CHAPTER 5

GENERAL SUMMARY

5.1 OVERALL SUMMARY

Chromosomal rearrangements, such as deletions, duplications, inversions and translocations, account for a broad spectrum of human genetic disorders, including Triple-X, Klinefelter, Turner, Down, Edwards, Patau, DiGeorge, Smith-Magenis, Williams, Prader-Willi and Angelman syndrome, to name just a few examples (Iliopoulos 2006; Kesler 2007; Tucker 2007; Mégarbané 2009; Tartaglia 2010; Wikström 2011). In order to unravel dosage-sensitive genomic regions and genes, and to gain better understanding of the development and pathophysiology of these human diseases, chromosomal rearrangements need to be generated in model organisms. The mouse is an excellent organism of choice because it shares many similarities with humans, both in terms of biology and genetics, and because its genome can be easily modified using chromosome engineering techniques, allowing the generation of defined chromosomal rearrangements. To date, many mouse models carrying defined genomic rearrangements have been successfully developed (Corral 1996; Jiang 1998; Sago 1998; Yang 1998; Kimber 1999; Lindsay 1999; Tsai 1999; Zheng 1999; Puech 2000; Lindsay 2001; Merscher 2001; Walz 2003; Walz 2003; Olson 2004; Yan 2004; Bi 2005; Skryabin 2007; Li 2009), giving new insights into dosage-sensitive genes involved in these human genetic disorders, and unravelling the molecular and cellular mechanisms underlying these pathologies.

During my PhD I have used chromosome engineering techniques to develop two monosomic mouse models carrying defined chromosomal deletions syntenic with 21q11.2–q21.1 and 5q35.2–q35.3 in humans.

The first mouse model, carrying a deletion of the *Lipi–Usp25* region, was develop to model clinical features diagnosed in patients with Monosomy

21, a disorder associated with intellectual disability, craniofacial, skeletal and/or cardiac abnormalities, and respiratory complications (Chettouh 1995; Riegel 2005; Lyle 2008; Katzaki 2010; Lindstrand 2010; Roberson 2010). Monosomic mice displayed impaired long-term memory retention in a socially relevant testing paradigm. Thus these monosomic mice have broadened our understanding of genes involved in Monosomy 21-associated intellectual disability and will be of great importance in future studies of genotype-phenotype correlations in Monosomy 21 patients and in identifying the molecular causes underlying this phenotype. Moreover, monosomic mice fed on a HFD exhibited a significant increase in fat mass/fat percentage estimate, severe fatty changes in their livers, and thickened subcutaneous fat. Thus a gene (or genes) within the *Lipi–Usp25* interval is also involved in the regulation of fat deposition. The identification of the HFD-induced increase in fat deposition in our monosomic mice was somewhat surprising, as to date only one study has reported obesity in patients with Monosomy 21 syndrome (Roland 1990). Thus further studies will be required to understand the molecular mechanisms linking deletions of (or encompassing) the 21q11.2–q21.1 region and HFD-induced increased fat deposition.

The second mouse model, carrying a deletion of the *4732471D19Rik–B4galt7* region, was developed to model clinical features diagnosed in patients with Sotos syndrome, an overgrowth disorder associated with advanced bone age, intellectual disability, hypotonia, facial, cardiovascular and/or urinary/renal abnormalities (Cole 1994; Tatton-Brown 2007). Monosomic mice showed dilation of the pelvicalyceal system in the kidneys, which models the hydronephrosis observed in patients with Sotos syndrome (SoS). Thus these monosomic mice have recapitulated the abnormality of the urinary/renal system observed in patients with Sotos syndrome, contributed new insights into genes involved in SoS-associated urinary/renal abnormalities, and will play an important role in establishing genotype-phenotype correlations in patients with Sotos syndrome and identifying molecular causes of this phenotype.

