosome; females have two X chromosomes. During the mitotic division, each chromosome divides into two; this ensures that each daughter nucleus has the same number of chromosomes as its parent cell. During gametogenesis, the number of chromosomes is halved with meiosis so that after conception the number of chromosomes remains the 2 same and not doubled. Hence, gametes are haploid cells. Chromosomes can be classified according to their size and shape, the largest being chromosome 1. The constriction in the chromosome is the centromere, which divides the chromosome into a short arm and a long arm, which are referred to as the p arm and the q arm respectively. o A metacentric chromosome has centromere right in the middle. So p and q arms are of equal length. o If it is placed at one end, it is called as an acrocentric or submetacentric where the arms are of unequal length. o If centromere is at the tail of a chromosome, it is called telocentric. With holocentric chromosomes, the entire length of the chromosome acts as the centromere. These latter two types are not seen in humans.

When cells possess chromosomal numbers different from normal diploid status, they are called aneuploid cells. Aneuploidy can occur in single numbers e.g. trisomy 21, trisomy 18, monosomy of Turner’s, etc. Very rarely, the entire chromosome set will be present in more than two copies, so the individual may be triploid rather than diploid and have a chromosome number of 69. Triploidy and tetraploidy (four sets) result in spontaneous abortion. These aberrations result from the failure of chromosome or chromatids to separate ('non-disjunction') in meiosis, with one gamete receiving two copies of that chromosome and one another with no copies of the chromosome. This can produce (i) an extra chromosome, so resulting in a fetus that is 'trisomic' and has three instead of two copies of the chromosome; or (ii) no chromosome, so the fetus is 'monosomic' and has one instead of two copies of the chromosome. Nondisjunction can occur with autosomes or sex chromosomes. However, only individuals with trisomy 13, 18 and 21 survive to birth, and most children with trisomy 13 and trisomy 18 die in early childhood.

05 - Important trisomiesmonosomies

Important trisomies/monosomies

© SPMM Course Sometimes, non-disjunction can occur during mitosis immediately after two gametes have fused. This leads to the formation of two cell lineages, each with a different chromosomal make-up. This occurs more frequently with the sex chromosomes and results in a 'mosaic' individual. Mosaics exhibit milder malformations than those who carry complete aneuploidies. Important trisomies/monosomies Down’s syndrome: Trisomy 21 is the most common chromosomal disorder that occurs at a rate of 1:700 causing congenital mental retardation. Prominent findings are reduced maternal levels of αfetoprotein, increased β-hCG and increased nuchal fold thickness in fetal ultrasound. The child shows mental retardation, flat facial profile, prominent epicanthal folds, simian palmar crease, duodenal atresia, hypothyroidism and heart disease (most common malformation is septum primum–type ASD due to endocardial cushion defects). Alzheimer’s disease and leukaemia are common in affected adults who survive childhood difficulties. 95% of Down’s is attributed to meiotic nondisjunction of homologous chromosomes. This is associated with an advanced maternal age (rates are 1:1500 in women < 20 but 1:25 in women > 45). 4% of cases due to Robertsonian translocation and 1% of cases are attributed to Down’s mosaicism (no maternal age association is seen in these). The features of mosaic Down syndrome are milder but similar to the features of full Down syndrome. However, the clinical phenotype varies according to the level and distribution of trisomic cells. Thus, the affected individuals may range from completely normal to presenting the full expression of Down syndrome. Edwards’ syndrome is characterised by severe mental retardation and rocker bottom feet, low-set ears, micrognathia (small jaw), congenital heart disease, clenched hands, the a prominent occiput. It is a result of trisomy 18. It occurs at a frequency of 1:8000 and often death occurs within 1 year of birth. It is three times more common in girls than boys. Patau’s syndrome is due to trisomy 13, and it is characterised by severe mental retardation, microphthalmia, microcephaly, cleft lip/palate, coloboma eye, abnormal forebrain structures, polydactyly, and congenital heart disease. The rate of occurrence is 1:6000. Metafemale – trisomy X Turner’s syndrome: Low hairline, broad chest, short stature, retrognathism and webbed neck are features of Turner’s syndrome. In 80% cases the origin of the aneuploidy is from paternal X chromosome; hence the single X chromosome present in a subject with Turner’s is of maternal origin. The incidence of Turner’s syndrome is approximately 1 in 2000 live-born female infants. Random inactivation (see

below) does not occur in cells with a single X chromosome. In general, girls with Turner show a disharmonic IQ profile. The full scale IQ is either comparable to general population or lower by a mean of 10 points (nearly one standard deviation) mostly due to reduced performance IQ (at least 20 points or 1 standard deviation lower) though verbal IQ is preserved. Specific subtests assessing visuospatial processing such as ‘Block Design’ and ‘Object Parental origin of meiotic error leading to aneuploidy. Aneuploidy Paternal % Maternal % Patau 13 85 Edward’s 18 90 Down’s 21 95 Turner’s 45X 20 Klinefelter’s 47 XXY 55

06 - 4. DNA & RNA structure

4. DNA & RNA structure

© SPMM Course Assembly’ may be affected more than others. Mathematical ability may also be lower than expected. This specific profile persists into adulthood. Females with a 45X karyotype (Turner syndrome) may have higher verbal skills if their only X chromosome is paternally derived instead of being maternal origin (most commonly it is maternal). This suggests the existence of an imprinted gene that is inactive if carried on a maternally derived X chromosome. 4. DNA & RNA structure Genetic information is stored in the form of double-stranded DNA. DNA and RNA are the most important nucleic acids in the cellular machinery. These nucleic acids are composed of many nucleotides. Nucleotides are phosphorylated versions of nucleosides. Each nucleoside consists of two components: A nitrogenous base and a pentose sugar. Each strand of DNA is made up of a deoxyribose-phosphate backbone and a series of purine (adenine (A) and guanine (G)) and pyrimidine (thymine (T) and cytosine (C)) bases of the nucleic acid. The length of DNA is generally measured in numbers of base-pairs (bp). Each nucleotide is a base joined to a sugar-phosphate unit. The two strands of DNA are held together by hydrogen bonds between the bases. There are only four possible pairs of nucleotides - TA, AT, GC and CG. The two strands twist to form a double helix structure for DNA. RNA is single stranded in human cells. A gene is a sub-portion of DNA. It contains codes for a polypeptide sequence. The length of each gene is variable depending on the size of the polypeptide coded. A set of three adjacent nucleotides is called as a codon; each codon codes for a specific amino acid. There are only 20 amino acids of which around 10 are ‘essential’ (i.e. those aminoacids not found in food and so need to be synthesized), but 64 possible codon combinations that make up the genetic code. This means that most amino acids are encoded for by more than one triplet; other codons are used as signals for 'initiating' or 'terminating' polypeptide-chain synthesis. The polypeptide coding sequences in a DNA are called exons; these are interrupted by intervening EXON INTRON

07 - 5. Synthesis of DNA, RNA & Protein

5. Synthesis of DNA, RNA & Protein

© SPMM Course sequences that are non-coding (called introns) at various positions. The introns contain three types of sequences (satellite, mini and microsatellite: see the graph below). All introns are removed from the mRNA before it leaves the nucleus and start protein synthesis. Humans have 3 × 109 bp of total chromosomal DNA but among these the protein-coding genes constitute only 32 000 bp. 5. Synthesis of DNA, RNA & Protein Replication refers to the production of new DNA copies from template copies of DNA. Synthesis of RNA from nuclear DNA is called transcription. This takes place in the nucleus of the cell. Such transcripted RNA initially contains the ‘junk’ sequences – introns – that do not code for polypeptides. This unprepared RNA is called heterogeneous nuclear RNA or hnRNA. This hnRNA then undergoes splicing aided by nucleosomes in the nucleus to remove non-coding sequences and results in messenger RNAs (mRNA). tRNAs (transfer RNAs) are also synthesized from DNA in the nucleus in a separate process. Translation refers to the production of proteins from RNA. This takes place in the cytoplasm, aided by ribosomes. Ribosomes can be seen attached to rough endoplasmic reticulum. As tRNAs that are synthesized in the nucleus enter the cytoplasm, they are attached to specific amino acids according to the codon sequences. This energy dependent process is called amino acid activation, catalyzed by a specific amino acid activating enzyme (aminoacyl-tRNA synthetase) in the presence of Mg2+. There is a separate aminoacyl-tRNA synthetase enzyme for each kind of amino acid. The energy stored in such activated amino acids is used in making peptide bonds during protein translation. Translation takes place in the cytoplasm on ribosomes where specific mRNAs are involved. tRNAs with their aminoacids, sequentially bind to various sites along the mRNA in a zipper like fashion. Translation includes three steps – initiation, elongation and termination. The ribosome contains two sites – Peptidyl P site where methionine-containing tRNA initially binds and aminoacyl A site where each new incoming tRNAs with activated amino acids can bind. In elongation step, amino acids are added one by one in a sting like fashion to produce proteins. Chain termination is signaled by one of the three codons UAA, UGA or UAG. Modification refers to posttranslational changes in a protein molecule before it becomes functionally active. Following protein synthesis (sometimes simultaneously as the protein is being synthesized) posttranslational modifications take place to transport the synthesized proteins to appropriate cellular sites. These modifications take place in endoplasmic reticulum and golgi bodies. The Golgi complex is a

dynamic system acting as a temporary protein repository that gives off vesicles and vacuoles for further processing and transport. These processes include covalent modifications, protein folding and tagging with signal peptides to dispatch to appropriate cellular destinations. Glycosylation, proteolysis, phosphorylation, gamma carboxylation, prenylation, ubiquitation, polyamination and nitration are some of the recognized posttranslational chemical modifications. This process is essential in tagging wrongly

08 - 6. Types of mutations

6. Types of mutations

© SPMM Course folded or aberrant proteins to enter lysosomes for destruction. Study of mRNAs using microchip arrays is called transcriptomics.

Note that microsatellite tandem repeats give rise to trinucleotide sequences: these are linked to a group of non-Mendelian disorders called trinucleotide repeat disorders.