5.2 CORRELATION BETWEEN THE RESULTS GATHERED FROM MONOSOMIC *Df*$^{Lipi\text{-}Usp25}$ AND *Df*$^{4732471D19Rik\text{-}B4galt7}$ MICE AND SYMPTOMS IN PATIENTS WITH MONOSOMY 21 AND SOTOS SYDNROME

The detailed phenotypic analysis of two monosomic mouse models led to the identification of a range of phenotypic abnormalities. Some of the defects, such as dilation of the pelvicalyceal system in monosomic *Df*$^{4732471D19Rik\text{-}B4galt7}$ mice, fully recapitulated clinical symptoms observed in patients with Sotos syndrome. Similarly, both monosomic *Df*$^{Lipi\text{-}Usp25}$ and *Df*$^{4732471D19Rik\text{-}B4galt7}$ mice displayed long-term memory deficit and learning impairment, and so seem to model, at least to a certain extent, the intellectual disability observed in individuals with Monosomy 21 and Sotos syndrome. However, we need to be aware of the broadness of the term “intellectual disability” since, in addition to long-term memory deficit and learning impairment, other areas, such as speech development, might also be impaired in humans. These other characteristics cannot be captured by the tests used here. Further, some of the other identified anomalies, such as increased fat deposition and liver steosis in HFD-fed monosomic *Df*$^{Lipi\text{-}Usp25}$ mice, have never been reported in Monosomy 21 indviduals. Conversely, some of the symptoms observed in humans with Sotos syndrome, including cardiac abnormalities and advanced bone age, were not identified in monosomic *Df*$^{4732471D19Rik\text{-}B4galt7}$ mice.

The inability to recapitulate some of the clinical phenotypes identified in patients with deletion syndromes clearly demonstrates the existence of significant developmental differences between mice and humans, and so places certain limitations on the use of mice as a model organism (at least in some cases). Also, we need to be aware that certain phenotypic features that are observed in humans with genomic disorders might be impossible to model or reliably identify in mice. For example, facial abnormalities, such as large/simple ears, nose or broad mouth observed in Monosomy 21 patients or high, broad forehead (the head is said to resemble an inverted pear), fronto-temporal hair sparsity, malar flushing, down-slanting palpebral fissures and a pointed chin observed in Sotos syndrome individuals, cannot be reliably

modelled in mice due to significant differences in facial appearance between mice and humans.

5.3 STRENGTHS AND WEAKNESSES OF THE APPROACH TAKEN AND THE RESULTS GENERATED

We have used chromosome engineering to generate monosomic *Df*$^{Lipi\text{-}Usp25}$ and *Df*$^{4732471D19Rik\text{-}B4galt7}$ mice to model human Monosomy 21 and Sotos syndromes, respectively. The approach taken has both strengths and weaknesses.

One of the important strengths is the similarity between mice and humans, namely they show about 99% gene identity, and have similar developmental programs and physical form. This means that human syndromes, caused by various genetic alterations, can often be accurately modelled in mice.

The other strength is the relative ease of working with mice. There are well-established methods for introducing particular mutations into the mouse genome (such as the deletions described here) which, because of the short mouse gestation period, can be achieved in reasonable time. Mice are also easy to house, as they require little space. Moreover, whilst using mice, many of the ethical considerations related to working with higher mammals can be avoided.

The approach used here offers an efficient method of determining the exact genes that are responsible for a given human phenotype. By initially deleting a chromosomal segment containing multiple genes, it is possible to construct models with successively smaller and smaller deletions, each time narrowing down the region containing the relevant gene. Ultimately, as happened for example in PWS syndrome, it is often possible to finally identify the causative gene accounting for a given syndrome.

Despite these advantages, there are certain disadvantages of working with mice and to the approach applied here. Firstly, although many features of development and anatomy are shared between mice and humans, there are also significant differences and it cannot be assumed that a given feature will be identical between these two organisms. This means that even an exact

model of a given human deletion in mice is never guaranteed to recapitulate the same syndrome. Even if certain phenotypes are shared between human patients and the mouse model, many other phenotypes may differ, and so we can never be sure that the model really represents the human syndrome. Further, certain human phenotypes can never be fully recapitulated in mice due to differences in body shape, anatomy and development. Perhaps it would be also interesting to model human deletions in another organism, perhaps one closer to humans, which might better capture the observed human phenotype.

A further problem is that some of the human genes do not have syntenic counterparts in mice, as is the case with two genes in our mouse model of Monosomy 21. Thus the deletion in mice is only an approximation to the deletion in humans. It is possible that the human genes that do not have mouse counterparts might play an important role in the human syndrome and may explain many of the observed human manifestations.

Another weakness of our approach is that we only use a few selective tests to test a particular phenotypic feature. For example, we use a social recognition test to attempt to search for such a complex condition as intellectual disability. Although it is difficult to devise better-suited tests, using such selective tests can result in misleading findings. For example, just because a group of mice performs normally in the social recognition test, does not necessarily means that they do not have other intellectual disabilities.