6. Types of mutations

 A mutation is a sudden, permanent and heritable change in the DNA sequence. Such changes in DNA will be transcripted to mRNA and can get translated into proteins leading to disease expression. Point mutation refers to single-base alteration in DNA. Point mutations are usually substitutions where one base is replaced by another. It could be termed as transition if a purine Telomeric repeats (necessary for integrity of chromosomes) Satellite (10-15% large series of simple repeats) Microsatellite (single, di or tri nucleotide repeats) Tandem Repeats INTRONS (noncoding) Minisatellite Hypervariable repeats (used in DNA fingerprinting) Interspersed Short Interspersed Nuclear Elements Long Interspersed Nuclear Elements DNA Sequences EXONS (coding)

09 - Some deletion syndromes of psychiatric releva

Some deletion syndromes of psychiatric relevance

© SPMM Course is replaced by another purine or a pyrimidine replaced by another pyrimidine (e.g. A to G). It is called transversion if a purine is replaced by a pyrimidine or vice versa (e.g. A to T). According to the effect on triplet sequence, mutations could be frame shift or in-frame. In frame shift mutations, the deletion or insertion is not in multiples of three codons e.g. a segment of 5 bases deletion mutations. This leads to a shift in triplet reading frame with variable results. In frame, mutation refers to changes happening in multiples of 3 bases, with no disturbances in actual reading frame. According to the effect of a mutation on protein product, mutations could be silent, mis-sense or nonsense. A silent mutation causes no change in protein product – this is possible because a single amino acid is often coded by more than one triplet sequence. In a silent mutation one triplet sequence is replaced by a different sequence but without changing amino acid product. In mis-sense mutation, the new mutant codon specifies a different amino acid with variable effects on final protein product. For example, haemophilia, sickle cell anaemia. In non-sense mutation the new codon is UUA UGA or UAG, which signals ‘stop’ to the amino acid sequence resulting in nonfunctional protein. Point substitutions do not shift the reading frame; they often occur in non-coding regions and go unnoticed. Even at coding regions they are often silent or mis-sense mutations. Translocation refers to exchange of chunks of genetic materials from one chromosome to another. These are essentially mutations occurring at ‘larger’ dimensions. These are mostly reciprocal so one segment is exchanged for another segment among chromosomes. Robertsonian translocation is a non-reciprocal (i.e. unequal exchange) that results in a single fused chromosome from 2 acrocentric (non homologous) chromosomes. Following a Robertsonian translocation, the small 'p' arms are discarded, and a metacentric fusion chromosome results. Thus from 2 chromosomes a single chromosome is formed with no significant (only trivial) loss of genetic material. Hence, these are viable and ‘balanced’ within the individual in whom they occur. But when gametes are formed, only one of the two gametes can have the whole translocated metacentric fusion chromosome, effectively resulting in monosomy (unbalanced translocation) for

one gamete if fertilized and trisomy for the gamete with fused chromosome (extra load of genes now). This is one of the mechanisms for Down’s syndrome. Due to the mother being a carrier of such translocation, the recurrence rate of Down’s is extremely high in such cases compared to sporadic Down’s due to non-disjunction.

Some deletion syndromes of psychiatric relevance

10 - 7. Mendelian inheritance

7. Mendelian inheritance

© SPMM Course Disorder Location and mode of transmission Features DiGeorge (Velocardiofacial) 22q11.2 Autosomal dominant, 50% risk to offsprings, 5-10% risk of deletion in parents. If offspring has the deletion, then 25% chance of schizophrenia, if not then general population risk ~1%. Mild to moderate learning disability, facial deformities esp. cleft palate, absent or malformed parathyroids resulting in hypocalcemia, broad nasal bridge, articulatory speech and swallowing problems, >25% have psychosis Williams syndrome 7q11 microdeletion Hypercalcemia at birth, supra valvular aortic stenosis, moderate learning disability, disinhibited disposition, speech that appears superficially fluent, hyperacusis. Smith Magenis syndrome 17p11.2 microdeletion Moderate to severe learning disability, self harming behaviours e.g., pulling off nails (onychotillomania) and inserting foreign bodies into body orifices. Sleep disturbances and self hugging are also noted. Angelman syndrome Deletion of 15q11-13 maternally inherited (see genomic imprinting below) Developmental delay, low IQ, jerky movements especially hand-flapping, frequent smiling, and seizures. Prader-Willi syndrome Deletion of 15q11-13 paternally inherited (see genomic imprinting below) Obesity, short stature, small limbs, decreased IQ with hyperphagia and skin picking. Cri-du-chat syndrome Deletion of chromosome 5p (the locus 5p15.2 is responsible for the phenotype) Feeding problems due to difficulty swallowing and sucking, cat-like cry with poorly developed facial features.

7. Mendelian inheritance

Johann Mendel was a Catholic priest who was interested in horticulture and botany. He studied garden peas and proposed ‘laws’ of inheritance. The first law is the law of uniformity. According to this law, if two plants that differ in just one trait (black and white) are crossed, then the resulting hybrids will be uniform in the chosen trait (either black or white, not blue). This is not entirely true as later geneticists demonstrated intermediate phenotypes resulting from co-dominant heterozygous expression. The second law is called the principle of segregation. It states that “for any particular trait, the pair of alleles of each parent separate and only one allele passes from each parent on to an offspring. Which allele in a parent's pair of alleles is inherited is a matter of pure chance”. For example if there are two alleles one determining black colour and the other determining white in mother and two alleles with one

11 - A. Single gene inheritance (Mendelian) disord

A. Single gene inheritance (Mendelian) disorders

12 - Autosomal dominant disorders

Autosomal dominant disorders

 determining white colour and one determining black colour in the father, then these two alleles segregate and only one of them could be passed on to the second generation from each parent. This will produce three possible types of offsprings as shown in the table. This was later proved to be true by studying chromosomes during cell division. The third principle is the principle of independent assortment. It states that “different pairs of alleles are passed to offspring independently of each other. The result is that new combinations of genes present in neither parent are possible”. As a very simplistic example, if a man with blue eyes and brown hair mates a woman with brown eyes and black hair; their child can have blue eyes and black hair. The inheritance of blue eyes does not take brown hair ‘with it’; these traits are independently assorted. Thus Mendelian principles are applicable to human genetics as well. Note that all traits studied using Mendelian genetics refer to categorical, all or none traits i.e. black vs. brown, blue vs. brown, tall vs. short, etc. It does not apply with same simplicity to dimensional traits such as IQ or blood pressure. Mendel’s Laws (aide memoir) Explanations Law of uniformity: DD X dd □ Dd Two alternative alleles at one locus Two homozygous parents (with a double dose of either one). All off springs are of uniform type (all Dds) Law of segregation Dd X Dd □ DD | Dd Dd | dd Two heterozygous parents Three possible types of offsprings 1DD.2Dd.1dd Law of independent assortment DdHh (blue-eye:brown hair) X ddhh (brown eye: black hair) □ DdHh | ddHh | Ddhh | ddhh Two loci with alleles D,d and H,h. Double heterozygote X Double homozygote parent Four possible types of offspring, each with equal probability (blue eye/brown hair, blue eye/black hair, brown eye/black hair, brown eye/brown hair). Adapted from McGuffin et al. (ed) Psychiatric genetics and genomics. Oxford Press: P37 A. Single gene inheritance (Mendelian) disorders Autosomal dominant disorders Each cell contains two copies of all the autosomes. An autosomal dominant disorder occurs when one of the two copies has a mutation and the protein produced by the normal form of the gene cannot compensate. So the mutant allele becomes dominant over the normal allele and results in disease expression. In this case, a heterozygous individual who has two different forms (or alleles) of the same gene will manifest the disease. The offspring of heterozygotes have a 50% chance of inheriting the chromosome carrying the disease allele, and therefore also of having the disease. If both parents are heterozygous, the recurrence risk is 75%. 'Incomplete penetrance' may occur if patients have a dominant disorder but it does not manifest

itself clinically in them. This gives the appearance of the gene having 'skipped' a generation. Having incomplete

13 - Autosomal recessive disorders

Autosomal recessive disorders

14 - Sex linked disorders

Sex-linked disorders

© SPMM Course penetrance increases the likelihood of having an unaffected child. The variable expression refers to differences in severity of the disease expressed. A mildly affected parent may have a severely affected child. Spontaneous disease-causing mutations can often present as diseases that are known to occur in autosomal dominant fashion. For example, achondroplasia and tuberous sclerosis are commonly due to spontaneous mutations, but families show AD pattern. Often the abnormal gene in autosomal dominant diseases codes for structural proteins such as receptors or cytoskeleton proteins. Sometimes such aberrant production of an autosomal dominant disorder without family history may be due to a phenotypically indistinguishable disorder without the genotype – this is called phenocopy. (Goldschedt, 1935) e.g., anti-psychotic medication causes patients to manifest the same symptoms as the genetically determined Parkinson’s disease. Another example is genotypically determined Pendred syndrome being mimicked by endemic cretinism. Autosomal recessive disorders These disorders manifest themselves only when an individual is homozygous for the disease allele; i.e. both chromosomes carry the mutated gene. In this case, the parents are generally unaffected, healthy but carriers (heterozygous for the disease allele). There is usually no family history, although the defective gene may be passed from generation to generation (skipping). The offsprings of an affected person are healthy heterozygotes unless the other parent is also a carrier. If carriers marry each other, the offspring has a 1 in 4 chance of being homozygous and affected and a 1 in 2 chance of being a carrier, and a 1 in 4 chance of being genetically normal. Consanguinity increases the risk. Often the abnormal gene in autosomal recessive diseases codes for enzymatic proteins. Sex-linked disorders Genes carried on the X chromosome are said to be 'X-linked', and can be dominant or recessive in the same way as autosomal genes. Normally males inherit an X chromosome from their mother and a Y chromosome from their father, whereas normal females inherit an X chromosome from each parent. The Y chromosome contributes very less genetic material to a man’s genetic makeup. Hence, there must be a mechanism to simulate this deficiency in females too to preserve natural equality. This phenomenon is now known to be ‘X inactivation’. This occurs very early in the development of female embryos. When an X chromosome is inactivated, it could be visualized under the microscope as a highly condensed Barr body in the nuclei of interphase cells. An inactivated X chromosome does not get transcripted to produce mRNA. X inactivation is random process. In other words, some cells of the female embryo have paternally inherited X inactivated while the other cells have maternally inherited X inactivated. It is an irreversible, fixed process and once inactivated these chromosomes do not get reactivated life long. The entire cell’s progeny will have same inactivation replicated. All X chromosomes in a cell are inactivated except one, irrespective of original number of X chromosomes in a cell. Thus females with trisomy X will have two Barr bodies. X inactivation occurs via DNA methylation.

15 - X linked recessive disorders

X-linked recessive disorders

© SPMM Course X-linked recessive disorders If a recessive disease-causing mutation occurs on the single X chromosome of a man, this is sufficient to cause disease, as another X chromosome is not existent to compensate any deficiencies. As females have two copies of the X chromosome, they need a double identical mutation for disease expression, which is extremely rare. But during random X inactivation if most X chromosomes carrying normal alleles are inactivated (called unfavourable Lyonisation), then these females can manifest the disease phenotype – termed as manifesting heterozygotes. But nevertheless the severity of expressed disease is mild and can go unnoticed too. Skipped generations are commonly seen because an affected male can transmit the disease-causing mutation to a heterozygous daughter, who remains normal phenotypically but carries and transmits the disease-causing allele to her sons. From McGuffin et al. (ed) Psychiatric genetics and genomics. Oxford Press: 2002 Male-to-male transmission is not seen in X-linked inheritance. Affected male mates with a homozygous normal female, all of the daughters will be heterozygous carriers; all of the sons will be homozygous normal. If a carrier female mates with normal male (which is often the case in this transmission), then half Disorder Location and mode of transmission Features Tuberous sclerosis 9q34 / 16p13 Auto.dominant (but most are spontaneous) 1 in 30 000 Adenoma sebaceum, normal to sever MR, ash leaf macules, brain hamartomas, heart and kidney cysts Treacher Collins syndrome 5q31 Auto.dominant 1 in 40 000 Maxilla-mandibular hypoplasia, malformed pinna, down slanting palpebrae, mild to moderate MR Apert syndrome 10q Auto dominant Variable MR, cranio synostosis, shallow orbits, trapezoid mouth, ‘mitten’ hands and feet. Noonan syndrome Chr 12 Auto.dominant 1 in 1 500 Mild MR, short stature, nuchal edema/webbed neck, pulmonary stenosis, cryptorchidism Hurler syndrome 4p16 Auto. recessive 1 in 100 000 Deteriorating IQ after age 2, coarse facies, clouded cornea, joint stiffness. Lesch-Nyhan syndrome Xq 26-27 X linked recessive Deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) Poor muscle control, and moderate mental retardation – year 1. Self-mutilating behaviors, characterized by lip and finger biting – by year 2. Hyperuricemia and hyperuricosuria -severe gout and kidney problems – can present anytime.

16 - X linked dominant disorders

X-linked dominant disorders

17 - B. Non Mendelian inheritance

B. Non Mendelian inheritance

18 - Mitochondrial inheritance

Mitochondrial inheritance

© SPMM Course of the sons will be affected, and half of the daughters will be carriers. e.g. haemophilia A/B, Duchene muscular dystrophy, and androgen insensitivity syndrome. X-linked dominant disorders These are rare. Similar to X-linked recessive pattern, male-male transmission of the disease-causing mutation is not seen. Because females have higher gene frequency for X chromosomes compared to males, females have twice as much chance than males to inherit an X-linked disease-causing mutation. Vitamin D-resistant rickets is the best-known example. Females who are heterozygous for the mutant gene and males who have one copy of the mutant gene on their single X chromosome will manifest the disease. As in autosomal dominant inheritance, the disease phenotype is seen in multiple generations making ‘skipped generations’ relatively unusual. If the affected male mates with homozygous normal female, none of the sons will be affected but all of the daughters will be affected. Heterozygous female mating a normal male will result in 50% of sons being affected and 50% of daughters being affected. An atypical pervasive developmental disorder called Rett’s syndrome is inherited in X-linked dominant fashion. B. Non Mendelian inheritance Mitochondrial inheritance, mosaicism, trinucleotide expansions and genomic imprinting do not follow normal Mendelian principles and so are called non-Mendelian inheritance. Polygenic and multifactorial disorders too, do not obey Mendelian principles in strict sense. Mitochondrial inheritance Mitochondrial DNA is wholly inherited from the ovum. The sperm has no mitochondria in its ‘head’; ‘head’ is made of nuclear material and acrosomal cap. The ‘body’ of sperm has many mitochondria that provide energy in propelling the ‘tail’. The ‘body’ and ‘tail’ are shed on entry of sperm into the ovum. Hence the mitochondria of an embryo are completely maternal-derived. The mitochondrial chromosome has no introns in the genes. Therefore any mutation has a high chance of having an effect. Most mitochondrial diseases are myopathies and neuropathies. This is important in clinical genetics as mitochondrial DNA abnormalities result in various diseases such as MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and recurrent stroke syndrome) and Leber hereditary optic neuropathy. X-LINKED MENTAL RETARDATION (XLMR) Learning disability is significantly more common in males than in females. So X linked genes are a suspect in their aetiology.

XLMR is a heterogenous condition - subdivided into syndromic (1/3rd) and nonsyndromic (2/3rd) forms, depending on the presence of further abnormalities.

The most common form of XLMR is the Fragile X syndrome.

Mutations in MECP2 gene in X chromosome give rise to a wide range of disorders, including female-specific Rett syndrome. MECP2 mutations also lead to other phenotypes such as severe encephalopathy, progressive spasticity, Angelman and PraderWilli like phenotypes and nonsyndromic XLMR in males

19 - Trinucleotide expansions

Trinucleotide expansions

© SPMM Course Leber's hereditary optic neuropathy (LHON) is the commonest cause of blindness in young men, with bilateral loss of central vision and cardiac arrhythmias. These diseases are purely maternally inherited. Mitochondrial DNA codes for 13 proteins involved in the respiratory chain in addition to 22 tRNAs and 2 ribosomal RNAs. Many other syndromes have been described. Myopathies include chronic progressive external ophthalmoplegia (CPEO); encephalomyopathies include myoclonic epilepsy with ragged red fibres (MERRF) and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS). Kearus-Sayre syndrome includes ophthalmoplegia, heart block, cerebellar ataxia, deafness and mental deficiency due to long deletions and rearrangements. Trinucleotide expansions Trinucleotides repeat disorders are a set of genetic disorders caused by trinucleotide repeats (codons – e.g. CGG, CTG, CAG, etc.) in certain genes exceeding the normal number of repeats. The mutation results in an unstable site, which is often fragile. Anticipation refers to a pattern of inheritance in which individuals in the most recent generations of an affected family develop a disease at an earlier age and with greater severity than those in previous generations. This is mostly due to the gradual expansion of trinucleotide repeat polymorphisms (this instability is called a dynamic mutation).

Fragile X genetics: This X-linked condition accounts for more cases of mental retardation in males than any condition except Down syndrome with the frequency of 1 in 4000. It can affect females but 50% less frequently than in males. A fragile site near the tip of the long arm of the X chromosome was initially suspected. Now it is known that fragile X results from the an expansion of a trinucleotide repeat (CGG) proximal to FMR1 gene. If the number of CGG repeats in this location increases beyond 52, this destabilizes this sequence allowing further expansion during spermatogenesis or oogenesis. Being born with one FMR1 allele with 200 or more repeats results in lower IQ in most men and ~ 60% of women. The phenomenon of anticipation is seen. Unlike men, heterozygous women usually have the other X chromosome that can compensate to some extent; thus they show no physical signs other than early menopause, mild learning difficulties and rarely frank retardation. Affected males suffer from enlarged testes, prominent ear lobes and a protracting jaw, a high-pitched voice, and mental retardation. Some men carry an increased number of CGG repeats in the FMR1 locus but do not show a full-blown clinical phenotype; these individuals are called premutation carriers. Though premutant carriers were long thought to be free from clinical features, it is now known that they are at increased risk for developing intention tremor and ataxia especially after middle age. Women who are premutation carriers (55–200 CGG

repeats) are at increased risk of premature ovarian failure and/or mild cognitive or behavioral abnormalities. The fragile site at first exon of FMR1 is called FRAXA, a second site at Xq28 called FRAXE Frag(g)ile X syndrome Frag(g)ile X syndrome •cGG Friedreich AtaxiA Friedreich AtaxiA •gAA Huntington ChoreA Huntington ChoreA •CAg MyoTonic dysTrophy MyoTonic dysTrophy •cTg

© SPMM Course is also linked to mental retardation. FRAXF is the third fragile site sensitive to folate, but not linked to MR. Similar to Myotonic Dystrophy (but in contrast to Huntington's), anticipation rates are higher in maternal than paternal inheritance. This is because further trinucleotide expansion occurs during oogenesis rather than spermatogenesis. Huntington's genetics: Huntington's disease is inherited in an autosomal dominant manner with full penetrance and a prevalence rate of about 5 per 100,000. The gene responsible is an expanded and unstable CAG trinucleotide repeat on the short arm of chromosome 4 - 4p16.3. This results in translation of an extended glutamine sequence in huntingtin, the protein product of the gene. Huntingtin is expressed throughout the body. Its function is unclear. Though slightly unusual for a genetic disease; the onset is usually between 30 and 50 years of age. Most adult-onset HD cases have CAG expansions of 40-55 repeats while greater expansions (>70 repeats) are seen in childhood-onset HD. The phenomenon of anticipation is seen here too. But unlike other X-linked disorders (see myotonic dystrophy below), inheritance of HD from the father is associated with the greater repeat expansion and earlier age of onset. Nearly one-third of father-to-offspring cases show an expansion resulting in juvenile-onset HD. Characteristic protein deposits form nuclear inclusions in neurons of HD patients. Myotonic dystrophy is another neurological disease with trinucleotide repeat expansion. Here CTG repeats are expanded. The anticipation resulting from trinucleotide instability is higher if the inherited expansion comes from the mother than the father in MD. This is because oogenesis, due to its inherently long dormancy compared to spermatogenesis, results in much higher instability. As a result anticipation is more prominent in maternal transmissions. Genomic imprinting Though no structural differences exist between maternal and paternally inherited chromosomes in humans, there are some subtle functional differences, which are increasingly being appreciated. For example, a deletion of part of the long arm of chromosome 15 (15q11-q13) will give rise to the Prader-Willi syndrome (PWS) if it is paternally inherited. A deletion of a similar region of the chromosome gives rise to Angelman's syndrome (AS) if it is maternally inherited. This may be due to differential regional expression of the chromosomes. Maternal chromosome 15q11-13 is expressed in the brain and hypothalamus, leading to neuronal damage in its absence. This phenomenon is called genomic imprinting. It is thought to be due to DNA methylation effects.

In genomic imprinting, the disease phenotype expressed depends on whether the allele is of maternal or paternal lineage. This parent-of-origin phenomenon is an important exception to the Mendelian inheritance patterns. Approximately 70% of patients with Prader Willi syndrome have a deletion in their paternally derived 15q11-q13. Maternal uniparental disomy (inheriting both copies from mother when embryo is formed) occurs in most of the remaining patients (25%). Most patients with Angelman's syndrome have a deletion in their maternally derived 15q11-q13. Paternal uniparental disomy occurs in about 4% of Angelman's syndrome.