Finally, it is worth mentioning that, in our monosomic $Df^{4732471D19Rik\text{-}B4galt7}$ mice, a large number of genes were deleted, and so using this approach to try to determine the causative gene, by generating progressively smaller and smaller deletions, can be both difficult and time-consuming.

5.4 FURTHER STUDIES THAT COULD BE UNDERTAKEN IN BOTH MICE AND HUMANS

In order to better understand and further analyse the initial results obtained from *Lipi-Usp25* and *4732471D19Rik–B4galt7* monosomic mice, further studies in both mice and humans could be undertaken.

Both Monosomy 21 and Sotos syndrome patients are described with intellectual disability. However, this term is very broad and encompasses various intellectual impairments, such as mental retardation, learning disability and speech development. Thus, it would be interesting to re-examine Monosomy 21 and Sotos syndrome patients to clarify the type of intellectual disability. This would be a great help in designing behavioural tests that could be used to test monosomic $Df^{Lipi\text{-}Usp25}$ and $Df^{4732471D19Rik\text{-}B4galt7}$ mice more adequately in order to be able to properly correlate results obtained from mouse models with human data.

Both monosomic $Df^{Lipi\text{-}Usp25}$ and $Df^{4732471D19Rik\text{-}B4galt7}$ mice have shown hippocampal-dependent long-term memory deficit and learning abnormalities in a social recognition paradigm. Further behavioural tests could be conducted, not only to confirm these findings, but also to expand on these results by applying behavioural tests that would be able to model other aspects of human intellectual disability and would be suitable for detecting impairments in different areas of the brain. For example, to confirm the existence of a long-term memory deficit and learning abnormalities in monosomic $Df^{Lipi\text{-}Usp25}$ and $Df^{4732471D19Rik\text{-}B4galt7}$ mice in a non-social context, a novel object recognition test could be applied. For instance, Morris water maze could be used to test for anomalies in spatial learning and memory, or a cued fear conditioning test could be applied to check amygdalar activity, or a contextual fear conditioning test to investigate both amygdalar and hippocampus functioning.

Monosomic $Df^{Lipi\text{-}Usp25}$ mice have shown increased fat deposition when being fed on a high-fat diet. In our study, we were unable to determine a reason for this phenomenon. In order to understand the cause of increased fat deposition, further test could be conducted, including indirect calorimetry testing at a later stage of life of monosomic $Df^{Lipi\text{-}Usp25}$ mice. Also, it might be worth carrying out a microarray analysis on brain samples collected from HFD-fed monosomic $Df^{Lipi\text{-}Usp25}$ mice to try to find genes that might be up- or down-regulated, and so to find the genes that might be contributing to the observed phenotype. Also, it would be of interest to re-examine Monosomy 21 patients for the presence or absence of obesity. Subsequently, if any of the patients are indentified with obesity, it would be important to look into their diet

to investigate whether the observed obesity in these patients was caused by the interaction between the genetic component (deletion of the 21q11.2–q21.1 region) and an environmental factor (such as consumption of food highly-enriched in fat).

Further, in order to establish whether overgrowth, a cardinal feature diagnosed in patients with Sotos syndrome, could be observed in monosomic *Df*$^{4732471D19Rik\text{-}B4galt7}$ mice at pre- and/or postnatal stage of their development, the growth curves should be generated.

Finally, as a long term goal, mouse models carrying smaller and smaller deletions of the initial *Lipi-Usp25* and *4732471D19Rik–B4galt7* intervals could be generated to find the causative genes responsible for, on one hand, long-term memory deficit, learning abnormalities and increased fat percentage in monosomic *Df*$^{Lipi\text{-}Usp25}$ mice, and, on the other hand, for long-term memory deficit, learning abnormalities and a dilation of pelvicalyceal system in monosomic *Df*$^{4732471D19Rik\text{-}B4galt7}$ mice.

To sum up, generation and analysis of monosomic mouse models of Monosomy 21 and Sotos syndrome have broadened our understanding of these human pathologies by recapitulating Monosomy 21-associated intellectual disability and SoS-associated hydronephrosis, as well as revealing previously unreported phenotypes, such as the HFD-induced increase fat deposition in Monosomy 21 mice.