20 - Multifactorial inheritance

Multifactorial inheritance

21 - Polygenic inheritance

Polygenic inheritance

22 - 8. Polymorphisms

8. Polymorphisms

© SPMM Course Multifactorial inheritance It is a complex inheritance in which multiple genes are involved jointly with environmental influences. Most common psychiatric disorders such as schizophrenia do not show a Mendelian pattern of inheritance. But these disorders are categorically defined as present or absent hence cannot be regarded as continuous variables too. But these conditions could be regarded as quasi-continuous in that those who are affected can be graded along a continuum of severity. So we can also assume that there is an underlying liability to develop the disorder, which is continuously distributed in the population. Those who pass a certain threshold manifest the condition. This is known as the liability/threshold model. If the underlying liability to develop the disorder is inherited in a multifactorial fashion, one can assume that the distribution will be approximately distributed along a normal distribution curve. But compared to the normal population, the genetic liability of relatives of affected individuals will be increased, and their liability distribution will be shifted to the right. Thus, the proportion of relatives above the disease threshold will be greater compared with the general population. If we know the proportion of affected relatives of probands and the proportion of those affected in the general population, it is possible to calculate the correlation in liability between pairs of relatives using this model. Recurrence risks to relatives for multifactorial disorders are influenced by the disease severity, the degree of relationship to the index case, the number of affected close relatives and, if there is a higher incidence in one particular sex, the sex of the index case. Polygenic inheritance Polygenic inheritance is again a complex inheritance in which multiple genes but no environmental factors are involved. Both polygenic and multifactorial inheritances defy normal Mendelian principles. The additive effects of many genes, i.e. polygenic inheritance, probably cause characteristics such as height and intelligence, which show a normally distributed continuous distribution in the general population. 8. Polymorphisms Polymorphism refers to variations in genetic make-up at a particular locus noted in general, apparently healthy population. To be defined as polymorphism the variant must occur in at least 1% of the total population and must be associated with normal but varied (not disease causing) expression of final phenotype. This excludes spontaneous mutations that are random and so cannot simultaneously occur in such significant (1%) proportion of total population. ABO blood groups are good examples of polymorphism expressed in protein products of genes. Restriction fragment length polymorphisms are variations that change the sites at which restriction enzymes can act on a DNA molecule, rendering differences in the final ‘restricted’ or cleaved DNA when these enzymes are applied in vitro (Southern Blotting). If polymorphisms are due to changes in single nucleotide in a sequence, then these are called SNPs or single nucleotide polymorphisms. These single-base polymorphisms can be assayed by DNA sequencing or through the use of DNA chips. If the variations are due to changes in length of the genetic sequence, these are termed length polymorphisms.

23 - 9. Cytogenetic techniques

9. Cytogenetic techniques

© SPMM Course VNTRs (variable number of tandem repeats). These polymorphisms are the result of varying numbers of repeats in a specific region of a chromosome. These Polymorphisms can be classified according to the length of polymorphic fragments; Short tandem repeat polymorphisms (STRPs) or microsatellites range in size from 2 to 6 bases. The minisatellites vary between 20 to 70 bases each. Microsatellites are currently preferred as genetic markers in disease mapping because they can be detected using the polymerase chain reaction. Polymorphisms arise out of mutations originally but are maintained in population due to number of factors such as founder effect, genetic drift and natural selection. Note that most polymorphisms occur in non-coding areas (introns) – as coding sequences or exons on mutation often produce disease phenotypes. Serotonin transporter polymorphisms are noted in promoter region, which is a non-coding part of DNA (5HTTLPR – 5HT transporter linked promoter region). 5HTTLPR can be of a short variant or long variant (length polymorphism). 55% of Europeans carry the long allele. In those with short variant, the serotonin transporter expression is low; short variant is speculated to be associated with higher incidence of affective disorders, neuroticism, anxiety and PTSD. But the evidence is inconclusive as most studies are case control design with significant heterogeneity. In an interesting study of environment-gene interaction, Caspi et al (2003) noted that individuals with one or two copies of the short allele of the 5-HT T promoter polymorphism exhibited more depressive symptoms, diagnosable depression, and suicidality in relation to stressful life events than individuals homozygous for the long allele. 9. Cytogenetic techniques

 Blotting techniques Southern blotting is a widely used method for the detection of a specific sequence in DNA. This method was named after Dr. E. M. Southern who introduced this method in 1975. Western blotting is another widely used method for the detection of specific protein after electrophoresis. The sample is electrophoresed on a polyacrylamide gel, then, blotted to a membrane. The membrane is incubated with the antibody to the specific protein. Northern blotting is a detection method for a specific RNA after electrophoresis.

 Polymerase chain reaction (PCR) Minute amounts of DNA can be amplified over a million times using an in vitro technique called polymerase chain reaction. Using this technique, minute amount of DNA such as those from buccal cell scrapings, blood spots, or single embryonic cells can be analysed. The DNA is amplified between two short single-stranded DNA fragments called oligonucleotide primers, which are complementary to the sequences at each end of the DNA of

interest. Hence the exact DNA sequence to be amplified needs to be

24 - 10. Heritability & concordance

10. Heritability & concordance

© SPMM Course known to carry out PCR. It is not error free as laboratory contaminants can have DNA which gets amplified erroneously. The technique has three steps. (1) Double-stranded genomic DNA is denatured by heat into singlestranded DNA. The reaction is then cooled to favour DNA annealing, and the primers bind to their target DNA. (2) DNA polymerase is used to extend the primers in opposite directions using the target DNA as a template. After one cycle there are two copies of double-stranded DNA, after two cycles there are four copies, and this number rises exponentially with the number of cycles. (3) The cycling is set to produce necessary number of amplifications. FISH – Fluorescent in situ hybridisation FISH is a cytogenetic technique to detect and localize specific DNA sequences on chromosomes. It uses fluorescent probes that bind to only those parts of the chromosome with which the probes have high degree of sequence similarity. Fluorescence microscopy is employed to detect the location where the fluorescent probe binds to the chromosome. FISH is often employed to detect specific features in DNA. DNA Cloning Plasmid is a bacterial DNA, which is extra chromosomal, and independently replicating (similar to mitochondrial DNA in humans). Any particular DNA fragment of interest can be isolated and inserted (using a DNA ligase enzyme) into the genome of such self-replicating plasmids. When used for such a purpose the plasmids are called vectors (vehicles for DNA replication). Bacteriophages and other viruses can also be used as vectors. Replication by the millions of the vectors results in multiple copies or clones of the inserted sequence. Removal of inserted gene sequences from the host vector results in large quantities of the required genes. 10. Heritability & concordance

Concordance: A twin pair is said to be concordant when both co twins have the same disease expression (or both are disease free). The pair can be discordant if one of them harbours a disease while the other does not. Due to higher degree of genetic sharing among homozygous individuals, one would expect higher concordance among monozygotes compared to dizygotes if the disease being studied has a significant genetic component. In contrast, a trait that has no genetic basis should have equivalent concordance rates for MZ and DZ twins. Heritability is the main measure of genetic variation in polygenic (quantitative) traits. The total variation of a trait in a population can depend on genetic variation or environmental variation, so heritability is the proportion that is genetic, not environmental, out of that total. The relative influence of genetic factors in defining

the variance in a trait is expressed as heritability. If this is defined as the proportion of the total phenotypic variance attributable to additive genetic variance, then it is known as narrow-sense heritability. Heritability is also sometimes used to describe the proportion of variance explained by the total genetic variance (additive and non-additive genetic variance). Here it is called broad-sense

25 - Specific heritability factors

Specific heritability factors

© SPMM Course heritability. Non-additive genetic influences include phenomena such as epistasis – gene-gene interaction, and dominance effects where presence of one gene mitigates the expression of other gene. Heritability can be calculated from concordance rates using the mathematical formulae. Interpretation of heritability: (from Visscher et al., 2008) STATEMENT ACCURACY A high heritability means that most of the variation that is observed in the present population is caused by variation in genotypes. CORRECT. So, in the current population, the phenotype of an individual is a good predictor of the genotype A heritability of 80% means that 80% of the variability in whether an individual becomes affected is inherited, while 20% is not. CORRECT. It does not mean that genes account for 80% of the causative factors – as inheritance is not same as genetic causation. High heritability implies genetic determination FALSE. It does not mean that the phenotype is determined once we know the genotype, because the environment can change or can be manipulated to alter the phenotype Heritability is the proportion of a phenotype that is passed on to the next generation FALSE: Phenotype is not passed on – only the genotype. There are many modifiers in the environment and cellular machinery between a genotype and phenotype. Heritability is informative about the nature of between-group differences FALSE. Heritability is measured within a specified population – differences among groups may not be due to genetic differences but due to nature of studied population A large heritability implies genes of large effect FALSE. Not true for polygenic disorders. There is no strong relationship between heritability and the number or size of genes affecting the trait. An exception is Mendelian single gene disorders – they all have heritability of 100%.

Specific heritability factors Some common and highly regarded as environmental disorders such as obesity have been demonstrated to have high familial loading. 80% of offspring with both parents obese, 40% of offspring with one parent obese are obese themselves compared to 10% obesity in children with both lean parents. Reported estimates of heritability for IQ from twin studies are remarkably consistent in the range of 0.5–0.8, with differing estimates for the various components of cognitive abilities.

Disorder Heritability estimate* Schizophrenia

26 - 11. Hardy Weinberg equilibrium

11. Hardy Weinberg equilibrium

> 80 Major depression Generalized anxiety Panic disorder Phobia Alcohol dependence *Based on DSM-IIR diagnosis. The estimates must be treated as approximations only. Autism and Tourette's may have around 90% heritability. (From Owen, MJ., Cardno, AG. &O'Donovan, MC. Psychiatric genetics: Back to the future Molecular Psychiatry (2000) 5, 22–31) The Big Five personality traits have following heritability: Openness: 57%; Extraversion: 54%; Conscientiousness: 49%; Neuroticism: 48%; Agreeableness: 42%

11. Hardy Weinberg equilibrium In the absence of mutation, non-random mating, selection and genetic drift, the genetic constitution of the population remains the same from one generation to the next. This principle can be used mathematically to determine frequency of an abnormal gene or genotype in the population. If p is the frequency of the normal gene in the population, q is the frequency of the abnormal gene, p2 is the frequency of the normal homozygote, q2 is the frequency of the affected abnormal homozygote, 2pq is the carrier frequency, and p + q = 1. The equation can be used, for example, to find the frequency of heterozygous carriers in an autosomal recessive disease XYZ. If the incidence of disease XYZ is 1 in 3600 live births, then q2 = 1/3600, and therefore q = 1/60. Since p = 1 - q, then p = 59/60. The carrier frequency is represented by 2pq, which in this case is 1/30. Thus 1 in 30 individuals in the whole population is a heterozygous carrier for disease XYZ. Hardy Weinberg equilibrium does not always hold true. Consider the following circumstances; Natural Selection: Genes which hinder survival and fertility are not maintained in the genetic pool of a population. This is because the abnormal genes are not passed on to next generation when reproductivity is low or if the patient dies at very young age. Similarly some mutations that offer survival benefits are maintained in higher than expected rates in the population. For example, GENOTYPE

FREQUENCY For a given locus, the genotype frequency measures the proportion of each genotype in a population. In a population of 100 individuals assume 33 have AA, 45 have AB and 22 have BB genotypes. The genotype frequency is obtained by dividing the count for each genotype by the total number of individuals. i.e genotype frequency for AA = 0.33, AB = 0.45 and BB = 0.22. The term gene frequency refers to the proportion of chromosomes in a population that contain a specific single allele. In the above example, frequency of allele A = 2x33 (where A occurs twice) + 45 expressed as percentage = 111% or 1.11. Similarly the gene frequency of B is 2X22 + 45 = 89% or 0.89.

© SPMM Course sickle cell carriers are protected against sever falciparum malaria, cystic fibrosis carriers may have an advantage against typhoid, etc. Genetic Drift: Genetic drift refers to gene frequency change caused by limitations in population size. Genetic drift explains why some genetic diseases are unusually common in small, isolated populations. In a small population, the chances of random distribution is limited as probabilities of the combination are restricted. This is very close to what is termed as 'founder effect'. Gene Flow: Gene flow refers to the exchange of genes between populations. Due to migration or other social reasons, the populations studied are not 'closed' populations anymore. Consanguinity: Non-random mating occurs, and mutations are preserved within a closed pedigree due to consanguinity. Autosomal recessive diseases are more often seen in consanguineous families. High frequency of mutations: Environmental exposure can provoke mutations at a higher frequency than expected in a stable population e.g. living near a nuclear reactor leak.

EPISTASIS, HETEROGENEITY & PLEIOTROPY Gene- gene interaction particularly between different alleles at different genes is called epistasis. This can occur at the same step or at different stages of the same biochemical pathway. Locus heterogeneity exists when the same disease phenotype can be caused by mutations in different loci. It becomes especially important when genetic testing is performed by testing for mutations at specific loci. For example early onset Alzheimer's could be caused by mutations in chromosome 1, 14 or 21. Allelic heterogeneity refers to the same disease phenotype resulting from different types of mutations at the same loci. Consider cystic fibrosis, here nearly 600 different mutations at the same site of chromosome 7 results in same disease. Pleiotropy exists when a single disease-causing mutation affects multiple organ systems. Pleiotropy is a common feature of genetic diseases. For example, consider Marfan's syndrome. Cardiovascular system, connective tissue, skeletal system etc. are affected by a single genetic aberration.

27 - 12. Types of genetic studies

12. Types of genetic studies

28 - A. Classical genetic studies

A. Classical genetic studies:

29 - Twin Studies

Twin Studies

© SPMM Course 12. Types of genetic studies Genetic methods can be classified into four paradigms

1. Basic genetic epidemiology: to quantify degree of familial aggregation and heritability estimates
2. Advanced genetic epidemiology: to explore the mechanism of action of genetic risk factors
3. Gene finding: to determine the genomic location and identity of offending genes
4. Molecular genetics: to trace biological pathways from DNA to disorder. Gene mapping refers to any strategy that permits finding the chromosomal location of one or more genes, often related to a disease. Genetic mapping of disease genes is a very useful method because it does not require any knowledge of a gene's function to find the chromosomal location initially. Once located then the identity of the disease gene could be dissected. Not all genetic studies are aimed at gene mapping; certain simpler designs are primarily aimed at demonstrating the presence or absence of a genetic influence in the aetiology of a disease or trait. These include family studies, twin studies, and adoption studies. Gene mapping studies involve linkage analysis, sib-pair analysis and to some extent allelic association studies. A. Classical genetic studies: Twin Studies Monozygotic (MZ, or "identical") twins are formed when an embryo is cleaved during early development. The result is two genetically identical embryos wherein 70% sharing even the same chorion. Dizygotic (DZ, or "fraternal") twins are the result of the fertilization of two different ova by two different sperm cells. DZ twins are genetically the same as siblings, sharing 50% of their genes. A pairwise concordance rate is estimated as the number of twin pairs who both have the disorder divided by the total number of pairs. However, where there has been systematic ascertainment, one can report a probandwise concordance rate, which is calculated as the number of affected twins divided by the total number of co-twins. This is possible if a twin register is maintained; it is also more useful method as this allows comparison of general population risk with the rate in co-twins of probands. Challenges in interpreting twin studies Monozygotes are often treated more closely than dizygotes as they look identical; so they share more environment than dizygotes. So a higher concordance may be due to higher environmental effect. Zygosity assignment done via anatomical similarity is far from perfect. Somatic mutations may occur in MZ twins after the cleavage event that forms them, causing "identical" twins to be at least somewhat different genetically. Chorionicity i.e. how many amnions and chorions are present for both foetuses determines shared uterine environment. Twin studies assume that the risk of disorder is same in monozygotic and dizygotic pairs, and in

singletons at the outset. This assumption holds good for most major psychiatric disorders, while it may not be the case for some physical disorders.

30 - Family studies

Family studies

© SPMM Course Family studies There are two types of family studies. The family history method is simple but unreliable; here psychiatric history is taken from the probands himself/herself. A comparison can be then made as to how many relatives are affected in one group compared to another. A more thorough but more timeconsuming approach is the family study method. Here all available relatives are directly interviewed. See below for other major disorders. From McGue M & Bouchard TJ Jr. Genetic and environmental influences on human behavioral differences. Annu Rev Neurosci. 1998;21:1-24.

Complete case ascertainment refers to the identification of all affected individuals in a given population. This is rarely possible. In multiple incomplete ascertainments consecutive referrals are identified; there is a chance that more than one probands may come from same family. Most genetic studies are concerned with in the proportion of individuals who have ever had the disorder (lifetime prevalence). But not all family members may have reached the age of risk for the disorder, and some may have died prematurely before the age of risk. Hence, age correction is important while ascertaining cases. There are many methods of age correction; Weinberg's shorter method is the often used as it is simpler. (note that such standard age correction methods do not exist for twin studies; it is a problem in MZ twins with psychiatric disorders as there is a high correlation between age of onset; sometimes survival analysis can be used for non-psychiatric phenotypes in twins) Relative risk of common psychiatric conditions derived from family studies Adapted from Johnstone, EC. Et al (Ed)Companion to Psychiatric studies Page 158

Disorder MZ Concordance DZ concordance Male alcoholism 41% 22% Female alcoholism 34% 31% Panic disorder 24% 11% Bulimia 23% 9% ADHD 58% 31% Autism 64% 9% Tourette’s 53% 8%

Advantages Disadvantages Family History Method Practical Many false negatives Few false positive

Family Study Few false positives or negatives Expensive Disorder Relative risk ADHD 55 times Autism 45 times Schizophrenia 10 times Bipolar disorder 7- 11 times Alcoholism 4 to 6 times Anorexia 2-4 Somatisation 3 times Unipolar depression 1.5-3 Generalised anxiety disorder 2-5 Alzheimer’s (late onset) 2 times Panic disorder 3 -8 (summarized as 5 by Hettema, 2001)

31 - B. Molecular genetic studies

B. Molecular genetic studies

32 - Linkage analysis

Linkage analysis

© SPMM Course Adoption studies Adoption studies are useful to differentiate the effects of genes and environment. The basic method of the adoption study lies in comparing the rates of disorder in biological relatives and adoptive relatives. There are many types of adoption studies.

Types Compared groups

Group1 Group2 Parent as proband (1 and 2) Adopted away children of ill parents (biological or adoptive) Adopted away children of well parents (biological or adoptive) Adoptee as proband (3) Biological relatives of (ill and well) adoptees Adoptive relatives of (ill and well) adoptees Crossfostering (4) Children with ill biological parents but raised by well adoptive parents Children with well biological parents but raised by ill adoptive parents

Adoption studies have certain potential problems. (1) There is a tendency for higher rates of some psychiatric difficulties amongst adopted children as adoption itself occurs due to various difficult social circumstances. (2) Adoptive parents are more likely than not to resemble biological parents as social agencies attempt to match the families of origin to families of adoption. B. Molecular genetic studies Linkage analysis During prophase I of meiosis, homologous chromosomes line up and occasionally exchange portions of their DNA. This process is termed crossover or synapsis. When a crossover event occurs between two loci, x and y, the resulting chromosomes may contain a new combination of alleles at loci x and y. This new combination is called a recombination. Because crossover events occur more or less randomly across chromosomes, loci that are located farther apart are more likely to experience an intervening crossover and thus a recombination of alleles. This offers a means of assessing the distance between loci on chromosomes. Alleles of loci that are close together on the same chromosome are likely to be inherited together; these loci are said to be linked. To be linked, these alleles must be syntenic i.e. on the same chromosome. If two loci are on different chromosomes, or if they are far apart on the same chromosome, their alleles will be transmitted independently. As crossing over is an independent event for each locus, if an allele at one locus is transmitted, there is a 50% chance (as in coin tossing) that a given allele at the

33 - LOD Scores

LOD Scores

© SPMM Course other locus will also be transmitted to the daughter cell. But linked loci are close enough together so that the chance of a recombination is less than 50%. Thus, their inheritance is not independent. The distance between two loci can be inferred by estimating the frequency with which cross-overs occur among them. The lesser the cross-over, the closer the loci. Because this is done by looking at recombination in families, this is called as recombination frequency. The recombination frequency provides a measure of the genetic distance between any pair of linked loci. Genetic distances are often expressed in centiMorgans (cM). One centiMorgan is equal to a 1% recombination frequency between two loci. 1 cM is approximately equal to 1 million base pairs of DNA (1 Mb). But crossovers occur more commonly at telomeres and less common near centromeres. LOD Scores To estimate the likelihood that two loci are truly linked with a specific recombination frequency, an LOD score is used. The LOD ("log of the odds") is estimated using the following expression LOD = log 10 (probability that recombination frequency is the observed value Ø) (probability that the recombination frequency is 50% i.e. chance) A logarithm is used because it allows LOD scores from different individual families studied to be added together later to obtain an overall LOD score. An LOD score greater than 3 is usually interpreted as statistical evidence of linkage (i.e., the numerator is 1,000 times greater than the denominator, indicating that linkage is 1,000 times more likely than nonlinkage). Conventionally an LOD score of -2 or less is taken as evidence that two loci are not linked (i.e., nonlinkage is 100 times more likely than linkage). Two loci are said to be in linkage disequilibrium if specific combinations of alleles at the loci are seen together on chromosomes more often than expected by chance. Because recombination is rare for very closely linked loci, such loci are more likely to exhibit linkage disequilibrium. Such linkage disequilibrium can be analysed in association studies too. Two different approaches can be adopted in linkage studies:

1. Candidate gene approach: A protein is suspected to be involved, then the gene is traced from this pathogenetic knowledge.
2. Positional cloning approach: Genes are identified through their positions in the genome rather than functions. Supported by human genome project. A prerequisite for successful linkage analysis (see below) is the availability of a large number of highly polymorphic markers dispersed throughout the genome. Sib pair analysis In this method several hundred DNA markers roughly evenly spaced along the 23 pairs of human chromosomes are taken and genotyping is carried out in a series of concordant sibling pairs. ‘The probability that siblings share 0, 1, or 2 alleles at any marker locus is respectively, 0.25, 0.5, and 0.25. However, if a marker locus is close to (and therefore linked with) a locus conferring susceptibility to

34 - Whole genome scan

Whole genome scan

35 - Association studies

Association studies

© SPMM Course the disease this will be detectable as increased allele sharing at the marker. This approach has been successful in identifying susceptibility loci for disorders such as type 1 diabetes. The main drawback is that susceptibility loci of very small effect (such as conferring a relative risk of less than 2) may require large numbers of sib pairs in the region of 600 to 800 to be detected. In a disorder such as schizophrenia the relative risk in a sibling of an affected individual is about 10; thus, if several additive genes are involved, none may individually have a relative risk of more than 2’. (Excerpts from McGuffin & Martin, BMJ. 1999 Jul 3; 319(7201): 37–40) Whole genome scan It is a type of linkage analysis in which markers placed at regular intervals covering the whole genome are typed. It is tedious but often the first approach when no genetic information is available about a particular phenotype. A good example is that of neuregulin. Stefansson et al. typed 950 microsatellite markers covering the whole genome in 110 Icelandic patients with reconstructed genealogical relationships, and found that neuregulin-1 is a candidate gene for schizophrenia (Malats & Calafell, 2003). Association studies Association studies are more straightforward to carry out than linkage studies. Here a case control design is often adapted, and a sample of cases affected by a disorder is compared with controls. The frequency of alleles at the marker locus is then compared in the two groups. This method, though increasingly used, cannot make strong causal inferences. The locus chosen for study must predispose to illness. Thus, loci chosen for association studies are often known as candidate genes. If the locus does not predispose to illness, then the results of an association study should be negative. However, false positive results can occur if the two populations are not carefully matched for ethnic background. One alternative control group is the parents or relatives of affected individuals (the alleles not transmitted to the affected child compose the "control group"—this is known as the Transmission Disequilibrium Test or TDT). In Genome Wide Association Studies (GWAS), ‘candidate gene’ approach is not used. Instead, several thousands of single nucleotide polymorphisms are assayed in thousands of individuals. This is the new ‘hot’ study technique in psychiatric genetics. Questions Most appropriate method Is the phenotype familial? Family study What is the relative contribution of genetic and environmental factors? (Heritability) Twin studies, adoption studies What is the mode of transmission? Segregation analysis Where might be the ‘culprit’ genes? Linkage analysis (known ancestries) What are the actual genes responsible? Association analysis (population level)

36 - Linkage vs. association

Linkage vs. association

37 - C. Alternative approaches in genetic studies

C. Alternative approaches in genetic studies

© SPMM Course Linkage vs. association Linkage studies Association studies Uses families Uses cases and controls or families with ‘internal controls’ Detectable over large distances >10cM Detectable only over small distances <1cM Can usually only detect large effects i.e. RR>2 Capable of detecting small effects e.g. OR<2 From McGuffin et al. (ed) Psychiatric genetics and genomics. Oxford Press: 2002 C. Alternative approaches in genetic studies Transgenic studies: Transgenesis is a term that describes the transfer of a gene from one species to another. In practice, this term often refers to the insertion of a modified mouse gene into the mouse genome to study gene function. Transgenesis is a direct and powerful approach for analysing gene function. Epigenetics: A discrepancy exists between the information provided by the DNA sequence (i.e. number of genes) and what is translated and produced by cellular machinery (messenger RNA and proteins). Though the DNA sequence provides a blueprint for synthetic activities of the cell, a number of ‘epigenetic’ modifications occur resulting in a second, equally complex layer of information. Waddington coined the term epigenetics to explain such mechanisms. DNA methylation and histone modification explain most of the epigenetic variations discovered to date. Crow has argued for long that epigenetic defects explain most of the concordance seen in schizophrenia; according to Crow, the hemispheric laterality and language specialisation unique to human brains is the source of schizophrenic defect and it can be ascertained only by an epigenetic enquiry. Position effects: gene activity can be dependent upon the precise chromosomal location of the gene and its ‘neighbourhood’. Such genes will show altered activity during translocation, even if the gene itself is not disrupted by chromosomal breakage. Endophenotypes: This term was coined by Gottesman and Shields in 1975. An endophenotype is an unseen but measurable phenomenon that is present in the distal genotype to disease pathway. It can be a biochemical, neuroimaging, electrophysiological, pathological, neuropsychological or sociofunctional marker. To be termed as an endophenotype, Gottesman suggested certain criteria to be satisfied by an identified disease marker. These are as follows:

1. Must be associated with a candidate gene or region
2. Must be present with a high relative risk in relatives, thus cosegregating with actual illness
3. Must be a parameter associated with disease with biological plausibility
4. Must be independently expressed in clinical state (i.e. must not be a state but a trait marker)
5. Must be heritable
6. Must be present in relatives more often than general population It is anticipated that the genetics of a complex construct such as schizophrenia can be studied easily in more or less Mendelian fashion if the constructs are broken down to constituent endophenotypes. The simpler a construct under study, the less number of genes will be on the causal pathway. Working

38 - 13. Psychiatric genetics

13. Psychiatric genetics

39 - A. Causal models

A. Causal models

© SPMM Course memory defects, information processing defects such as prepulse inhibition, smooth pursuit defects, glial cell changes and certain other putative neurocognitive markers are termed as probable endophenotypes for schizophrenia. To be an endophenotype, a character must be observable independent of clinical state and must be measurable in relatives at a higher degree than the general population. In spite of their simplicity, there are some important problems that need to be overcome while studying endophenotypes. The endophenotypic expression could be well under the influence of the developmental environment. An endophenotype can be differentially expressed in different brain regions. Often patients have multiple endophenotypic deficits with significant interaction among these. In spite of hard toil, researchers are unable to narrow down genetic linkages of suspected endophenotypes to achieve better than modest LOD (log of odds) scores.

13. Psychiatric genetics A. Causal models Several notable features regarding psychiatric genetics are listed here (excerpted from Craddock et al., BJPsych, 2007:190;3)
14. Families with clear Mendelian inheritance patterns are rare: There are no clear demonstrations of Mendelian pattern of inheritance of schizophrenia or other psychiatric disorders in families.
15. Single genes of major effect have not been found: Even in extended pedigrees with multiple cases of psychiatric illnesses, intensive molecular genetic studies have not demonstrated mutations of major effect (LOD scores are meager). The odds ratio in most psychiatric genetic association studies are in the order of 1 to 2; median being 1.3. This is insufficient to prove a genetic cause for most disorders. These findings are suggestive of multiple risk alleles of modest effect.
16. Mathematical modelling of familial risk is inconsistent with single genes of large effect: According to Craddock et al., “for both schizophrenia and bipolar disorder there is a very rapid, non-linear decrease of risk when moving from a genetically identical individual (i.e. monozygotic co-twin where the risk is 50– 60%), to an individual who shares half the genes (e.g. sibling, parent, dizygotic co-twin where risk is around 10%)”. This rapid, non-linear decrease of risk is compatible with multiple interacting risk factors, albeit of unknown frequency, that individually have modest effects.
17. The causal pathway from an identified genetic abnormality to actual disease expression is too complex and not fully explored in any known genetic markers of psychiatric diseases. For example it is unclear how mutant dysbindin gene that is implicated in schizophrenia can lead to a belief that aliens are invading earth. The association between genes and diseases are very non-specific and weak with respect to psychiatric diseases.

© SPMM Course 5. Contingent models of association: Non-contingent gene–disorder association refers to the fact that the relationship is not influenced by other factors such as environment or presence of other genes i.e. not polygenic or multifactorial. But most psychiatric disorders do not follow non-contingent association models. 6. Practical difficulties in conducting genetic enquiries in psychiatry: a. Wide ethnic, geographical variations are seen in psychiatric disorders. b. Ascertainment method. The spectrum of clinical features (symptoms, severity, functioning, illness course, etc.) of individuals recruited depends upon the mode of ascertainment. These variations can reduce or increase the modest effect sizes noted. c. Unknown phenotypic model. Reliance on DSM–IV or ICD–10 categories is a huge challenge for psychiatric genetics. These are arbitrary classifications, and it is possible that we have been missing many etiological factors due to these empirical categories. For example, the distinct DSM-based categories of affective disorders may not breed true as strong overlap exists between the genetic risk of unipolar and bipolar disorders. Two views exist concerning the causal modeling of genetic factors in psychiatric disorders (Craddock et al., 2007):

1. Common disease–rare variant model: Rarely occurring mutations cause diseases such as schizophrenia. There are various different mutations that can explain the disease (locus and allelic heterogeneity). But each mutation is sufficient but not necessary to cause the disease. Each family inherits one such mutation explaining higher risk in the relatives. These mutations are rare, but when present they commonly cause the disease.
2. Common disease–common variant model: Here a disease such as schizophrenia is thought to be a result of the co-action of multiple (ranging in principle from a few to many thousand) common variants (`polymorphisms'), each of which has a small effect on illness susceptibility – see table below. When an individual inherits several, or many, susceptibility variants together, they have a sizable influence on disease risk. Hence, the mutations or polymorphisms are not sufficient by themselves to cause disease, but they occur very commonly so they can interact in combinations and produce the disease. This model is more popular currently and forms the basis of association and linkage studies being carried out widely. Characteristics Mendelian disorders Most psychiatric disorders Diagnostic boundaries Clear Vague Phenocopies Absent Multiple Penetrance Usually complete/ predictable Incomplete / unpredictable Association Non-contingent models Contingent models Modelling familial risk Linear change in risk Non-linear changes in risk MZ concordance Nearly 100% 30-70% only Locus heterogeneity Never within families; often absent across families too Likely

40 - B. Genetics of Schizophrenia

B. Genetics of Schizophrenia

How important is the genetic contribution to schizophrenia? The relative risks for first-degree relatives / twins of probands are higher than relative risks due to any individual environmental factors. Without genetic contribution, schizophrenia cannot be explained. Risk to family members: In the attached chart ‘parents’ refer to one parent having schizophrenia, where the risk to the child is 13%. If both parents have schizophrenia, then the risk is 46% - close to monozygotic twin risk. The risk to a half sibling is 4%. Note that for the children and siblings of individuals with schizophrenia, the increase in risk is around 10-fold, but it is somewhat less than this in parents. This is probably ‘explained by a reduction in the reproductive opportunities, drive, and possibly fertility of affected individuals’ (Craddock et al. 2005).

 Monozygotic (MZ) concordances = 41–65% Dizygotic (DZ) concordances = 0–28% Broad heritability = 80% The most frequent personality disorder in relatives of schizophrenia patients is schizotypal personality disorder (DSM)-nearly 15% can be diagnosed with it. Twin studies had shown significantly higher MZ concordance rates for schizophrenia when probands had hebephrenic or nonparanoid subtypes than paranoid subtypes.

 Psychotic symptom dimensions consistently show only modest familial aggregation in affected sibling pairs, and rather weak and inconsistent relationships with the familial risk of psychoses. So the severity of schizophrenia is not directly associated with a family history or genetic loading. Gene suspected in schizophrenia

Locus

NRG1 Neuregulin 8p12-p21 DTNBP1 dysbindin 6p22 G72 13q34 DAAO (interacts with G72) D amino acid oxidase 12q24 RGS4 Regulator of G protein signalling 4 1q21-22 COMT Catechol-o-methyl transferase 22q11 DISC1 Disrupted in Schizophrenia 1q42

41 - C. Genetics of Mood disorders

C. Genetics of Mood disorders

42 - Bipolar disorder

Bipolar disorder

© SPMM Course Murray et al. (2002) point out a number of studies that have shown a higher familial risk to be associated with earlier age of onset. Sham et al. (1994) showed that the morbid risk of schizophrenia is greater among the relatives of those probands who had an onset before rather than after age 21 years. Most case-control studies have not provided evidence in support of COMT associations, but association studies with family design provide greater evidence for COMT in schizophrenia. In Down's syndrome, the risk of schizophrenia is same as or lower than the general population. The exact figure is unknown, but an estimate of less than 0.6% is quoted.

C. Genetics of Mood disorders If one parent has a mood disorder, a child will have a risk of between 10 and 25 percent for mood disorder. If both parents are affected, this risk roughly doubles. The presence of more severe mood disorder in the family conveys a greater risk. Bipolar disorder A family history of bipolar disorder conveys a greater risk for mood disorders in general and bipolar disorder in particular. This may be due to common genetic underpinnings between these two forms of mood disorder. Estimates of broad heritability are high: nearly 85-90%. The lifetime risk in relatives does not vary according to the sex of relative or sex of proband. Because of its higher prevalence, the unipolar disorder is typically the most common mood disorder in families of bipolar probands. According to Craddock et al. (2005), lifetime risk of narrowly defined bipolar disorder in relatives of a bipolar proband are: o unrelated member of the general population: 0.5–1.5%; o first degree relative 5–10% (relative risk = 8); o monozygotic co-twin 40–70% (relative risk = 60); Lifetime risk of unipolar disorder in relatives of a bipolar proband are: o unrelated member of the general population: 5-10%; o first degree relative 10-20% (relative risk = 2-3times); o monozygotic co-twin 15-25% (relative risk = 3-5 times); o Note: You can get the risk of major mood disorder by adding the absolute risk of unipolar and bipolar from the above data.

Genes suspected in Bipolar Disorder

Locus

BDNF (Brain-derived neurotrophic factor) 11p13 DAO G72/G30 D aminoacid oxidase 13q33 COMT Catechol-o-methyl transferase Breakpoint cluster region (BCR) gene 22q11

43 - Unipolar depression (MDD)

Unipolar depression (MDD)

44 - Schizoaffective disorder

Schizoaffective disorder

45 - Molecular associations (Schizophrenia and Bip

Molecular associations (Schizophrenia and Bipolar disorder)

© SPMM Course Other implicated chromosomes – Chr 18 - nearly 4 loci, affective disorders in general;? parent of origin effect) Chr 21q - both in scz and BPAD. An X-chromosomal locus to BPAD has been suggested on the basis of the cosegregation of BPAD in some families with color blindness, the glucose-6-phosphate dehydrogenase deficiency, and the coagulation factor IX deficiency. In an extended Finnish pedigree, Xq24-q27.1 was demonstrated to segregate with bipolar disorder. Low activity allele in COMT gene may be associated with rapid cycling. Serotonin transporter gene (hSERT) and 5HT2A gene may be associated with modest statistical significance in Seasonal Affective Disorder.

Unipolar depression (MDD) Age-adjusted risk of MDD to first-degree relatives: 5-30%, relative risk 1.1-4.0. MZ Twin concordance for MDD: 40%. DZ Twin concordance for MDD: 11%. Heritability: Unclear (~20-80%); meta-analysis reports 31-42%. (Data from NCHPEG Empric Risk Data: Retrieved from www.nchpeg.org) Early onset and recurrent episodes likely increase risks to first-degree relatives. Recurrence risks for unipolar depression could be 50 percent or higher for probands with early-onset and recurrent episodes. While the definition of “early onset” is not entirely clear, research suggests that family members of probands who had onset before age 25-30 years have the highest risk; relatives of probands with onset between ages 25-40 years have an intermediate risk; and relatives of probands with onset after age 40 years have a risk that is only slightly increased over the population risk Schizoaffective disorder The risk to first-degree relatives for ANY psychiatric disorder is higher in SA disorder than any other psychiatric disorder. The extent of heritability is unclear, although likely in the range of schizophrenia. Relatives have a higher rate of schizoaffective illness, schizophrenia and bipolar disorder. The rate of bipolar disorder is high if proband has a schizoaffective-manic presentation. The rate of schizophrenia is high if proband has schizoaffective-depressive presentation. In depressive subtype no elevation in bipolar risk has been noted in a large cohort (Andreasen 1987).

Molecular associations (Schizophrenia and Bipolar disorder) G72: The function of G72 (also sometimes referred to as DAOA) may be to, oxidize serine, a potent activator of glutamate transmission via a modulatory site on the NMDA (n-methyl-d-aspartate) receptor. Inadequate DAOA function might be hypothesized to lead to problems in modulating the glutamate signal in areas of the brain such as the prefrontal cortex. A new suggestion is that the major role of G72 may be in maintaining neuronal structure. Brain-Derived Neurotrophic Factor (BDNF): Several studies have shown that antidepressant administration is associated with increased central BDNF levels in experimental animals, and administration of BDNF itself has been associated with the antidepressant-like activity. Depression has Shared genes – BPAD and Schizophrenia DAO & BDNF – seen more in mood disorders than schizophrenia DISC 1 & NRG – shared with schizophrenia; seen in schizoaffective disorder Dysbindin – seen more in schizophrenia than mood disorders CREB1 (chr2) – unipolar depression

46 - D. Genetics of dementias

D. Genetics of dementias

© SPMM Course been postulated to be associated with decreased neurogenesis in the hippocampus, which is dependent on neurotrophic factors, including BDNF. Disrupted in Schizophrenia 1 (DISC1): This gene on chromosome 1q was identified in a Scottish family with a genetic translocation and with multiple cases of psychiatric disorders, primarily schizophrenia. This gene is expressed in multiple brain regions, including the hippocampus, where it is differentially expressed in neurons. It is associated with microtubules; in mice, disruption of DISC1 leads to abnormal neuronal migration and dendritic organization in the developing cerebral cortex. DISC1 appears to interact with phosphodiesterase 4B, which may play a role in mood regulation. 5HTT, MAOA, COMT: These three genes have been shown in meta-analyses to be associated with BP disorder. The effect size for each appears to be in the range of 10–20% increase in risk. Each of these genes is associated with other behavioral phenotypes, and each has been reported to interact with the environment to increase the risk of specific disorders (major depression, antisocial personality disorder, and schizophrenia respectively). Recent data in BP illness are more positive for 5HTT than for MAOA or COMT. Dysbindin: Also known as dystrobrevin binding protein 1 - involved in the formation of synaptic structures Neuregulin: Involved in neuronal migration and in the genesis of glial cells and subsequent myelination of neurons by these cells GRK3: This is the only candidate identified using animal model studies (a mouse model employing methamphetamine). This gene participates in the down-regulation of G-protein coupled receptors and is associated with Bipolar disorder. D. Genetics of dementias Alzheimer’s disease (AD) Mutations in the amyloid precursor protein (chr 21) and presenilin 1 (chr 14) and 2 (Chr 1) genes may be responsible for as much as 50% of familial (ie, autosomal dominant) AD beginning before 60 years of age (presenile). But this accounts for less than 1% of patients worldwide. The genetic factor with the highest attributable risk for AD is apolipoprotein E (APOE). The APOE gene on chromosome 19q has 3 codominant alleles, 2, 3, and 4, differing by single-base substitutions in the coding region of the gene. The ancestral allele, 4, is overrepresented, and 2 is underrepresented in AD (from Graff-Radford et al.: Arch Neurol. 2002;59(4):594-600). In Caucasian subjects, the odds of AD for those homozygous for 4 and for 3/ 4 heterozygotes are 14.9 and 3.2 times, respectively, greater than the odds associated with 3 homozygosity. The mean age of onset of AD is 2 decades earlier in 4 homozygotes. The APOE 4 allele has also been found to increase AD risk in nonwhite populations, including Afro-Caribbean, Chinese and Japanese. The increased risk associated with the 4 allele is greater in women than in men though this is not replicated in African Americans. Chr 21 harbours mutant APP (Amyloid Precursor Protein) – this is related to Down’s

syndrome and explains the higher prevalence of AD in patients with Down’s syndrome

Male Abs. risk% Female Abs. Risk % Relative risk (both sexes)

47 - Frontotemporal dementia

Frontotemporal dementia

© SPMM Course ApoE status unknown (general population) 6.3% 12%

No Apo 4 4.6% 9.3% 0.75 times (less) Apo 4 heterozygote 12% 23% 3.2 times (up to 5 times in some studies) Apo 4 homozygote 35% 53% 14.9 times Modified from McGuffin et al. (ed) Psychiatric genetics and genomics. Oxford press: 2002

 An actually predicted risk of developing Alzheimer's disease in the first-degree relatives of probands with Alzheimer's disease is 15-19%, compared with 5% in controls. Thus, the risk to the first-degree relatives of patients with Alzheimer's disease who developed the disorder at any time up to the age of 85 years is increased some 3 – 4 times relative to the risk in controls. This translates to a risk of developing Alzheimer's disease of between one in five and one in six (from Liddell et al., 2001). In the case of patients with Alzheimer's disease who became demented late in old age, say by their 80s, relatives probably run the same 30-50% risk of developing dementia as anyone else who live to the age of 90 years and beyond (from Liddell et al., 2001). Like other disorders that reflect the combined action of several genes, the risk to relatives drops rapidly as the degree of genetic relatedness falls. Data are limited, but the risk to second-degree relatives, such as grandchildren, is probably less than twice the population levels (from Liddell et al., 2001) Probandwise concordance rates of about 40% for DZ and 84% for MZ twins are seen. Frontotemporal dementia Frontotemporal lobar degeneration (FTLD) refers to the 3 different syndromes of frontotemporal dementia (FTD), progressive non-fluent aphasia and semantic dementia. Some patients with FTLD show tau protein based pathological changes. In familial cases, mutations have been identified in the microtubule-associated protein tau gene (MAPT) on chromosome 17q21. Many cases are tau-negative but show ubiquitin-immunoreactive neuronal cytoplasmic inclusions. In some of these tau negative cases mutations have been identified in

progranulin (PGRN) gene, also on chromosome 17q21. Progranulin is a widely expressed growth factor that plays a role in wound repair and inflammation by activating signalling cascades in cell cycle. Progranulin has also been linked to tumorigenesis CADASIL CADASIL is a form of amyloid angiopathy that can present with Alzheimer's like features. NOTCH3 is the only gene currently known to be associated with CADASIL. Most mutations in the NOTCH3 gene in individuals with CADASIL are located in exon 4. The mutation detection rate is up to 96% in individuals with well-defined or biopsy-proven CADASIL. The defective gene is identified as NOTCH3 in 19p13.1-13.2

48 - Lewy Body dementia

Lewy Body dementia

49 - E. Other disorders

E. Other disorders

50 - Autism

Autism

© SPMM Course Lewy Body dementia No specific genetic associations have been established for Lewy Body Dementia. Certain mutations have been reported inconsistently at alpha-synuclein locus. DLB is considered as a part of ‘synucleinopathies’ where synuclein molecules aggregate in presynaptic terminals producing Lewy bodies. Other diseases included are Parkinson’s and Multisystem atrophy. Parkinson’s disease LOCUS POSITION/Protein Clinical features/inheritance PARK1, PARK4 4q21 Alpha-synuclein gene Dominant inheritance; not seen in sporadic cases. Onset in 40s. nigral degeneration with Lewy-bodies. PARK2 6q25 Parkin gene Recessive inheritance; nigral degeneration without Lewy-bodies. Onset 40 – 60. (most early onset cases, l-dopa responsive) PARK8 cen (pericentromeric) LRRK2 gene Dominant. Onset around 60. Variable - synuclein and tau pathology. PARK6 1p35-37 PTEN-INduced Kinase (PINK1)

in mitochondria Autosomal recessive; onset 30-40 (12% of early-onset cases, l-dopa responsive) PARK7 1p38 DJ-1 Autosomal recessive; onset 30-40 α-synuclein is a protein that is expressed throughout the brain and has potential roles in learning, synaptic plasticity, vesicle dynamics and dopamine synthesis. E. Other disorders Autism The recurrence rate in siblings of autistic children is 2% to 8% (higher than the rate in the general population but lower than in single-gene disorders). This translates to 50 times (range: 30 – 120) relative risk in siblings. Risk of autistic disorder in a sibling of 2 autistic children: 25-30% (nearly 300 times higher) Twin studies reported 60% concordance for classic autism in monozygotic (MZ) twins versus 0 in dizygotic (DZ) twins. If a broader autistic phenotype that included communication, and social disorders is considered, the concordance increased remarkably from 60% to 92% in MZ twins and from 0% to 10% in DZ pairs. This translates to 90% heritability. The identity and number of genes involved remain unknown. Chromosomes 2, 7 and 15 are implicated. A segregation analysis in a series of multiplex families was consistent with autosomal recessive inheritance with sex-specific modifications The striking feature is the association of the genetics of autism with multiple single-gene disorders. The most clearly documented of these disorders is the fragile X syndrome. Perhaps 8% of autistic subjects have the cytogenetic fragile X; 16% of fragile X males are autistic. There are also probable associations between autism and tuberous sclerosis, neurofibromatosis, and phenylketonuria

51 - ADHD

ADHD

52 - Personality disorders

Personality disorders

53 - Panic disorder

Panic disorder

54 - Social phobia

Social phobia

55 - Alcoholism

Alcoholism

© SPMM Course There appears to be as much variability in the phenotypic symptom expression within monozygotic twins as between MZ pairs. This suggests non-genetic influences play an important role in determining the pattern of phenotype in autism (LeCouteur, 1996). Risk for broader phenotype (delayed speech, reading/spelling difficulties, social reticence/awkwardness, poor social language abilities) in first-degree relatives and dizygotic twins: 30%. In monozygotic twins, the spectrum phenotype has 82% concordance. (All data excerpted from http://pediatrics.aappublications.org/content/113/5/e472) ADHD Risk to first-degree relatives: 15-60%, 2-6 relative risk Risk to second-degree relatives: 3-9%, 0.5-0.8 relative risk Heritability: ~70-80% Risks are higher for male relatives and lower for female. It is unclear if recurrence risks are higher when the proband is female. Continuation of illness into adulthood may indicate increased risk to relatives. Personality disorders The largest factor accounting for nearly 50% or more of the variation in most personality traits is nonshared, person-specific environmental variation. Among personality disorders, antisocial PD has the highest heritability (60-70%). Emotional dysregulation has high heritability among various features of borderline PD. A variant of the tryptophan hydroxylase gene (which codes for the synthetic enzyme for serotonin) is associated with low 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid and suicide attempts in violent criminal offenders. Panic disorder Lifetime prevalence of panic disorder (+/- agoraphobia) is around 4.7%. In a metaanalysis of family studies, Hettema et al. (2001) found OR of 5 for panic disorder in firstdegree relatives (absolute risk 8-31%). Early onset panic disorder confers increased risk than lateronset disease. Nearly 17 fold increase in risk is seen if the onset is before 20 years compared to the only 6-fold increase in relatives of probands with onset after 20 years. The heritability is estimated to be around 0.43. Social phobia The 10-fold increase in risk is seen in first-degree relatives of probands with generalized social phobia. Non-generalised discrete social phobia does not show familial transmission. Specific phobias are 3-4 times more common in 1st degree relatives of probands (OR 4). Nevertheless, twin data suggest that individual-specific environmental influences are more important in the development of simple phobias. Alcoholism Genetic influences play an important role in alcoholism: the risk in families may be 4 to 6 times higher than in the general population.

56 - OCD

OCD

© SPMM Course Majority of adoption studies show that the risk of alcoholism in adopted children is strongly correlated with their biological parents rather than adoptive parents (3- 4 times higher); no protective effect was noted in being raised away from drinking biological parents (Goodwin 1973). The genetic risk is clearly higher in males and weak in females. Variants in GABRA2 on chromosome 4p have been shown to be associated with alcohol dependence - particularly strongly related to problems with impulse control; the risk allele is also seen in adolescents with conduct disorder and in alcohol dependent persons who are drug dependent. ADH (alcohol dehydrogenase) is the major metabolic enzyme for alcohol, catalyzing its breakdown into acetaldehyde, which is then further metabolized by aldehyde dehydrogenase (ALDH). Both ADH and ALDH have variants associated with the "flushing" reaction to alcohol. The strongest finding with regard to alcoholism is in ADH4, which appears to be associated with the early onset of regular drinking. A meta-analysis of 21 studies shows an increased risk of alcoholism of 50–100% of persons carrying the A1 allele of DRD2. However, recent work has questioned whether this polymorphism may actually be reflecting variation in a gene next to DRD2. OCD Early onset suggests higher genetic risk for family members; some studies suggest increased risk only in the case of early age at onset (generally defined as before 18 years) [www.nchpeg.org]. Fathers were three times as likely as mothers to receive a diagnosis of OCD for probands with severe childhood OCD. Increased severity and chronicity appear to increase risk Risk to 1st degree relatives: Onset before age 18: range of ~10-35% Onset after age 18: no increased risk to ~15% MZ Twin concordance: 53-87% DZ Twin concordance: 22-47%

Family History Increased Risk for Offspring General Population Risk Unipolar depression Unipolar 2-fold (16%); Bipolar 4-fold (4%) 6% Bipolar depression Unipolar 2-3 fold (16%); Bipolar 8 to 9-fold (9%) 1% Schizophrenia (SZ) Unipolar - 2-fold (16%); Bipolar -4-fold (4%) 1% Alcoholism 5-fold (27% for males, 5% for females) 5% males, 1% females Panic disorder 12-fold (6%) 0.5% Tourette's syndrome 100-fold (25%) 0.25% Alzheimer's disease 5-fold (15%) at age 75 3% Attentiondeficit/hyperactivity disorder 5-fold (15%) 3% Anorexia nervosa 10-fold (5%) 0.5% Adapted from Tsuang D, Faraone SV, Tsuang MT. Psychiatric genetic counseling. In: Floyd EB, David JK (eds). Psychopharmacology: The Fourth Generation of Progress. New York: Raven Press, 1995.

57 - 14. Clinical genetics

14. Clinical genetics

© SPMM Course 14. Clinical genetics When an individual approaches a genetic clinic for genetic testing, 2 approaches can be employed. Direct testing: This is very much like any other lab test. A sample is tested for the presence of a certain genotype. Only one individual is tested, and the abnormality that is being sought is already known to have an association with the illness studied. Gene tracking: Many family members are tested to discover whether or not the suspected patient seeking the test has inherited the high-risk chromosome from a heterozygous parent. The test is based on Mendelian principles and seeks information about the segregation of a chromosome within a family. It can be used even if the exact genetic locus associated with a disease is unknown. . Prenatal identification: Prenatal test is the test of a fetus to identify a suspected genotype. It is often initiated on the basis of family history or maternal factors (e.g. older mothers at risk of Down's). Maternal serum screening to identify neural tube defects and Down's is offered routinely in many countries. In general, adult-onset genetic conditions are not usually tested prenatally. . Genetic counseling is routinely offered to individuals seeking genetic tests. The counseling service provides information about risks and probabilities before the test and also provides support (but not psychological) services after the testing. Within the NHS Regional Genetic Centres that incorporate cytogenetic, molecular and clinical genetic services operate and offer familial (carrier) testing, diagnostic and prenatal (presymptomatic) testing. . DNA banks provide secure, long-term storage for an individual's genetic material. While this is seen as beneficial for biomedical research the possibility of misuses has raised several ethical issues. .

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. DISCLAIMER: This material is developed from various revision notes assembled while preparing for MRCPsych exams. The content is periodically updated with excerpts from various published

sources including peer-reviewed journals, websites, patient information leaflets and books. These sources are cited and acknowledged wherever possible; due to the structure of this material, acknowledgements have not been possible for every passage/fact that is common knowledge in psychiatry. We do not check the accuracy of drug related information using external sources; no part of these notes should be used as prescribing information.

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